8.2 Intestinal Amoeba – *Entamoeba Histolytica*

8.2.1 Geographical Distribution

It is Worldwide in distribution they are more common in the tropics than elsewhere. It is found wherever sanitation is poor.

8.2.2 Habitat

Trophozoites of *E.histolytica* live in the mucous and submucous layers of the large intestine of human.

8.2.3 Morphology

E.histolytica occurs in 3 forms as Trophozoite, Precyst and Cyst.

Trophozoite: It is the growing or feeding stage of the parasite. It is the only form present in tissues. It has no fixed shape. They vary in size from 18 to 40µ, average being 20 to 30µ. The cytoplasm is usually described as outer ectoplasm and inner endoplasm (Figure 8.1). The endoplasm contains nucleus, food vacuoles, erythrocytes, occasionally leucocytes and tissue debris. The nucleus is characterised by evenly arranged chromatin on the nuclear membrane and the presence of a small, compact, centrally located karoyosome (It is a DNA containing body, situated peripherally or centrally within the nucleus). Trophozoites exhibits active crawling or gliding motility by forming finger-like projections called Pseudopodia.

The trophozoite reproduce by binary fission in every 8 hours. Trophozoites survives upto 5 hours at 37°C and are killed by heat, drying and chemical sterilization. Even if live trophozoites from freshly passed stools are ingested, they are rapidly destroyed in stomach and cannot initiate infection. Therefore, the infections is not usually transmitted by trophozoites.

Precyst

Trophozoites undergo encystment in the intestinal lumen. Encystment does not occur in the tissue or in feces outside the body. Precyst is smaller in size about 10 -20 µm in size. It is round or oval in shape. The endoplasm is free of red blood cells and other ingested food particles (Figure 8.1). The nuclear structure retains the characteristics of the trophozoite.

Cyst

Precyst secretes a highly refractive cyst wall around it and becomes a cyst. A mature cyst is a quadrinucleate spherical body. The cyst begins as a uninucleate body but soon divides by binary fission and develops into binucleate and quadrinucleate bodies (Figure 8.1). The cytoplasm of the cyst is clear and hyaline (translucent) and the nuclear structure retain the characteristic of the trophozoites.

The mature quadrinucleate cyst, passed in the stool, do not undergoes any further development and remain alive for several months in the soil or in environment where they were deposited. The mature quadrinucleate cysts are the infective forms of the parasite.

8.2.4 Life – Cycle of Entamoeba Histolytica

E. histolytica passes its life cycle only in one host, the human.

Infective form: Mature quadrinucleate cyst.

Mode of transmission: Ingestion of food and water contaminated with cyst.

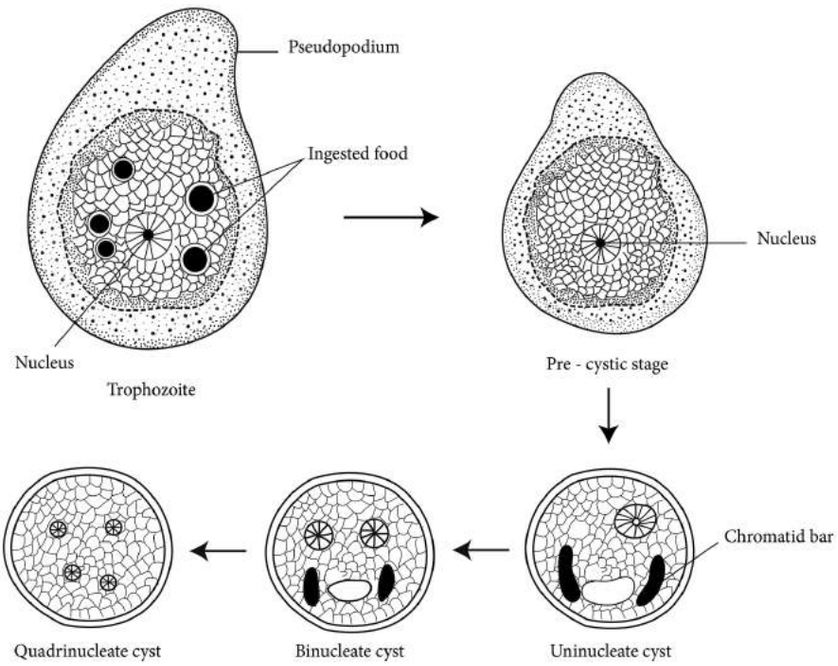


Figure 8.1: Trophozoite, precyst and cyst of *Entamoeba histolytica*

- The cyst that are swallowed along with food and water enters into the alimentary canal. The cyst wall is resistant to action of gastric juice. The cyst pass through the stomach undamaged and enters the small intestine (Figure 8.2).
- When the cyst reaches caecum or lower part of the ileum, due to alkaline medium, the cyst wall is damaged by trypsin leading to excystation.
- The cytoplasm gets detached from the cyst wall and an amoeboid movement appear causing a tear in the cyst wall, through which quadrinucleate amoeba is liberated. This stage is called the metacyst.
- The nuclei in the metacyst immediately undergo division to form 8 nuclei, each of which gets surrounded by its own cytoplasm to become 8 small amoebulae or metacystic trophozoites.
- These metacystic trophozoites are carried to the caecum and colon. They invade the tissues and lodge in the submucous tissue of the large intestine which is their normal habitat.
- Trophozoite grow and multiply by binary fission. The trophozoite phase of the parasite is responsible for producing the characteristic lesion of amoebiasis.

The amoeba infecting man may be classified according to their pathogenicity and habitat.

A. Pathogenic

Intestinal Amoeba: *Entamoeba histolytica*

B. Non pathogenic

1. Mouth Amoeba: *Entamoeba gingivitis*
2. Intestinal Amoeba: *Entamoeba coli*
   Entamoeba nana

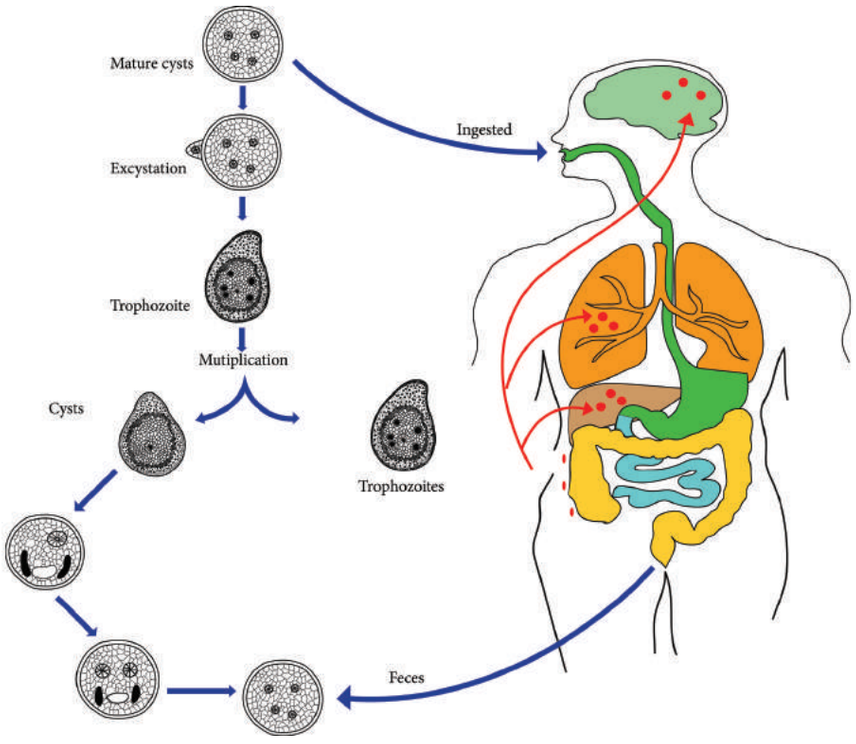


Figure 8.2: Life cycle of *Entamoeba histolytica*

- Some of the trophozoites in colon develop into precystic forms and cysts, which are passed in feces to repeat the cycle.

8.2.5 Pathogenesis

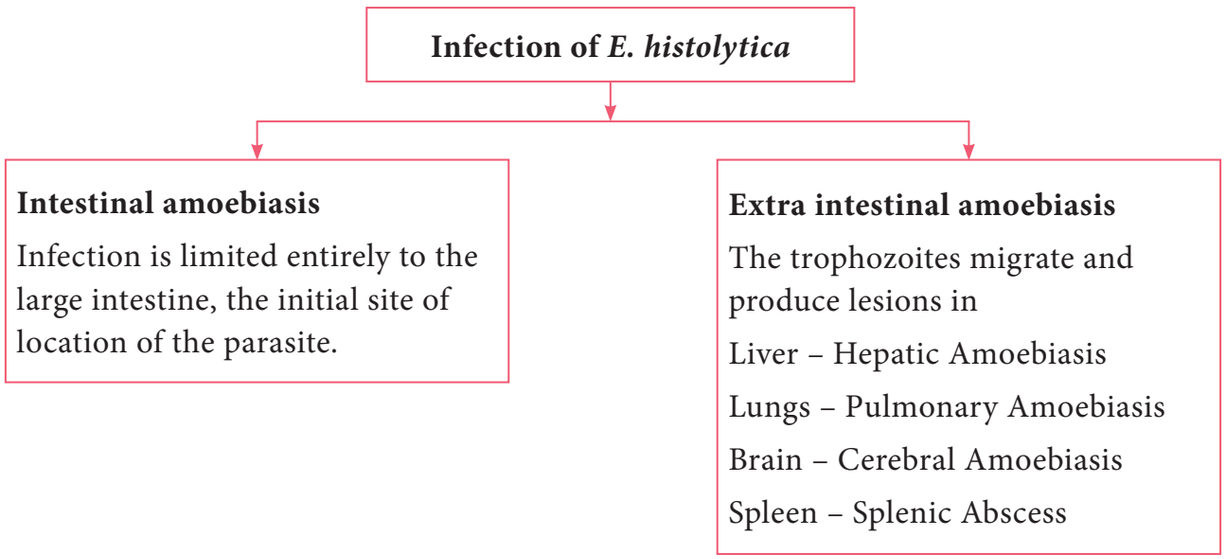
E. histolytica causes intestinal and extra intestinal amoebiasis (Flowchart 8.3).

E. histolytica can live in the intestine without causing symptoms. But, they can also cause severe disease. These amoebas may invade the wall of the intestine leading to amoebic dysentery, an illness that causes intestinal ulcers, bleeding, increased mucus production and diarrhoea. The ulcers are strictly confined to the large intestine being most numerous in the caecum and next in the sigmoid-rectal regions. The lesions may be generalized or localised. A typical amoebic ulcer varies from pin's head to one inch or more in diameter in size. The shape of ulcer may be round or oval. On vertical section, the ulcer appears like flask, with mouth and neck being narrow and base being large and rounded (Figure 8.3 shows the flask – shaped ulcer). The base of ulcer is generally formed by the muscular coat and filled up by the necrotic material. The ulcers generally do not extend deeper than submucosal layer.

8.2.6 Clinical Features

Incubation period is highly variable, but is generally 4 to 5 days.

A wide spectrum, from asymptomatic infection (luminal amoebiasis), to invasive intestinal amoebiasis (dysentery, colitis, appendicitis, toxic mega colon, amoebomas), to invasive extraintestinal amoebiasis occurs. Flowchart 8.4 classifies the clinical outcomes of infection with *Entamoeba histolytica*. Only about 10% to 20% of people who are infected with *E. histolytica* become sick from the infection.



Flowchart 8.3: Infection Caused by E.*histolytica*

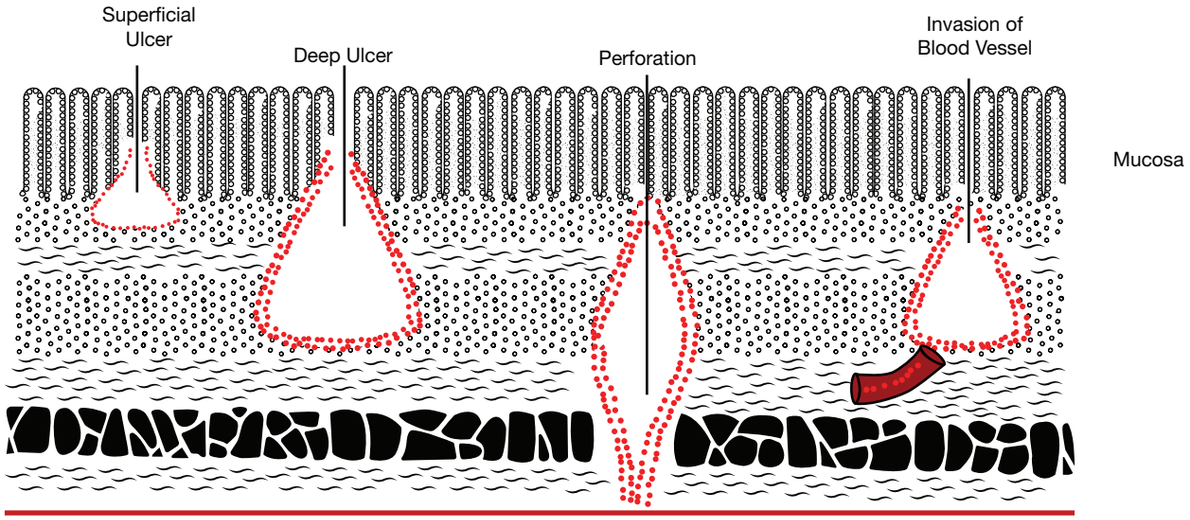
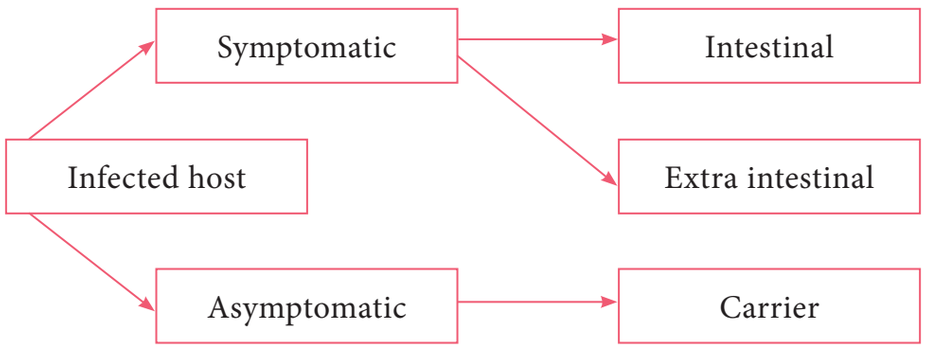


Figure 8.3: Ulcers in intestinal amoebiasis



Flowchart 8.4: The clinical outcomes of infection with *Entamoeba histolytica*

The typical manifestation of intestinal amoebiasis is amoebic dysentery. The symptoms are often quite mild and can include loose feaces, stomach pain and stomach cramping. In acute amoebic dysentery, the symptoms include abdominal pain, bloody stool, fever, tenderness, rectal tenesmus and hepatomegaly (enlargement of liver). People affected may develop anemia due to loss of blood. On clinical and laboratory ground, amoebic dysentery should be differentiated from bacillary dysentery. A Table 8.1 shows the difference between the stools of amoebic and bacillary dysentery.

Extra intestional amoebiasis

1. **Hepatic amoebiasis:** This is the most common form of extra intestinal invasive amoebiasis. Liver abscess may be multiple or more often solitary, usually located in the upper right lobe of the liver (Figure 8.4). Amoebic liver abscess (ALA) contains an odour less and thick chocolate brown pus called anchovy sauce pus. ALA is associated with an abrupt onset of high fever, right upper abdominal pain and tenderness. Anorexia (loss of appetite for food), nausea (the sensation to vomit), vomiting, fatigue (extreme tiredness) and weight loss are also frequent.

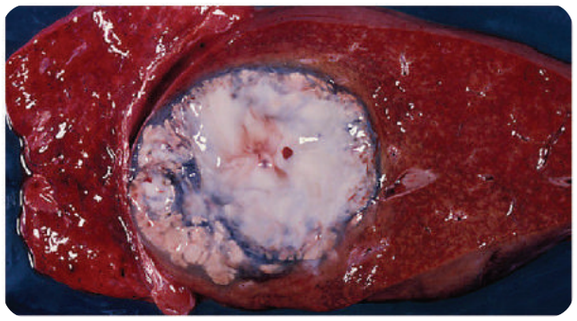


Figure 8.4: Amoebic liver abscess

2. **Pulmonary Amoebiasis:** It is very rare, but this may occur when direct hematogenous spread from the colon. The patient presents with severe chest

Table 8.1: Difference between the stools of amoebic and bacillary dysentery

	Amoebic dysentery	Bacillary Dysentery
Macroscopic observation		
Number	6–8	Over 10 motions a day
Amount	Relatively large	Small
Odour	Offensive	Odorless
Colour	Dark red	Bright red
Nature	Blood and mucus mixed with faeces	Blood and mucus no faeces
Microscopic observation		
RBC	In clumps, reddish – yellow in colour	RBC in rouleaux, bright red in colour
Pus cells	Scanty	Numerous
Parasite	Trophozoites of *E. histolytica*	Nil
Charcot – Leyden crystals	Present	Nil

pain and have dyspnoea (shortness of breath). The sputum of patient is chocolate brown. Amoebic trophozoites may be demonstrated in the sputum.

3. **Cerebral amoebiasis:** The condition is unusual. In cerebral amoebiasis, the abscess is single, small and is located in the cerebral hemisphere. The patient may die of rupture or involvement of cerebellam within 12–72 hours. Biopsy of the brain shows the amoebic trophozoites.
4. **Cutaneous amoebiasis:** It can be caused by perforation of an amoebic abscess or surgical wound infected with amoebae. It is less frequent condition.
5. **Genitourinary Amoebiasis:** This condition includes amoebiasis of the kidney and genital organs. Amoebiasis of the genital organs is a rare condition. Lesions of amoebiasis is shown in Figure 8.5.

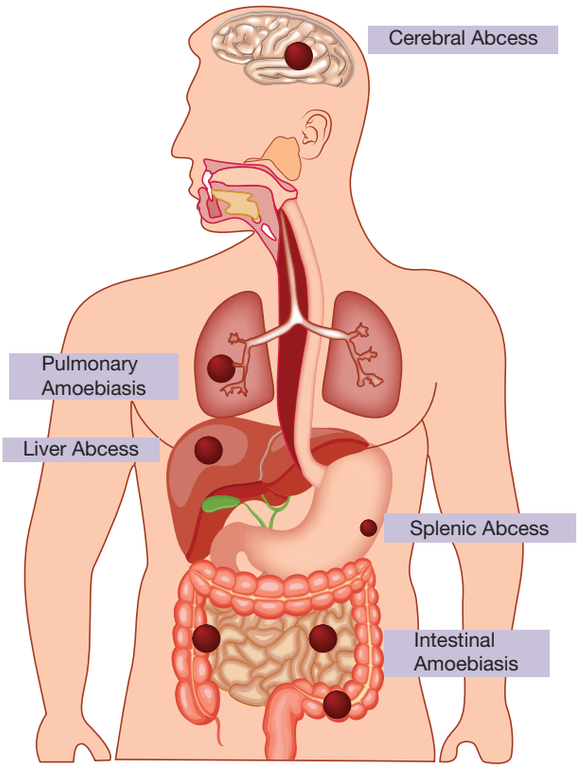


Figure 8.5: Extra-intestinal amoebiasis

8.2.7 Laboratory Diagnosis

Specimens: Stool is the specimen of choice. Other specimens collected includes blood, rectal exudates and rectal ulcer tissue collected from the base by endoscopies.

Methods in examination of stool

A. Direct wet mount examination of stool: Demonstration of mature quadrinucleate cysts or trophozites in stool is diagnostic of intestinal amoebiasis. The wet mount of stool is prepared in the saline, iodine or lacto phenol cotton blue.

B. Examination of stool after concentration: Demonstration of amoebic cysts by Formalin – ether is the method of choice.

C. Examination of stained stool smears: Staining by iron haematoxylin, Periodic Acid – Schiff (PAS) stains demonstrate the presence of both trophozoites and cyst.

Amoebic liver abscess (ALA): Demonstration of amoebic trophozoites in the aspirated liver pus establishes the diagnosis of ALA.

Serology: Detection of amoebic antigens in the serum by Enzyme Linked Immunosorbent Assay (ELISA).

Molecular diagnosis: PCR (Polymerase chain reaction) is employed to detect amoebic genome in the aspirated liver pus for the diagnosis of ALA.

Imaging methods: X – Ray magnetic resonance imaging (MRI) scan and computerized Axial Tomography (CAT) Scan are the imaging methods used.

Treatment: Eradication of amoebae by the use of amoebicidal drugs and replacement of fluid and electrolyte is the treatment for amoebiasis. Listed below the drugs used in the treatment for amoebiasis.

- Paramomycin and iodoquinol acts in the intestinal lumen but not in tissues.
- Emetine, chloroquine are effective in systemic infection. They act only on trophozoites.

Metronidazole is the drug of choice which acts as both luminal and tissue amoebicides. It is low in toxicity and is effective against intestinal as well as extra -intestinal amoebic infections.

8.2.8 Prevention and Control

- Proper sanitation is the key to avoid amoebiasis. Washing hands with soap and water after using the bathrooms and before handling food.
- Drinking safe and boiled water.
- Avoid eating unwashed fruits and vegetables.
- Prevention of water supplies from faecal contamination.
- Early rapid detection of diseased people and subsequent treatment with amoebicidal drugs. No vaccine is available yet against amoebiasis in humans.

8.3 Intestinal Flagellates – *Giardia Lamblia*

(Also known as *Giardia duodenalis, Giardia intestinalis*)

8.3.1 Geographical Distribution

It is the most common protozoan pathogen and is worldwide in distribution. The disease is very high in areas with low sanitation, especially tropics and subtropics.

8.3.2 Habitat

Giardia lamblia lives in the duodenum and upper jejunum of human. It is the only protozoan parasite found in the lumen of the human small intestine.

8.3.3 Morphology

It exists in two forms

- Trophozoite and
- Cyst

Antoine van Leewenhock observed and illustrated *Giardia lamblia* in his own loose stool. This was the first protozoan parasite of human that is recorded and the first to be seen under a microscope.

Trophozoite

The trophozoite is in the shape of a tennis or badminton racket. It is rounded anteriorly and pointed posteriorly. The size of the trophozoite is 14 µ long by 7µ broad. Dorsally, it is convex and ventrally, it has a concave sucking disc which helps in its attachment to the intestinal mucosa. It is bilaterally symmetrical. All the organs of the body are paired. Trophozoite of Giardia possess,

- 1 pair of nuclei
- 4 pairs of flagella
- Parabasal body (Blepharoplast), from which the flagella arise (4 pairs)

- 1 pair of axostyles, running along the midline
- Two sausage – shaped parabasal or median bodies lying transversely posterior to the sucking disc
- The trophozoite is motile, with a slow oscillation about its long axis, often resembling falling leaf (Figure 8.6a).

Cyst

It is the infective form of the parasite. The cyst is small and oval, measuring 12 µm × 8 µm and is surrounded by a hyaline cyst wall.

Its internal structure includes 2 pairs of nuclei grouped at one end. A young cyst contains 1 pair of nuclei. The axostyle lies diagnonally, forming a dividing line within cyst wall (Figure 8.6b).

8.3.4 Life Cycle: Giardia Passes Life Cycle in one Host, the Human

Infective form: Mature cyst

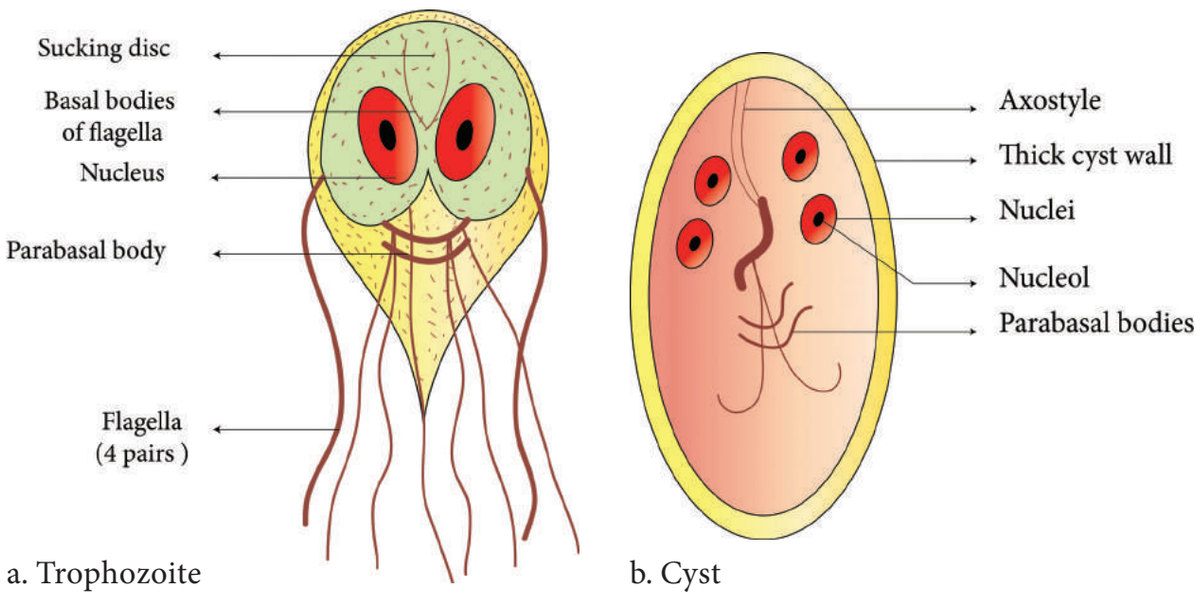
Mode of transmission: Human acquires infection by ingestion of cyst in contaminated water and food. Direct person – to person transmission occurs in children. Transmission occurs through oral-anal and oral-genital route in sexually active homosexual males. Within half an hour of ingestion, the cyst hatches out into two trophozoites, which multiply by binary fission and colonize in the duodenum. The trophozoites live in the duodenum and upper part of jejunum, feeding by pinocytosis. When conditions in duodenum are unfavourable, encystment occurs, usually in large intestine. Cysts are passed in stool and remain viable in soil and water for several weeks (Figure 8.7).

8.3.5 Pathogenicity

Giardia lamblia does not invade the tissue, but remains attached to intestinal epithelium by means of the sucking disc. It causes a disturbance of intestinal function leading to malabsorption of fat.

8.3.6 Clinical Manifestations

Incubation period is variable, but is usually about 2 weeks.



a. Trophozoite b. Cyst

Figure 8.6: Trophozoite and cyst of *Giardia*

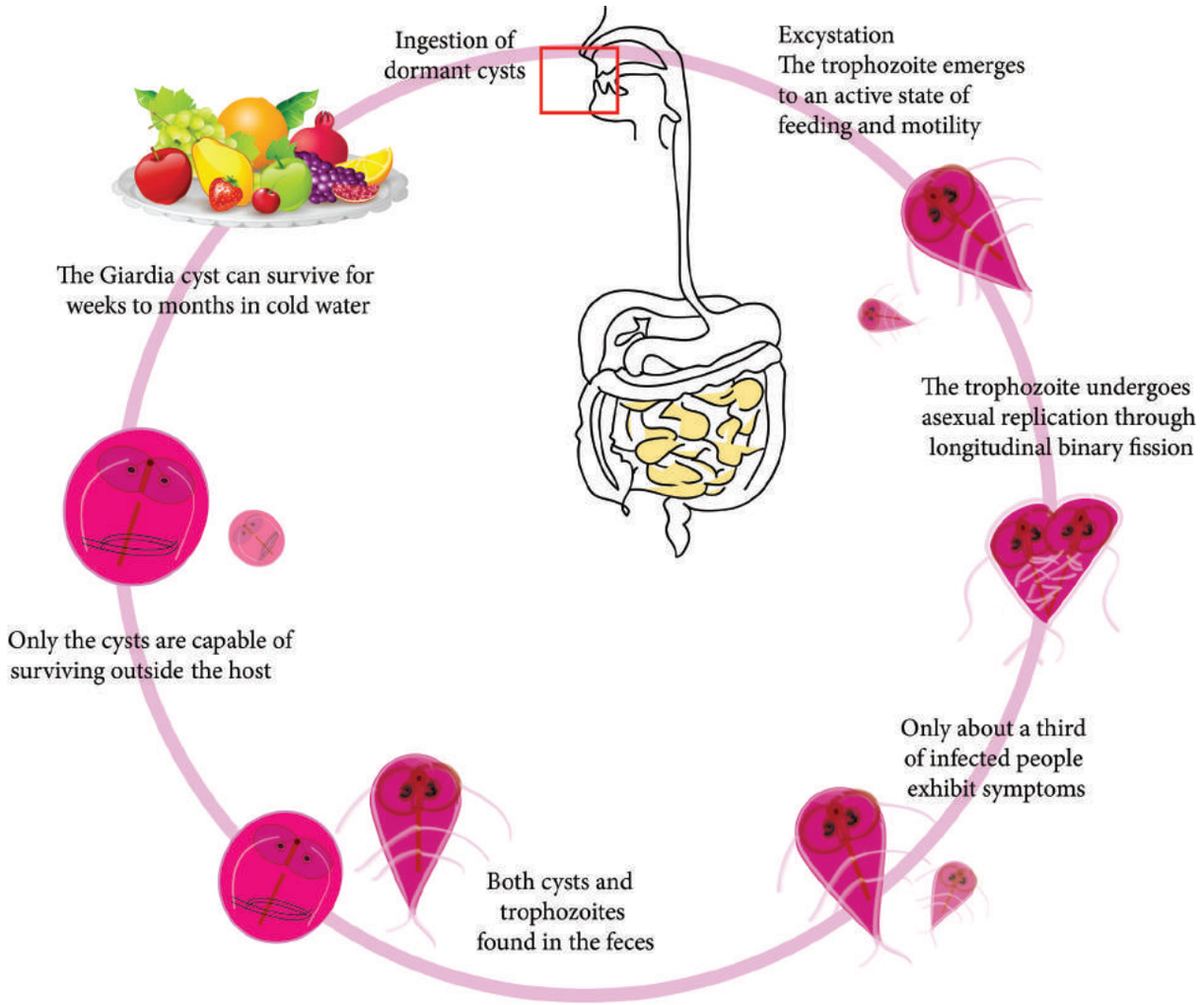


Figure 8.7: Life cycle of *Giardia lamblia*

The disease is asymptomatic, but in some cases it may lead to abdominal cramps, flatulence, looseness of bowels, foul smelling stool and mild steatorrhoea (passage of yellowish and greasy stools in which there is excess of fat). The stool contains excess mucus and fat but no blood and pus. Children may develop chronic diarrhoea, malaise (discomfort), nausea, anorexia (loss of appetite for food), malabsorption of fat, vitamin A and protein. Occasionally, Giardia may colonize the gall bladder causing biliary colic and jaundice.

8.3.7 Laboratory Diagnosis

Specimens: Stool and blood

Examination of stool sample: Giardiasis can be diagnosed by identification of cysts of *Giardia lamblia* in the formed stools and the trophozoites and cyst of the parasite in diarrhoeal stools.

Macroscopic examination of stool: Fecal specimens containing *Giardia lamblia* may have an offensive odor. It is pale coloured with fatty substance floating in water.

Microscopic examination of stool: Cysts and trophozoites can be found in

diarrheal stools by saline and iodine wet preparations (Figure 8.8).

Serodiagnosis: Immuno chromatographic strip tests and indirect immunofluroscence (IIF) tests are readily available. For antigen and antigen detection ELISA, Commercially available ELISA kits detects Giardia – Specific antigen.

Molecular methods: DNA probes and polymerase chain reaction (PCR) have been used to demonstrate parasitic genome in the stool specimen.

8.3.8 Treatment

Metronidazole and Tinidazole are the drugs of choice.

8.3.9 Prevention and Control

Giardiasis can be prevented and controlled by,

- Proper disposal of human faeces, maintenance of food and personal hygiene and health education.
- After using the bathroom and before eating, the hands should be washed thoroughly with soap and warm water. Boiling of water is the best and effective method in killing the viable cysts.
- To reduce the risk of venereal transmission, patients should avoid risky sexual behavior.
- No vaccine or effective chemo prophylactic drug is available for prevention of Giardiasis.

8.4 Tissue Flagellates – *Leishmania donovani*

The genus is named after the scientist Leishman, who first described the parasite in London in May 1903.

8.4.1 Geographical Distribution

Leishmania species is found in the Mediterranean, the Middle East, Africa and Asia including India.

8.4.2 Habitat

Leishmania donovani is an obligate intracellular parasite of human and other mammalian hosts. They are always found as intracellular amastigotes in the reticuloendothelial cells of the spleen, bone marrow, liver, intestinal mucosa and mesenteric lymph nodes of hosts.

8.4.3 Morphology

The parasite exists in two forms:

Amastigote: It is the form found in human and other mammalian hosts. They are found inside monocytes, polymorphonuclear leucocytes or endothelial cells. They are small, round to oval bodies measuring 2–3µm in length (Figure 8.8). They are also known as LD (Leishman donovan) bodies.

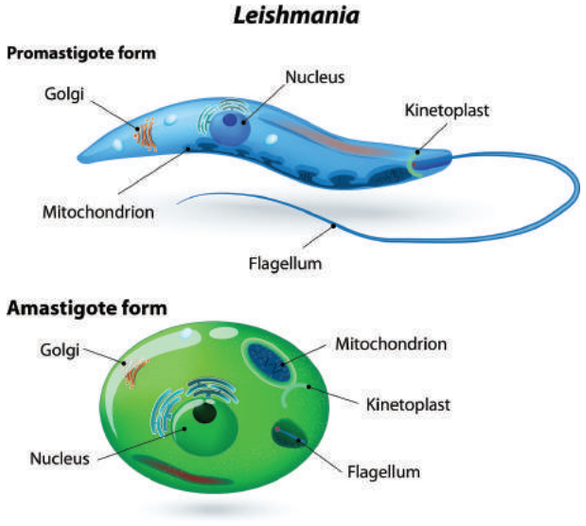


Figure 8.8: Promastigote and Amastigote form of Leishmania

Promastigote: This forms are found in the mid-gut of sand fly and in the culture media. The fully developed promastigotes are long, slender and spindle – shaped. They measure 15µm to 25µm in length and 1.5µm to 3.5µm in breadth. A single nucleus is situated at the centre. The kinetoplast lies near the anterior end. The flagellum is single, delicate and measures 15µm–28µm (Figure 8.8).

Infobits

There are 3 main forms of Leishmaniases – Visceral (also known as Kala-agar and the most serious form of the disease), cutaneous (the most common) and mucocutaneous. The disease affects some of the poorest people on earth, and is associated with malnutrition, population displacement, poor housing, a weak immune system and lack of financial resources. Leishmaniasis is linked to environmental changes such as deforestation, building of dams, irrigation schemes, and urbanization.

PKDL occurs in all areas endemic for L.donovani but is commonest in East Africa and on the Indian subcontinent, where up to 50% and 10% of patients with kala-azar, respectively develop the condition. The frequency is reported to be declining in India.

Cutaneous Leishmaniasis

The clinical spectrum of cutaneous leishmaniasis (oriental sore) is broad and may mimic that of other skin conditions, such as staphyloccal or streptococcal infection, mycobacterial ulcer, leprosy, fungal infection, cancer, sarcoidosis and tropical ulcer.

8.4.4 Life – Cycle of Leishmania donovani

Leishmania donovani completes its life cycle in two different hosts. The complete life cycle is given in Figure 8.9.

Host	Forms
Human and other mammals (Example: Dogs)	Amastigote
Sandfly of Genus *Phlebotomus*	Promastigote

Development in Human

The parasite is transmitted to human and other vertebrate hosts by the bite of blood sucking female sandfly. During the blood meal, the sandfly deposists promastigotes on surface of the skin. These promastigotes are immediately phagocytosed by fixed macrophages of the host, in which they are transformed into amastigotes. The amastigotes multiply by binary fission within the macrophages. As many as 50 to 200 amastigotes may be present inside the enlarged cell. These are called LD bodies. The rupture of cell releases amastigotes in large numbers which inturn are free to infect other cells. Free amastigotes are subsequently carried by circulation. These forms invade monocytes of the blood and macrophages of the spleen, liver, bone marrow, lymph nodes and other tissues of the reticuloendothelial cells.

Development in sandfly

Female sandfly during a blood meal ingest free, as well as intracellular amastigotes in the blood. In the mid gut of the sandfly, the amastigotes are transformed within 72 hours to flagellated promastigotes.

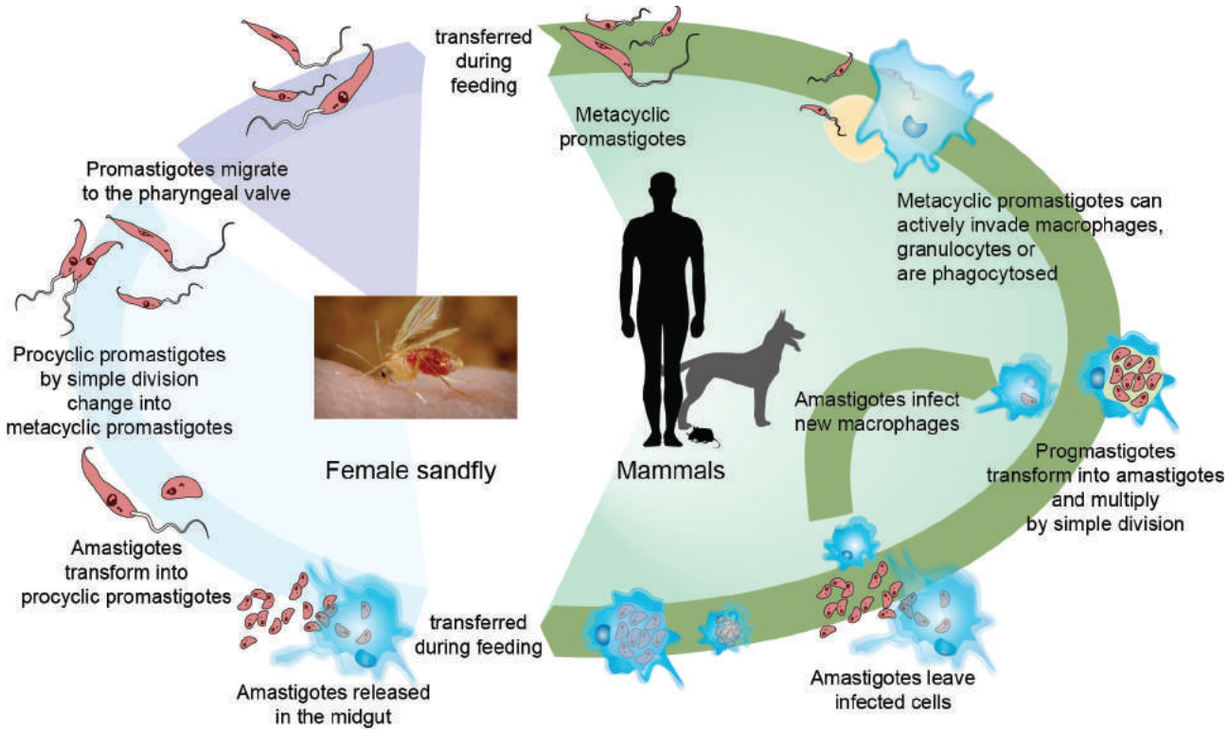


Figure 8.9: Life cycle of *Leishmania donovani*

These promastigotes multiply by binary fission. After a period of 6 to 9 days, these forms migrate from the midgut to the pharynx and buccal cavity of sandfly. Bite of the infected sandfly transmits infection to susceptible persons and the life – cycle is repeated.

8.4.5 Pathogenesis

Leishmania donovani causes visceral Leishmaniasis. The disease is also known as Dum – Dum fever, Asian fever, Assam fever, or infantile splenomegaly. Leishmaniasis is a disease of the reticuloendothelial system. Proliferation and destruction of reticuloendothelial cells of the internal organs are responsible for the pathological changes in visceral leishmaniasis.

Spleen, liver and lymphnodes are enlarged in this condition. Bone marrow is dark red in colour and shows extensive proliferation of reticuloendothelial cells. Kidney shows cloudy swelling and is invaded by macrophages parasitized by amastigotes.

8.4.6 Clinical Features

Incubation period: It is usually 3–6 months but can be months or years.

Visceral Leishmaniasis is a serious and fatal systemic disease. In India, the disease is called Kala – azar meaning "black disease".

The disease is characterized by the presence of fever, hepatosplenomegaly (Figure 8.10) (the simultaneous enlargement of both liver and the spleen), hypergammaglobulinemia (a condition in which increased levels of a certain immunoglobulin in blood serum), Leucopenia, Thrombocytopenia (deficiency of platelets in the blood), Cachexia (a condition that causes extreme weight loss)

with marked anemia, emaciation and loss of weight. Epistaxis (bleeding from nose) and bleeding from gums are common. In Indian patients, the skin on the hands, feet, abdomen, around the mouth and fore – head becomes grayish and dark coloured. This hypo – pigmentation of the skin is unique in Indian patients giving the disease name Kala – azar.

Figure 8.10: Splenomegaly

Post kala – azar dermal leishmaniasis (PKDL): It is a non – ulcerative lesion of the skin, which is seen after completion of treatment of the kala – azar. This condition is characterized by multiple, hypopigmented, erythematous macules involving the face and trunk (Figure 8.11). In Indian forms, PKDL appears after a latent period of 2 years and may even persist as long as 20years, creating a persistent human reservoir of infection.

Laboratory diagnosis

Specimens: Aspiration from spleen, bone marrow, lymph node, liver biopsy and peripheral blood.

Figure 8.11: Post kala – azar dermal leishmaniasis -PKDL

Methods of examination: This includes, microscopy and culture

1. Direct microscopy

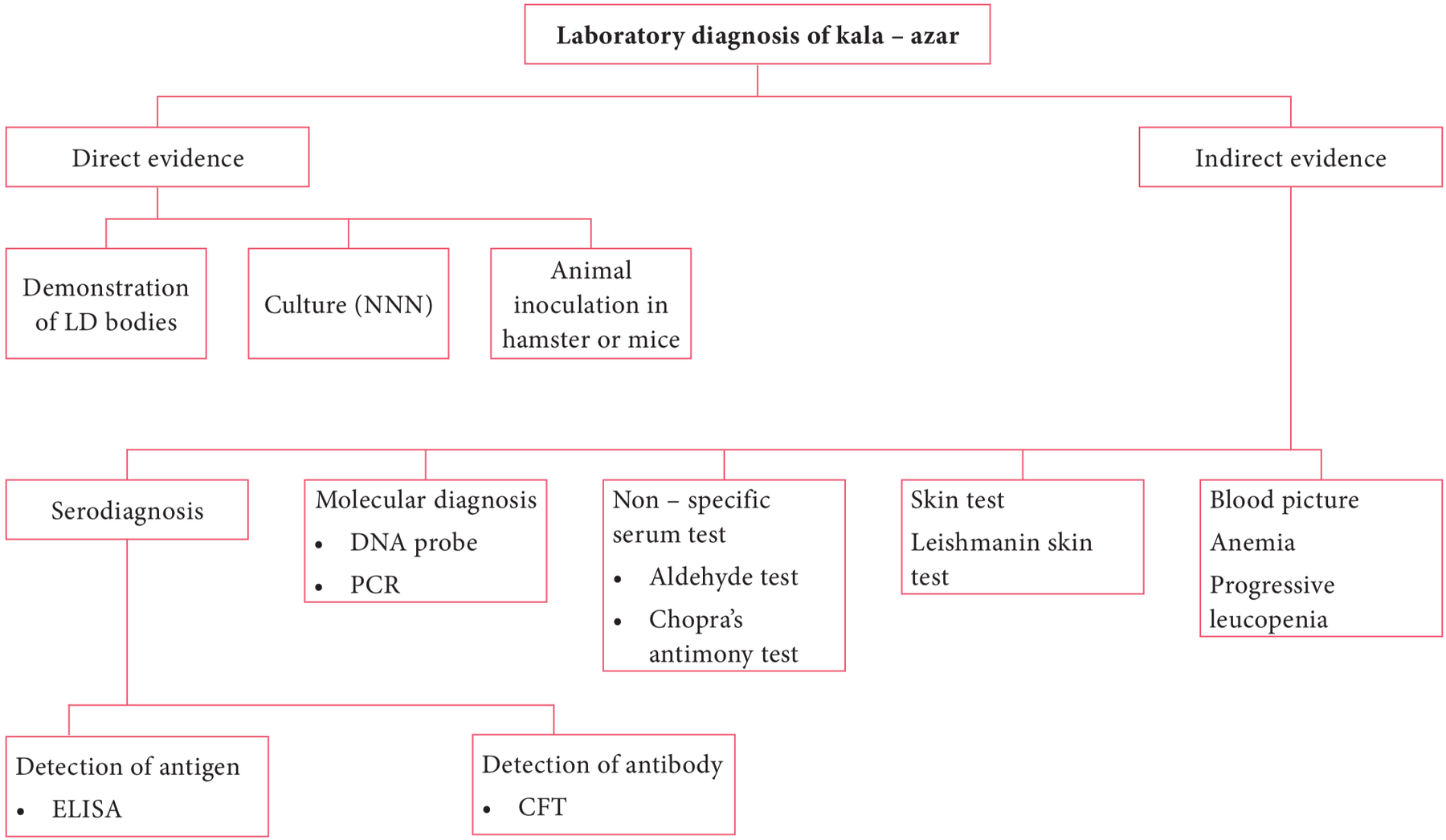
The amastigotes of *Leishmania donovani* (known as LD bodies) can be demonstrated in the smears of spleen, bone marrow, liver, lymph node and peripheral blood stained in Leishman, Giemsa or wright stains. Splenic aspiration is the most sensitive method to detect LD bodies. Examination of peripheral blood smear and buffy coat smear is more commonly used to find LD bodies in the circulating monocytes.

2. Culture

Promastigotes are found in the culture media. Tissue samples and aspirates are inoculated in the NNN (Novy–MacNeal–Nicolle) medium for demonstration of promastigotes.

Laboratory diagnosis of kala – azar is briefly discussed in Flowchart 8.5.

Treatment: Pentavalent antimonials are the drugs of choice. Pentamidine, Amphotericin B and Miltefosine (oral drug) are recommended.



Flowchart 8.5: Laboratory diagnosis of kala-azar

8.4.7 Prevention and Control

Integrated insecticidal spraying (DDT and Malathion) to reduce sandfly population.

Reduction of reservoir by killing all the infected dogs.

Personal prophylaxis by using anti – sandfly measures like using thick clothes, bed nets, window mesh or insect repellants and keeping the environment clean.

No vaccine is available against kala – azar.

8.5 Sporozoa – *Plasmodium*

Protozoan parasites characterised by the production of spore – like oocysts containing sporozoites were known as sporozoa. The parasites belonging to this group of protozoa do not possess any special organs of locomotion, such as flagella or cilia. The medically important parasite of this class that is given in the text is malaria parasites.

Malaria

It is the disease condition with seasonal intermittent fevers, chills and shivering. The name malaria (Mal: bad, aria: air) was given in the 18th century in Italy. The specific agent of malaria was discovered in RBC's of a patient in 1880 by Alphonse Laveran. In 1897, Ronald Ross identified the developing stages of malaria parasites in mosquitoes in Secunderabad, India. This led to various measures for the control and possible eradication of malaria by mosquito control. Both Ross (1902) and Laveran (1907) won the Nobel Prize for their discoveries in malaria.

The single most important protozoan disease is malaria, which causes 1.5 million deaths each year.

Different species of malaria parasites can develop in the same mosquito and such an infected mosquito can transmit the infection to man giving rise to cases of "mixed infection" the commonest being *P. falciparum* with *P. vivax*.

Infobits

Three basic types of malaria

1. Benign tertian (P. vivax and P. orale) with a fever every 2nd day (Example: Monday – fever, Tuesday – no fever, Wednesday – fever).
2. Benign quartan (P. malariae) with a fever every 3rd day. (Example: Monday; fever, Tuesday – no fever, Wednesday – no fever, Thursday – fever.
3. Malignant tertian (P. falciparum), in which the cold stage is less pronounced and the fever stage is more prolonged and severe. This type of malaria is more dangerous because of the complications caused by capillary blockage (i.e, convulsion, coma, acute pulmonary insufficiency and cardic failure). Large numbers of erythrocytes are parasitized and destroyed, which may result in dark-coloured urine. (black water fever); intravascular hemolysis, hemoglobinuria, and kidney failure).

   Two species of plasmodium, P. vivax and P. ovale, can remain in the liver, if not treated properly. The organism leave the liver and re-infect erythrocytes, causing the symptoms.

Causative agents of human malaria:
The organisms: Four species of Plasmodium cause malaria in humans.

- *Plasmodium vivax:* (Benign Tertian malaria)
- *Plasmodium falciparum:* (Malignant tertian malaria)
- *Plasmodium malaria:* (Benign Quartan malaria)
- *Plasmodium ovale:* (Benign tertian malaria)

The two most common species are P. vivax and P. falciparum, WHO reports (2018) that falciparum being the most pathogenic of all.

8.5.1 Geographical Distribution

Malarial parasites are found in all countries. In India, malaria continues to be a major public health threat.

8.5.2 Habitat

The malarial parasites infecting man, after passing through a developmental phase in the parenchyma cells of the liver, reside inside the red blood corpuscles and are carried by the circulating blood to all the organs.

8.5.3 Vectors

Human malaria is transmitted by over 60 species of female Anopheles mosquito.

Human malarial parasite – *Plasmodium falciparum*

Of all the human malaria parasites, *P. falciparum* is the most highly pathogenic and responsible for malignant tertian malaria. This is a form of disease which runs an acute course in non-immune patients and is frequently fatal if untreated.

8.5.4 Life Cycle

The malaria parasite passes its life cycle in two different hosts and comprises of two phase as follows,

Definitive host: Female Anopheles mosquito (a sexual phase of parasite occurs).

Intermediatehost: Human (an asexual phase of parasite occurs).

Thus, life cycle of malaria parasite show alternation of generations- asexual and sexual generation in two different hosts (Figure 8.12).

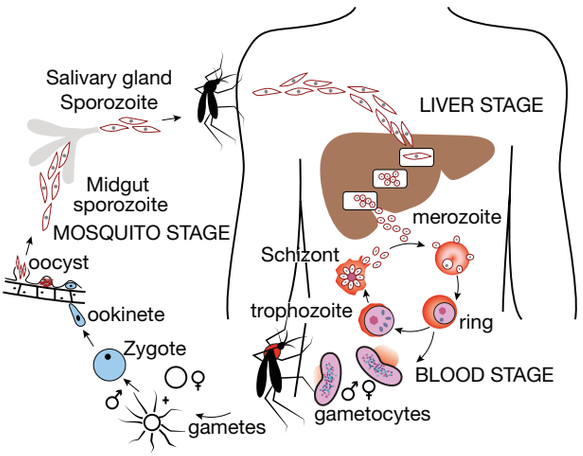


Figure 8.12: Lifecycle of Plasmodium spp

8.5.5 Human Cycle (Asexual Phase – Schizogony)

Human infection occurs when the sporozoites (the infective forms of the parasite are present in the salivary gland of the mosquito) are injected into blood capillaries when the mosquito feeds on blood after piercing the skin. The malarial parasite multiplies by division and the process designated as Schizogony (schizo: to split, gone: generation).

Sporozoites are minute thread-like curved organisms with tapering ends. Measuring 9–12μ in length with a central elongated nucleus while, the cytoplasm reveals no pigment as seen with a light microscope. In human, schizogony occurs in two locations. One in the red blood cells (erythrocytic schizogony) and other in the liver cells (pre – or exoerythrocytic schizogony).

A. Pre–erythrocytic or Exoerythrocytic schigony

- Sporozoites do not directly enter the RBC's to initiate erythrocyticschizogony, but undergo developmental phase in other human tissues.
- This cycle lasts for about 8 days in *Plasmodium vivax*, 6 days in *P. falciparum* and 9 days in *P. ovale*.
- This pre–erythrocytic schizogony occurs within parenchymal cells of the liver.
- The Sporozoites, which are elongated spindle – shaped bodies, become rounded inside the liver cells.
- They enlarge in size and undergo repeated nuclear division to form several daughter nuclei, each of which is surrounded by cytoplasm.
- This stage of the parasite is called the pre–erythrocytic or exoerthrocytic schizont or merozoites.
- The heptocyte is distended by the enlarging schizont and the liver cell nucleus is pushed to the periphery.
- Mature liver stage schizonts are spherical multinucleate and contain 2000–50,000 uninucleate merozoites.
- These normally rupture in 6–15 days and release thousands of merozoites into the blood stream.
- They do not return from red blood cells to liver cells.

Plasmodium vivax and *P. ovale* – parasites in liver tissue are called hypnozoites.

B. Erythrocyticstage

- The merozoites released by pre–erythrocytic schizonts invade the red blood cells (Parasitaemia).
- Merozoites are pear – shaped bodies, about 1.5 μm in length.
- In the erythrocyte, the merozoite loses its internal organelles and appears as rounded body having a vacuole in the center with the cytoplasm pushed to the periphery and the nucleus at one pole. These forms are called ring forms or young trophozoites.
- The parasite feeds on the hemoglobin of the erythrocyte. They incompletely metabolize hemoglobin therefore, hematin – globin pigment or haemozoin pigment is left behind.
- The malaria pigment released when the parasitized cells rupture is taken up by recticuloendothelial cells.
- The ring form develops and becomes irregular in shape and shows amoeboid motility. This is called the amoeboid form.
- When the amoeboid form reaches a certain stage of development, its nucleus starts dividing by mitosis followed by a division of cytoplasm to become mature schizonts or merozoites.

- A mature schizont contains 8–32 merozoites and haemozoin. The mature schizont bursts releasing the merozoites into the circulation.
- The merozoites invade fresh erythrocytes within which they go through the same process of development. This cycle is called erythrocytic schizogony.
- The rupture of the mature schizont releases large quantities of pyrogens. This is responsible for the febrile paroxysms characterising malaria.
- In *P. falciparum*, erythrocytic schizogony always takes place inside the capillaries and vascular regions of internal organs. Therefore, in these infections, schizonts and merozoites are usually not seen in the peripheral blood.

C. Gametogony

- Some of the merozoites, after a few erythrocytic cycles do not develop into trophozoites and schizonts but they undergo sexual differentiation to develop into the gametocytes.
- Development of gametocytes takes place within the internal organs and only the mature forms appear in circulation.
- The mature gametocytes in *P. falciparum* are crescent shaped.
- Female gametocytes are generally more numerous and larger.
- Male gametocytes and female gametocytes are called micro gametocytes and macro gametocytes respectively.
- Gametocyte appears in 10–12 days in *P. falciparum*.
- The gametocytes do not cause any clinical illness in the host, but are essential for transmission of the infection.
- A person who harbors the gametocytes is referred to as a carrier or reservoir.

Infobits

Sir Ronald Ross Institute of Parasitology is a malaria research institute located in Begumpet, Secunderabad, Hyderabad, India. Established in 1955, the institute is a division of Osmania University. The institute is named after Sir Ronald Ross, winner of Nobel Prize for Physiology or Medicine, 1902. Though he was a surgeon by qualification, Ross was attracted towards research in tropical diseases, especially malaria. During his posting, he worked on his research from a laboratory in the old Begumpet military hospital building. It was in this building on 20 August 1897 that he made the discovery of the malarial parasite inside the body of a mosquito.His study confirmed that mosquitoes were the carriers of malaria parasite.

8.5.6 Mosquito Cycle (Sexual Cycle – Sporogony)

- A Female Anopheles mosquito during its blood – meal from an infected person, sucks up both the sexual and asexual forms of parasite. But, only the mature sexual forms develop and the rest die.
- The gametocytes are set free in the midgut (stomach) of mosquito and undergo further development.

- The nuclear material and cytoplasm of the male gametocyte divides to produce long, actively motile, whip – like forms of 8 microgametes. This process is called exflagellation of male gametocytes.
- The Exflagellation is completed within 15–30 minutes for *P. falciparum*.
- The female gametocyte does not divide but maturation involves by condensation of nucleus to become the female gamete.
- Female gamete is fertilized by one of the microgametes to produce the zygote. The zygote is formed in 20–120 minutes after the blood meal. The zygote is initially is a non – motile round body, but within 18–24 hours, it gradually elongates into a vermicular motile form. This is called the ookinete.
- Ookinete penetrates the epithelial lining of stomach wall. Their anterior end comes in close contact to the cell membrane by secretion of some proteolytic substances which causes lysis of cell membrane. Later, the ookinete come to lie just beneath the basement membrane.
- It becomes rounded into a sphere with an elastic membrane. This stage is called the oocyst. The oocyst increase in size and undergo numerous nuclear multiplication which develops a large number of sickle shaped bodies known as sporozoites.
- Number of oocysts in the stomach wall varies from a few to over a hundred.
- Around the 10th day of infection the oocyst ruptures, releasing sporozoites in the body cavity of the mosquitos.
- The sporozoites are distributed through the circulating fluid into various organs and tissues of the mosquito except the ovaries.
- The sporozoites have a special affinity towards the salivary glands. The mosquito at this stage is capable of transmitting infection to man.

8.5.7 Pathogenesis

In malaria, typical pathological changes are seen primarily in the spleen, liver, bone marrow, lungs, kidney and brain.

Liver: The liver is enlarged. The organ becomes more firm and pigmented. Pigments are found in parenchymal cells.

Spleen: The spleen is markedly enlarged. If the infection lasts over a long period, the spleen is usually grayish, dark brown or even black and is commonly known as 'ague cake'.

Bone marrow, Lungs, Kidneys and Brain are enlarged and pigmented. They are filled with parasitized erythrocytes. Anemia is caused by destruction of large number of red cells by complement mediated and autoimmune hemolysis. It is also due to the increased clearance of both parasitied and parasitized RBCs by the spleen.

8.5.8 Clinical Manifestations

The incubation period is generally 9–14 days but, it can be as short as 7 days. The most malignant form of malaria is caused by P. *falciparum* hence, variable clinical syndromes are associated with falciparum malaria. That include,

1. Prodromal (initial indication of the onset of disease) period: Non – specific symptoms such as malaise (condition of

general weakness or discomfort), myalgia (severe muscle pain) headache and fatigue (feeling of tiredness) are usually seen during the prodromal period.

2. Malarial paroxysm (sudden onset of disease): It is the classical manifestation of acute malaria. It is characterised by fever, chill and rigor (sudden feelings of cold with shivering).The fever is caused by rupture of red blood cells that contain malarial parasites. The fever occurs every 48 hours in falciparum malaria.
3. Anemia (A condition in which the blood does not have enough healthy Red Blood cells) and
4. Hepatosplenomegaly (simultaneously enlargement of both the liver and the spleen)

The symptoms are non – specific with headache, pains in back and limbs, anorexia, nausea and a feeling of chill rather than a distinct cold phase. Hyponatremia (A condition that occurs when the level of Sodium in the blood is too low) occur in both uncomplicated and severe malaria.

Infobits

Transfusion Malaria

Malaria can be transmitted by transfusion of blood from infected donors. First reported in 1911, transfusion malaria is one of the most common transfusion-transmitted infections today. Blood transfusion can accidentally transmit malaria, if the donor is infected with malaria. The parasites may remain viable in blood bank for 1–2 weeks. As this condition is induced by direct infection of red cells by the merozoites. Pre-erythrocytic schizogony and hypnozoites are absent.

8.5.9 Complications of Severe Falciparum Malaria

1. Black water fever

The syndrome is the manifestation of repeated infections of falciparum malaria, which were inadequately treated with quinine. The condition is associated with haemoglobinaemia (excess of hemoglobin in the blood plasma) and haemoglobinuria (excretion of free haemoglobin in the urine). The syndrome is known as black water fever due to the dark red to brown – black appearance of the urine in this condition (Figure 8.13). It is dark due to presence of free haemoglobin as methaemoglobin or oxyhaemoglobin in it. Kidney failure is the immediate cause of death.

2. Cerebral malaria

Cerebral malaria is the most common presentation of severe malaria in adult. Cerebral malaria may be sudden in onset. Clinically, the condition manifests with fever for 4–5 days, slowly lapsing into coma, with or without convulsions. It is marked by a severe headache, high fever even above 180°F, and changes in mental status. Death may occur within few hours. Algid malaria and septicemic malaria are also other serious complication of falciparum malaria.

3. Pernicious malaria

The term pernicious malaria is referred to as a series of phenomena that occur during the course of an in treated *P. falciparum* infection within 1 to 3 days.

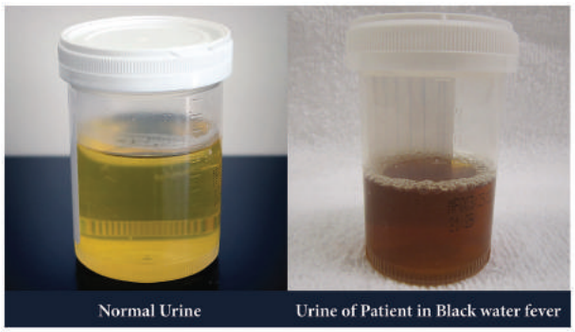


Figure 8.13: Urine in Black water Malaria

4. Anaemia: An individual suffering from an attack of malaria, after a few paroxysms becomes temporarily anaemic. The reduction in red blood cells is greater in *P. falciparum* infection than in infection with *P. vivax* and *P. malariae*. This is because *P. falciparum* invades young and mature erythrocytes and the infection rate of red blood cells is also greater.

HOTS

Which stage is infective in Blood transfusion malaria?

8.5.10 Recrudescence

In *P. falciparum* and *P. malariae* infections after the primary attack, sometimes there is a period of latency, during which there is no clinical illness. But some parasites persist in some erythrocytes and gradually increase in numbers. Fresh malarial attacks then develop. It appears after a period of latency usually within weeks after the primary attacks. Persistence of the erythrocytic cycle of the parasites are called recrudescences. In *P. falciparum* infections, recrudescences are seen for 1–2 years, while in *P. malariae* infection, they may last for long periods, even upto 50 years.

Infobits

The global technical strategy for malaria 2016-2030 was adopted by the World health Assembly in May 2015. It provides a comprehensive framework to guide countries in their efforts to accelerate progress towards malaria elimination. The strategy sets the target of reducing global malaria incidence and mortality rates by at least 90% by 2030.

8.5.11 Plasmodium vivax

P. vivax shows a similar life cycle in humans and mosquitoes like that of *P. falciparum*. Except in *P. vivax*, a latent tissue stage, the hypnozoites present in the liver parenchyma.

Relapse in vivax malaria is caused by these hypnozoites. Hypnozoites are the dormant stages of the parasites. These are single – nucleated parasites measuring 4µm–6µm in diameter. These become active and develop into tissue schizonts after a short period of dormancy. This relapse may occur at intervals up to 3 years or more after the first attack. *P. vivax* merozoites invade only young erythrocytes and the reticulocytes.

8.5.12 Clinical Manifestations

P. vivax is the most wide spread species causing malaria in man. However, unlike

Table 8.2: Comparison of course of infection – *P. falciparum* and *P. vivax* in man

Stage	P. falciparum	P. vivax
Pre – erythrocytic schizogony	Stage lasts for 6 days. Each Schizont produces about 40,000 merozoites approximately	Lasts for 8 days. Each Schizont produces about 12,000 approximately
Erythrocytic schizogony	Each cycle lasts for 36–48 hours. First temperature peak occurs by 12thday of infection. Primary attack last for 10–14 days.	Each cycle lasts for 48 hours. First fever peak occur by 16th day of infection. Primary attack lasts for 3–4 weeks.
Gemotogony	Gametocytes in peripheral blood may be seen on 21st day of infection.	Gametocytes in peripheral blood may be seen on 16th day of infection.
Exo – erythrocytic schizogony	Absent. Relapses do not occur	Present. Can continue for up to 3 years. Relapses often occur.

falciparum malaria, vivax malaria, is less severe and death from the condition relatively is less common. Table 8.2 describes the comparison of course of infection in Falciparum malaria with Vivax malaria

8.5.13 Laboratory Diagnosis

Diagnosis of malaria includes:

a. Parasitic diagnosis

b. Serodiagnosis, and

c. Molecular diagnosis

Parasitic diagnosis – Demonstration of parasite by microscopy

Specimen: Blood

Conventional light microscopy of stained blood smear is the gold standard for confirmation of malaria.

Two types of smears are prepared from the peripheral blood. They are thin and thick smears (Figure 8.14). Ring forms and gametocytes are most commonly seen in the peripheral blood smear.

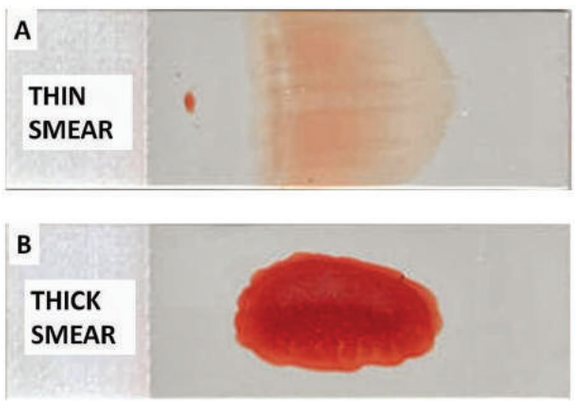


Figure 8.14: Blood smear

Thin smear

They are prepared from capillary blood of fingertip and spread over a good quality slide by a second slide (spreader slide) held at an angle of 30°–45° from the horizontal such that a tail is formed.

Thin smears thus prepared are air dried, fixed in alcohol and stained by one of the Romanowsky stains such as Leishman, Giemsa or JSB (Jaswant singh and Bhattacharjee) stain.

Thin smears are used for:

a. Detecting parasites, and

b. For determining the species of the infecting parasite.

Thick smear

They are prepared usually with 3 drops of blood spread over a small area of about 10mm. The thick film is dried. This smears consist of a thick layer of dehemoglobinized (lysed) red blood cells. It is not fixed in methanol.

Thick film is stained similar to thin film. Thick smears have the advantage of concentrating the parasites and therefore increase the sensitivity of diagnosis. Thick smears are used for:

a. Defecting parasites,

b. Quantitating parasitaemia, and

c. Demonstrating malarial pigments.

Fluoroscence microscopy

The method is mainly used for mass screening in field laboratory. Fluorescent dyes like acridine orange is used to stain the blood smears. It stains DNA as fluorescent green and cytoplasmic RNA as red.

QBC (Quantitative Buffy coat smear)

This is a sensitive method for detection of malaria parasites. In this method, blood is collected in a capillary tube coated with fluorescent dye and is subjected to centrifugation. After centrifugation, the Buffy coat in the centrifuged capillary tubes is examined under a fluorescent microscope. Acridine orange – stained malaria parasites appear brilliant green.

Serodiagnosis

It is not helpful in clinical diagnosis. It is used mainly for epidemiological survey and to identify the infected donors in transfusion malaria. The test used are indirect haemagglutination (IHA), Indirect fluorescent antibody (IFA) and Enzyme – linked immunosorbent assay (ELISA) for the defection of serum antibodies.

Rapid Antigen defection tests kits are available commercially like the dipstick, card and cassette bearing monoclonal antibody. These tests are based on the detection of antigens using immune chromatographic methods. These tests can detect plasmodium in 15 minutes.

Molecular diagnosis

DNA probe and PCR are highly sensitive methods for the diagnosis of malaria. It is more sensitive than that of thick blood smear. It is highly species specific.

Other tests includes the measurement of hemoglobin, total WBC and platelet count in severe falciparum malaria, urine can be tested for free hemoglobin, if black water fever is suspected. Blood urea and serum creatinine has to be monitored for renal failure.

8.5.14 Treatment

The most commonly used drugs are Chloroquine, Quinine, Pyrimethamine and Doxycycline.

8.5.15 Prevention and Control

The preventive measures to control malaria mainly depend on treatment of

infected individuals and reducing the transmission of malaria.

The control measures include the use of insecticides such as DDT (Di chlorodiphenyl tri chloromethane) or Malathion for controlling the populations of adult mosquitoes.

Proper use of mosquito nets, wearing protective clothings and use of mosquito repellants can prevent the mosquito bite.

Introduction to Helminths

General characteristics of Helminthic parasite:

1. Helminths are multicellular worms. They are bilaterally symmetrical animals having 3 germ layers and belong to the kingdom Metazoa.
2. They are invertebrates characterised by elongated, flat or round bodies.
3. Helminths develop through egg, larval and adult stages. Flowchart 8.1 describes the classification of helminthes.

8.6 Nematode: *Ascaris Lumbricoides*

8.6.1 Geographical Distribution

It is the most common of human helminthes and is distributed worldwide.

8.6.2 Habitat

The adult worms lives in the small intestine particularly in jejunum and in ileum.

8.6.3 Morphology

Adult worm

Ascaris lumbricoides resembles and sometimes confused with the earthworm. Its specific name *lumbricoides* means earthworm in Latin. Male and Female worm of Ascaris lumbricoides are shown in Figure 8.15.

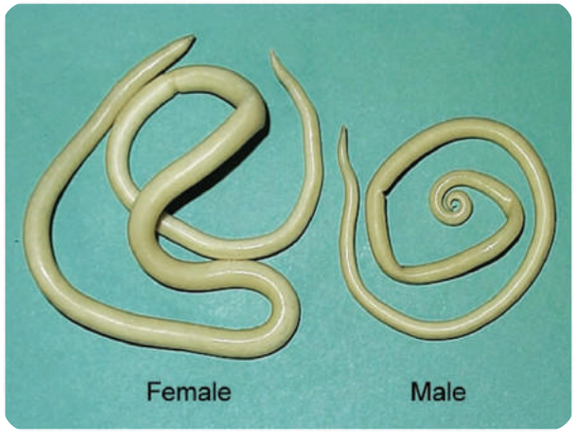


Figure 8.15: Adult worms of *Ascaris Lumbricoides*

- They are large cylindrical worms with tapering ends. The anterior end being thinner than the posterior end. It is the largest intestinal nematode parasitizing man.
- The life – span of the adult worm is less than a year.

Male worm

- The adult male worm is smaller than female worms.

DO YOU KNOW?

The roundworm, Ascaris lumbricoides is the largest nematode parasite in the human intestine. An editorial in the lancet in 1989 observed. That if all the round worms in all people worldwide were placed end-to-end. They would encircle the world 50 times. Soil-transmitted intestinal nematodes are called Geohelminths.

- The tail – end (Posterior end) of the male worm is curved ventrally to form a hook and 2 curved copulatory spicules.

Female worm

- The adult female worm is larger (20–40 cm) and thicker (3–6 mm) than male worm.
- The posterior end is conical and straight. The anus is in the sub terminal part and opens like a transverse slit on the ventral surface.
- The vulva is situated mid – ventrally, near the junction of the anterior and middle thirds of the body. This part of the worm is narrow and is called the vulvar waist.
- A single worm lays up to 200,000 eggs per day.

Egg: Two types of eggs are passed in feces by the worms.

Fertilized Egg

- The fertilized eggs are produced by fertilized females.
- The eggs are round or oval in shape and measures 45 µm in length and 35µm to 50µm in breadth.
- They are bile – stained and appear as golden brown (brownish) in colour.
- The egg is surrounded by a thick smooth shell with an outer albuminous coat (corticated eggs). Sometimes this outer coat is lost in few eggs. Those eggs are called as decorticated eggs (Figure 8.16).
- Each egg contains a large unsegmented ovum with a clear crescentic area at each pole. The eggs float in saturated solution of common salt.

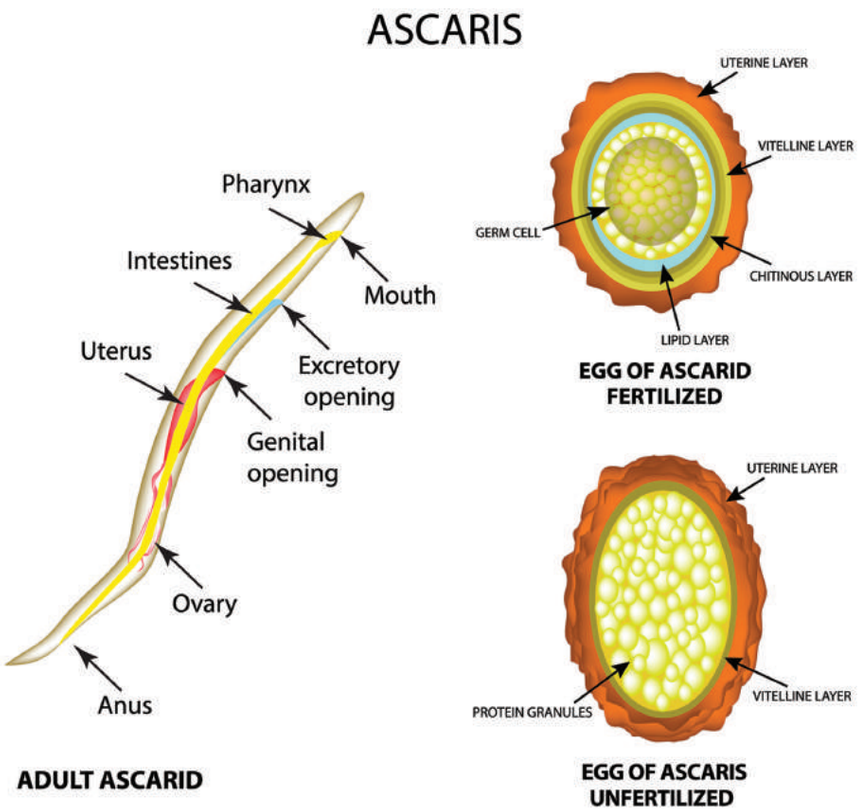


Figure 8.16: Fertilized and Unfertilized egg of *Ascaris lumbricoides*

Unfertilized egg

- The female even not fertilized by male is capable of liberating eggs. These unfertilized eggs are narrower, longer and elliptical in shape.
- These are heaviest of all the helminthic eggs – It measures about 80µm × 105µm in size.
- The eggs have a thinner shell with an irregular coating of albumin (Figure 8.16).
- These eggs do not float in saturated solution of common salt.

HOTS

What makes worm's egg float or sink?

8.6.4 Life – Cycle

The life – cycle of *A. lumbricoides* is completed in a single host, human (Figure 8.17).

Infective form: Ermbryonated eggs. The fertilized egg passed in feces is not immediately infective. It has to undergo a period of development in soil. The development usually takes from 10–40 days. The embryo moults twice during the time and becomes the infective rhabditiform larva.

Mode of transmission: Man acquires the infection by ingestion of food, water or raw vegetables contaminated with embryonated eggs of the round worm.

The ingested eggs reach the duodenum to liberate the larvae by hatching. These

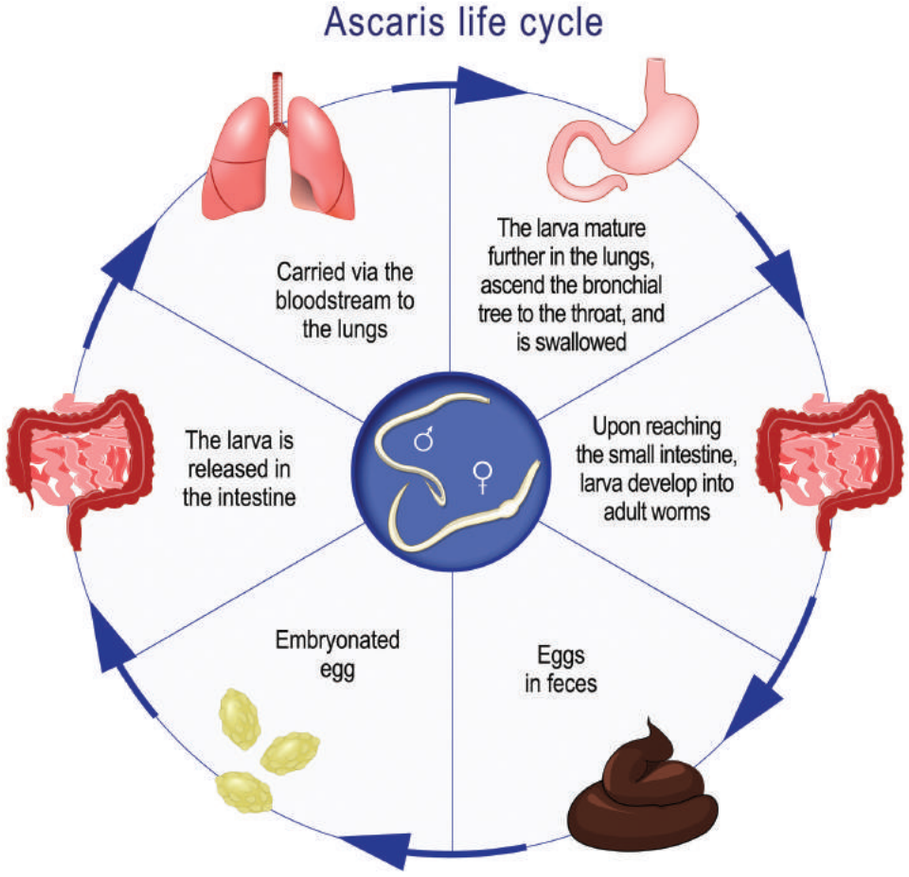


Figure 8.17: Lifecycle of *Ascaris lumbricoides*

larvae then penetrate the intestinal wall and are carried by the portal circulation to the liver. They live in liver for 3 to 4 days. Then they are carried to the right side of the heart, then to lung. In the lung, they grow and moult twice.

After development in the lungs, in about 10–15 days, the larvae pierce the lung capillaries and reach the alveoli. Then they are carried up the respiratory passage to the throat and swallowed back to the small intestine.

In the small intestine, the larvae moult finally and develop into adults. They become sexually mature in about 6–12 weeks. The fertilized female start laying eggs which are passed in the faces to repeat the cycle.

8.6.5 Pathogenesis

Infection of *A. lumbricoides* in human is known as ascariasis. The adult worm may produce its pathogenic effects in the following ways.

a. The spoliative or nutritional effects is usually seen when the worm burden is heavy. Presence of enormous numbers (sometime exceeds 500) often interferes with proper digestion and absorption of food. Ascariasis may contribute to protein – energy malnutrition and vitamin A deficiency.

b. The toxic effects is due to the metabolites of adult worm. Ascaris allergens produce various allergic manifestations such as fever, urticaria and conjunctivitis.

c. The mechanical effects are the most important manifestations of ascariasis. In heavy infections, adult worms can cause obstruction and inflammation of intestinal tract, particularly of the terminal ileum.

d. Ectopic ascariasis (Wander lust) is due to the adult male worms. They are restless wanderers. The wandering happens when the host temperature rises above 39°C. The worm may wander up or down along the gut. It may enter the biliary or pancreatic duct causing acute biliary obstruction or pancreatitis. It may enter the liver and lead to liver abscesses. The worm may go up the esophagus and come out through mouth or nose. It may crawl into the trachea and the lung causing respirator obstruction or lung abscesses. Migrating downwards, the worm may cause obstructive appendicitis. The worm may also reach kidneys. "Larva migrans" is a term used when the larval sworms migrate to various parts of the body.

8.6.6 Clinical Manifestations

Incubation Period is 60–70 days. Clinical manifestations due to adult worm vary from asymptomatic to severe and even fatal infection. Clinical manifestation in ascariasis can be caused either by the migrating larvae or by the adult worms.

Symptoms due to the migrating larvae: leads to ascaris pneumonia and larvae may enter the general circulation, disturbances have been reported in the brain, spinal cord, heart and kidneys.

Symptoms due to the adult worms: Diffuse or epigastric abdominal pain, abdominal cramping, abdominal swelling (especially in children), fever, nausea,

vomiting and passing roundworms and their eggs in the stool.

8.6.7 Laboratory Diagnosis

Specimen collected: Stool, sputum and blood.

Detection of parasite

Adult worm: It can be detected in stool or sputum of patient by naked eye. Pancreatic or biliary worms can be detected by ultra-sound and endoscope.

Larvae: Larvae can be detected in sputum and often in gastric washings. Chest X – ray may show pulmonary infiltrates.

Eggs: Detection is through demonstration of eggs in feces. Detection of both fertilized and unfertilized eggs are made after staining. Eggs may be demonstrative in the bile obtained by duodenal aspirates.

Blood Examination

Complete blood count may show eosinophilia in early stage of infection.

Serological tests

Ascaris antibody can be detected by IHA, IFA and ELISA

8.6.8 Treatment

Commonly used drugs are Albendazole and Mebendazole.

8.6.9 Prevention and Control

a. Proper health education should be given for improved sanitation and personal hygiene.

The National Deworming Day (Febuary 10th) is an initiative of India to make every child in the country worm free. This is one of the largest public health programs reaching large number of children during a short period.

More than 836 million children are at risk of parasitic worm infections worldwide. According to World Health Organization 241 million children between the ages of 1 and 14years are at risk of parasitic intestinal worms in India, also known as Soil-Transmitted Helminths (STH).

b. Avoid eating of uncooked green vegetable, food preparation and fruits that may contain faecal eggs.

c. Treating infected persons especially children. Deworming of school children have been found effective in control of ascariasis.

Summary

Medical Parasitology deals with the study of parasites infecting humans. The diseases caused by them and the clinical manifestations produced in infected humans. It is also concerned with various methods of their diagnosis, treatment and their prevention and control. There are different types of parasites and hosts. Parasites lives on its host for its nourishment and survival. The relationship between host and the parasite can be symbiotic,

commensalic or parasitic. Parasites of medical importance comes under the kingdom called Protista and Animalia. Protista includes the microscopic single – celled eukaryotes known as protozoa. In contrast, helminths are macroscopic, multicellular worms possessing well differentiated tissues and complex organs belonging to the kingdom Animalia. Protozoa includes *Entamoeba* and *Giardia* which cause intestinal infections (dysentery and diarrhoea) Leishmania donovani, the unicellular tissue flagellatis causes Leishmaniasis. Plasmodium spp., the protozoan parasite which causes malaria are transmitted by female Anopheles mosquito carring sporozoites forms of the parasite. The four species infective to humans are *P. falciparum*, *P. malariae*, *P. vivax* and *P. ovale*. Multicellular organisms and intestinal worms. The helminths such as Ascaris lumbricoides causes Ascariasis, an infection of the small intestine. Ascariasis is the most common roundworm infection.

Evaluation

Multiple choice questions

1. A host in which a parasite undergoes asexual reproduction is _____.
   a. Definitive host
   b. Intermediate host
   c. Reservoir host
   d. perfect host

2. Which of the following statement is true concerning Entamoeba histolytica.
   a. It has no cystic stage
   b. It is non – pathogenic
   c. It is not transmitted through faecal – oral route
   d. Trophozoites live in large intestine of human

3. Animals that are routinely infected with a protozoan or parasite are termed as _____.
   a. Definitive b. Intermediate
   c. Reservoir d. Parasite

4. The schizonts enter which body part?
   a. Blood stream b. Spleen
   c. Mouth d. Liver

5. Leishmania organism are transmitted to human by _____
   a. Sandflies b. tsetse files
   c. Mosquitoes d. Reduviid bug

6. The _____ parasite is capable of producing disease in an immune deficient host.
   a. Entamoeba histolytica
   b. Toxoplasma gondii
   c. Ascaris
   d. Taenia

7. Which of the following parasitic infection can lead to malabsorption syndrome.
   a. Amoebiasis
   b. Ascariasis
   b. Hookworm infection
   d. Giardiasis

8. Flask shaped ulcers in human intestine are related to _____.
   a. Giardiasis b. Amoebiasis
   c. Leishmanian d. Chaga's disease

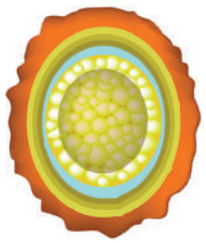
9. This disease was first observed by Leeuwenhoek often he discovered parasitic organisms in his stool under the microscope.
   a. Chaga's diease b. Gardiasis
   c. Malaria d. Ascariasis

10. The common name for A. Lambricoides is round worm.
    a. Round worm b. Pin worm
    c. Tape worm d. Whip worm

Answer the following

1. Which laboratory findings are diagnostic for leishmaniasis?
2. How is amoebiasis diagnosed.
3. Following ingestion, what is the life cycle of E.histolytica?
4. What is the clinical spectrum of amoebiasis?
5. What is the role of microscopy in the diagnosis of amoebiasis.
6. What is ALA?
7. How Female Ascaris worm is differentiable from male worm?

8. Why do some parasites need definitive and intermediate hosts rather than just one host to complete its life-cycle.
9. What is the difference between reservoir and paratenic hosts?
10. Why is the mosquito a definitive host in malaria?
11. A fecal sample was subjected to Saturated salt flotation from a 14 year old boy according to doctor's advice and check-up. The results of this test are shown under the microscope. Identify the parasite egg and comment on it.



12. With neat diagram describe the trophozoite of *Giardia*.
13. Explain the erythrocytic stage of Plasmodium falciparum.
14. What complication arises due to inadequately treated patient with quinine suffering from malignant malaria?
15. Describe the life-cycle of large roundworm which grows to a length of up to 40cm that infects humans.
16. For which parasite mosquito acts as definitive and intermediate host?
17. Fill in the blanks in the column given below.

Human Nematode Infection			
Parasite	Acquired by	Site in humans	Transmitted through
Ascaris lumbricoides	Ingestion of eggs	_____	Person to person

Chapter 9

Medical Mycology

Learning Objectives

After studying this chapter the students will be able to,

- *Identify the pathogenic fungi most commonly causing disease by using advanced techniques. Fungal infection is common in developing countries so we should be aware to prevent and treat the fungal infection.*
- *Study the taxonomy, structure and classification of medically important fungi.*
- *Study about the mycoses and its pathogenesis, clinical feature, and its treatment and prophylaxis.*
- *Study about the collection, processing of the sample and its molecular diagnosis.*

Chapter Outline

5N9S8R

The branch of biology that deals with the study of fungi is known as **"Mycology"**. The term is derived from Greek work **'Mykes'** means **mushroom** and **'Logos'** means **study**. Medical Mycology is the study of fungal epidemiology, ecology, pathogenesis, diagnosis and treatment in human beings**. Raymond Jacques Sabouraud (1864–1936)** is the father of Medical Mycology.

9.1 Classification of Fungi based on the Host Parasitic Relationship

Based on the host parasitic relationship the fungi are grouped into three types.

a. Commensalism: The fungus neither gets benefit nor harmed by the host parasitic relationship.

b. Mutualism: The fungus benefited from the host parasitic relationship.

c. Parasitism: The host is harmed by the fungus in host parasitic relationship.

Based on their wide spectrum of adaptability, fungi causing human mycoses can be categorized into:

a. Pathogenic fungi: The ability of the fungi to adapt to skin flora and cause infection.

b. **Opportunistic fungi:** When the immune status of the host is reduced, fungi will induce or cause infection.

c. **Toxigenic fungi:** Toxins produced by fungi are responsible for the illness or death of patients after ingestion of the contaminated food.

d. **Allergenic fungi:** Allergens are secreted by the fungi which cause allergic reaction in the human beings.

9.1.1 Mycoses

Diseases caused by the medically important fungi are called Mycoses. Mycoses are classified according to the specific site of involvement.

a. **Superficial Mycoses:** The infection is limited to the outer most layers of the skin and its appendages. Example: Malassezia and Piedra infection

b. **Cutaneous Mycoses:** The infection extends deeper into the epidermis and it also invades hair and nails. Example: Dermatophytoses.

c. **Sub cutaneous Mycoses:** The infection extends to dermis, subcutaneous tissue and muscles by any traumatic injury. Example: Mycetoma

d. **Systemic Mycoses**: The infection originates from lungs and later spreads systemically to other organs. Systemic mycoses along with the opportunistic fungal infection are known as deep mycoses. Example: Cryptococcosis

e. **Opportunistic Mycoses:** The infection occurs when the immune status of the individuals is altered. It is common among immune compromised and immune suppressed patients. Example: Candidiasis

Aeromycology

The Aeromycology is the study of air borne fungi, its types and the seasonal variations of allergenic fungal spores in the environment.

There are certain fungal pathogens which cause infections associated with workers in mycological laboratories. To avoid this safety procedures and equipments safety levels or bio safety levels (BSL) are used. BSL - 1 is used for low - risk microorganisms and BSL - 4 is used for highly risk pathogens.

Infobits

Medical Mycology in India

In India, the fungal infections are known since the ancient civilization mentioned in **Aryan documents such as Atharva Veda** Mycetoma is described as Padavamikam meaning ant hillfoot this was observed by John Gill in Madurai district of Tamil Nadu in 1842 which was designated as '**Madura foot**'.

9.1.2 Characteristics of Fungi

Fungi are heterotrophic organisms that exist as saprophytes, commensal or parasites. They are found on decaying vegetative matter and also in soil. Morphological features, cell structure, reproduction, nutritional requirement and thermal dimorphism in the pathogenic fungi are described as follows:

i. Morphological Features

Fungi are eukaryotic with well defined cell wall and internal membrane bound organelles. The cell wall is composed of polysaccharides and chitin. A fungus

varies in size and shape. They are broadly divided into two main groups.

a. **Yeasts:** The yeasts are unicellular organisms which reproduce by asexual process known as **budding** or by **fission**. The cell develops a protuberance that enlarges and separates from the parental cell. The yeasts produce chains of elongated cells known as **Pseudohyphae**. Some yeasts are reproduced by sexual process Example: *Cryptococcus neoformans*. Germ tube is special morphology found in *Candida albicans*. Some are commensal without any medical significance.

b. **Molds:** The molds grow by apical extension, forming an interwoven mass called as **Mycelium,** branching filaments known as **hyphae**. Hyphae that grow on the surface are called **vegetative hyphae**. They are responsible for the absorption of nutrients. The hyphae that project above the surface are called **aerial hyphae** and they produce specialized reproductive structures called as **conidia**.

HOTS

Can you cultivate the molds at home?

Depending on cell morphology fungi are divided into four types, they are **Yeasts:** These are unicellular organisms that divide by budding (Figure 9.1a & b). Example: *Cryptococcus neoformans* (Pathogenic), *Saccharomyces cerevisiae* (Non pathogenic). **Yeast – like fungi:** These fungi reproduce by budding but fails to separate and hence elongation takes place forming pseudohyphae. Example: *Candida* species (Pathogenic). **Molds:** These fungi produce spores which germinate to form vegetative hyphae (Figure 9.2). Example: Dermatophytes, *Aspergillus, Penicillium, Mucor*. **Dimorphic fungi:** These Fungi exist in both yeast at 37°C and filamentous form at 25°C. This Phenomenon is known as **Fungal dimorphism** (Figure 9.3). Example: *Histoplasma capsulatum*, *Blastomyces dermatitidis*.

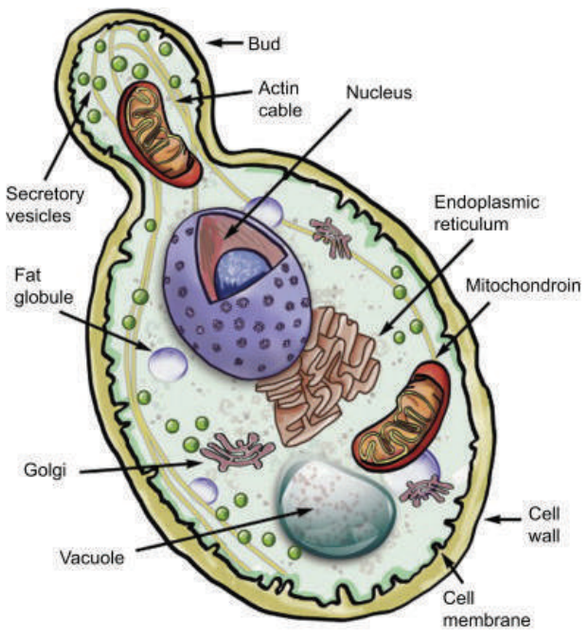
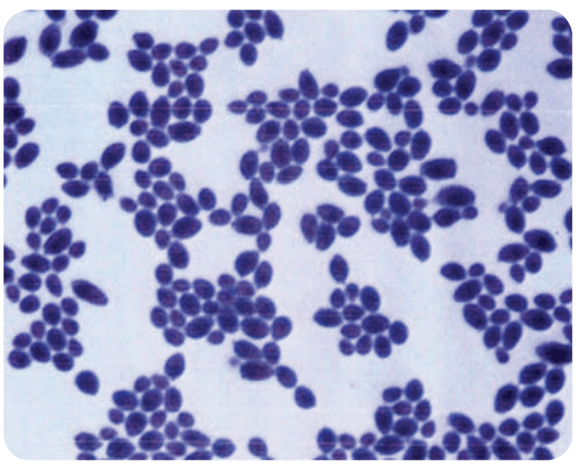


Figure 9.1: (a) Morphology of yeast



(b) Microscopic view of yeast

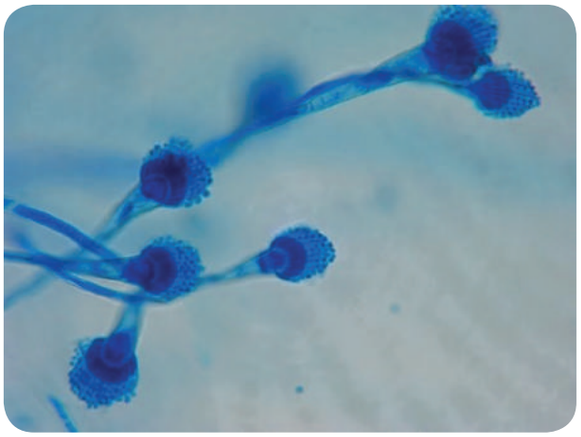


Figure 9.2: Microscopic view of yeast Moulds

Phaeoid fungi: Most of true pathogenic fungi are dimorphic fungi which are composed of darkly coloured hyphal form known as **dematiaceous fungi**. Some are yeast like and also known as black yeasts.

Vegetative Structures: Several structures are formed by the vegetative mycelia that have no reproductive value but are important for the differentiation of fungi eg. Chlamydospores and Arthrospores. Chlamydospores are thick walled, resistant to adverse conditions and are larger than other cells. Arthrospores are rectangular spores which are thick walled that are disposed on maturity.

ii. Cell structure

a. Capsule: Fungi produce an extra cellular polysaccharide in the form of capsule. Example: *Cryptococcus*.

b. Cell wall: Fungi possess a multilayered rigid cell wall exterior to the plasma lemma. The cell wall is made up of chitin, a water insoluble, homo polymer of N-acetyl glucosamine. Chitin synthase is responsible for the bio synthesis of chitin.

c. Plasma lemma: Cytoplasmic membrane or plasma lemma encloses complex cytosol. It is composed of glycoprotein, lipids and ergosterol.

d. Cytosol: Cytosol comprises of mitochondria, microtubules ribosome's, golgi apparatus, double membrane endoplasmic reticulum and Nucleus. The nuclei of the fungi are enclosed by a membrane and contain most of cellular DNA.

iii. Reproduction of fungi

Spores play a major role in reproduction. There may be asexual or sexual cell divisions.

a. Asexual Reproduction: The asexual reproduction involves, budding or fission or mitosis. Fungi produce more than one type of asexual spores. They are microspores (micro conidia) and macrospores (macro conidia). Spores that are present inside sporangium are

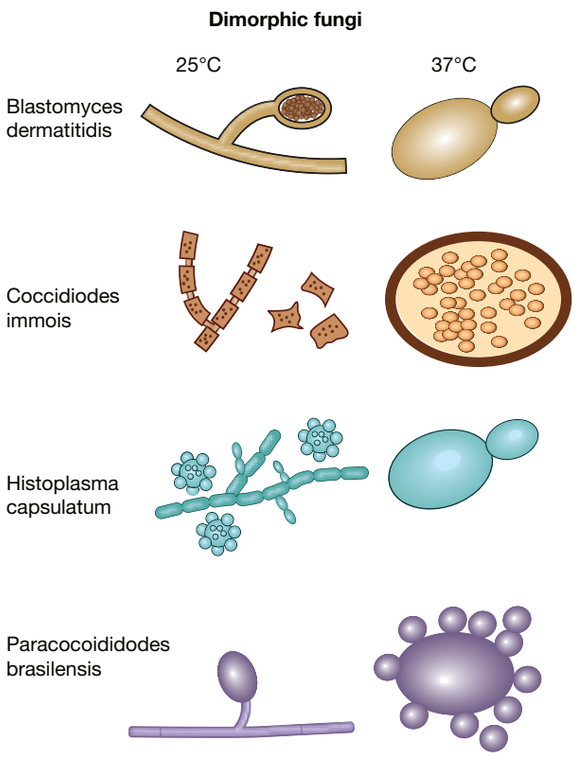


Figure 9.3: Dimorphic Fungi

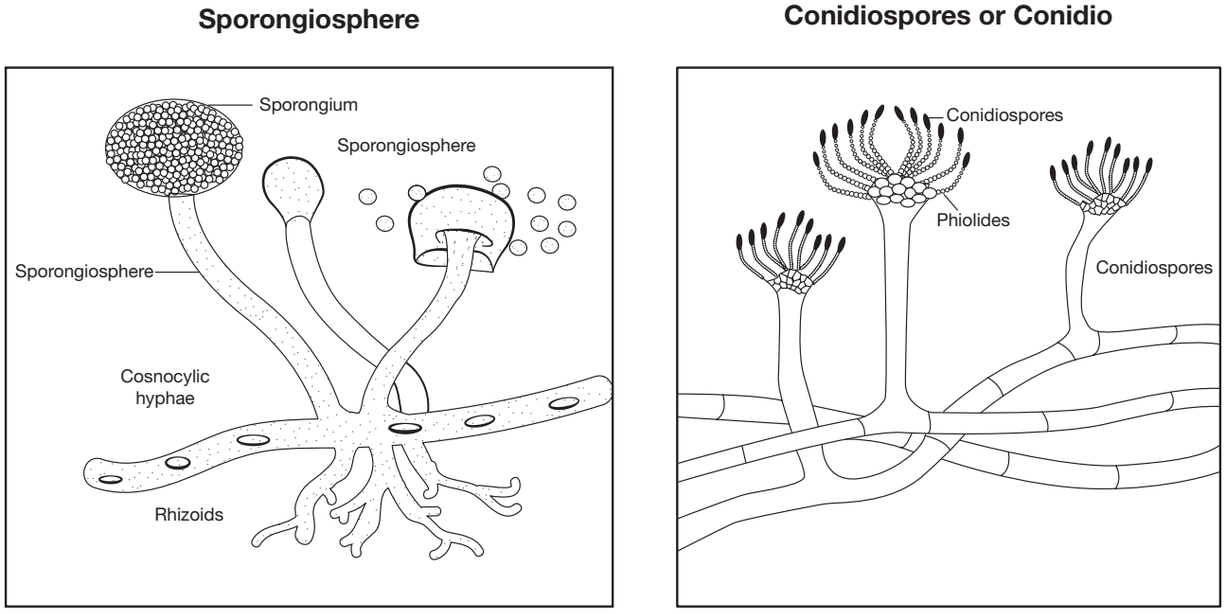


Figure 9.4: Asexual Spores of fungi

known as **sporangiospores** and those that are borne exogenously are called **conidiospores** (Figure 9.4)**.** Based on the arrangement of conidia they are classified as Acropetal, Basipetal and Sympodial.

b. Sexual Reproduction: The process of sexual reproduction typically consists of plasmogamy (cytoplasmic fusion), Karyogamy (union of two nuclei) and meiosis (haploid formation). Anamorphs and Telomorphs are the 2 phases of sexual reproduction

c. Mycelia Sterile: Mycelia sterile are fast growing molds that do not produce spores or conidia. They are medically significant fungi and are difficult to identify

iv. Growth and nutrition

Fungi are ubiquitous in nature and grow readily in the presence of nitrogen and carbohydrates. Medically significant fungi are Mesophilic. The optimum temperature *invitro* for majority of the pathogenic fungi is between 25°C and 37°C. The fungi prefer acidic pH; do not require light for their growth. All fungi are heterotrophs requiring organic nutrients. They absorb their nutrient and do not ingest food. Medically significant fungi are facultative parasites, capable of causing disease or living on dead organic matter.

9.2 Superficial Cutaneous Mycoses

The superficial cutaneous fungal infections involve the outer most layers of skin and its appendages like hair and nails. The causative agents colonize on epidermis or supra - follicular portions of hair and do not penetrate into deeper layers.

The genus ***Malassezia*** is responsible for the superficial infection of the skin. *Malassezia furfur* is lipophilic yeast. It is a commensal of normal skin in the sebaceous glands of warm - blooded vertebrates. It may be pathogenic under certain conditions usually causing skin

conditions like Pityriasis versicolor, Seborrheic dermatitis, Atopic dermatitis, Malassezia folliculitis and systemic infection. Symptoms include macular, erythematous, hyper pigmented or hypo pigmented lesions with fine scaling.

Tinea nigra is responsible for the superficial cutaneous infection of the skin. *Hortaea werneckii* is the phaeoid (dematiaceous) fungi causes infection on the palms and soles. It is also commonly termed as *Tinea nigra palmaris* and *Tinea nigra plantaris*. Symptoms includes brown to black deeply pigmented non - scaly, macular lesions affecting skin of the palms and occasionally soles.

Piedra causes superficial infection of hair shaft. The word **Piedra** is derived from Spanish word **Stone**. There are two types of Piedra based on causative fungi and characteristics of nodules. They are **Black piedra** caused by *Piedraia hortae* and **White piedra** caused by *Trichosporon* species. The symptoms include development of firm, irregular nodules of fungal elements cemented to the hair. The piedra can be distinguished on the basis of shape, size and pigmentation of fungal cells of nodules which are found around hair cortex.

9.2.1 Dermatophytoses

Dermatophytoses are the most common cutaneous fungal infection seen in man and animals affecting skin, hair and nails. The fungi can invade the keratinized tissues of skin and its appendages and they are collectively known as **Dermatophytes** or **Tinea** or **ring worm** infection. The dermatophytes are hyaline septate molds. They are divided into three main anamorphic genera depending on their morphological characteristics.

i. ***Trichophyton*** [Cause infection in skin, hair and nails]

ii. ***Microsporum*** [Cause infection skin and hair]

iii. ***Epidermophyton*** [cause infection skin and nail]

The fungal species affecting humans are known as **anthropophilic**. Those inhabitating domestic and wild animals as well as birds are called **zoophilic**. Fungi species from soil are known as **geophilic** dermatophytes.

HOTS

What are the sources of dermatophytes?

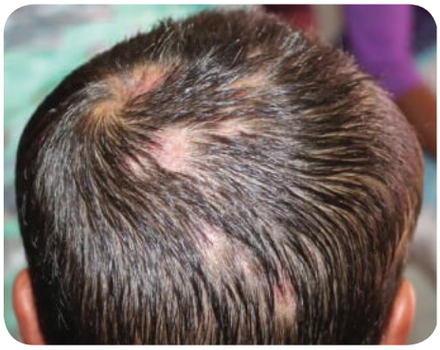
9.2.2 Pathogenesis and Pathology

The dermatophytes grow within dead keratinized tissue and produce keratinolytic proteases, which provide means of entry into living cells. Fungal metabolic products cause erythema, vesicles and pustule on the site of infection. Some dermatophytes species like soil saprobes digest the keratinaceous debris in soil and are capable of parasitizing keratinous tissues of animals.

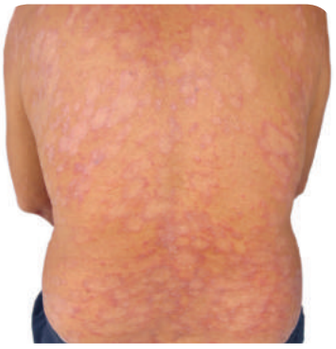
9.2.3 Clinical Features

The clinical manifestations of Dermatophytoses are also called **Tinea or Ringworm** depending on the anatomical site involved. Following are the common clinical conditions produced by dermatophytes:

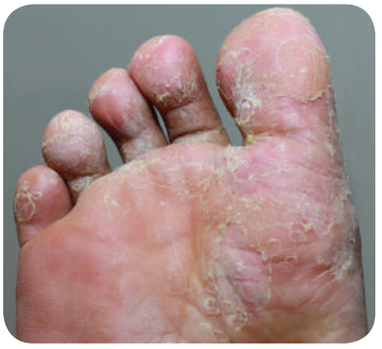
1. **Tinea Capitis:** This is an infection of the **shaft of scalp hairs**. It can be inflammatory (eg. Kerion, Favus) or non - inflammatory (Black dot, Seborrheic dermatitis). The infected hairs appear dull and grey (Figure 9.5a). Breakage of hair at follicular orifice which creates patches of alopecia with black dots of broken hair. It is caused by *Trichophyton* species.
2. **Tinea Corporis:** This is an infection on the **glabrous (non - hairy) skin** of body. Erythematous scaly lesions with sharply marginated raised border appear on the infected areas (Figure 9.5b). It is caused by *Trichophyton rubrum*.
3. **Tinea Imbricata:** It forms concentric rings of scaling on the **glabrous skin**, leading to lichenification. It is caused by *Trichophyton concentricum*
4. **Tinea Gladiatorum:** This infection is common among wrestlers and athletes. Lesions are seen on **arms, trunk or head and neck**. It is caused by *Trichophyton tonsurans*.
5. **Tinea Incognito:** It is steroid modified Tinea caused as a result of misuse of corticosteroids in combination with topical antimycotic drugs.
6. **Tinea Faciei:** This is an infection of **skin of face** except beard. Erythematous annular plaques are formed. It is one of the forms of Tinea incognito.
7. **Tinea Barbae:** This is the infection of the **beard and moustache** areas of the face. This is also called **barber's itch.** It is caused by *Trichophyton mentagrophytes*, *Trichophyton rubrum* and *Microsporum canis*. Erythematous patches on the face with scaling appear and these develop folliculitis.
8. **Tinea Pedis:** This is an infection of the **foot, toes and interdigital web spaces**. This is seen among the individuals wearing shoes for long hours and known as **Athlete's foot** (Figure 9.5c). Erythema and scaling associated with itching and burning sensation appear with thin fluid discharging from small vesicles. It is caused by *Trichophyton mentagrophytes*, *Trichophyton rubrum* and *Epidermophyton floccosum*.
9. **Tinea Cruris:** This is an **infection of the groin** in men who use long term tight fitting garments. Erythematous



(a) Tinea Capitis



(b) Tinea Corporis



(c) Tinea Pedis

Figure 9.5: Clinical conditions of Dermatophytes

sharp margin lesions known as Jock itch. It is caused by *Trichophyton rubrum* and *Epidermophyton floccosum*.

10. **Tinea Manuum:** This is an infection of the **skin of palmar** aspect of hands. It causes hyperkeratosis of the palms and fingers. It is caused by *Trichophyton mentagrophytes*, *Trichophyton rubrum* and *Epidermophyton floccosum*.
11. **Tinea Unguium:** This is an infection of the **nail plates**. The infection spreads on the entire nail plate infecting the nail bed. It results in opaque, chalky or yellowish thick ended nail. It is caused by *Trichophyton mentagrophytes*, *Trichophyton rubrum* and *Epidermophyton floccosum*.

Figure 9.6 shows the microscopic view of major determatophytes

Infobits

How do dermatophytes cause disease in humans?

Dermatophytosis is a common contagious **disease caused** by fungi **known as dermatophytes**. **Dermatophytes** belong to a group of **organisms** that are able to break down the keratin in tissues such as the epidermis, hair, nails, feathers, horns and hooves.

9.2.4 Laboratory Diagnosis

i. Samples

Skin scrapings, hair and nail samples were collected

a. Direct Examination

Samples are subjected to KOH (10%) wet mount, the affected site were disinfected with alcohol before collecting the clinical specimen.

b. Fungal culture

The samples are inoculated on Sabouraud dextrose agar (SDA) with antibiotics and cycloheximide and are incubated at 25°C–35°C. The colony morphology can be identified.

The three genera of dermatophytes are *Trichophyton*, *Microsporum* and *Epidermophyton* (Table 9.1). Based on morphology of the macro conidia, micro conidia, their shape, position on the spore bearing hyphae such as spiral hypha, racquet hypha, nodular pectinate body, they are identified.

ii. Special Techniques

1. Wood's Lamp Examination

Clinical samples are exposed to Wood's lamp. Wood's glass consists of Barium silicate containing 9% Nickel oxide. It transmits long wave ultra violet light with a peak of 365nm that shows a characteristic fluorescence produced by the samples. The patterns of fluorescence are bright green, golden yellow and coral red. *Microsporum* species and *Trichophyton* species are differentiated using this technique.

2. Hair brush sampling Technique

It involves brushing the scalp with a sterile plastic hair brush, which is then inoculated into an appropriate culture medium by plates, is incubated at 25°C–35°C. The colony morphology can be identified.

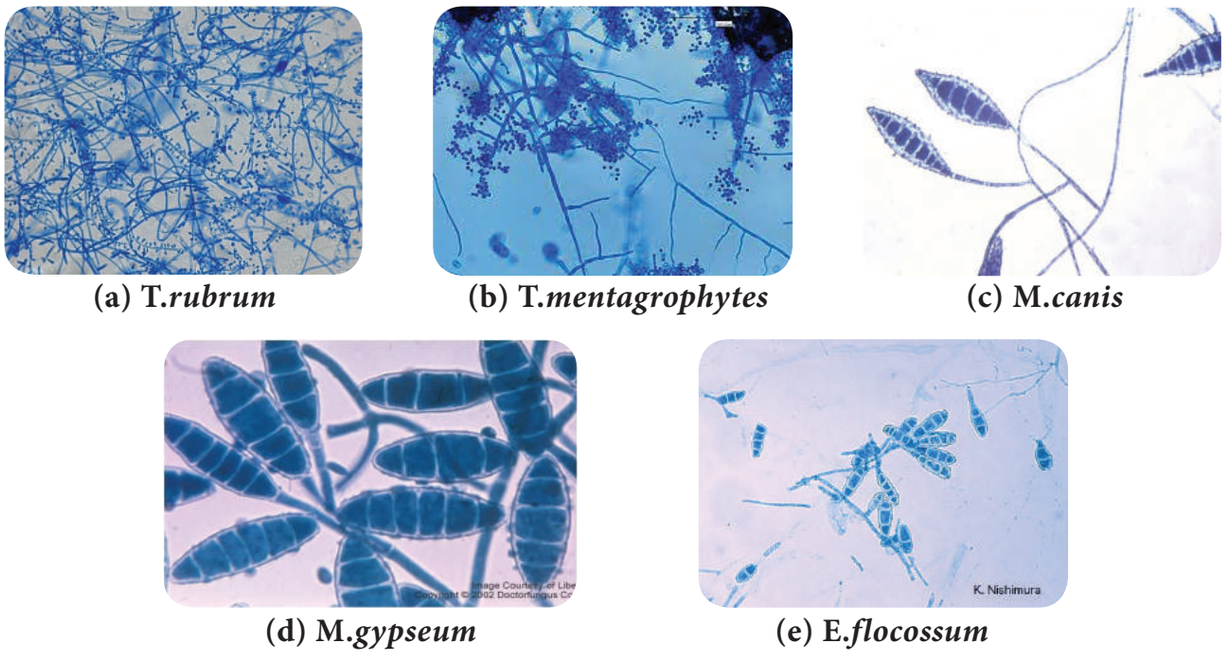


Figure 9.6: LPCB wet mount of major Dermatophytes

Table 9.1: Microscopic and macroscopic characteristics of Dermatophytes.

S.No	Dermatophytes	Macro conidia	Micro conidia	Macroscopic Morphology – SDA
1.	*Trichophyton*	Rare, thin-walled, smooth	Abundant	
2.	*Microsporum*	Numerous, thick-walled, rough	Rare	
3.	*Epidermophyton*	Numerous, smooth-walled	Absent	

Dermatophyte infections, also known as tinea, are the most common fungal **infections** of the skin, hair, and nails. The term "**dermatophyte**" refers to fungal species that infect keratinized tissue, and includes members of the *Trichophyton, Microsporum*, and *Epidermophyton* genera

3. Hair perforation Test

It is used to differentiate *T. mentagrophytes and T. rubrum*. Wedge-shaped perforations in the hair shaft are observed in hair infected with *T. mentagrophytes*.

4. Urease Test

It is used to differentiate between *T. mentagrophytes* and *T. rubrum*. *T. mentagrophytes* hydrolyzes urea and becomes deep red, showing positive result.

iii. Treatment

Whitfield's ointment is used for all Tinea infections. Oral griseofulvin is the drug of choice for nails and scalp infections. Itraconazole and terbinafine may be given as pulse therapy.

9.3 Subcutaneous Mycoses

The fungal infections are characterized by development of lesions at the site of infection by the traumatic inoculation in the subcutaneous tissues. Examples are Mycetoma, Sporotrichosis, Chromoblastomycosis and Rhinosporidiosis.

9.3.1 Mycetoma

Mycetoma is a slowly progressive, chronic granulomatous infection of skin and subcutaneous tissues with involvement of under lying fasciae and bones usually affecting the extremities. Mycetoma is commonly called **Madura foot or Maduramycosis** (Figure 9.7). They are classified into two categories, namely **eumycetoma** cased by **fungi** and **actinomycetoma** caused by higher bacteria of class **actinomycets**.

9.3.2 Pathogenesis and Pathology

The causative agent of Mycetoma is commonly present in saprobic soil source and is transmitted by accidental trauma by thorns or by injury into the subcutaneous tissue. It is common among farmers with minor trauma and abrasions of the skin. Use of wicks for removal of earwax is responsible for Mycetoma of the ear.

HOTS

Is mycetoma occupational disease?

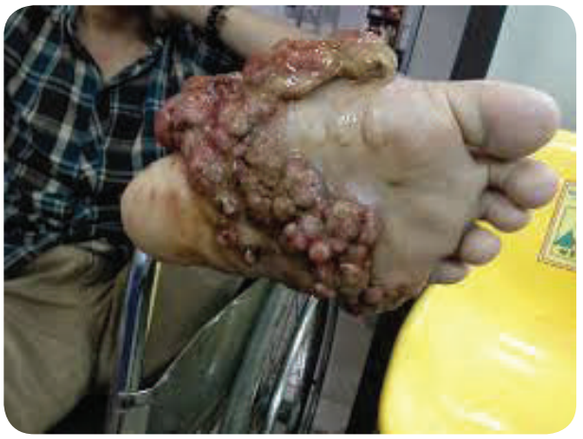


Figure 9.7: Madura foot

9.3.3 Classification of Mycetoma

The Mycetoma is classified on the basis of the causative agent **aerobic actinomycetes** causes **actinomycetoma** whereas **hyaline and phaeoid fungi** cause **eumycetoma.**

9.3.4 Clinical Features

The clinical entity depends upon the age of the lesions and to size, shape and color of the grains. The painless localized swollen lesions with purulent fluid lead to the secondary bacterial infections. Important features of Mycetoma are as follows:

i. Tumor like swelling

ii. Multiple draining sinuses

iii. Presence of grains or granules in sinuses.

9.3.5 Laboratory Diagnosis

i. Samples

The clinical sample in Mycetoma is usually grains, pus exudates or biopsy were collected.

a. Direct Examination

Grams staining, modified Ziehl – Neelson staining, LPCB and KOH wet mount are used to visualize the organisms.

The grains should be washed, crushed and cultured on different media. Crushed grains are examined (Figure 9.8a).

KOH mount

Eumycotic grains show thick 2–6 µm hyphae with large globose swollen cells with or without chlamydospores. Actinomycotic grains show thin filaments of 0.5–1 µm with coccoid or bacillary forms.

Gram stain

Actinomycetoma grains show Gram-positive branching filamentous bacteria with branches (Figure 9.8b).

Ziehl - Neelson stain

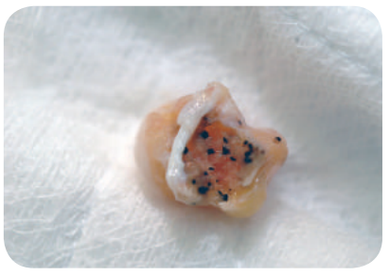
Nocardia species show red pink acid fast filamentous bacteria.

b. Culture

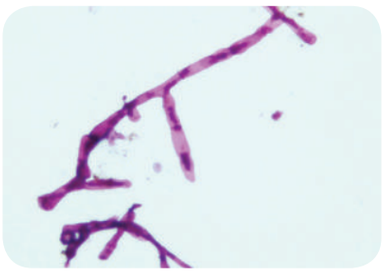
Crushed grains are washed several times with normal saline without antibiotics and inoculated on to **Sabouraud dextrose agar, blood agar, Lowenstein -Jonson** media and brain-heart infusion agar. The plates are incubated at 25°C, 37°C and 44°C for various organisms (Figure 9.8c).

ii. Treatment

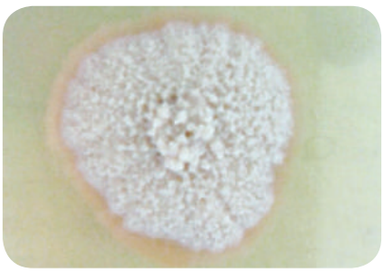
1. Ketoconazole 200 mg and Itraconazole 100mg are given for 8–24 months to treat eumycetoma.
2. Sulfonamides, tetracylines, streptomycin, amoxicillin are administered to treat actinomycetoma.



(a) Biopsy-Black grains



(b) Microscopic Morphology



(c) Macroscopic Morphology

Figure 9.8: Laboratory Diagnosis of Mycetoma

9.4 Systemic Mycoses

Systemic mycoses are caused by dimorphic fungi; these infections are acquired by inhalation of spores. These primarily involve the respiratory system and are self-limiting and asymptomatic. If symptomatic, it spreads to other parts of body through circulation. These infections are caused by **true fungal pathogens**. Systemic and opportunistic infections together caused **Deep mycoses**.

The organisms have a mycelial form when grown on fungal culture and have yeast form in the tissue. The examples of systemic mycoses are Histoplasmosis, Blastomycosis.

9.4.1 Histoplasmosis

Histoplasmosis is caused by dimorphic fungus ***Histoplasma capsulatum***. The fungi live inside the cells of the reticuloendothelial system, where they grow within macrophages and giant cells. This infection is also known as **Darling's disease**.

9.4.2 Pathogenesis and Pathology

The infection with H. *capsulatum* develops when conidia or mycelial fragments are in haled and converted into yeasts in alveolar macrophages in the lungs. The oval yeast cells parasitize macrophages, which are activated by T lymphocytes resulting in localized granulomatous inflammation.

HOTS

H. *capsulatum* is dimorphic fungi - justify

9.4.3 Clinical Features

The disease is mostly asymptomatic. The development of symptom or symptomatic disease appears to depend on the intensity of exposure to conidia and cellular immune response of the host. The disease may be classified as follows.

1. Acute pulmonary Histoplasmosis – Fever, headache, chills, sweating, chest pain, cough and dyspnoea
2. Chronic pulmonary Histoplasmosis – Ulcerative lesions of the lips, mouth, nose, larynx and intestines
3. Cutaneous, mucocutaneous Histoplasmosis – Mucous lesions on skin, abdomen wall and thorax.
4. Disseminated Histoplasmosis – Fever, anoxia, anemia, leucopenia constant hepatosplenomegaly and multiple lymphadenopathies.

9.4.4 Laboratory Diagnosis

i. Samples

Specimens collected are sputum, bone marrow and lymph nodes, cutaneous and mucosal lesions and peripheral blood film.

a. Direct Examination

Thick and thin smears should be prepared from peripheral blood, bone marrow and stained with Calcofluor white, Giemsa or Wright stains.

The fungus is small, oval yeast like cells, 2–4 µm in diameter, within the mononuclear or polymorpho nuclear cells and occasionally in giant cells.

b. Fungal culture

The clinical samples is inoculated on Sabouraud dextrose agar (SDA) and

Brain-heart infusion (BHI) agar with antibiotics and actidione at 25°C and 37°C. On Sabourad dextrose agar the colonies appear albino or brown. The albino type consists of white, fine aerial hyphae and brown type consists of flat colonies with light tan or dark brown in color in seven days. At 37°C the colonies grow as granular to rough, mucoid and cream-colored turning tan to brown in 14 days.

ii. Treatment

Amphotericin B is given for the treatment of disseminated and other severe forms of Histoplasmosis.

9.5 Opportunistic Mycoses

The opportunistic systemic mycoses are infections found in patients with underlying pre disposing conditions. It is produced by non pathogenic or contaminant fungi in a host, where the immunological defense mechanisms are weakened by endogenous causes like cancer, leukemia or exogenous causes like immunosuppressive therapy and AIDS. The examples of opportunistic mycoses are Candidiasis, Cryptococcosis, Aspergillosis and zygomycosis.

9.5.1 Candidiasis

Candidiasis is the commonest fungal disease found in humans affecting **mucosa, skin, nails and internal organs** of the body. It is caused by yeast like fungi called *Candida albicans*. The infection may be acute or chronic, superficial or deep and found mainly as secondary infection in individuals with immune compromised condition.

The **fungus *candida albicans*** is responsible for most **vaginal** yeast infections. Your vagina naturally contains a balanced mix of yeast, including candida, and bacteria. Certain bacteria (lactobacillus) act to prevent an **overgrowth** of yeast. But that balance can be disrupted.

Pathogenesis and Pathology

Some of the virulence factors contributing to pathogenicity are toxins, enzymes and adhesion. The organism adheres to the epithelial and endothelial cells by proteinase production. Then the yeast cells of *Candida* encounter a particular host tissue and colonization takes place at the local site or they invade deeper into the host tissue and induce various clinical symptoms.

Clinical Features

The *Candida* species are found as commensal on mucosal surfaces of the body. They cause disease as and when conditions are favourable. This yeast like fungi colonizes mucocutaneous surfaces, which can be portals of entry into deeper tissues when the host defenses are compromised. They may cause a simple lesion to event the life threatening systemic infection.

The clinical manifestations of Candidiasis are divided into two broad categories. They are:

1. Infectious Diseases

a. Mucocutaneous Involvement

i. Oral Candidiasis – Most common form *Candida* colonizes on the oral

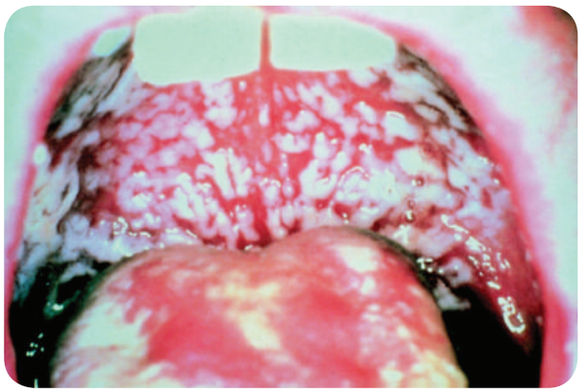


Figure 9.9: Oral candidiasis

cavity (oral thrush) infection on the buccal mucosa, gums, tongue, reddening of the mucous membrane gives dry, smooth metallic taste and burning at the local site (Figure 9.9).

ii. Alimentary Candidiasis – *Candida* colonizes on the oesophagus causing oesophagitis. It is mostly asymptomatic or it may cause burning pain in the epigastrium or throat.

b. Cutaneous Dermatitis

i. Diaper Dermatitis – *Candida* colonize on the cutaneous layer causes cutaneous Candidiasis leads to maculopapules vesicles with erythematous rash. This is common among infants and known as **Diaper rash**.

ii. Intertrigo – This is an **i**nflammatory lesion of the skin folds due to candidal infection.

c. Systemic Involvement

The *Candida* colonizes in various organs and causes various manifestations through the blood stream. Clinical features are found to be Urinary tract C**andidiasis, Candiduria, Endocarditis, Pulmonary Candidiasis, Arthritis, Osteomyelitis, Meningitis, Candidemia** and **Septicemia.**

2. Allergic Diseases

Allergic manifestation is caused due to the metabolites of *Candida*. The cutaneous allergies are **urticaria** and **eczema**, and **bronchial asthma**.

Laboratory Diagnosis

i. Samples

Specimens collected are mucous membrane from the mouth, vagina, skin and sputum based on the site of involvement.

a. Direct Examination

Gram staining LPCB, and KOH wet mount are used to visualize the yeast cells.

Presence of yeast cells approximately 4.8 µm with budding and pseudo hyphae are observed. Other stains like periodic acid - Schiff stain and Gomori's methylamine silver stain are also used to observe the fungal elements in tissue.

b. Fungal culture

The clinical specimens can be cultured on Sabouraud dextrose agar (SDA) with antibiotics and incubated at 25°C and 37°C (Figure 9.10). The colonies appear in 3–4 days as cream coloured, smooth and pasty.

The some of the species of *Candida* are *Candida albicans, Candida tropicalis, Candida krusei* and *Candida glabrata*.

ii. Special Test

Germ tube test

The culture of Candida species is treated with sheep or normal human serum and inoculated at 37°C for 2 to 4 hours. A drop of suspension is examined on the slide. The germ tubes are seen as long tube–like projections extending from

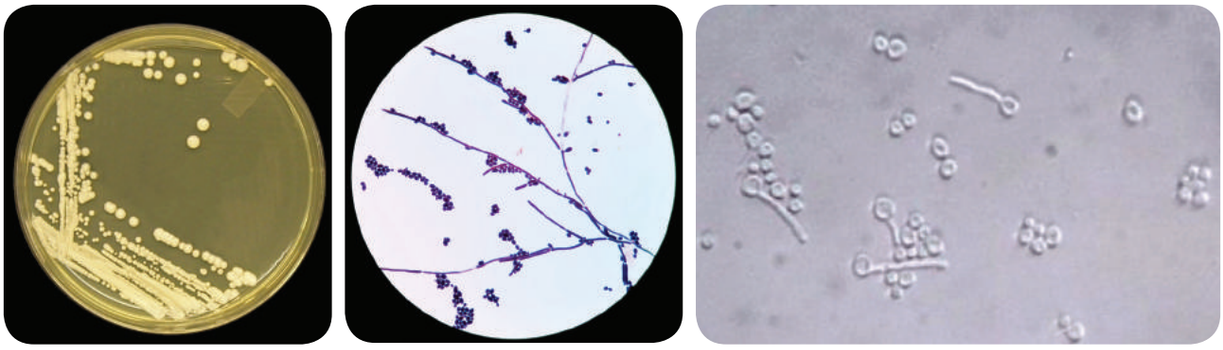


Figure 9.10: Candida **(a)** Macroscopic Morphology **(b)** Microscopic Morphology **(c)** Germ tube

the yeast cells. The demonstration of the germ tube is known as Reynolds – Braude phenomenon.

Biochemical tests

Sugar fermentation and assimilation tests are used for the identification of Candidal species. C.*albicans* ferments Glucose and Maltose and assimilates Glucose, Maltose, Sucrose, Lactose and Galactose.

Chlamydospores formation

Candida isolates are grown on corn meal, agar (CHN) or rice starch agar (RSA) and incubated at 25°C for 2–3 days. The formation of large, thick walled terminal chlamydospores is demonstrated in C.*albicans* and C. *dubliniensis*.

iii. Treatment

1. 1% gentian violet is locally applied to the affected areas.
2. The azole creams like Clotrimazole, Miconazole, Ketoconazole and Econazole are also used.

9.5.2 Cryptococcosis

Cryptococcosis is an acute, sub acute or chronic fungal disease caused by encapsulated yeast called *Cryptococcus neoformans*. It is pathogenic to man and animals. It causes opportunistic infection, involving the lungs and disseminates to extra pulmonary sites through circulation to different body organs particularly to central nervous system causing **Meningoencephalitis**.

Infobits

What does Cryptococcus cause?

Meningitis can be caused by different germs, including bacteria, **fungi**, and viruses. Two types of fungus can cause **cryptococcal meningitis** (CM). They are called Cryptococcus neoformans (C. *neoformans*) and Cryptococcus gattii (C. *gattii*). This disease is rare in healthy people.

Pathogenesis and Pathology

Cryptococcal infection occurs through inhalation of small forms or basidiospores. The fungus may remain dormant in the lungs until the immune system weakens and then can disseminate to the central nervous system and other body sites.

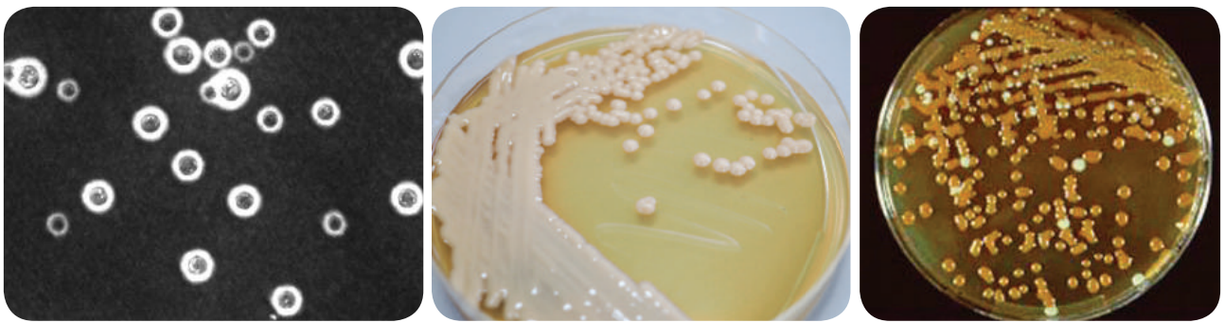


Figure 9.11: Cryptococcus **(a)** capsule staining **(b)** On SDA **(c)** On BSA

Clinical Features

The clinical features of Cryptococcosis depend upon the anatomical sites.

i. Pulmonary Cryptococcosis

The respiratory route is usually the portal of entry for propagules in Pulmonary Cryptococcosis that subsequently disseminate to extra pulmonary sites. The symptoms are dry cough, dull chest pain and milder or no fever with small gelatinous granules all over the lungs.

ii. CNS Cryptococcosis

This is an infection of brain and meninges leading to Meningoencephalitis. Nitrogenous source such as asparagines and creatinine present in cerebrospinal fluid enrich the yeast. The symptoms are nausea, dizziness, impaired memory, blurred vision and photophobia. The enlarged granulomatous cerebral lesions are called **cryptococcoma**.

iii. Visceral Cryptococcosis

This infection usually spreads from a primary focus to invade the optic nerve and meninges. Visual loss in patients is due to intra cranial pressure. There are two distinct patterns of visual loss namely; rapid visual loss (within 12 hrs) and slow visual loss (within weeks to months).

Laboratory Diagnosis

i. Samples

Specimens collected are mainly serum, CSF and other body fluids.

a. Direct Examination

10% Nigrosin or India ink staining, Gram staining and LPCB are used to visualize the yeast cell.

Biopsy material is stained with periodic acid - Schiff and Gomoris's methylamine silver stain to observe the fungal cells in the tissue. Round budding yeast cells with a distinct halo gelatinous capsule can be seen (Figure 9.11a). Gram positive budding yeast cells are demonstrated by Gram staining.

b. Fungal Culture

The clinical specimens can be cultured on Sabouraud dextrose agar, Bird Seed agar and incubated at 37°C. The colonies are mucoid, cream to buff - colored in SDA (Figure 9.11b), whereas brown colored due to conversion of the substrate into melanin by Phenoloxidase in BSA (Figure 9.11c).

ii. Treatment

1. Amphotericin B, Flucytosine is given together as induction and maintenance therapy.
2. Fluconazole is also recommended.

Saccharomyces cerevisiae Fungemia: An Emerging **Infectious** Disease. Saccharomyces cerevisiae is well known in the baking and brewing industry and is also used as a probiotic in humans. However, it is a very uncommon cause of infection in humans.

Summary

This chapter dealt with the general classification of fungi in relationship with the host cells, classification of mycoses and also about the vegetative and reproductive structure, its growth and nutrition. Medically important fungi such as Dermatophytoses, Mycetoma, Histoplasmosis, Candidiasis and Cryptococcosis its pathogenesis, clinical features and laboratory diagnosis were discussed.

Evaluation

Multiple choice questions

1. _____ is the father of Medical Mycology
   a. Pasteur
   b. Raymond Jacques Sabouraud
   c. Robert koch
   d. Anton de Bary

92XUG2

2. _____ is an example for deep mycoses
   a. Systemic b. Opportunistic
   c. Both d. None of these
3. _____ is an example for dimorphic fungi
   a. *Histoplasma* b. *Mucor*
   c. Cryptococcus d. None of these
4. Phaeoid fungi is also called as _____
   a. Black yeast b. White yeast
   c. Moulds d. None of these
5. Tinea Barbae is also called as _____
   a. Athlete's foot b. Onchomycosis
   c. Barber's itch d. None of these
6. Tear shaped micro conidia is found in _____
   a. *T.mentagrophytes* b. *T.rubrum*
   c. *T.vercossum* d. None of these
7. _____ is called as Madura foot
   a. Piedra b. Tinea pedis
   c. Mycetoma d. None of these
8. _____ is known as Darling's disease
   a. Cryptococcosis b. Histoplasmosis
   c. Candidiasis d. None of these
9. Diaper rash is caused by _____
   a. Dermatophytes b. Histoplasmosis
   c. Candidiasis d. None of these
10. Demonstration of _____ is called as Reynolds – Braude phenomenon
    a. Arthrospore b. Chlamydospore
    c. Germ tube d. None of these

Answer the following

1. Define Mycology.
2. What is host parasitic relationship?

3. What are toxigenic fungi?
4. Discuss about the types of mycoses?
5. Define Aeromycology?
6. What is Pseudohyphae?
7. What are Dimorphic fungi?
8. Brief note on vegetative structure of fungi.
9. Account on reproduction of fungi.
10. Short note on taxonomy of fungi?
11. What are the clinical features of dermatophytes?
12. Define Ringworm infection.
13. Differentiate dermatophytic fungi based on macroscopic and microscopic morphology
14. What is maduramycosis.
15. Short note on classification of mycetoma.
16. Define deep mycoses
17. Elaborate on clinical feature of histoplasmosis.
18. What is Opportunistic mycosis.
19. Discuss on Lab diagnosis of Candidiasis?
20. Define Germtube.
21. What is Cutaneous dermatitis?
22. Note on Meningoencephalitis.
23. What is Capsulated yeast?
24. Brief account on clinical feature of Cryptococcosis.
25. Differentiate between *Candida* and *Cryptococcus.*

Chapter
10

Medical Virology

Learning Objectives

After studying this chapter the students will be able to,

- *Study the importance of viruses causing disease in humans and from Animals. Viral infection are very common so its our main concern to protect us from the viral infection.*
- *Study the classification, structure and cultivation of Viruses.*
- *Study about the virus its pathogenesis, clinical feature and its treatment and prophylaxis.*
- *Study about the collection, processing of the sample and its molecular diagnosis.*

Chapter Outline

UGHGMI

Viruses are **infectious agents** with size ranging from about 20 nm to about 300 nm is diameter and contain only **one type of nucleic acid** (RNA or DNA) as their genome. The nucleic acid is encased in a protein shell, which may be surrounded by a lipid containing membrane. The entire infection unit is termed as **virion**. Viruses are obligate intracellular parasite, they replicate only is living cells. The study of virus is called as **virology**. **Martinus Beijerinck** is known as the Father of Virology.

10.1 Evolutionary Origin of Viruses

The origin of viruses is not known, but two theories of vital origin can be summarized as follows;

i. Viruses may be **derived from (DNA or RNA)** nucleic acid components of host cells to replicate and evolve independently.

ii. Viruses may be **degenerate forms** of intracellular parasites.

Morphology

Size

Viruses are smaller than bacteria, known as filterable viruses vary widely in size.

The **largest** among them is the **Pox virus** measuring about 300nm. The **smallest** virus is **Parvo virus** measuring about 20 nm.

Structure and Shape

The virion consists of nucleic acid surrounded by a protein coat, **the capsid**. The capsid with the enclosed **nucleic acids** is known as the nucleo capsid. The capsid is composed of a large number of **capsomers**. The functions of the capsid are to **protect** the **nucleic acid** from the deleterious agents and also to introduce viral genome into host cells by adsorbing readily to cell surfaces (Figure 10.1). Two kinds of symmetry encountered in the virus are **icosahedral** (cubical) and **helical**. Virions may be **enveloped or non enveloped** (naked). The envelope or outer covering of viruses is derived from the host cell membrane when the progeny virus is released by budding. The envelope is **lipoprotein** in nature. The lipid is of host cell origin while the protein is virus coded. **Protein subunits** may be seen as **projecting spikes** on the surface of the envelope and are known as Peplomers.

Overall shape of the virus particle varies; mostly animal viruses are roughly spherical. Some are irregular and pleomorphic. The rabies virus is **bullet shaped**, Ebola virus is **filamentous** and pox viruses are **brick shaped**.

Chemical Properties

Viral protein determines the **antigenic specificity** of the virus. Some viruses contain small amounts of carbohydrates. Most Viruses do not possess any enzymes but **retro virus** has a unique enzyme, such

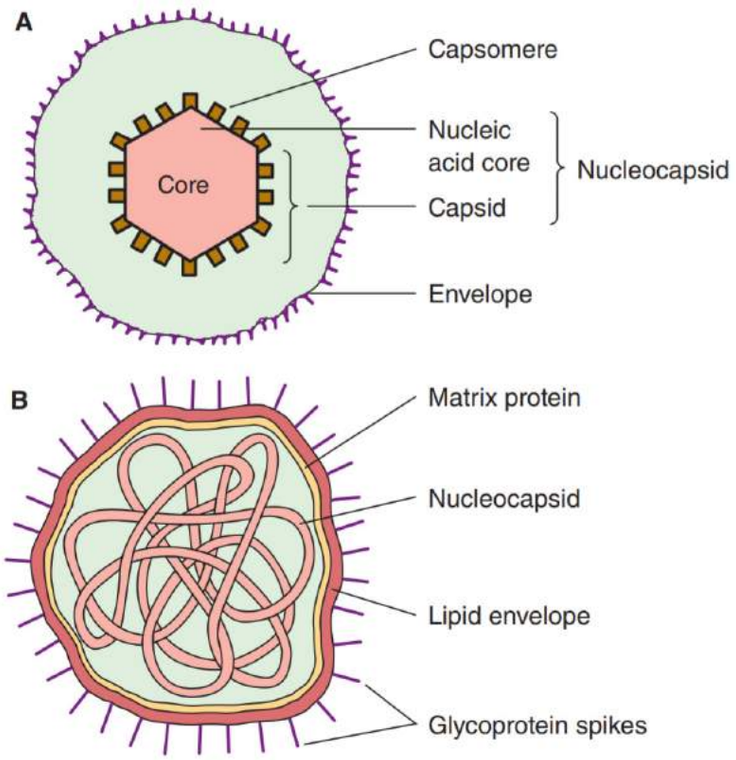


Figure 10.1: Structure of Virus

as **RNA dependent DNA polymerase or transcriptase** which can transcribe RNA into DNA.

Resistance

Viruses are **inactivated** by sunlight, UV rays and ionizing radiations. The most active **antiviral disinfectants** are oxidizing agents such as hydrogen peroxide, potassium permanganate and hypochlorites. Organic iodine compounds are actively **virucidal**. Chlorination of drinking water kills most viruses but its efficacy is influenced by the presence of organic matter. Some viruses such as hepatitis virus, polio viruses are relatively resistant to chlorination.

Antibiotics active against bacteria are completely ineffective against viruses.

Viral Multiplication

The genetic information necessary for viral replication is contained in the viral nucleic acid, and also depends on the synthetic machinery of the host cell for replication. The Viral multiplication cycle can be divided into **six steps** and they are as follows, **1. Adsorption or attachment, 2. Penetration, 3. Uncoating, 4. Bio synthesis, 5. Maturation** and **6. Release.**

1. Adsorption

Virions may come into contact with cells by random collision but adsorption takes place only if there is an **affinity** between the **virus and the host**. The cell surface should contain specific receptor site for the virus to attach on to it.

2. Penetration

Bacteria possess rigid cell walls, only the viral nucleic acid is introduced intracellularly by a complex mechanism. Animal cells do not have rigid cell walls and the whole virus can enter and virus particles may be engulfed by a mechanism resembling **phagocytosis**, a process known as **'Viropexis'**. In case of the **enveloped viruses**, the viral envelope may **fuse** with the plasma membrane of the host cell and release the nucleocapsid into the cytoplasm.

3. Uncoating

Release of the viral nucleic acid from the capsid into the host cell with most viruses, uncoating is affected by the action of lysosomal enzymes of the host cell.

4. Biosynthesis

Virus can synthesise viral nucleic acid, capsid protein and also the enzymes necessary in the various stages of viral synthesis, assembly and release. In addition certain regulator proteins are also synthesized. Most **DNA viruses** synthesise their nucleic acid in the **host cell nucleus**. Most **RNA viruses** synthesise all their components in the cytoplasm.

Biosynthesis consists of the following steps:

i. Transcription of messenger RNA (mRNA) from the viral nucleic acid.

ii. Translation of the mRNA into 'early proteins'

iii. Replication of Viral nucleic acid.

iv. Synthesis of late or structural proteins, which are the components of daughter virion capsids.

5. Maturation

Assembly of daughter virions follows the synthesis of viral nucleic acid and proteins. Virions assembly may take place in the host cell nucleus or cytoplasm. Herpes and adeno viruses are assembled in the **nucleus**, while picorna and pox viruses are assembled in the **cytoplasm**.

6. Release

In case of bacterial viruses, the release of progeny virions takes place by the lysis of the infected bacterium. However, in the case of **animal viruses**, release usually occurs **without cell lysis**. **Eclipse phase** is from the stage of penetration till the appearance of mature daughter virions. The virus cannot be demonstrated inside the host cell. The virus seems to disappear (Figure 10.2).

Viroids

Viriods are small, single stranded covalently closed circular RNA molecules existing as highly base paired rod like structure. The viroid depends on the host for replication. These are responsible for some of the transmissible plant diseases.

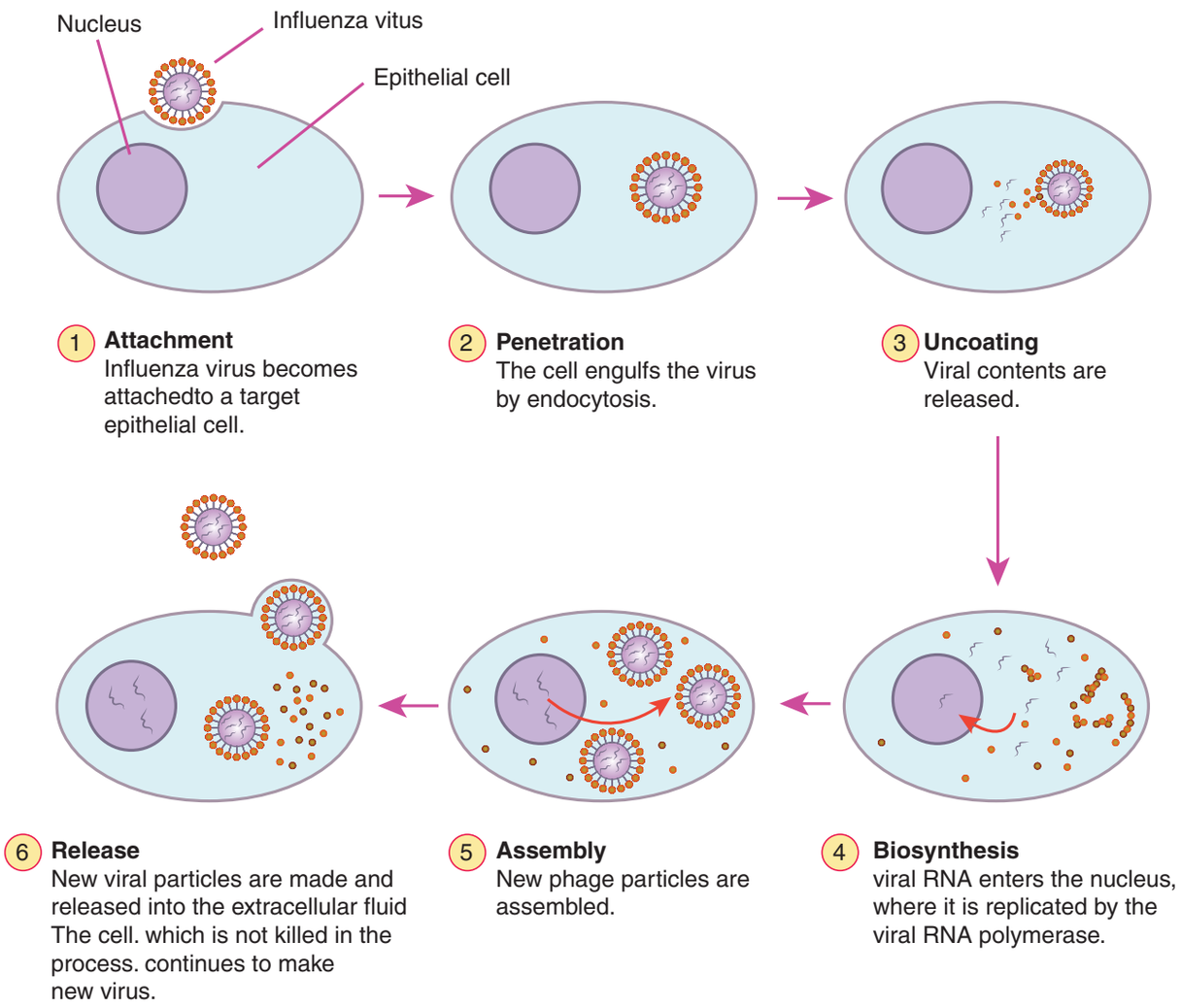


Figure 10.2: The Viral Life cycle

HOTS

Which is more dangerous – bacteria or Virus?

Prion

Prions are small proteinaceous infectious agents without genetic material. These are responsible for a number of degenerative brain diseases (Example: Creutzfeldt) and hereditary dementia.

10.2 Cultivation of Viruses

Viruses are **obligate intracellular** parasites; they cannot be grown on any inanimate culture medium. **Three methods** are employed for the cultivation of viruses – **inoculation into animals, embryonated eggs and tissue culture or cell culture**.

i. Animal Inoculation

The earliest method for the cultivation of viruses causing human diseases was inoculation into human volunteers. Monkeys were used for the isolation of the polio virus by **Landsteiner and Popper (1909)**. The embryonated hen's egg was first used for cultivation of viruses by **Good pasture (1931)**. The embryonated egg offers several sites for the cultivation of viruses. Non human primates provide the only method for virus cultivation. Mice are most widely employed animals in Virology.

ii. Embryonated Eggs

a. Chorioallantonic Membrane (CAM)

Inoculation on **the chorioallantonic membrane** produces visible lesions (pocks). Different viruses have different pock morphology. Example: varcola or vaccinia

b. Allantonic Cavity

Inoculation on the **allantonic cavity** provides a rich yield of influenza and some paramyxo viruses.

c. Amniotic Sac

Inoculation into the **amniotic sac** is for the primary isolation of the influenza virus.

d. Yolk Sac

Inoculation into the **yolk sac** is for the cultivation of some viruses like Chlamydiae and Rickettsiae.

Allantonic inoculation is employed for growing influenza virus for vaccine production (Figure 10.3).

iii. Tissue Culture

First tissue culture in Virology was maintained by **Steinhardt and colleagues (1913)** for the vaccinia virus in fragments of rabbit cornea. Bacterial contamination was the major limitation. Different types of culture used are:

a. Organ culture

Small bits of organs can be maintained, used for the isolation of some viruses. Example: Corona virus (respiratory pathogen) cultured on tracheal ring organ culture.

b. Explant culture

Fragments of minced tissue are grown as 'explants'. This is also known as tissue culture. Example: Adeno virus cultured on Adenoid tissue explants.

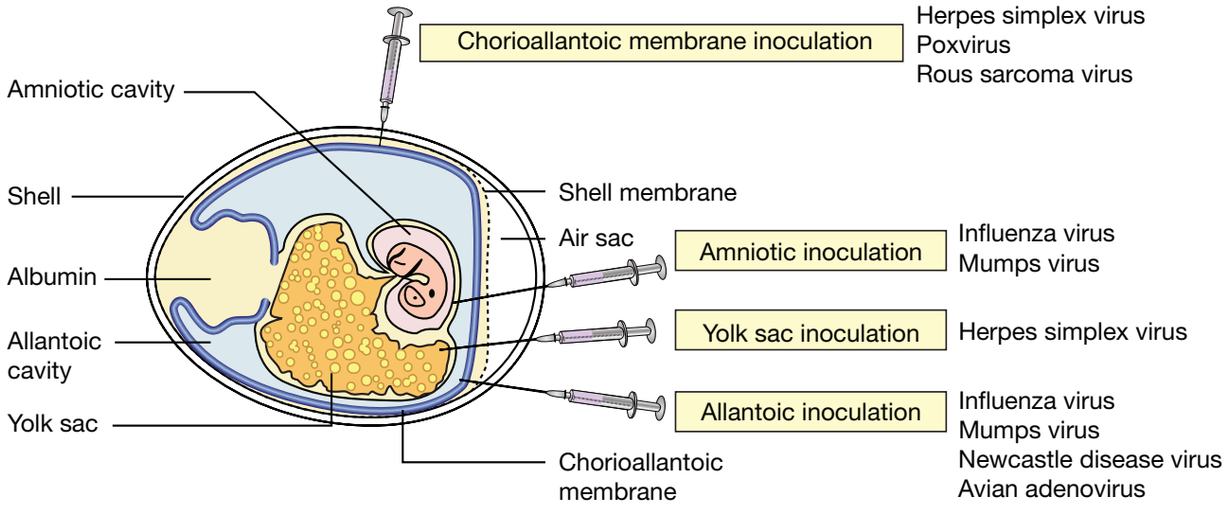


Figure 10.3: Cultivation of virus in Embryonated Egg

iv. Cell Culture

Tissues are dissociated into the component cells by the **action of enzymes** (trypsin) or by mechanical process and are suspended in a growth medium (amino acids, vitamins, salts, glucose) **supplemented** with fetal calf serum of antibiotics and **indicator** (Phenol red). This media is dispensed in bottles, tubes or petridishes. The cells adhere to the glass surface and on incubation divides to form a confluent monolayer sheet of cells covering the surface within about a week. The cell culture is classified into three types.

a Primary cell cultures

In this culture, **normal cells** are taken from the body and cultured. They are capable of only limited growth in culture. Example: Monkey kidney, Human embryonic kidney, Chick embryo cell culture.

b. Diploid cell strains

These are cells of a **single type** that retain the original **diploid chromosome number** and serotype during serial sub cultivation for limited number of times. Example: Human fibroblast.

c. Continuous cell lines

These are **single type, derived from cancer cells** that are capable of continuous serial cultivation. Example: Cells derived from cancers, such as Hela, Hep-2 and KB cell lines.

Detection of virus Growth in Cell Cultures

Virus growth in cell cultures can be detected by the following methods.

1. Cytopathic effect

Many viruses cause morphological changes in cultured cells in which they grow. These changes are known as **cytopathic effects (CPE) and** the viruses causing CPE are called as **'cytopathogenic viruses'.**

2. Metabolic inhibition

In normal cell cultures, the medium **turns acidic** due to cellular metabolism. This can be made out by the colour of the indicator (Phenol red) incorporated in the medium.

3. Haemadsorption

When **haemagglutinating viruses** grow in cell cultures, their presence can be identified by the addition of guinea pig

erythrocytes. The erythrocytes will adsorb on to the surface of viral cells multiplying in the culture. This is known as **'haemadsorption'**.

4. Interference

The growth of **non cytopathogenic virus** in cell culture can be tested with the cytopathogenic virus. The growth of the first will inhibit infection by the second virus by **'interference'**.

5. Transformation

Tumour forming viruses **induce cell transformation** and loss of contact inhibition, so that growth appears in a piled up fashion producing **'microtumours'**.

6. Immuno fluorescence

Cells from virus infected cultures are **stained by fluorescent** conjugated antiserum and examined under the UV microscope for the presence of virus antigen.

10.3 Herpes Viruses

The herpes virus family contains more than a hundred species of **enveloped DNA** viruses that affect humans and animals.

Structure

The herpes virus **capsid is icosahedral**, composed of 162 capsomers and enclosing the core containing the linear **double stranded DNA** genome. The nucleocapsid is surrounded by the lipid envelope derived from the host cell. The envelope carries **surface spikes** (Figure 10.4). **Teguments** are present in between the envelope and capsid. The **enveloped virion** measures about 200nm and the **naked virion** about 100 nm in diameter.

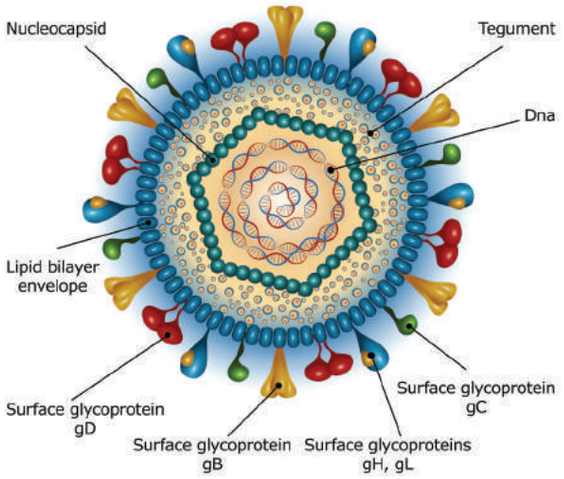


Figure 10.4: Structure of Herpes Simplex Virus

Classification

The family Herpesviridae is divided into **three subfamilies** based on biological, physical and genetic properties (Table 10.1).

Table 10.1: Classification of Human Herpes Viruses

Species	
Name	**Common name**
Human herpes virus type1	Human Simplex virus type1
Human herpes virus type 2	Human Simplex virus type2
Human herpes virus type 3	Varicella Zoster virus
Human herpes virus type 4	Epstein - Barr Virus
Human herpes virus type 5	Cytomegalovirus
Human herpes virus type 6	Human B cell lymphotropic virus*
Human herpes virus type 7	R K Virus *
Human herpes virus type 8	-

i. Alpha herpes viruses

They have relatively **short replicative cycle** (12–18 hours) and a variable host range. They cause latent infection in sensory ganglia. Example: Herpes simplex virus and varicella zoster virus.

ii. Beta herpes viruses

They **replicate slowly** (more than 24 hours) and have a narrow host range, grow well in fibroblasts. They cause latent infection of salivary gland and other organs. Example: Cytomegalovirus.

iii. Gamma herpes viruses

They have a **narrow host range** and replicate in lymphoblastoid cells. They are specific for either B or T lymphocytes and causes latent infection in lymphoid tissue Example: Epstein - Barr Virus. Eight different types of herpes viruses are known whose primary hosts are humans. They have been designated as 'Human herpes virus type 1–8.

1. Herpes Simplex

The herpes simplex virus (HSV) occurs **naturally only in humans**, but it can produce experimental infection in laboratory animals. There are **two types** of the herpes simplex virus. **HSV type 1** (Human herpes virus type 1 or **HHV type 1**) is isolated from lesions in and around the mouth and is transmitted by direct contact or droplet spread from carrier. **HSV type 2** (Human herpes virus type 2 or **HHV type 2**) is responsible for the genital herpes infections transmitted venereally.

Pathogenesis

Herpes simplex is one of the most **common viral infection** humans, the sources of infection are saliva, skin lesions or respiratory secretions. In type 2, transmission occurs by close contact and may be venereal in genital herpes.

The virus enters through defects in the skin or mucous membranes and multiples locally, with cell to cell spread. The **herpes lesions** are thin walled, umbilicated vesicles, the roof of which breaks down, leaving tiny superficial ulcers. They heal without scarring.

Clinical features

The clinical manifestations depend on the site of infection, age and immune status of the host and the antigenic type of the virus. They are

- Cutaneous infections
- Mucosal infections
- Ophthalmic infections
- Nervous system infections
- Visceral infections
- Genital infections

Laboratory diagnosis

Microscopy

Smears are prepared from the lesions, from the vesicles and stained with 1% aqueous solution of **toluidine blue 'O'** for 15 seconds. Multinucleated giant cells with faceted nuclei with ground glass chromatin (**Tzanck cells**) was observed.

Virus isolation

Inoculation in mice and on chick embryo CAM is insensitive. Primary human embryonic kidney, human amnion cells are susceptible, but **human diploid fibroblasts** are preferred vesicle fluid, spinal fluid, saliva and swabs may be used. Cytopathic changes may appear as early as 24–48 hrs.

Serology

Antibodies develop within a few days of infection and **rise in titre of antibodies** may be demonstrated by ELISA, neutralization or complement fixation tests.

Chemotherapy

Indoxyuridine used topically in eye and skin infection, acyclovir and vidarabine are given for deep and systemic infections.

2. Varicella Zoster

In 1889, Von Bokay had suggested that varicella (Chicken pox) and herpes zoster are **different manifestations** of the same virus infection. The virus is therefore called **Varicella zoster virus** (VZV). The chicken pox follows **primary infection** in a non immune individual, while herpes zoster is a **reaction of the latent virus** when the immunity has fallen to infective levels.

VZV is **similar** to the herpes simplex virus in its morphology. It can be grown in cultures of **human fibroblasts human amnion** or HeLa cells. Chicken pox is one of the mildest and **most common of child hood infections**. The disease may, occur at any age.

Herpes gladiatorum is **spread** through skin-on-skin contact. If you kiss someone with a herpes cold sore on their lips, you could become infected.

3. Cytomegaloviruses

Cytomegaloviruses (CMV) formerly known as **salivary gland viruses** are a group of ubiquitous herpes viruses of humans and animals. They are characterized by **enlargement of infected cells** and intranuclear inclusions. In 1926, cytomegalia presumed to be due to viral infection was reported in the salivary glands of guinea pigs and children and the viral agent was called the **'salivary gland virus'.**

Infobits

CMV is the **largest viruses** in the herpes virus family, being 150–200 nm in size.

4. Epstein – Barr Virus

A number of different viruses apparently **'Passenger Viruses'** were isolated from cultured lymphoma cells. **Epstein, Barr and Achong in 1964** observed a new type of herpes virus named it has **'EB Virus'** affecting cells of B lymphocyte. Only human and some sub human primate **B cells have receptors** (CD21 molecules) for the virus.

The source of infection is usually the **saliva of infected persons** who shed the virus in orophryngeal secretions. Intimate oral contact, as in kissing, appears to be the predominant mode of transmission. This accounts for infectious mononucleosis being called as **'The kissing disease'**.

5. Human Herpes Virus Types 6,7,8

A herpes virus, first isolated in 1986 from the **peripheral blood** of patients with lympho proliforative disease called as **human B lymph tropic virus**, renamed as HHV - 6. HHV- 7 was isolated in 1990 from **peripheral CD4 cells** of a healthy

Does HSV shorten your lifespan?

Becoming infected with **the herpes virus** seriously complicates your social, emotional and sexual life, but it is not otherwise a terribly dangerous condition to have. Having **genital herpes does** make it easier to get HIV (and thus AIDS), but otherwise, the condition is not disabling, and does not reduce lifespan.

person appears to be widely distributed and transmitted through saliva.

In 1994, DNA sequences presumed to represent a new herpes virus from **tissues of Kaposi's sarcoma** from AIDS patients named as HHV8. Later identified in Kaposi's sarcoma in persons not infected with HIV and referred to as **Kaposi's Sarcoma-associated herpes virus** (KSHV).

10.4 Hepatitis Viruses

The term viral hepatitis refers to a **primary infection of the liver**, hepatitis viruses consists of types A, B, C, D, E and G. Except for **type B** which is a DNA virus all the others are RNA viruses.

Two types of viral hepatitis had been recognised **type one** affects mainly children and young adults and transmitted by the fecal-oral route called **as infective or infectious hepatitits or type A hepatitis. Second type** transmitted mainly by receiving serum inoculation or blood transfusion named as **homologous serum jaundice, serum hepatitis transfusion hepatitis or type B hepatitis**.

Type A Hepatitis (HAV)

HAV is a 27nm non enveloped RNA virus belonging to the **picorna virus family**. It is designated as **'entero virus 72'**, HAV is recognised as new genus **'Hepatovirus'**. It can be grown in human and simian cell cultures and is the only human hepatitis virus can be cultivated in vitro.

HAV transmission is by the **fecal oral route**. Infection is by ingestion. The virus multiplies in the intestinal epithelium and reaches the liver by **hematogenous spread**. Once jaundice develops, it is rarely detectable in feces. The incubation period is 2- 6 weeks. The clinical disease consists of two stages the **prodromal and the icteric stage**. The onset may be acute with fever, malaise, anorexia, nausea, vomiting and liver tenderness. These usually subside with the onset of jaundice. Recovery is slow, over a period of **4–6 weeks**. The disease is milder in children. Type A hepatitis caused by **contaminated food, water or milk**. Over crowding and poor sanitation favour its spread.

Laboratory Diagnosis

Diagnosis of type A hepatitis may be made by **demonstration of the virus** or its **antibody**. Virus can be visualized by **Immunelectron Microscopy (IEM)** in fecal extracts during the late incubation period.

IgM anti-HAV antibody appears during the late incubation period disappears after 3-4 months. **IgG** appears peaks in 3-4 months and persists much longer for life. ELISA kits for detection of IgM and IgG antibodies are available.

A safe and effective formalin inactivated, **alum conjugaged vaccine** containing HAV grown in human diploid cell culture. Course consists of **two intra muscular injections** of the vaccine. Protection begins **4 weeks** after injection and lasts for **10 to 20 years**. No specific antiviral drug is available.

Type B Hepatitis (HBV)

HBV is a 42nm **DNA virus** with an outer envelope and an inner core 27nm in diameter. Enclosing the **viral genome** and a **DNA polymerase**. It belongs to the family Hepadna Viridae HBV is **'Hepadna Virus type 1'**. **Australia antigen** was found to be associated with serum hepatitis. It was the surface component of HBV, so named as **hepatitis B surface antigen (HBsAg)**.

3 types of particles are visualized, most abundant form is a **spherical particle**, 22nm in diameter. The second type of particle is **filamentous** or **tabular** with a diameter of 22nm both are antigenically identical. Third type of particle for fewer in number, is a **double walled spherical** structure 42 nm in diameter. This particle is the complete **hepatitis B virus**, known as **Dane particle**.

The envelope proteins expressed on the surface contains **hepatitis B surface antigen (HBsAg)**. HBsAg consists of two major polypeptides, one of which is glycosylated. The nucleocapsid or core contains **hepatitis B core antigen (HBcAg A)** (Figure 10.5)**.** Third antigen called the **hepatitis B e antigen (HBeAg)** is a soluble non particulate nucleocapsid protein.

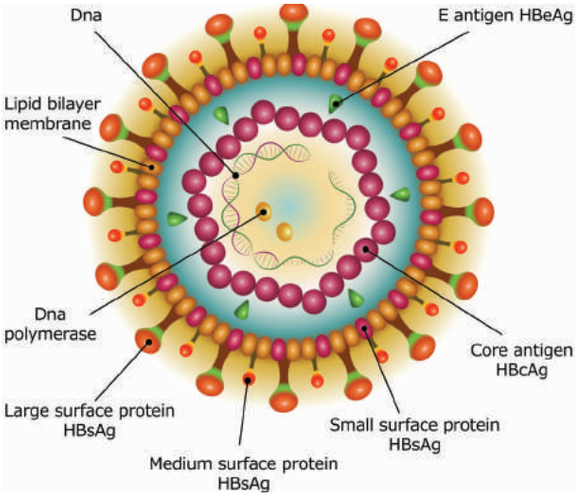


Figure 10.5: Structure of Hepatitis B Virus

The **nucleocapsid** encloses the viral genome consisting of **two linear strands of DNA** held in a circular configuration. One of the strands **incomplete** (+ strand) DNA appears partially **double stranded** and partially **single stranded**. Associated with the + strand is a **viral DNA polymerase** (has both DNA dependent DNA polymerase and RNA dependent reverse transcriptase functions). This polymerase can **repair the gap** in the plus strand and render the **genome fully double straned**.

Natural infection occurs **only in humans**. The virus is maintained in carriers whose blood contains **circulating virus** for long periods carriers are of two categories, the highly infectious **super carriers** and the simple carriers. Former have high titre HBsAg along with HBsAg, DNA polymerase and HBV in ciruculation. Simple carriers have low infectivity and low titre HBsAg in blood.

HBV is a **blood borne virus** and the infection is transmitted by **parenteral, sexual and perinatal models**. The virus may also be present in other **body fluids and excreations** such as saliva, breast

milk, semen, vaginal secretions, urine bile and feces of these semen and saliva are known to transmit the **infection** very commonly. Transfusion of carrier blood once the most widely known mode infection has largely been eliminated by donor screening is strictly enforced. Infection by direct contact with open skin lesions such as pyoderma, eczema, cuts and scratches is very common among **young children** in developing countries. Certain groups and **occupations** carry a high risk of infection. These include medical and paramedical staff of blood banks, dialysis units, barbers, box workers.

The incubation period is long about 1- 6 months. The onset is insidious and fever is not prominent. Extra hepatic complications like arthralgia, urticaria and glomerulonephritis may occur. About 90-95% of adults with acute hepatitis B infection recover within 1-2months of onset and eliminate the virus from the body. They may be **Asymptomatic carriers** or may progress to recurrent or chronic liver disease.

Laboratory Diagnosis

Serology

Diagnosis of hepatitis B depends on the **serological demonstration** of the viral markers. **HBsAg** is the first marker to appear in **blood** after infection, being detectable. It remains in circulation throughout the symptomatic course of the disease (2- 6months). Anti HBs is the protective antibody.

HBcAg is **not demonstrable** in circulation because it's **enclosed** within the **HBsAg coat** but its antibody, anti HBc appears in serum a week or two after the appearance of HBsAg. As anti HBc remains life long, it serves as a useful indicator of prior infection with HBv.

HBeAg appears in blood concurrently with HBsAg, indicating the **high infectivity**. Molecular methods such as **DNA: DNA hybridization and PCR** at present used for HBV DNA testing are highly sensitive and quantitative.

Immunization

Both passive and active methods of immunization are available. **Active immunization** is more effective. The currently preferred vaccine is genetically engineered by cloning the **S gene for HBV** in **Baker's yeast**. A special vaccine containing all antigenic components of HBsAg (Pre-S1, Pre-S2 and s) has been developed. **No specific antiviral treatment** is available for acute HBV infection.

10.5 Rabies Virus

The Family Rhabdoviridae contains viruses that **infects mammals, reptiles, birds, fishes, insects and plants**. The disease in human being is called **hydrophobia** because the patient exhibits **fear of water**, being **incapable** of drinking though subject to **intolerable thirst**.

Pasteur established that the rabies virus was present in the **brain of infected animals**. By serial **intracerebral passage** in rabbits, he demonstrated **fixed virus** that could be **rendered immune** by a series of injections. **Vaccine was prepared** by drying pieces of **spinal card** from rabbits infected with the **fixed virus**.

Joseph Meister a nine year old boy, severely bitten by a rabid dog and in grave risk of developing rabies, was given a course of **13 inoculations** of the infected cord **vaccine** by Pasteur. The boy **survived**. This dramatic event was a **mile stone** in the development of medicine.

Morphology

The rabies virus is **bullet shaped**, with one end rounded or conical and the other planar or concave. The lipoprotein envelope, carries **knob like spikes**, composed of **glycoprotein G** responsible for pathogenesis, virulence and immunity beneath the envelope is the **matrix (M) protein layer** which may be invaginated at the planar end. The membrane may project outwards forming a **bleb**. The genome is unsegmented linear RNA (Figure 10.6).

The rabies virus isolated from natural human or animal infection is termed **'the street virus'**. Rabies has been recognized from very ancient times as a disease transmitted to humans and animals by the **bite of 'mad dogs'**. The name rabies comes from the **Latin** word rabidus, meaning **'mad'**, derived from the **Sanskrit** root rabhas, for **frenzy**.

Pathogenesis

Human infection is usually caused by the **bite of rabid dogs** or other animals. The virus present in the saliva of the animal is **deposited** in the wound (Figure 10.7). **Rarely**, infection can also occur following non-bite exposures such as licks or aerosols.

The virus appears to **multiply** in the muscles, connective tissue or nerves at

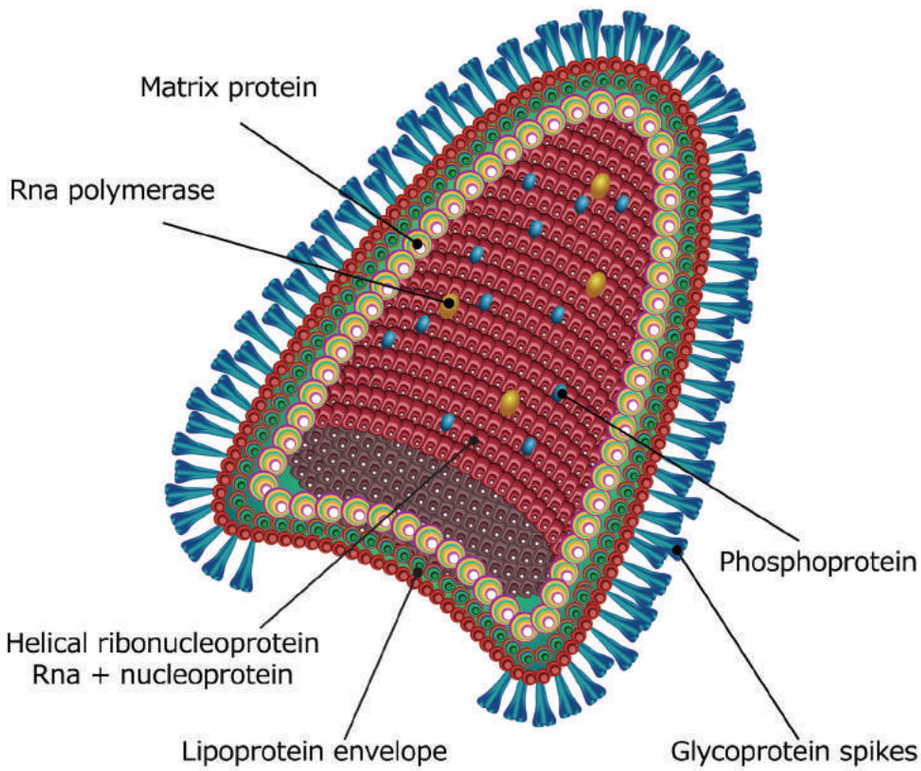


Figure 10.6: Structure of Rabies Virus

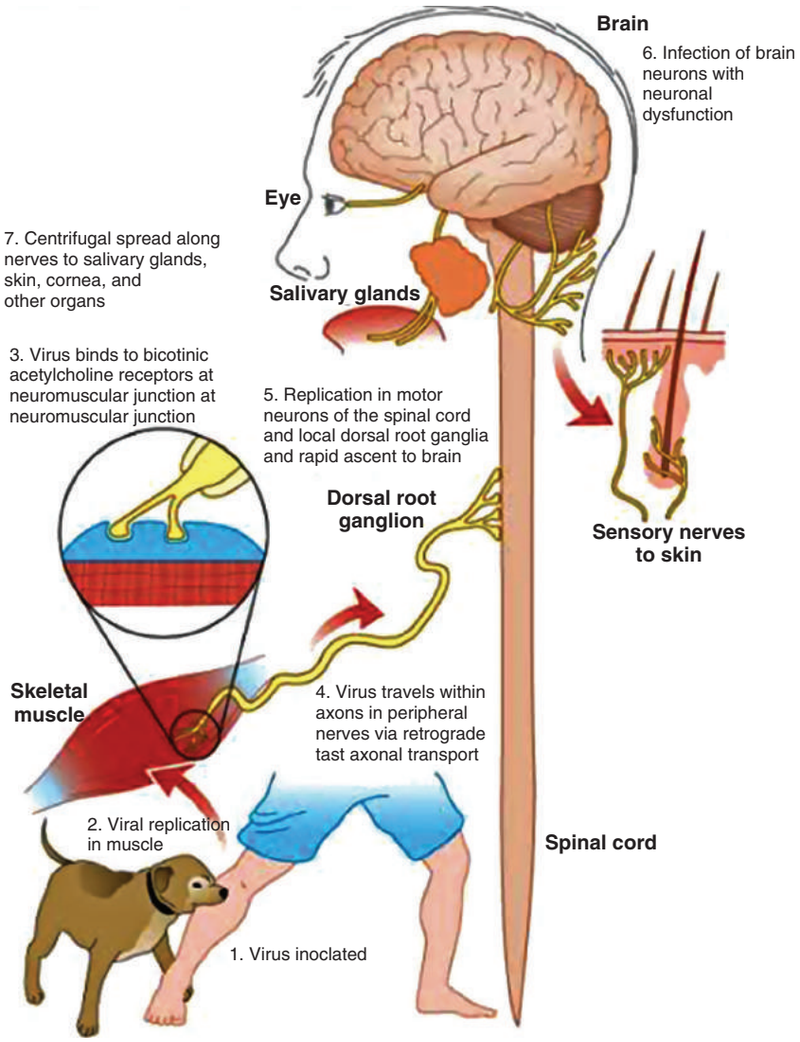


Figure 10.7: Mechanism of Rabies Infection

the site of deposition for 48-72 hours. It penetrates the **nerve endings** and travels in the axoplasm towards the **spinal cord and brain**, at speed of about 3 mm per hour. The virus multiples and **spreads centrifugally** along the nerve trunks to various parts of the body including the salivary glands. It multiplies in the salivary glands and is **shed** in the **saliva**. The virus reaches every tissue in the body and disseminated may be interrupted at any stage by death. In humans the incubation period is usually from **1–3 months**, short as 7 days or as long as three year. The incubation period is usually short in persons bitten on the face or head and long in those bitten on the legs. This may be related to the distance the virus has to travel to reach the brain. The incubation period is generally shorter in children than in adults.

The **four stages** of the disease are as follows, **prodrome, acute encephalitic phase, coma and death**. The onset is market by symptoms such as fever, headache, malaise, fatigue and anorexia. Anxiety, agitation, irritability, nervousness, insomnia or depression. The neurological phase begins with hyperactivity. Attempts to drink on such painful spasms of the

pharynx and larynx producing chocking or gagging that patients develop a dread of even the sight or sound of water (hydrophobia).

Animal Infection

In dogs, the incubation period is **usually 3–6 weeks** but it may range from 10 days to a year. The initial signs are an **alert, troubled air and restlessness, snapping at imaginary objects, licking** or gnawing at the site of the bite.

After 2–3 days of this prodromal stage, the disease develops into either the furious or dumb types of rabies. In **furious rabies**, dog runs biting without provocation, the lower jaw droops and saliva drools from the mouth. Paralysis convulsions and death follow. **In dumb rabies,** is the paralytic form, animals lies huddled, unable to feed. About 60% of rabid dogs shed the virus in saliva. Rabid dogs usually die in 3-5days.

Laboratory Diagnosis

Human Rabies

The specimens tested are **corneal smears and skin biopsy**. Commonly used method for diagnosis is the demonstration of **rabies virus antigens** by immuno fluorescence. **Direct immunofluorescence** is done using antirabies serum tagged with fluorescein isothiocyanate.

Negri bodies in the brain, are demonstrated, Isolation of the virus by intracerebral inoculation in **mice** can be attempted from the brain, CSF, saliva and urine. The mice are examined for **signs of illness**, and their brains are examined at dealth.

Animal Rabies

The **whole carcass** of the animal suspended to have died of rabies may be sent to the laboratory. The brain may be **removed** sent for biological test and microscopy respectively. The portion of brain sent should include the **hippocampus and cerebellum** as **negri bodies** are most abundant. The following tests are done in the laboratory.

1. Demonstration of rabies virus antigen by immuno fluorescence
2. Demonstration of inclusion bodies - Negri bodies are seen as intracytoplasmic, round or oval purplish pink with characteristic basophilic inner granules. Negribodies vary in size from 3.27 Mm.

Infobits

Local Treatment for rabies

- Prompte cauterization of the wounds helps to destroy the virus.
- Antirabic serum may be applied topically.
- Antitetanus measures and antibiotics to prevent sepsis.

Antirabic Vaccines

Antirabic vaccines fall into two main categories neural and non-neural.

Neural Vaccines

Suspension's of nervous tissues of animals infected with the fixed rabies virus. Following are the modified forms.

1. **Semple Vaccine:** Vaccine developed by semple (1911). It is a 5% suspension of **sheep brain infected with fixed virus**

and inactivated with phenol at 37°C leaving noresidual live virus.

2. **Beta propiolactone (BPL) Vaccine:** Beta propiolactone is used as the **inactivating agent** instead of Phenol.
3. **Infant Brain Vaccine:** The enceptalitogenic factor in brain tissue is a basic protein associated with myelin.

Vaccines were developed using infant mouse, rat or rabbit brain. Infant brain vaccine is impractical in India.

Non-neural Vaccines

Non-neural vaccines includes

1. Egg Vaccines
2. Tissue Culture Vaccines
3. Subunit Vaccine

Passive Immunisation

Human rabies immune globulin **(HRIG)** is free from the danger of sensitization but should be ensured free from HIV and hepatitis viruses.

Vaccines for Animals

Antirabies immunization in animals is to be done as pre-exposure prophylaxis concentrated cell culture vaccines – inactivated virus gives good protection after a single **Intramuscular** injection. Injections are given at 12 weeks of age and repeated at 1–3 years intervals.

10.6 Human Immuno Deficiency Virus

HIV is known as **Human Immuno Deficiency Virus**, the etiological agent of AIDS, belongs to the lentivirus subgroup of the family **Retroviridae**.

Infobits

Detecting HIV sooner

Fourth generation test helps to detect HIV in blood earlier than previously recommended antibody test. It identifies the viral protein, HIV-1 P24 antigen, which appears in the blood sooner than antibodies.

Source: CDC

Structure

HIV is a spherical **enveloped Virus**, about 90-120min size. The nucleo capsid has an outer icosahedral shell and an inner cone shaped core, enclosing the **ribonucleo proteins**. The genome is diploid, composed of two identical single stranded, **positive sense RNA copies**. When the virus infects a cell, the Viral RNA is transcribed by the **reverse transcriptase enzyme**, first into single stranded DNA and then to double stranded DNA (provirus) which is integrated into the host cell chromosome. The virus coded **envelope proteins** are the projecting knob like spikes which binds to the CD4 receptors on susceptible host cells (Figure 10.8).

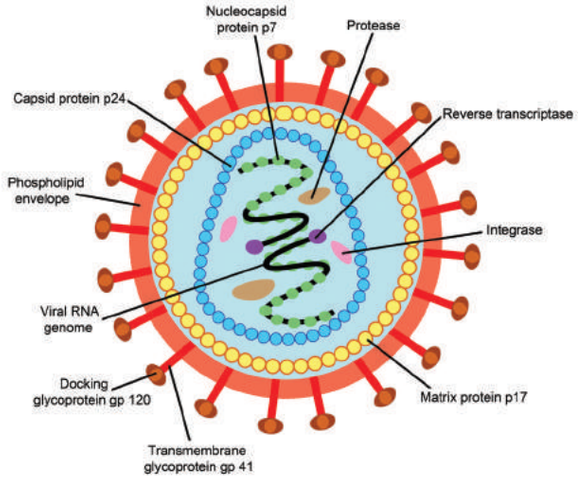


Figure 10.8: Structure of HIV

Viral Genes and Antigens

The genome of HIV contains the **three structural genes** (gag, pol and env) as well as other nonstructural and regulatory genes specific for the virus. These products of these genes, both structural and non structural act as antigens.

Genes coding for structural proteins

1. The gag gene ⟶ Determines the core and shell of the Virus. Precursor protein, p^{55} and it is cleaved into three proteins p^{15}, p^{18} and p^{24}. **Major core antigen p^{24}** can be detected in serum
2. The env gene ⟶ Determines the synthesis of envelope glycoprotein gp^{160}. Cleaved in to gp^{120} and gp^{41}

   The pol gene ⟶ Codes for the **polymerase reverse transcriptase** and other viral enzymes such as protease and endonucleases. It's expressed as a precursor protein, which is cleaved into protein p31, p51 and p66.

Pathogenesis

Infection is transmitted when the Virus **enters the blood or tissues** of a person and comes into contact with a suitable host cell, principally the CD4 lymphocyte. The receptor for the virus is the CD4 antigen and therefore the virus may infect any cell bearing the CD4 antigen on the surface. Specific binding of the **virus to CD4 receptor** is by the envelope **glycoprotein gp^{120}**. Cell fusion is brought about by transmembrane gp^{41}. After **fusion virus** with the host cell membrane, the HIV genome is uncoated and internalized into the cell. Viral reverse transcriptase mediate transcription of its RNA into double stranded DNA, which is **integrated into the genome** of the infected cell through the action of the viral enzyme integrase, causing a latent infection. The primary pathogenic mechanism in HIV infection is the damage caused to the CD4+T lymphocyte. The **T_4 cells decrease** is numbers. Infected T_4 cells do not release normal amounts of interleukin^{-2}, gamma interferon and other lymphokines, this is damping effect on cell mediated immune response.

Clinical Features

AIDS is only the last stage in the wide spectrum in HIV infection.

1. Acute HIV infection

3–6 weeks of infection, persons experience low grade fever, malaise, headache, lymphadenopathy, with rash. Antibodies are usually negative at the onset of the illness but become positive during its course called **'Sero conversion illness'**.

2. Asymptomatic or latent infection

All HIV infected persons, whether or not they experience Sero conversion illness, pass through a phase of **symptomless infection** which may last up to several years. The infection progresses in course of time through various stages, CD4 lymphocytopenia, minor opportunistic infections, ARC AIDS-related complex, ultimately terminating to AIDS.

3. Persistent generalized lymphadenopathy (PGL)

It's defined as the presence of **enlarged lymph nodes** at least 1cm, in diameter in two or more non contiguous extrainguina, sites persist for at least three months.

4. AIDS related complex (ARC)

This group includes patients with considerable **immuno deficiency**, suffering from various symptoms or minor opportunistic infections. eg. oral, Candidiasis, salmonellosis or tuberculosis.

5. AIDS

End-stage disease, poor immune defence mechanism leading to the opportunistic infection and malignancies.

a. Commonest symptoms

Drycough, dyspnea and fever. Pheumonia may be viral (cmv) or fungal (Cryptococcus, Histoplasma).

b. Gastrointestinal system

The mouth is often involved with thrush, stomatitis, gingivitis, hairy leukoplakia. Dysphagia due to esophageal Candidiasis. Intestinal pathogen in AIDS is cryptosporidium. Other pathogens are Salmonellae, Mycobacteria, CMV or adeno viruses. 'gay bowel syndrome' is common among the male homosexuals.

c. Central nervous system

The typical CNS opportunistic infections are toxoplasmosis and cryptococcosis. Lymphomas of the CNS are Common.

d. Malignancies

Kaposi's Sarcoma was the lesion seen in male homosexuals. The tumours commonly seen are lymphomas, both the Hodgkin and non Hodgkin types.

e. Cutaneous

Herpes lesions, Candidiasis, Dermatitis, impetigo are common cutaneous lesions.

6. Dementia

Direct **cytopathogenic damage** in the CNS. It cross the blood-brain barrier and cause encepthalopathy leading to dementia.

7. Pediatric AIDS

Viral transmission may occur to the **fetus in pregnancy**. Many of the infected children may not survive for a year. Children may also acquire the infection from blood transfusion or blood products.

Laboratory Diagnosis

Lab diagnosis of HIV infection include tests for immuno deficiency in HIV infection.

A. Immunological tests

i. Total leukocyte and lymphocyte count to demonstrate leucopenia and a lymphocyte count usually below 2000/ mm3.

ii. Platelet count will show thrombocytopenia.

iii. Raised IgG and IgA levels.

B. Specific tests for HIV infection

1. Antigen detection

Single massive infection, as by blood transfusion, the **virus antigens** may be detectable in blood after about two weeks. The major core antigen p24 is the virus marker to appear in blood.

2. Polymerase Chain reaction

It the most **senstitive** and specific test. Two forms of PCR have been used, DNA PCR and RNA PCR.

3. Antibody detection

Demonstration of **antibodies** is the simpest and widely employed technique.

2–8 weeks to months for antibodies to appear after infection, during part of this period, the individual may be highly infectious. This sero negative infective stage is known as the 'window period'. Antibody can be detected by

1. ELISA
2. Western blot test.

Treatment

The treatment of AIDS include:

1. The treatment and prophylaxis of infections and tumours.
2. General management
3. Immunorestrorative measure
4. Specific anti-HIV agents.

Effective drugs are available, they are Zidovudine, Didanosine, Zalcitabine, Lamivudine and Protease inhibitors like Saquinavir, Ritonavir, Indinavir used as monotherapy or in various combination.

10.7 Arbo Virus

Arbo Viruses (arthropod - borne viruses) are viruses of vertebrates biologically transmitted by **hematophagous insect vectors**. They multiply in blood sucking insects and are transmitted by bite to vertebrate hosts. Arbo viruses are worldwide in distribution. Arbo viruses have been named according to the disease caused (**yellow fever**), the place of isolation of the virus (**kyasanur forest disease**) or the local name for the disease (**chikungunya**). They are classified into Toga, Flavi, Bunya, Reo and Rhabdovirus families. Arbo viruses have a very wide host range including many species of animals and birds. The most important arbo virus vectors are **mosquitoes**, followed by **ticks**.

The virus enters the body through the **bite** of the insect vector. After multiplication in the **reticuloendothelial system, viremia** of varying duration occurs, or the virus is transported to the target **organs** such as central nervous system in **encephalitides**, the liver in **yellow fever** and the capillary endothelium in **hermorrhagic fever**.

Clinical syndromes are fever with or without rash encephalitis, hemorrhagic fever with or without rash encephalitis, hemorrhagic fever and systemic disease, yellow fever.

Diagnosis may be established by virus isolation or serology.

Samples (Blood, CSF) are inoculated intra cerebrally into sucking mice. The animal develop **fatal encephalitis**. Viruses may be isolated in tissue cultures or in eggs. Isolates are identified by hemagglutination inhibition, complement fixation, gel precipitation, immunofluorescence.

ELISA

Virus isolated from insect vectors and from reservoir animal.

Toga Viruses

Toga viruses are **spherical enveloped viruses** with a diameter of 50-70nm. Single stranded RNA genome. The virus replicates in the **cyloplasm** of the host cell and released by budding through host cell membranes. The name Toga Virus is derived from **'toga'** meaning the Roman Mantle refers to the **viral envelope**.

The genus Alpha Virus was formerly classified as **Group A arbo viruses** which explains the name Alpha Virus. The genus Alpha Virus contains 32 species of which 13 infect humans. All are **mosquito borne**.

10.7.1 Chikungunya Virus

The virus was first isolated from human patients of ***Aedes aegypti*** mosquitoes (Figure 10.9) from Tanzania in 1952. The name Chikungunya is derived from the native word for the disease in which the patient lies **'doubled up due to severe joint pains'**. The virus first appeared in India in 1963 in Calcutta, Madras and Other areas.

The disease presents as a sudden onset of fever, Crippling joint pains, lymphadenopathy and conjunctivitis. A **maculopapular rash** in common. The fever is typically biphasic with a period of remission after 1–6 days of fever. The vector is ***Aedes aegypti***. No animal reservoir has been identified. Antibody to the virus has been demonstrated in horses, cattle and other domestic animals.

Flavi viruses

The family flaviviridae contains only one genus **flavivirus**. They are smaller than alpha viruses, being 40nm in diameter. There are over 60 arthropod borne flava viruses classified as **mosquito-borne and tick borne viruses**. Examples of mosquito borne group known as encephalitis viruses they are St. Louis encephalitis Virus, Ilheus virus, west nile virus, murray valley encephalitis virus and Japanese encephalitis. Tick borne viruses ore classified in to tick brone encephalitis viruses and tick borne hemorrhagic fevers.

Figure 10.9: *Aedes aegypti*

10.7.2 Dengue

The name dengue is derived from the **'Swahili ki denga pepo'**, meaning a sudden **seizure by a demon**. Dengue fever is similar to the illness caused by chikungunya. Four types of dengue virus exist: **DEN1, DEN2, DEN3 and DEN4**.

HOTS

What is the best home remedy for dengue fever?

Dengue presents after an incubation period of 3-14 days as fever of sudden onset with headache, retrobulbarpain, conjunctival injection, pain in the back and limbs (break bone fever), lymphadenopathy and maculopapular rash. The fever is typically **biphasic** (saddle back) and lasts for 5–7 days. Dengue may be more serious forms with **hemorrhagic** manifestations (dengue Hemorrhagic fever) or with shock (dengue shock syndrome).

Dengue virus is transmitted from person to person by ***Aedes aegypti*** mosquitoes. The Incubation period is 8–10days. All four types of dengue virus are identified. Demonstration of circulating IgM antibody provides early diagnosis. IgM ELISA test offers reliable diagnosis. Difference between Dengue and Chikungunya is given in Table 10.2.

Table 10.2: Difference between Dengue and Chikungunya

S.No	Factors	Chikungunya	Dengue
1.	Vector	*Aedes aegypti*	*Aedes aegypti*
2.	Virus	Toga viridae - alphavirus	Flavi viridae - flavivirus
3.	Incubation Period	3-7 days	4-7 weeks
4.	Symptoms	Chikungunya begins as an **acute febrile** illness. Pain can be severe. Other common symptoms include headache, muscle pain, joint swelling, and rash. Some patients have persistence or relapse of rheumatologic symptoms in the months following acute illness.	Dengue is an **acute febrile** illness. **Febrile Phase:** Lasts 2–7 days Fever with Headache, retro-orbital pain, joint pain, muscle and or bone pain, rash, mild bleeding (nose or gums) **Critical Phase:** Lasts 24–48 hours. Most patients improve but severe disease requiring hospitalization can occur. **Recovery Phase:** Gradual reabsorption of extravasated fluid from plasma leakage over 48–72 hours. Dieresis, hemodynamic status stabilizes,patient can temporarily become bradycardic.
5	Major symptom	Tremendous Joint pain	Bleeding and breath discomfort
6	Person at risk	Neonates exposed intrapartum, older adults, and persons with underlying medical conditions.	Some patients may develop life threatening consequences and require hospitalization. Infection with each dengue virus type confers lifetime immunity for that specific virus type.

Infobits

Which fruit is good to increase platelet count?

Pomegranate is another great fruit you can eat to increase platelets. As with all **red**fruits, the seeds of this **delicious** fruit are packed with iron, an **essential** mineral for combating low platelet count. **Pomegranate** has been used since the ancient times for its healthy and medicinal properties.

10.7.3 Zika Virus

Zika virus is a mosquito-borne flavivirus that was identified in Uganda in 1947 in **monkeys**. Zika spreads by daytime-active Aedes mosquitoes, such as *A. aegypti* and *A. albopictus*. The infection is known as **Zika fever** or **Zika virus disease**. Zika is related to the dengue, yellow fever, Japanese encephalitis, and West Nile viruses

Zika virus is **enveloped and icosahedral** and has a non segmented,

single-stranded, positive-sense (+) **RNA genome** (Figure 10.10). A positive-sense RNA genome can be directly translated into **viral proteins**, the RNA genome encodes **seven nonstructural proteins** and **three structural proteins**. One of the structural proteins forms the envelope. The RNA genome forms a nucleocapsid along with copies of the 12-kDa capsid protein.

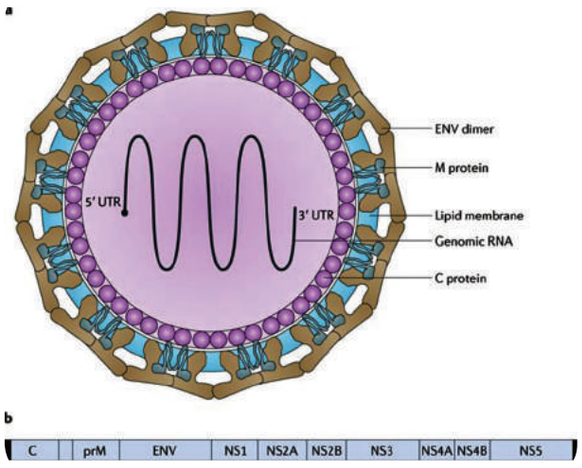


Figure 10.10: Structure of Zika Virus

Viral genome replication depends on the making of double-stranded RNA from the single-stranded, positive-sense RNA (ssRNA(+)) genome followed by **transcription** and **replication** to provide viral mRNAs and **new ssRNA(+) genomes**.

Pathogenesis and Clinical features

Zika virus replicates in the mosquito's **mid gut epithelial cells** and then its salivary gland cells. After 5–10 days, the virus can be found in the mosquito's saliva. If the mosquito's saliva is inoculated into **human skin**, the virus can infect epidermal keratinocytes, skin fibroblasts in the skin and the Langerhans cells. The pathogenesis of the virus is hypothesized to continue with a **spread to lymph nodes** and the **bloodstream**.

Zika virus is primarily transmitted by the **bite of an infected mosquito** from the *Aedes* genus, mainly *Aedes aegypti*. The mosquitoes usually bite during the day, peaking during early morning and late afternoon or evening. This is the same mosquito that transmits dengue, chikungunya and yellow fever. Zika virus is also transmitted from mother to fetus during pregnancy, through sexual contact, transfusion of blood and blood products, and organ transplantation.

The incubation period of Zika virus disease is estimated to be **3–14 days**. The majority of people infected with Zika virus **do not develop symptoms**. Symptoms are generally mild including fever, rash, conjunctivitis, muscle and joint pain, malaise, and headache, and usually last for 2–7 days. **Zika fever** (also known as Zika virus disease) is an illness caused by the Zika virus. Zika virus infection during pregnancy is a cause of **microcephaly** and other **congenital abnormalities** in the developing fetus and newborn. Zika infection in pregnancy also results in pregnancy complications such as fetal loss, stillbirth, and preterm birth.

Laboratory diagnosis

Virus can be demonstrated from the blood or other body fluids, such as urine or semen.

Zika virus grow well in a variety of mammalian and insect cell lines. Zika virus is identified by **NAAT–** Nucleic acid Amplification test, Zika Antigen is detected by ELISA and PCR. **Zika Antibody** IgM is detected by MAC - ELISA, IgG by ELISA and by **PRNT**-plaque reduction neutralization test.

Prevention and Treatment

Protection against **mosquito bites** during the day and early evening is a key measure to prevent Zika virus infection. It is important to eliminate these mosquito breeding sites, Health authorities may also advise use of **larvicides and insecticides** to reduce mosquito populations and disease spread. There is no treatment available for Zika virus infection or its associated diseases. **No vaccine is yet available for the prevention or treatment of Zika virus infection. Development of a Zika vaccine remains an active area of research.**

Summary

This chapter dealt with the history, morphology, chemical properties, viral replication, virus classification, cultivation and detection of cytopathic effects of virus. Most important viruses such as Adeno, herpes, hepatitis, Influenza, rabies, HIV and Arbo virus, its morphology, classification, pathogenesis and its laboratory diagnosis were discussed.

Evaluation

Multiple choice questions

1. _____ is an example for smallest virus
   a. Pox virus
   b. Parvo virus
   c. Rabies virus
   d. HIV virus
2. CPE stands for _____
   a. cytoplasmic effects
   b. cytopathogenic effects
   c. cytopathic effects
   d. None of these
3. Cytomegalo viruses also called as _____
   a. Salivary gland virus
   b. Thymus gland virus
   c. Endocrine gland virus
   d. None of these
4. _____ is an example for passenger viruses
   a. HIV virus b. EB Virus
   c. Rabies virus d. None of these
5. The process of elution is caused by _____ enzyme
   a. Neuramidase b. Isomerase
   c. Polymerase d. None of these
6. Beta propiolactone (BPL. Vaccine is given for _____
   a. HIV virus b. Influenza Virus
   c. Rabies virus d. None of these
7. _____ is an example for mosquito-borne and tick borne viruses
   a. Dengu virus b. Flavi virus
   c. Chikungunya virus d. None of these

Answer the following

1. What is Virology?
2. Define virion.
3. Which is the largest virus?
4. What is Nucleocapsid?
5. Brief note on Steps involved in viral multiplication.
6. What is Viropexis?
7. Define Abortive infective.

8. What are Prions?
9. Write Short note on cultivation of Virus.
10. What are Cytopathogenic Virus?
11. Classification of Herpes virus.
12. Discuss on HSV-1 and HSV-2
13. Expand VZV
14. What is EB Virus?
15. Define Dane particle
16. Genome structure of HBV
17. Give the Structure of Rabies Virus
18. What is Furious and dumb rabies?
19. Define Negri bodies
20. Discuss on Vaccine for rabies
21. Note on Viral gene and antigen of HIV
22. Write about the Clinical features of HIV
23. Give the Lab diagnosis of HIV
24. Define Arbo Virus
25. Give the Symptoms of hermorrhagic fever.
26. Short note on Chikungunya virus
27. Account on Mosquito borne virus
28. Write about the Structure of Zika Virus
29. What is Zika fever

Chapter 11

Immunology

Learning Objectives

After studying this chapter the students will be able to,

- *Understand the Antigen Antibody reactions*
- *Know the principle behind Western Blot techniques*
- *Learn about Hypersensitivity*
- *Gain knowledge about Transplantation*
- *Know Immunization/Vaccination*
- *Appreciate the Updated National Immunization Schedule chart.*

Chapter Outline

The immune system refers to a collection of cells and proteins that function to protect the skin, respiratory passages, intestinal tract and other areas from foreign antigens, such as microbes (organisms such as bacteria, fungi, and parasites), viruses, cancer cells, and toxins. The immune system works by recognising the difference between one's own body cells and alien cells, allowing it to destroy anything that could be potentially harmful. Immune deficiency diseases decrease the body's ability to fight invaders, causing vulnerability to infections. In the previous year, we have elaborately discussed with the main components and function of the immune system. This chapter deals with the role of Immune system in both health and disease.

11.1 Antigen Antibody Reactions

The interaction between antigen and antibody is called antigen-antibody reactions. It is abbreviated as Ag-Ab reaction. This reaction is the basis of humoral immunity. The antigen and the antibody react to form immune complex.

Ag + Ab ---------- Ag – Ab complex

The reaction between antigen and antibody is highly specific. It is compared to the lock and key system. The part of the antigen that combines with the antibody is called epitope or antigenic determinant. The part of antibody which combines with the antigen is called paratope or antigen

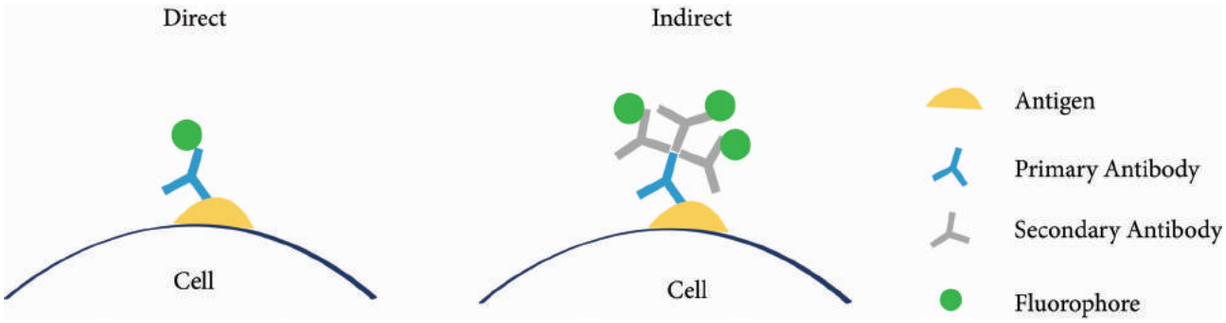


Figure 11.1: Methods in Immunofluorescence

determining site. Most of the antibodies have two binding sites and IgM has 5–10 binding sites.

Immunofluorescence

When antibodies are mixed with the fluorescent dyes such as fluorescein or rhodamine, they emit radiation. This phenomenon of emitting radiation by antibodies labelled with fluorescent dye is called immuno fluorescence. This reaction is well observed under fluorescent microscope. It is used to locate and identify antigens in tissues.

Types of Immunofluorescence

- Direct method
- Indirect method
- Sandwich method

Direct Method

In this method, the antibody labelled with fluorescent dye is directly applied on the tissue section. The labelled antibody binds with specific antigen. This can be observed under the fluorescent microscope.

Indirect Method

In this method, unlabelled antibodies are directly applied on the tissue sections which bind with the specific antigens. Then the antibody labelled with the fluorescent dye is added to the tissue. Anti-antibody specifically binds with already added or linked unlabelled antibody (Figure 11.1).

Sandwich Method

This is an immuno fluorescence method used to test the number of cells producing antibodies for a specific antigen. In this method, lymphocytes are fixed with ethanol. These fixed cells are treated with polysaccharide antigen of *Pneumococcus*. This antigen combines with those lymphocytes which have the capacity to produce antibody against pneumococcal antigen. Now fluorescent antibody is added. Antigen is sandwiched between antibodies.

ELISA (Enzyme Linked Immuno Sorbent Assay)

ELISA (Enzyme-Linked Immuno Sorbent Assay) is a plate-based assay technique designed for detecting and quantifying substances such as peptides, proteins, antibodies and hormones. It is also known as Enzyme Immuno Assay (EIA).

In 1971, after the descriptions of Peter Perlmann and Eva Engvall at Stockholm University in Sweden, ELISA has become the system of choice when assaying

soluble antigens and antibodies. All assays for antibody production depend upon the measurement of interaction of elicited antibody with antigen.

Principle

The **principle of ELISA** is very simple. The test is generally conducted in micro titre plates. (Figure 11.2 Micro titre plate).

If the antigen is to be detected the antibody is fixed in the micro titre plate and vice versa. Test sample is added in the microtitre plate, if there is presence of Ag or Ab in the test sample, there will be Ag-Ab reactions (with immobilized Ab or Ag). Later enzyme labelled antibody is added in the reaction mixture, which will combine with either test antigen or Fc portion of test antibody.

The enzyme system consists of:

1. **An enzyme**: Horse Radish Peroxidase(HRP), alkaline phosphatase which is labelled or linked, to a specific antibody.
2. **A specific substrate**:
   - O-Phenyl-diamine-dihydrochloride for peroxidase
   - P Nitrophenyl Phosphate- for Alkaline Phosphatase

Substrate is added after the antigen-antibody reaction. The enzyme hydrolyses the substrate to give a yellow colour compound in case of alkaline phosphatase (Figure 11.3). The intensity of the colour is proportional to the amount of antibody or antigen present in the test sample, which can be quantified using ELISA reader (Figure 11.4 ELISA reader)

Figure 11.2: Micro titre plate

Figure 11.4: ELISA Reader

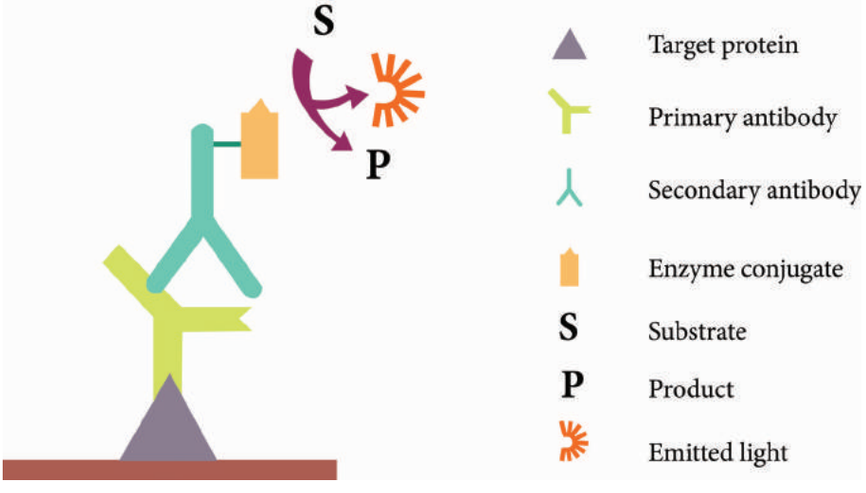


Figure 11.3: Basic steps in ELISA

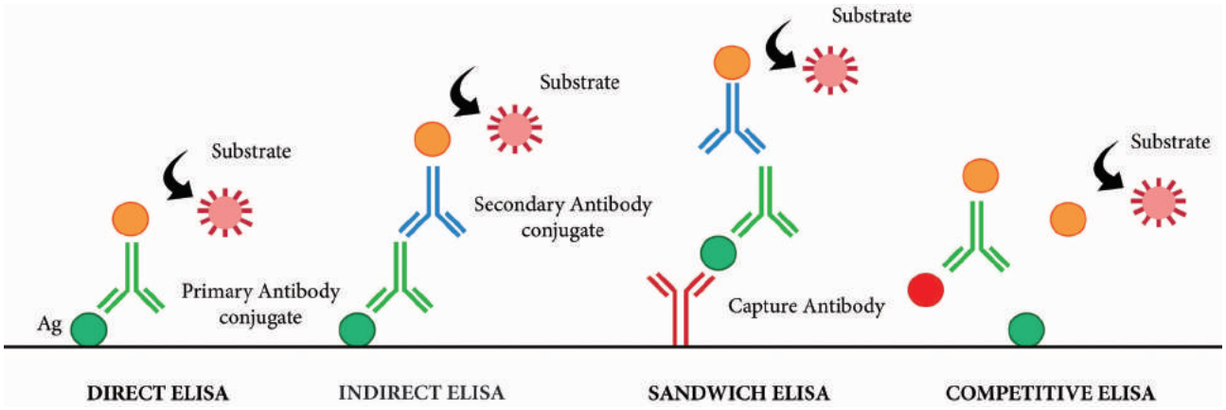


Figure 11.5: Types of ELISA

Types

There are **four kinds** of ELISA assay tests. They are: Direct ELISA, Indirect ELISA, Sandwich ELISA and Competitive ELISA (Figure 11.5).

i. Direct ELISA

An antigen is immobilized in the well of an ELISA plate. The antigen is then detected by an antibody directly conjugated to an enzyme such as HRP. Direct ELISA detection is much faster than other ELISA techniques as fewer steps are required. The assay is also less prone to error since fewer reagents and steps are needed, i.e. no potentially cross-reacting secondary antibody needed. Finally, the direct ELISA technique is typically used when the immune response to an antigen needs to be analyzed.

ii. Indirect ELISA

Indirect ELISA is used to detect antibody. A known antigen is coated on the micro titre plate. If the patient's serum contains antibody specific to the antigen, the antibody will bind to the antigen. After incubation the wells are washed and the enzyme labelled with anti Human Gamma Globulin (HGG) is added to the well. Anti-HGG can react with antigen antibody complex. The substrate for the enzyme is added finally which is hydrolysed by the enzyme which develops a colour.

iii. Sandwich ELISA

Sandwich ELISA is used to detect antigen. A known antibody is coated on the micro titre plate. A test antigen is added to each well and allowed to react with the bound antibody.

If the patient's serum contains antigen specific to the antibody, the antigen will bind to the antibody. Specifically bound antigen and antibody will remain in the wells even after washing. The second antibody is added and allowed to react with bound antigen. Substrate is added to measure colour reaction.

iv. Competitive ELISA

It is used for the detection of antigens. Antibody is first incubated with a sample-containing antigen. The antigen and antibody complex is added to the antigen coated microtitre well. If more antigen present in the sample, the less free antibody will be available to bind to the antigen coated well. Addition of an enzyme conjugated secondary antibody specific to the primary antibody can be used to determine the amount of primary antibody bound to the well. It is a quantitative test for the antigen detection.

Application

An ELISA test may be used to diagnose: HIV, Lyme disease, pernicious anaemia, Rocky Mountain spotted fever, rotavirus, squamous cell carcinoma, syphilis, toxoplasmosis, varicella-zoster virus, which causes chickenpox and Zika virus.

11.2 Western Blot techniques

Macromolecules immobilized or fixed on nitrocellulose membrane i.e., blotted can be subjected to a variety of analytical techniques more easily. Southern blotting was the first blotting technique developed which made the analysis and recording of DNA easy. Later the technique was extended for analysis of RNA and proteins and they have acquired the jargon terms Northern and Western Blotting respectively. Western blotting is also known as immunoblotting because it uses antibodies to detect the protein. Western blotting is a quantitative test to determine the amount of protein in sample.

Principle

Western blotting technique is used for the identification of a particular protein from the mixture of proteins. In this method, the proteins are first extracted from the sample. Extracted proteins are subjected to Poly Acryl amide Gel Electrophoresis (PAGE). Transfer of proteins from poly acryl amide to the nitrocellulose paper is achieved by applying electric field. When radio labelled specific antibody is added on such membrane it binds to the specific complementary protein. Finally the proteins on the membrane can be detected by staining or through ELISA technique.

Steps

Step I: Extraction of Protein

The most common protein sample used for Western blotting is cell lysate. The protein from the cell is generally extracted by mechanical means or by adding chemicals which can lyse the cell. The extraction step is termed as tissue preparation. Protease inhibitor is used to prevent the denaturing of proteins. Using spectroscopy the concentration of the protein sample is analysed and diluted in loading buffer containing glycerol. This will help the sample to sink in the well. Bromothymol blue is used as tracking dye and is used to monitor the movement of the sample.

Step II: Gel electrophoresis

The protein sample is loaded in well of SDS-PAGE Sodium dodecyl sulfate-poly-acryl amide gel electrophoresis. The proteins are separated on the basis of electric charge, isoelectric point, molecular weight, or combination of all these. Proteins are negatively charged, so they move toward positive (anode) pole as electric current is applied. Smaller proteins move faster than the larger proteins.

Step III: Blotting

Blotting refers to the transfer of the protein from the gel to the nitrocellulose paper by capillary action. Electro blotting is done nowadays to speed up the process. In electro-blotting nitrocellulose membrane is sandwich between gel and cassette of filter paper and then electric current is passed through the gel causing transfer of protein to the membrane.

Step IV: Blocking

The nitrocellulose membrane is non-specifically saturated or masked by using casein or Bovine serum albumin (BSA) before adding the primary antibody. This blocking step is very important in western blotting as antibodies are also proteins and they are likely to bind to the nitrocellulose paper.

Step V: Treatment with primary and secondary antibody

The primary antibody is specific to desired protein so it forms Ag-Ab complex. The secondary antibody is enzyme labelled and is against primary antibody (anti-antibody) so it can bind with Ag-Ab complex. Alkaline phosphatase or Horseradish peroxidase (HRP) is labelled with secondary antibody.

Step VI: Treatment with suitable substrate

Finally, the reaction mixture is incubated with specific substrate. The enzyme convert the substrate to give visible coloured product, so band of colour can be visualized in the membrane (Figure 11.6).

Application

1. The size and concentration of protein in given sample is determined by western blotting.
2. It is used in the detection of antibody against virus or bacteria in serum and helps in the disease diagnosis.
3. Western blotting technique is the confirmatory test for HIV. It detects anti HIV antibody in patient's serum.
4. Useful to detect defective proteins.

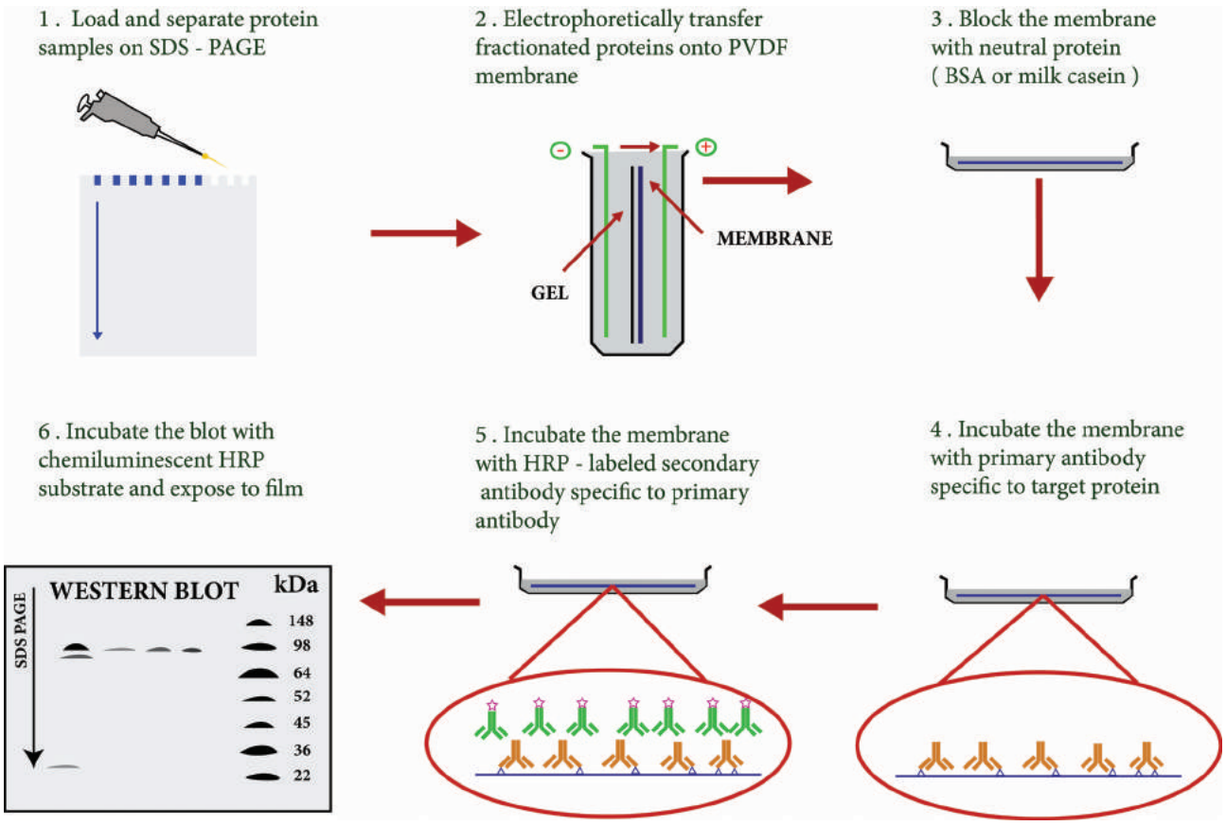


Figure 11.6: Western blot technique

11.3 Hypersensitivity

Hypersensitivity is defined as the exaggerated immunological response leading to severe symptoms and even death in a sensitized individual when exposed for the second time. It is commonly termed as allergy. The substances causing allergic/ hypersensitivity is known as allergens. Example:. Drugs, food stuffs, infectious microorganisms, blood transfusion and contact chemicals.

Classification of Hypersensitivity (Coombs and Gell Classification)

Type I: Immediate (Atopic or anaphylactic) Hypersensitivity

Type II: Antibody–dependent Hypersensitivity

Type III: Immune complex mediated Hypersensitivity

Type IV: Cell mediated or delayed Hypersensitivity

Type I: Immediate (Atopic or anaphylactic) Hypersensitivity

This type of hypersensitivity is an allergic reaction provoked by the re-exposure to a specific antigen. The antigen can make its entry through ingestion, inhalation, injection or direct contact. The reaction may involve skin, eyes, nasopharynx and gastrointestinal tract. The reaction is mediated by IgE antibodies (Figure 11.7). IgE has very high affinity for its receptor on mast cells and basophils. Cross linking of IgE receptor is important in mast cell trigerring. Mast cell degranulation is preceded by increased Ca^{++} influx. Basophils and mast cells release pharmacologically active substances such as histamines and tryptase. This causes inflammatory response. The response is immediate (within seconds to minutes). Hence, it is termed as immediate hypersensitivity. The reaction is either local or systemic.

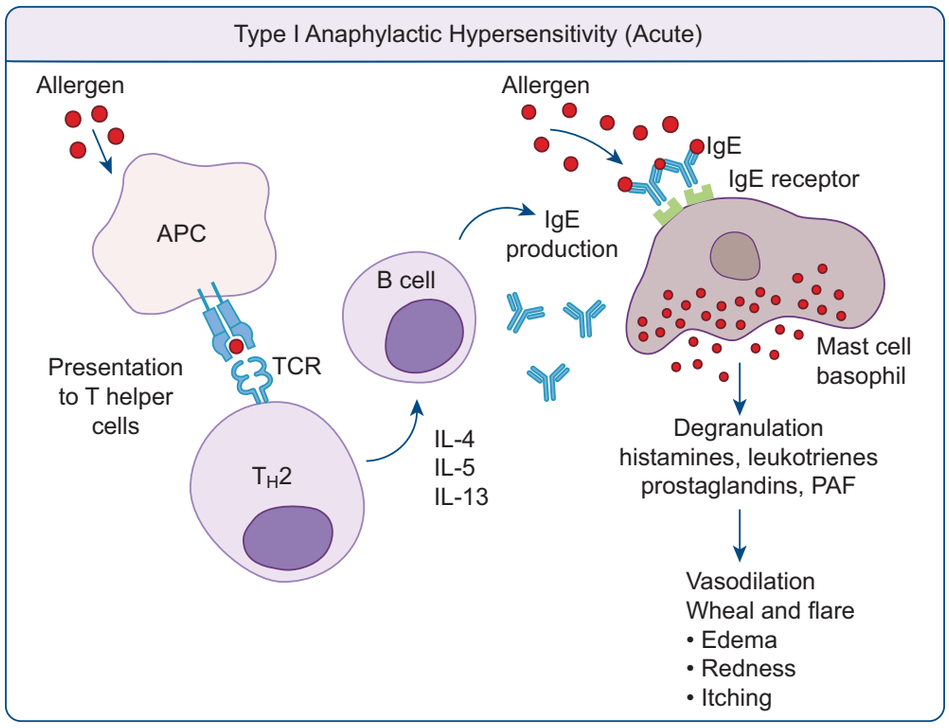


Figure 11.7: Type I hypersensitivity

Hay Fever

Allergic rhinitis is commonly known as hay fever. Allergic rhinitis develops when the body's immune system becomes sensitized and overreacts to something in the environment like pollen grains, strong odour of perfumes, dust etc. that typically causes no problem in most people. When a sensitive person inhales an allergen the body's immune system may react with the symptoms such as sneezing, cough and puffy swollen eyelids.

Type II Hypersensitivity: Antibody dependent hypersensitivity

In this type of hypersensitivity reactions the antibodies produced by the immune response binds to antigens on the patient's own cell surfaces. It is also known as cytotoxic hypersensitivity and may affect variety of organs or tissues. Ig G and Ig M antibodies bind to these antigens and form complexes. This inturn activates the classical complement pathway and eliminates the cells presenting the foreign antigen. The reaction takes hours to day (Figure 11.8).

Drug induced haemolytic anaemia

Certain drugs such as penicillin, cephalosporin and streptomycin can absorb non-specifically to protein on surface of RBC forming complex similar to hapten-carrier complex. In some patients these complex induce formation of antibodies, which binds to drugs on RBC and induce complement mediated lysis of RBC and thus produce progressive anaemia. This drug induced haemolytic anaemia is an example of Type II hypersensitivity reaction.

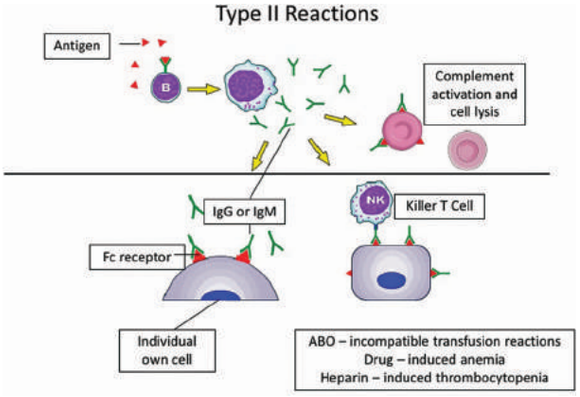


Figure 11.8: Type II hypersensitivity

Type III Hypersensitivity: Immune complex mediated hypersensitivity

When a huge amount of antigen enters into the body, the body produces higher concentrations of antibodies. These antigens and antibodies combine together to form insoluble complex called immune complex. These complexes are not completely removed by macrophages. These get attached to minute capillaries of tissues and organs such as kidneys, lung and skin (Figure 11.9). These antigen-antibody complexes activate the classical complement pathway leading to vasodilation. The complement proteins and antigen-antibody complexes attract leucocytes to the area. The leukocytes discharge their killing agents and promote massive inflammation. This can lead to tissue death and haemorrhage.

Arthus reaction

It was first observed by Arthus. It is a local immune complex reaction occurring in the skin. Horse serum and egg albumin are the antigens that induce the arthus reaction. It is characterized by erythema, induration, oedema, haemorrhage and

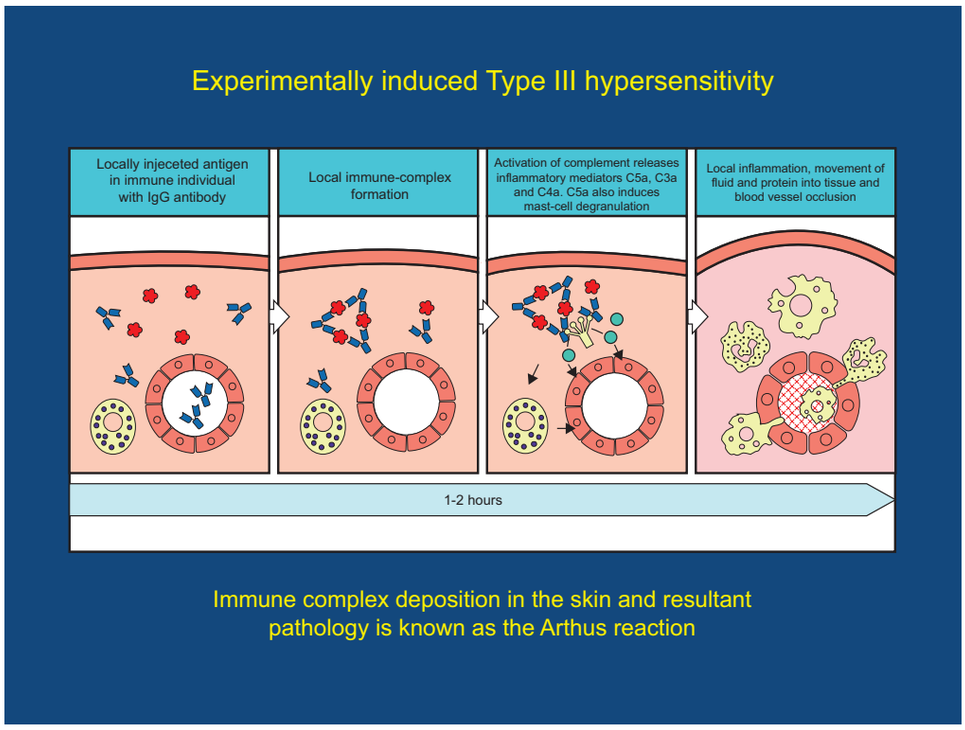


Figure 11.9: Type III hypersensitivity

necrosis. This reaction occurs when antibody is found in excess. It appears in 2–8 hours after injection and persists for about 12–24 hours (Table 11.1).

Type IV hypersensitivity: Cell Mediated Delayed Hypersensitivity

It is often called as delayed hypersensitivity reaction as the reaction takes two to three days to develop. Type IV hypersensitivity is involved in the pathogenesis of many autoimmune and infectious diseases such as tuberculosis and leprosy. T lymphocytes, monocytes and macrophages are involved in the reaction. Cytotoxic T Cells cause direct damage whereas the T helper cells secrete cytokines and activate monocytes

Table 11.1: Difference between Immediate Hypersensitivity and Delayed Hypersensitivity

Sn. No.	Immediate Hypersensitivity	Delayed Hypersensitivity
1.	It appears and disappears rapidly	It appears slowly and last longer.
2.	It is induced by antigens or haptens by any route	Induced by infection, injection of antigen intra dermally or with adjuvants of by skin contact.
3.	The reaction is antibody mediated B-cell response	The reaction is T-cell mediated response.
4.	Passive transfer is possible with serum	Cannot be transferred with serum but can be transferred by lymphocytes
5.	Desensitization is easy, but does not last long	Desensitization is difficult but long lasting.

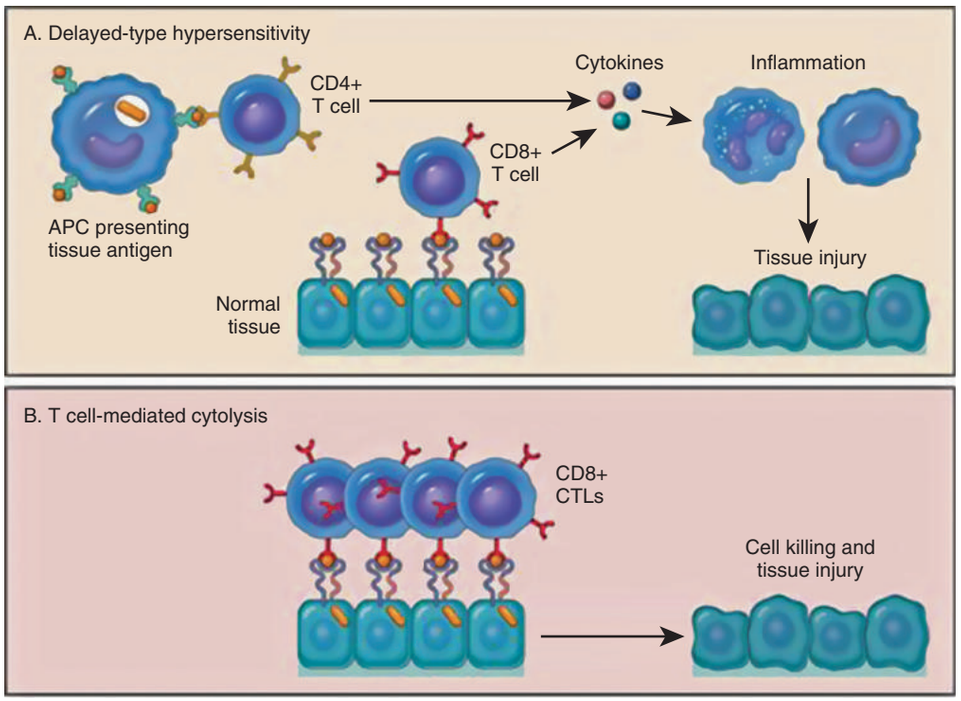


Figure 11.10: Type IV delayed hypersensitivity

and macrophages and cause the bulk damage (Figure 11.10).

Tuberculin reaction (Mantoux Reaction)

When a small dose of tuberculin is injected intra dermally in an individual already having tubercle bacilli, the reaction occurs. It is due to the interaction of sensitized T cell and tubercle bacterium. The reaction is manifested on the skin very late only after 48–72 hours.

11.4 Transplantation

Transfer of living cells, tissues or organs from one part of the body to another or from one individual to another is known as transplantation.

A tissue or organ that is removed from one site and placed to another site usually in a same or different individual is called graft. The individual who provides the graft is called donor and the individual who receives the graft is called host or recipient.

If the graft is placed into its normal anatomic location, the procedure is called orthotopic transplantation. If the graft is placed in a different site it is called heterotopic transplantation.

Transplantation is the only form of treatment for most end-stage organ failure. In clinical practice, transplantation is used to overcome a functional and anatomic deficit in the recipient. Transplantation of kidneys, hearts, livers, lungs, pancreas and bone marrow are widely done today.

Methods of Transplantation

- Auto grafting: The transfer of self tissue from one body site to another in the same individual
- Allografting: The transfer of organs or tissues from human to human
- Xenografting: The transfer of tissue from one species to another (Figure 11.11).

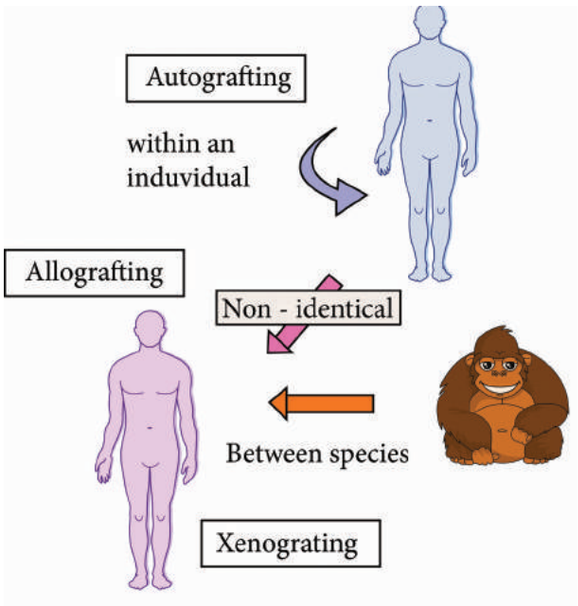


Figure 11.11: Types of Grafting

Graft Acceptance

When transplantation is made between genetically identical individuals the graft survives and lives as healthy as it is in the original places. When the graft tissue remains alive, it is said to be accepted and the process is called graft acceptance.

Graft Rejection

When transplantation is made between genetically distinct individual the graft tissue dies and decays. When the graft tissue dies, the graft is said to be rejected and the process is called graft rejection. It is of two types. They are

i. Host Verses Graft Reaction

ii. Graft Verses Host Rejection.

Host Verses Graft Reaction (HVG)

The graft tissue antigens induce an immune response in the host. This type of immune response is called host versus graft reaction.

Allograft Rejection

Types of allograft rejection

- Acute rejection–Quick graft rejection. It is due to stimulation of thymocytes and B lymphocytes
- Hyperacute rejection–It is a very quick rejection. It is due to pre-existing humoral antibodies in the serum of the host as a result of presensitization with previous grafts.
- Insidious rejection–It is a secret rejection due to deposition of immune complex on the tissues like glomerulus membrane that can be demonstrated in kidney by immune fluorescence.

Mechanism of Allograft Rejection

Immunological contact

When tissue is implanted the graft as can pass into local lymph nodes of the host. The graft antigens then make contact with the lymphocytes of the host. Production of sensitized T cells and cytotoxic antibodies are produced in the host. This brings about graft rejection.

First set rejection

When the graft is made between genetically different individuals, the graft gets blood supply from the host and it appears to be normal for the first 3 days. But on the 5th day, sensitized T cells, macrophages and a few plasma cells invade the graft. Inflammation starts in the graft. This leads to necrosis. It is similar to the primary immune response to an antigen.

Second set rejection

When a graft is implanted in an individual who has already rejected a graft is second set rejection. This is similar to the secondary immune response of our body.

Cell mediated cytotoxic reaction

The 1st set of rejection of allograft is brought about mainly by CMI response. In this process the cells involved in the cytotoxic mediated immunity involves. On stimulation of these cells interferon causes the lysis of the graft.

Antibody mediated cytotoxic reaction

The 2nd set rejection of graft is brought about mainly by HMI response. This is one of the hyperacute rejection brought about by the antibodies. Complement, macrophages, mast cells, platelets, B cells bring about this reaction.

Graft versus Host Rejection (GVH)

Sometimes the graft tissue elicits an immune response against the host antigens. This immune response is called graft versus host reaction. It occurs when:

- Graft remains inside the host and the host should not reject the graft.
- The graft should have immune competent T cells.
- The transplantation antigens of the host should be different from that of the graft.

A transplanted heart usually beats slightly faster than normal because the heart nerves are cut during surgery.

Mechanism of the graft

The graft lymphocytes aggregate in the host lymphoid organs and are stimulated by the lymphocytes of the host. The stimulated lymphocytes produce lymphokines. Lymphocytes in turn activate the host T cell. Activated T cell further activates the B cells. The stimulated B cell reacts with the self antigen and causes the damage.

How to prevent graft rejection?

Before transplantation the following things should be done to avoid graft rejection.

- Perform blood grouping and Rh grouping
- HLA typing should be done
- Immuno suppressive drugs should be administered
- Suitable graft should be used

11.5 Immunization/Vaccination

Father of Immunization is Edward Jenner. He produced the vaccine for small pox from cow pox virus.Vaccine is a substance that is introduced into the body to prevent the disease produced by certain pathogens. Vaccines consist of dead pathogens or live but attenuated (artificially weakened) organisms.

Immunization programmes and the development of new vaccines play an important role in protecting individuals against illness. Vaccination works by safely exposing individuals to a specific pathogenic microbe, artificially increasing their immunity to it.

Vaccines are made from

- Live micro-organisms that have been 'treated' so that they are weakened (attenuated) and are unable to cause disease.
- Dead micro-organisms.
- Some part or product of the micro-organism that can produce an immune response.

Principles of Vaccination

- The primary goal in vaccination is to provide protective immunity by inducing a memory response to an infectious microorganism using a non-toxic antigen preparation. It is important to produce immunity of the appropriate kind: antibody / or cellular immunity.
- Antibodies produced as a result of immunization are effective primarily against extracellular organisms and their products e.g., toxins. Passively administered antibodies have the same effect as induced antibodies.
- Cell-mediated immunity (T cells, macrophages) induced by vaccination is important particularly in preventing intracellular bacterial and viral infections and fungal infections.
- The ultimate goal of any immunization program is the eradication of the disease.
- This requires that the infection is limited only to humans, with no animal or environmental reservoir, and the absence of any subclinical or carrier state in humans.
- Achieving elimination requires a high level of herd immunity to prevent person to person spread.
- This requires considerable infrastructure support to ensure that all at-risk populations are targeted for immunization.
- This has been achieved for small pox, although we are close to the elimination of polio.

Vaccine Types

- **Live attenuated vaccines:** These vaccines contain modified strains of a pathogen that have been weakened but are able to multiply within the body and remain antigenic enough to induce a strong immune response. Example: Oral Polio vaccine
- **Heterologous vaccine:** These are a group of live attenuated vaccines produced from the strains that are pathogenic in animals and not in humans. It is a vaccine that confers protective immunity against a pathogen that shares cross-reacting antigens with the microorganisms in the vaccine. Example: Cow pox virus that protects against small pox in humans.
- **Killed inactivated vaccines:** These groups of vaccine are produced either by killing or inactivating the bacteria or virus by chemical treatment or heat. Example:; Polio virus
- **Sub unit vaccine:** Uses the antigenic determinant / epitope (the very specific part of the microbe) is used to prepare the vaccine.
- **DNA Vaccines**: When the genes for microbe's antigens are introduced into the body some cells will take up the DNA. The DNA then instructs those cells to make the antigen molecules. The cells secrete the antigens adn display them on their surfaces. The body's own cells become vaccine generating factories.

Routes of Administration

- Deep subcutaneous or intramuscular route – most vaccines
- Oral route – Oral BCG vaccine
- Intradermal route – BCG vaccine
- Scarification – Small pox vaccine
- Intranasal route – Live attenuated influenza virus

Types of Immunization

Immunization is of two types:

1. Passive Immunization
2. Active Immunization

1. Passive Immunization

- Passive immunization is produced without challenging the immune system of the body. It is done by administration of serum or gamma globulins from a person who is already immunized to a non- immune person.
- Passive immunization is the administration of preformed antibodies either intravenously or intramuscularly.
- It is used to provide rapid protection in certain infections such as diphtheria or tetanus or in the event of accidental exposure to certain pathogens such as hepatitis B.
- It is also used to provide protection in immune compromised individuals.

Passive natural immunization - acquired from the mother before and after birth. Before birth, immunity is transferred from mother to the fetus in the form of maternal antibodies through placenta. After birth, the antibodies (Ig A) are transferred through breast milk (Table 11.2).

Passive artificial immunization - developed by injecting previously prepared

Table 11.2: Passive Immunization

Infection	Source of Antiserum	Indications
Tetanus	Immune human; horse	Post exposure (plus vaccine)
Diptheria	Horse	Post-exposure
Gas gangrene	Horse	Post-exposure
Botulism	Horse	Post-exposure
Varicella-Zoster	Immune human	Post-exposure in immunodeficiency
Rabies	Immune human	Post exposure (plus vaccine)
Hepatitis B	Immune human	Post-exposure prophylaxis
Hepatitis A	Pooled human Ig	Prophylaxis
Measles	Immune human	Prophylaxis
Snakebite	Horse	Post-bite
Some autoimmune disease	Pooled human ig	Acute thrombocytopenia and neutropenia

antibodies using serum from humans or animals. This type of immunity is useful for providing immediate protection against acute infections like tetanus, measles etc.

2. Active Immunization

Active immunization is the administration of vaccines containing microbial products with or without adjuvants in order to obtain long term immunological protection against the offending microbe.

At present the normal route of vaccination in most instances is either intramuscularly or subcutaneously.

Oral immunization is the method of choice for polio and *Salmonella typhi* vaccines. However, there is an increasing awareness that this route of immunization may be the best for most immunizations since nearly all infectious agents gain entrance through the mucosal surfaces.

Active natural immunization involves activation of immune system in the body to produce antibodies. It is achieved in both clinical and subclinical infections.

Active artificial immunization is achieved by the administration of vaccines or toxoids.

Antigen preparations

Most vaccines consist of attenuated organisms, killed organisms, inactivated toxins, or sub cellular fragments and more recently genes for antigens in viral 'vectors', and DNA itself. Thus, vaccines must be capable of targeting the immune system appropriately i.e. cellular/or humoral mechanisms (Table 11.3).

Table 11.3: Antigen Preparations Used in Vaccines

Type of antigen	Examples	
	Viruses	**Bacteria**
Normal heterologous organism	Vaccinia (Cowpox)	
Living attenuated organism	Measles	BCG
	Mumps	Typhoid (New)
	Rubella	
	Polio (Sabin)	
	Yellow fever	
	Varicella-Zoster	
Whole killed oranism	Rabies	Pertussis
	Poli (Salk)	Typhoid
	Influenza	Cholera
Inactivated toxin (toxoid)		Diphtheria
		Tetanus
		Cholera (New)
Capsular polysaccharide		*Meningococcus*
		Pneumococcus
		Haemophilus
		Typhoid (New)
Surface antigen	Hepatitis B	

Adjuvants

Nonliving vaccines, especially those consisting of small molecules require the inclusion of agents to enhance their effectiveness.

These adjuvants include microbial, synthetic and endogenous preparations having adjuvant activity, but at present only aluminium or calcium salts are generally used in humans.

Adjuvants should enable antigens to be slowly released, preserve antigen integrity, target antigen presenting cells and induce cytotoxic lymphocytes.

Table 11.4: National immunization schedule

Sn. No.	Vaccine	Due age	Max age	Route
1.	BCG	At birth	till one year age	Intra dermal
2.	Hepatitis B-Birth dose	At birth	within 24 hours	Intra muscular
3.	OPV-O	At birth	within the first 15 days	Oral
4.	OPV 1, 2 & 3	At 6 weeks, 10 weeks & 14 weeks	till 5 years of age	Oral
5.	Pentavalent 1, 2 & 3 (Diphtheria + Pertuss is + Tetanus + Hepatitis B + Hib)	At 6 weeks, 10 weeks & 14 weeks	1 year of age	Intra muscular
6.	Inactivated polio vaccine	At 6 & 14 weeks	1 year of age	Intra muscular
7.	Rotavirus (where applicable)	At 6 weeks, 10 weeks & 14 weeks	1 year of age	Oral
8.	Pneumococcal conjugate vaccine (where applicable)	At 6 weeks & 14 weeks. At 9 completed months – booster	1 year of age	Intra muscular
9.	Measles/Rubella 1st dose	At 9 completed months – 12 months	5 years of age	Subcutaneous
10.	DPT Booster-1	16–24 months	7 years of age	Intra muscular
11.	Measles/Rubella 2nd dose	16–29 months	5 years of age	Subcutaneous
12.	OPV Booster	16–24 months	5 years	Oral
13.	DPT Booster – 2	5–6 years	7 years of age	Intra muscular
14.	TT	10 years & 16 years	16 years	Intra muscular

11.6 Updated National Immunization Schedule Chart

Immunization/vaccination produce a response in the body that is similar to the body's response in the body that is similar to the body's response to a natural infection (Table 11.4). Immunization or vaccines can therefore protect the body from a disease before the disease has a chance to cause illness. Immunization has helped to reduce the impact of communicable disease on health and well being. Some diseases have been well controlled and other has been eliminated from some parts of the world because of vaccination. Stopping vaccination may lead to epidemic.

Summary

The reaction between antigen and antibody is highly specific. It is compared to the lock and key system. **ELISA** (Enzyme-Linked Immuno Sorbent Assay) is a plate-based assay technique designed for detecting and quantifying substances such as peptides, proteins, antibodies and hormones. There are **four kinds** of ELISA assay tests. They are: Direct ELISA, Indirect ELISA, Sandwich ELISA and Competitive ELISA. Western blotting technique is used for the identification of particular protein from the mixture of proteins. The most common protein sample used for Western blotting is cell lysate. Blotting refers to the transfer of the protein from the gel to the nitrocellulose paper by capillary action. The substances causing allergic/hypersensitivity is known as allergens. Allergic rhinitis develops when the body's immune system becomes sensitized and overreacts to something in the environment like pollen grains, strong odour of perfumes, dust etc. Certain drugs such as penicillin, cephalosporin and streptomycin can absorb non-specifically to protein on surface of RBC forming complex similar to hapten-carrier complex.

Transfer of living cells, tissues or organs from one part of the body to another or from one individual to another is known as transplantation. The graft tissue antigens induce an immune response in the host. This type of immune response is called host versus graft reaction. The ultimate goal of any immunization program is the eradication of the disease. Active natural immunization involves activation of immune system in the body to produce antibodies. It is achieved in both clinical and subclinical infections Immunization has helped to reduce the impact of communicable disease on health and well being.

Evaluation

Multiple choice questions

1. Antibody reacts with ____ to give agglutination.
   a. Particulate antigen
   b. Hapten and antigen
   c. Antibody and soluble antigen
   d. Carrier and antibody

W6BZR6

2. Anaphylaxis refers to
   a. Immediate hypersensitivity
   b. Hyposensitivity
   c. Delayed hypersensitivity
   d. Auto sensitivity
3. Atopy occurs due to
   a. House dust b. Egg
   c. Pollen d. all the above
4. In type II reaction, _____ is involved.
   a. IgG antibody
   b. IgG and IgM antibodies
   c. IgM antibody
   d. IgE antibody
5. _____ acts as an ACP.
   a. Macrophage
   b. RBC
   c. T cells
   d. Mucosal cells
6. Phagocytosis is enhanced by _____ process.
   a. Pinocytosis
   b. Opsonisation
   c. Endocytosis
   d. None
7. _____ produce antibodies.
   a. T cells
   b. B cells
   c. Ts cells
   d. Plasma cells
8. Sabin is _____ vaccine.
   a. Injection
   b. Recombination
   c. Oral
   d. Subunit
9. _____ is an injectable polio vaccine.
   a. Salk
   b. TAB
   c. Sabin
   d. BCG
10. Immunisation done in a community is called _____ immunity.
    a. Combined
    b. General
    c. Local
    d. Herd

Answer the following

1. What do you understand by the term antigen presentation?
2. Define: Pathogenicity.
3. Match the following:
   a. MMR - Subunit vaccine
   b. Salk - Triple vaccine
   c. HBV - Recombinant vaccine
   d. Sabin - Killed vaccine
   e. Influenzae - Live vaccine
4. What is meant by attenuation?
5. Describe toxin with examples.

6. Match:

a. Mast cell	-	Myelomaprotein
b. Primary immune	-	IgG
c. Secondary immune response	-	IgM
d. Secretory antibody	-	IgA
e. Plasma cell tumor	-	IgE

7. Write a note on Lymphocytes.
8. Define the following:
   a. Immunity
   b. Innate immunity
   c. Acquired Immunity
   d. Active immunity
   e. Passive immunity

Chapter
12

Microbial Genetics

Learning Objectives

After studying this chapter the students will be able to,

- *Define gene, genome, genetic code, genotype, phenotype, mutagen, wildtype*
- *Describe transcription and translation*
- *Classify mutations and its types and Understand how mutants are formed*
- *Know the mode of action of physical and chemical mutagens*
- *Identify the purpose of and outline the procedure for Ames test*
- *Compare the gene transfer mechanisms*
- *Know the types of cloning vectors used in genetic engineering*
- *Describe how plasmids and bacteriophages are used to transfer foreign DNA*
- *Explain the role of restriction enzymes in recombinant DNA technology*
- *Know the types of restriction enzymes*
- *Understand agarose gel electrophoresis and PCR techniques*
- *Explain RAPD and RFLP*

Chapter Outline

12.1 Concept of Gene

The fundamental unit of information in living systems is the **gene**. **Genome** is the set of all genes and genetic signals of a cell. The information contained in genes is converted to molecules that determine the metabolism, structure and form of microorganisms. Gene is expressed through a sequence of events. A gene can be defined biochemically as a segment of DNA (or, in a few cases, RNA) that encodes the information required to produce a functional biological product. The final product is usually a protein. Not all genes are involved in protein synthesis; some code instead for rRNA and tRNA.

The central dogma of molecular biology, comprises the three major processes (Figure 12.1). The first is **replication**, the

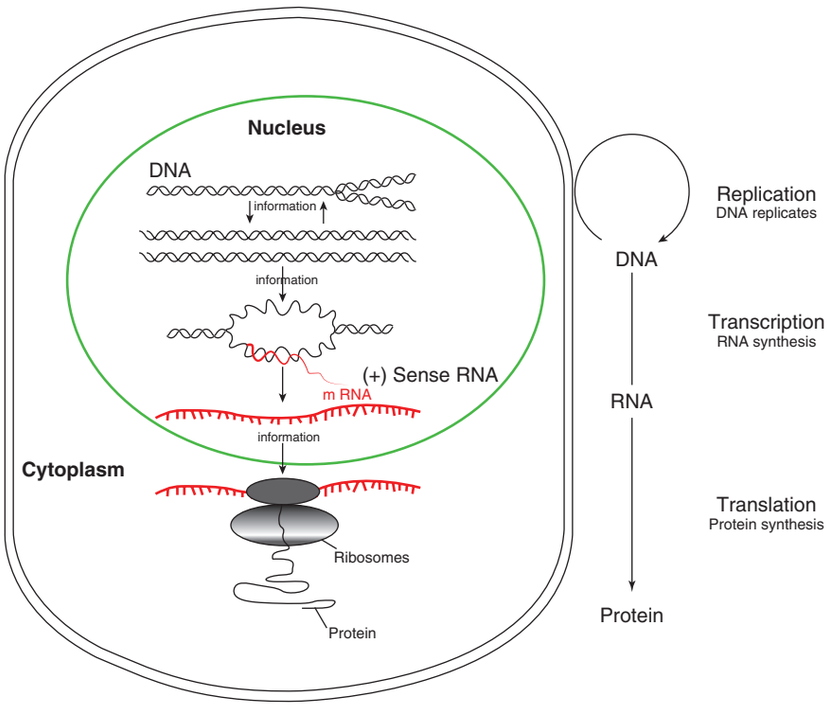


Figure 12.1: Central dogma of molecular biology

copying of parental DNA to form daughter DNA molecules with identical nucleotide sequences. The information contained in the base sequence of DNA is copied into protein molecule through an RNA molecule. The second is **transcription**, production of mRNA from DNA. It is the process by which the segment corresponding to a particular gene is selected and an RNA molecule is synthesized. The third is **translation**, The production of an amino acid sequence from an RNA base sequence. The genetic message encoded in messenger RNA (mRNA) is translated on the ribosomes into a polypeptide with a particular sequence of amino acids. The order of amino acid in a polypeptide chain is determined by DNA base sequence.

12.2 Transcription

An important feature of RNA synthesis is that even though the DNA molecule being copied is double stranded, in any particular region of DNA only one strand serves as a template. The DNA strand copied into RNA molecule is called CODING OR SENSE STRAND.

The synthesis of RNA consists of five discrete stage (Figure 12.2):

1. Promoter recognition: RNA polymerase binds to DNA within a specific base sequence (20–200 bases long) called a promoter. The sequence TATAAT (or a nearly identical sequence) often called a pribnow box or – 10 region is found as part of all prokaryotic promoters.

   The RNA polymerase of the bacterium *E.coli* consists of five protein subunits. Four of the subunits comprise the core enzyme (catalyzes the joining of the nucleoside triphosphates to the RNA) and fifth subunit, the σ subunit (required for promoter binding).
2. Local unwinding of DNA occurs and RNA polymerase forms an open promoter complex.

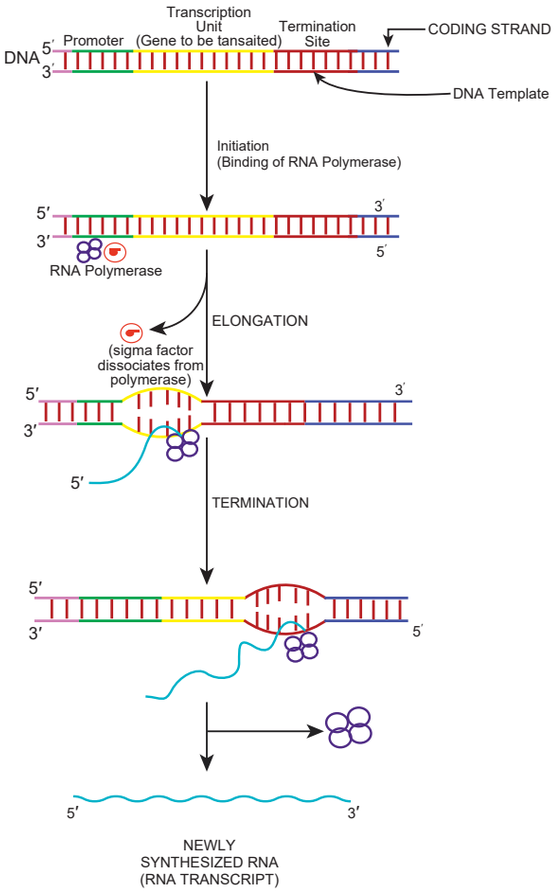


Figure 12.2: Major events in transcription

3. The first nucleoside triphosphate is placed at polymerization start site (near to the initial binding site) and synthesis begins.
4. RNA polymerase then moves along the DNA, adding ribonucleotides, to the growing RNA chain.
5. RNA polymerase reaches chain termination sequence and both the newly synthesized RNA and the polymerase are released. Two kinds of termination events are known those that are self – terminating (dependent on the base sequence only) and those that require the presence of the termination protein Rho.

Initiation of a second round of transcription need not await completion of the first, for the promoter becomes available once RNA polymerase has polymerized 50–60 nucleotides. In bacteria most mRNA molecules are degraded within a few minutes after synthesis. This degradation enables cells to dispense with molecules that are no longer needed.

In prokaryotes mRNA molecules commonly contain information for the amino acid sequences of several different polypeptide chains. In this case, such a molecule is called polycistronic mRNA. Cistron is a term used to mean a base sequence encoding a single polypeptide chain. The genes contained in polycistronic mRNA molecule (Figure 12.3) often encode the different portions of a metabolic pathway. For example, in *E. coli* the ten enzymes needed to synthesize histidine are encoded in one mRNA molecule.

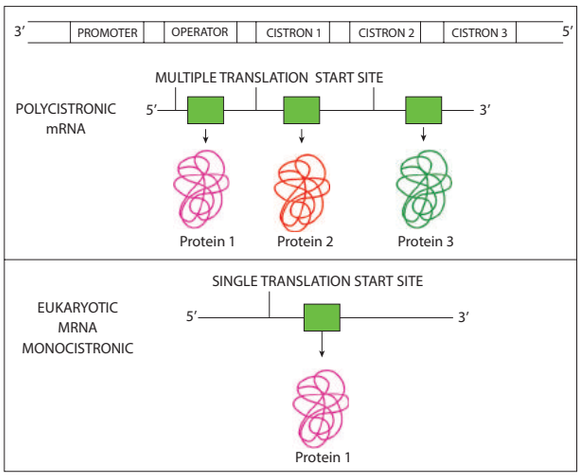


Figure 12.3: Polycistronic mRNA

In prokaryotes the immediate product of transcription (called the primary transcript) is mRNA, in contrast in eukaryotes the primary transcript must be converted to mRNA. This conversion called RNA processing consists of two types of events- modification of termini and excision of untranslated sequences (non-coding sequence or introns) embedded

within coding sequences (exons). Introns excision and the joining of exons to form an mRNA molecule is called RNA splicing. The introns are present in almost all eukaryotic transcripts but are rare in the free – living unicellular eukaryotes such as yeast. Some bacterial genes do contain introns.

Synthesis of rRNA and tRNA

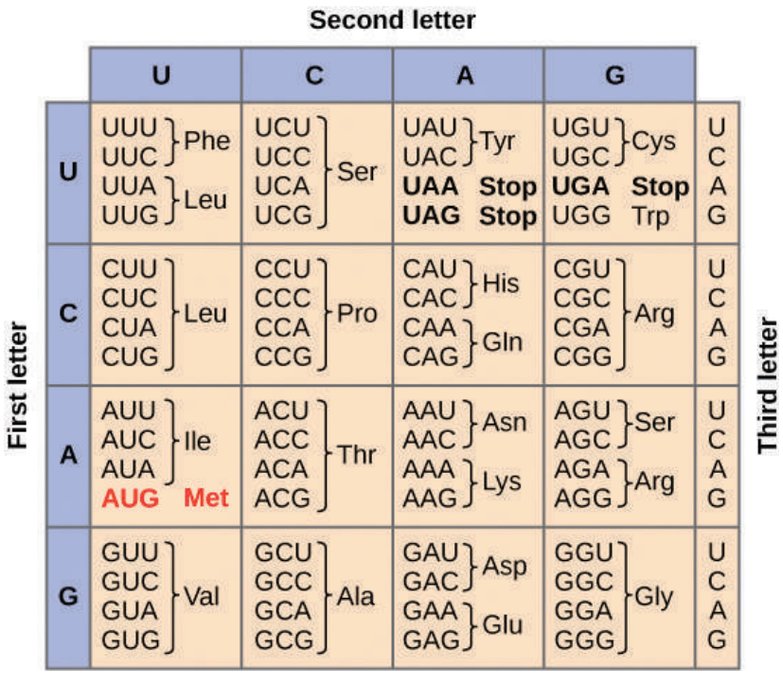
Ribosomal RNA and tRNA are also transcribed from genes. The production of these molecules is not as direct as synthesis of bacterial mRNA. The main difference is that these RNA molecules are excised from large primary transcripts. Highly specific RNA excise rRNA and tRNA from these large transcripts, and other enzymes produce the modified bases in tRNA.

12.3 Genetic Code

A tRNA molecule "reads" the base sequence of mRNA. The language read by the tRNA molecules is called the genetic code, which is a set of relations between sequences of three adjacent bases on an mRNA molecule and particular amino acids. An RNA base sequence (a set of 3 bases) corresponding to a particular amino acid is called a codon. The genetic code is the set of all codons. Only four bases in DNA serve to specify 20 amino acids in proteins, so some combination of bases is needed for each amino acid. Before the genetic code was elucidated, it was reasoned that if all codons were assumed to have the same number of bases, then each codon would have to contain at least three bases. Codons consisting of pairs of bases would be insufficient because four bases can form only $4^2 = 16$ pairs, and there are 20 amino acid. Triplets of bases would suffice because, these can form $4^3 = 64$ triplets. In fact, the genetic code is a triplet code, and all 64 possible codons carry information of some sort. Several different codons designate the same amino acid. Furthermore, in translating mRNA molecules the codons do not overlap but are used sequentially. The same genetic code is used by almost all biological systems and hence is said to be universal (exceptions are mitochondria and a few unusual microorganisms). The codons are by convention written with the 5′ end at the left. The complete code is shown in Table 12.1.

Features of the Code:

- Sixtyone codons correspond to amino acids. Four codons are signals. These are the three stop codons – UAA, UAG, UGA – and the one start codons, AUG. The start codons (initiation codon) also specifies the amino acid methionine. In rare cases, certain other codon (E.g. GUG) initiate translation. No normal tRNA molecule has an anticodon (a sequence of three bases on tRNA that can base – pair with a codon sequence in the mRNA) complementary to any of the stop codons UAG, UAA or UGA, which is why these codons are stop signals.
- The code is highly redundant i.e. more than one codons code for an amino acid. Only tryptophan and methionine are specified by one codon. The synonymous codons usually differ only in third base (except for serine, leucine and arginine).



The 20 amino acids and their abbreviations	
Amino acid	3-letter abbreviation
Alanine	Ala
Arginine	Arg
Asparagine	Asn
Aspatic acid	Asp
Aspartic acid & Asparagine	Asx
Cysteine	Cys
Glutamine	Gln
Glutamic acid	Glu
Glutaine or Glutamic acid	Glx
Glycine	Gly
Histidine	His
Isoleucine	IIe
Leucine	Leu
Lysine	Lys
Methionine	Met

Table 12.1: Genetic code

HOTS

1. How many of the 64 codons can be made from the three nucleotides A, U, and C?
2. If codons were four bases long, how many codons would exist in a genetic code?

12.4 Translation

RNA is translated from the 5′ end of the molecule toward the 3′ end. Polypeptides are synthesized from the amino terminus toward the carboxyl terminus, by adding amino acids one by one to the carboxyl end. Not all the base sequences in an mRNA is translated into amino acid sequences of polypeptides. Initiation of polypeptide synthesis may begin hundreds of nucleotides from the 5′ – P terminus of the RNA. The section of untranslated RNA before the region encoding the first polypeptide chain is called a **leader**, which in some cases contains regulatory sequences that influence the rate of protein synthesis. The major events in translation are (Figure 12.4).

1. An mRNA binds to the surface of a protein synthesizing particle, the **Ribosome**.
2. The tRNA – amino acid complexes (made by the aminoacyl tRNA synthetases) bind sequentially, one by one, to the mRNA molecule that is attached to the ribosome.

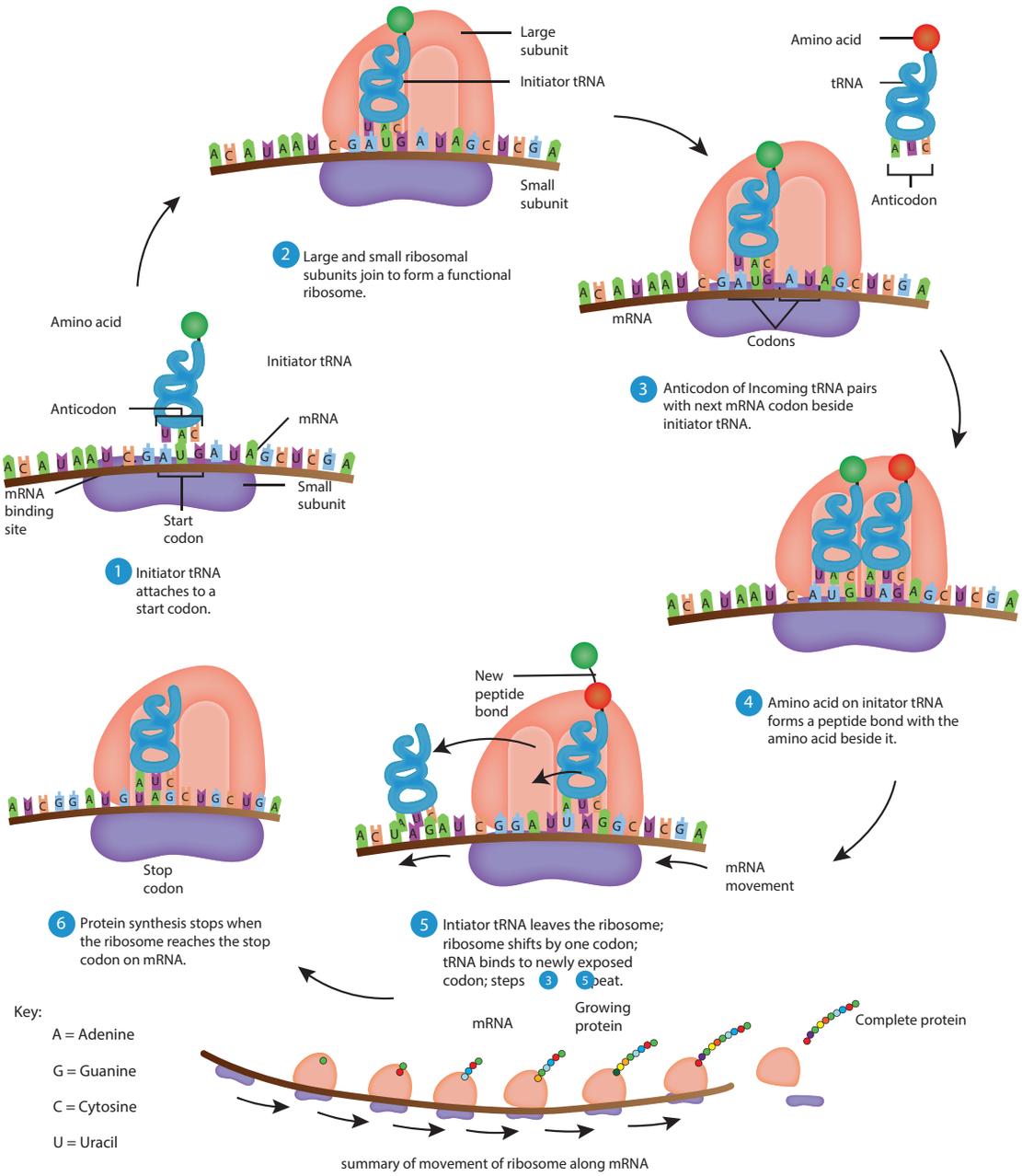


Figure 12.4: Major events in Translation

3. Peptide bonds are made between successively aligned amino acids.
4. Finally the chemical bond between the tRNA and its attached amino acids is broken and the completed protein is removed.

- The 3′ terminal of the tRNA molecule (Figure 12.5) is covalently linked to the amino acid corresponding to the particular mRNA codon
- When an amino acid has become attached to a tRNA molecule, the tRNA is said to be acylated or charged
- An important feature of initiation of polypeptide synthesis in both prokaryotes and eukaryotes is the use of a **specific initiating tRNA molecule**. In prokaryotes this tRNA molecule is acylated with the modified amino acid N – formyl methionine (fMet). This

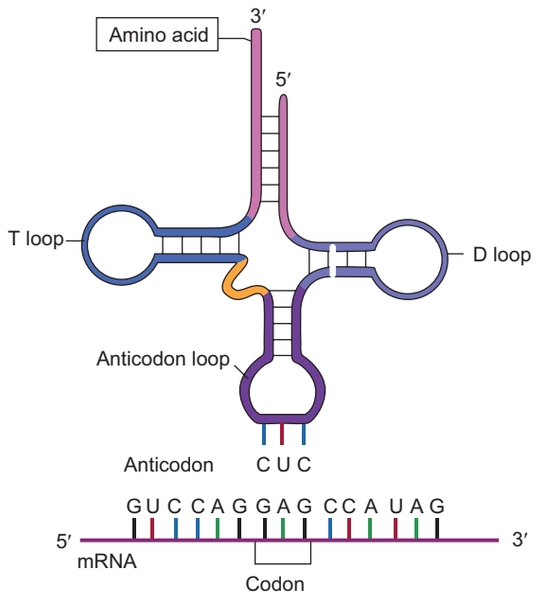


Figure 12.5: tRNA

tRNA is often designated **tRNA^{fMet}**. Both tRNA^{fMet} and tRNA^{Met} recognize the codon AUG, but only tRNA^{fMet} is used for initiation. All prokaryotic proteins while being synthesized have fMet at the amino terminus. However, this amino acid is frequently deformylated or removed later.

- The usual form of translation unit is a polyribosome or polysome wherein an mRNA is covered with ribosomes.

Redundancy and the Wobble Hypothesis

The identity of the third base of a codon appears to be unimportant. (The first base in a codon is at the 5′ end and the third base is at the 3′ end). Wobble refers to the less stringent requirement for base pairing at the third position of the codon than at the first two positions. That is the first two bases must follow Watson and Crick base pairing rule (A with U, or G with C), but the third base pair can be of a different type (for example, G with U). The Wobble hypothesis explains the pattern of redundancy in the code in that certain anticodons. (For example, those containing U and G in the first position of the anticodons) can pair with several codons during translation (Figure 12.6).

12.5 Types of Mutations

The base sequence of DNA determines the amino acid sequence of a protein. The chemical and physical properties of each protein are determined by its amino acid sequence, so a single amino acid change is capable of altering the activity of, or even completely inactivating, a protein. **Genotype** refers to the genetic composition

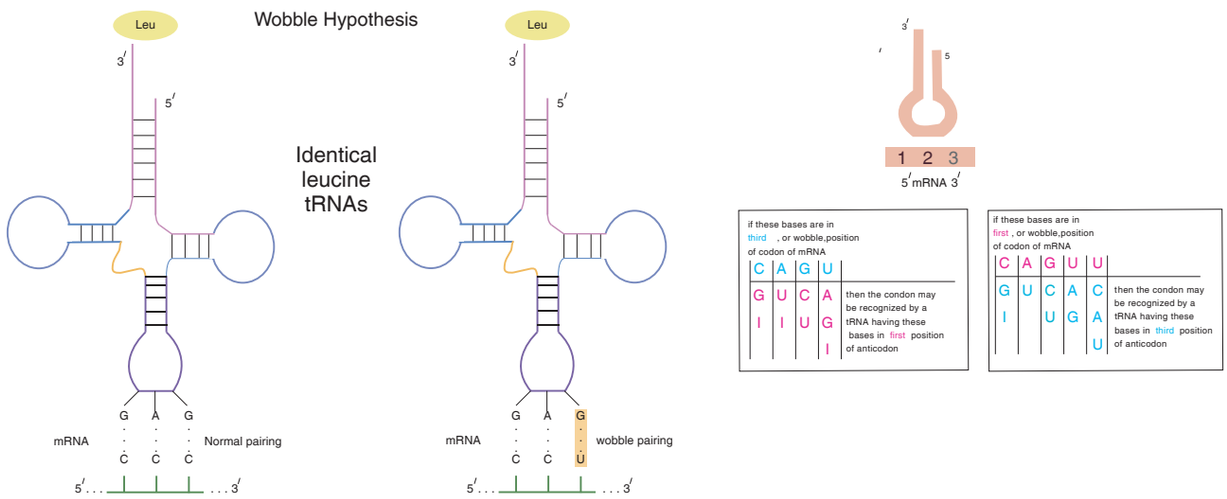
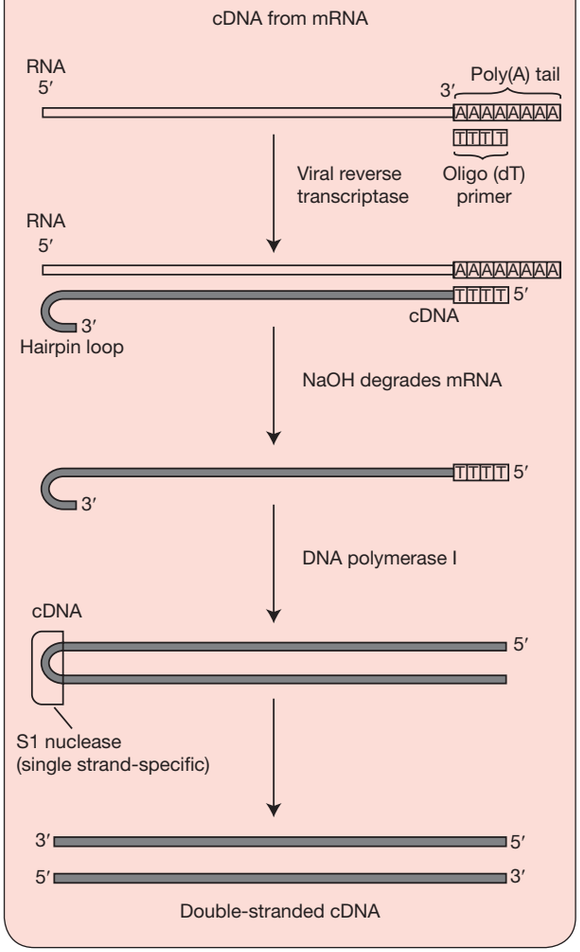


Figure 12.6: Wobble hypothesis

Infobits

In 1970 Howard Temin and David Baltimore independently discovered the enzyme **reverse transcriptase** that retroviruses use to produce DNA copies of their RNA genome. This enzyme can be used to construct DNA copy, called **complementary DNA** (cDNA), of any RNA as shown in figure below . Thus genes or major portions of the gene can be synthesized from mRNA.



of an organism. **Phenotype** is an observable property of organism. The functional form of a gene is called **Wildtype** because presumably this is the form found in nature.

Mutation is the process by which the sequence of base pairs in a DNA molecule is altered.The alteration can be a single base pair substitution, insertion or deletion.

Mutations can be divided into two general categories:

1. **Base–pair substitution mutation** involves a change in the DNA such that one base pair is replaced by another.
   - A mutation from one purine – pyrimidine base pair to the other purine –pyrimidine base pair is a transition mutation (Figure 12.7a). E.g. AT to GC, CG to TA.
   - A mutation from a purine - pyrimdine base pair to a pyrimidine – purine base pair is a transversion mutation (Figure 12.7b). E.g. AT to TA, CG to GC.
2. **Base pair insertion or deletions** involves the addition or deletion of one base pair. If one or more base pairs are added to or deleted from a protein coding gene, the reading frame of an mRNA can change downstream of the mutation. An addition or deletion of one base pair, for example, shifts the mRNA's downstream reading frame by one base, so that incorrect amino acids are added to the polypeptide chain after the mutation site. This type of mutation, called a frame shift mutation (Figure 12.8) usually results in a nonfunctional protein.

Frame shift mutations:

- May generate new stop codons, resulting in a shortened protein.
- May result in a read through of the normal stop codon, resulting in longer than normal proteins
- Or may result in a complete alteration of the amino acid sequence of a protein.

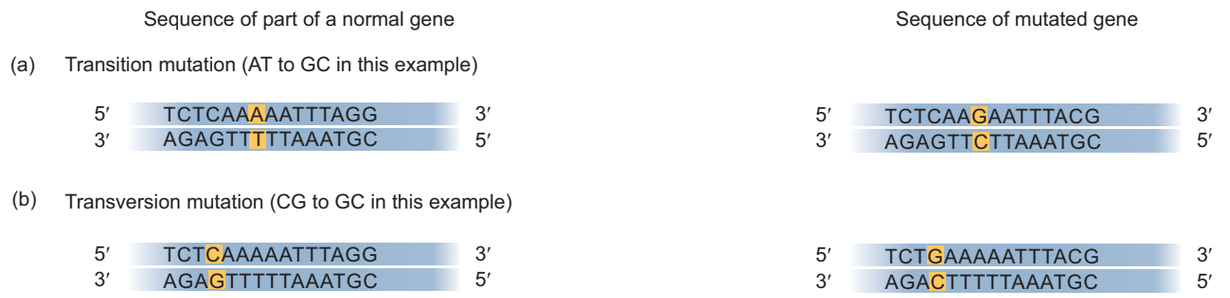


Figure 12.7: **(a)** Transition mutations **(b)** transversion mutations

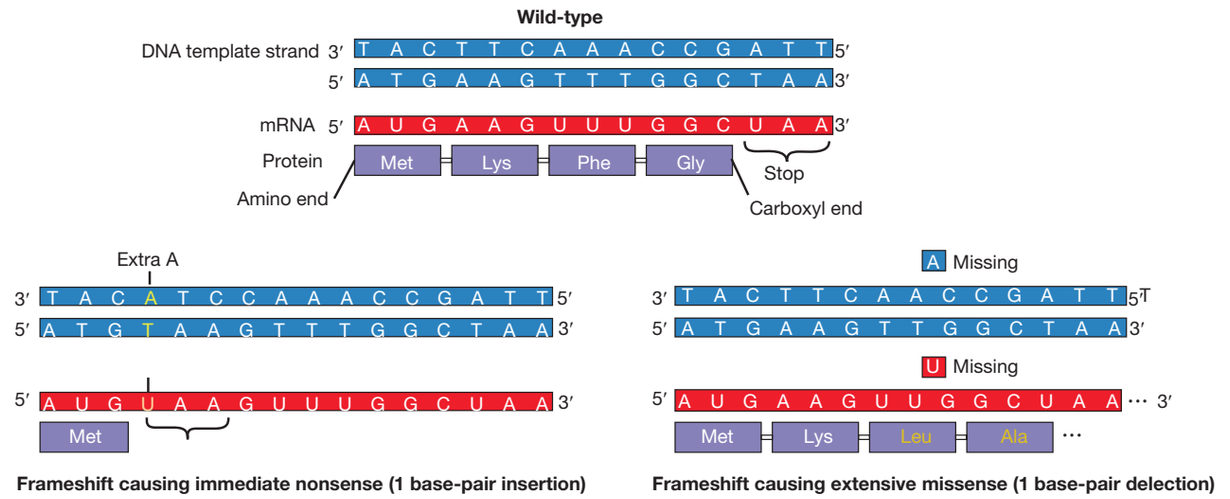


Figure 12.8: Frameshift mutations

Point mutations are single base changes, that do not affect the reading frame, that is, the mutation only makes a single change in a single codon, and everything else is undisturbed.

Mutations can also be defined according to their effects on amino acid sequences in proteins. They are

1. A missense mutation (Figure 12.9a) is a gene mutation in which a base – pair change in the DNA changes a codon in an mRNA so that a different amino acid is inserted into the polypeptide.
2. A neutral mutation (Figure 12.9b) is a subset of missense mutations in which the new codon codes for a different amino acid that is chemically equivalent to the original and therefore does not affect the proteins function. Consequently, the phenotype does not change.
3. A silent mutation (Figure 12.9c) is also a subset of missense mutations that occurs when a base – pair change in a gene alters a codon in the mRNA such that the same amino acid is inserted in the protein. In this case, the protein obviously has a wild type function.
4. A nonsense mutation (Figure 12.9d) is a gene mutation in which a base – pair change in the DNA, changes a codon in an mRNA to a stop (nonsense) codon (UAG, UAA or UGA). Nonsense mutation cause premature chain termination so instead of complete polypeptides, shorter than

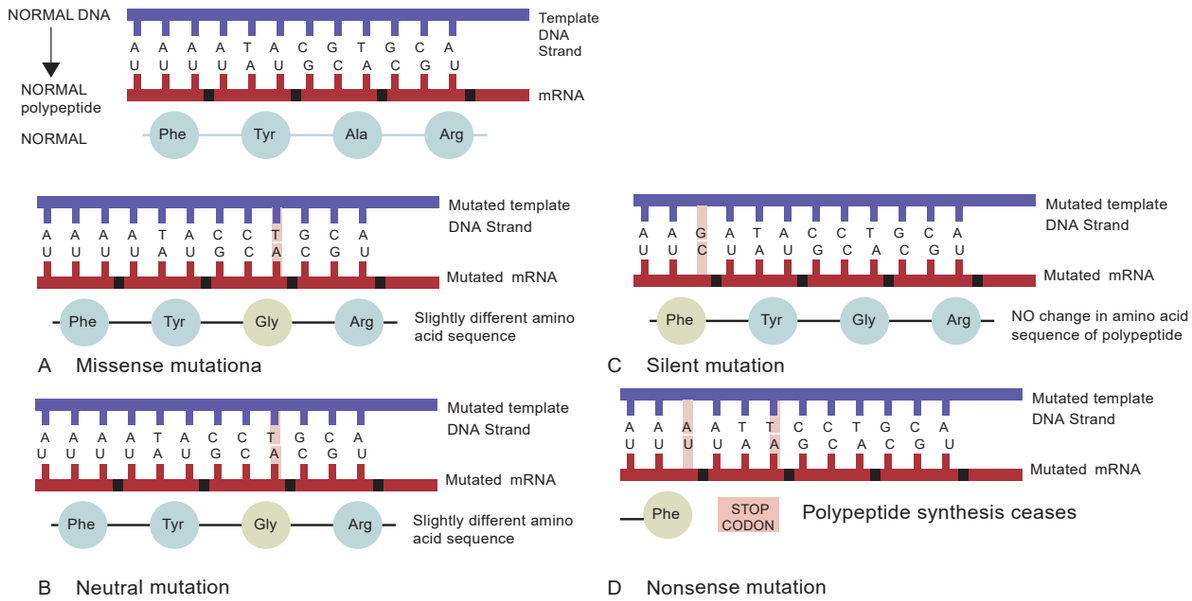


Figure 12.9: **(a)** Missense **(b)** neutral c) silent **(d)** nonsense mutation respectively

normal polypeptide fragments (often nonfunctional) are formed.

Forward mutations change the genotype from wild type to mutant and reverse mutations (or reversions or back mutations) change the genotype from mutant to wild type or to partially wild type. An organism which has reverted is Revertant. The effects of mutation may be diminished or abolished by a suppressor mutation. Suppressor mutation is a mutation at a different site from that of the original mutation. A suppressor mutation masks or compensates for the effects of the initial mutation, but it does not reverse the original mutation.

12.6 Formation of Mutants

The term mutant refers to an organism in which either the base sequence of DNA or the phenotype has been changed. A mutant is an organism whose genotype differs from that found in nature. The process of formation of mutant organism is called mutagenesis. In nature and in the laboratory, mutations sometimes arise spontaneously, without any help from the experimenter. This is called spontaneous mutagenesis. The two mechanisms that are most important for spontaneous mutagenesis are

1. Errors occurring during replication and
2. Spontaneous alteration of bases.

Mutations can also be induced experimentally by application of mutagens. Mutagens are agents that cause mutations.

Mutagens and their Mode of Action

Physical Mutagens

UV radiation: UV light causes mutations because the purine and pyrimidine bases in DNA absorb light strongly in the ultraviolet range (254 to 260 nm). At this wavelength, UV light induces point mutations primarily by causing photochemical changes in the DNA. One of the effects of UV radiation on DNA

is the formation of abnormal chemical bonds between adjacent pyrimidine molecules in the same strand, or between pyrimidines on the opposite strands, of the double helix. This bonding is induced mostly between adjacent thymines, forming what are called thymine dimmers (Figure 12.10), usually designated TT. This unusual pairing produces a bulge in the DNA strand and disrupts the normal pairing of T's (thymines) with corresponding A's(adenines) on the opposite strand. If UV induced genetic damage is not repaired, mutations or cell death may result.

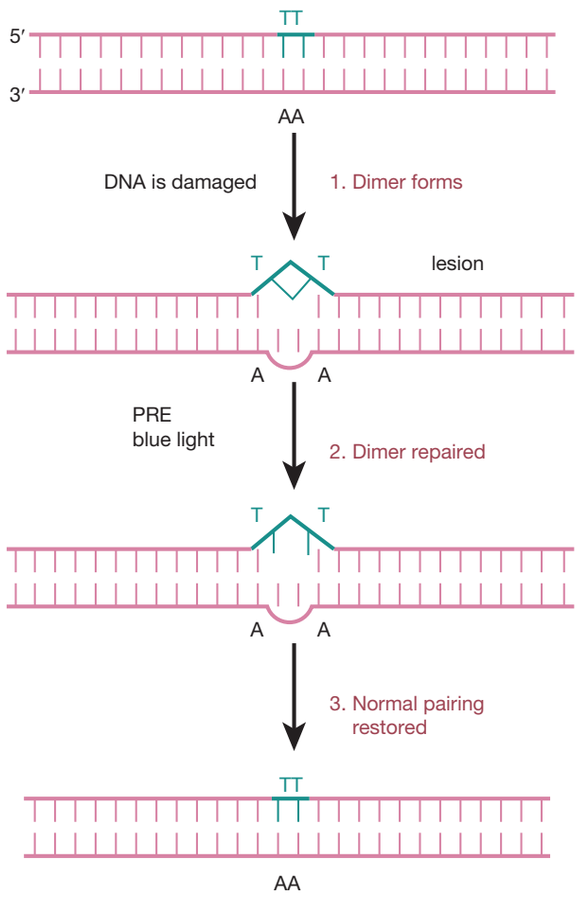


Figure 12.10: UV induced DNA damage

Chemical Mutagens

Chemical mutagens include both naturally occurring chemicals and synthetic substances. These mutagens can be grouped into different classes on the basis of their mechanism of action. They are

i. **Base analogs** are bases that are similar to the bases normally found in DNA. Like normal bases, base analogs exist in normal and rare tautomeric states. In each of the two states, the base analog pairs with a different normal base in DNA. Because base analogs are similar to the normal nitrogen bases, they may be incorporated into DNA in place of normal bases. E.g. 5 – bromouracil (5-BU). If 5-BU is incorporated in its more common normal state, it pairs with adenine. If it changes into its rare state during replication, it pairs with guanine instead. In the next round of replication, the 5-BU – G base pair is resolved into a C – G base pair instead of the T – A base pair. By this process a transition mutation is produced, from TA to CG (Figure 12.11).

Not all base analogs are mutagens. For example, AZT (Azidothymidine), one of the approved drugs given to patients with AIDS, is an analog of thymidine, but it is not a mutagen, because it does not cause base pair changes.

ii. **Base Modifying Agents** are chemical that act as mutagens by modifying the chemical structure and properties of bases. The three types of mutagens that work in this way are

1. A deaminating agent e.g.: Nitrous acid removes amino groups (- NH_2) from the bases guanine, cytosine, and

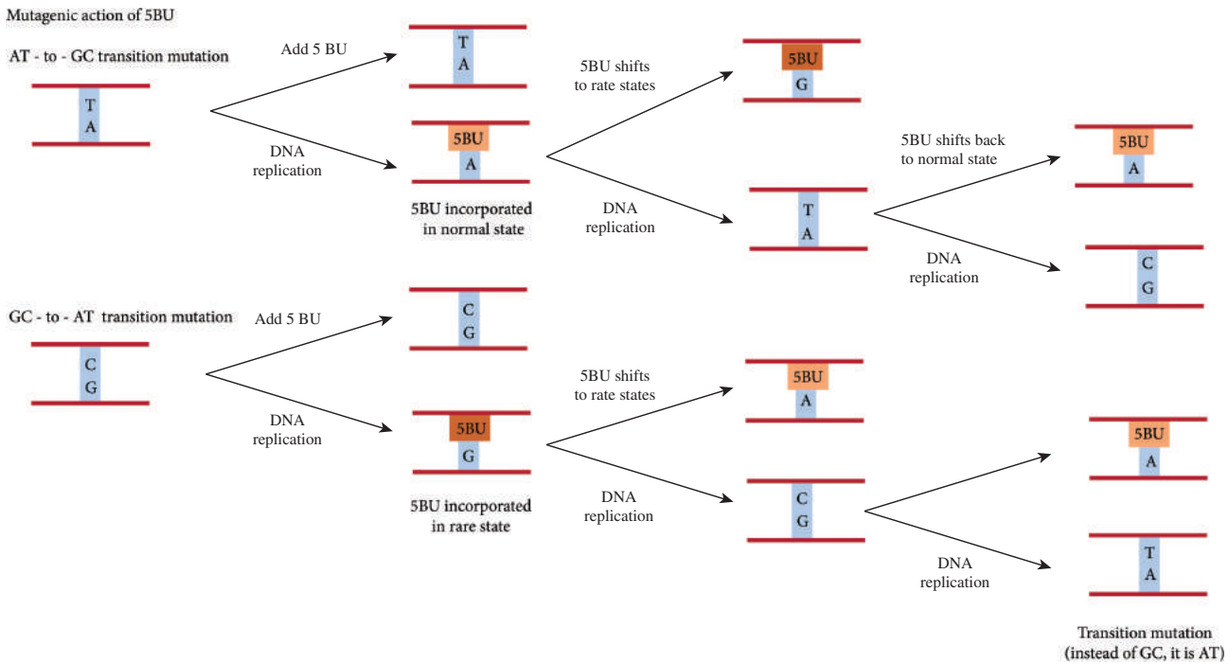


Figure 12.11: Mutagenic effects of 5-BU

adenine. When cytosine is treated with nitrous acid it removes amino group from cytosine which changes to uracil resulting in transition mutation (Figure 12.12a).

2. Hydroxylamine (NH_2 OH) is a hydroxylating mutagen that react specifically with cytosine, modifying it by adding a hydroxyl group (OH) so that it can pair solely with adenine instead of with guanine. Mutations induced by hydroxylamine can only be CG to TA transitions (Figure 12.12b).
3. Alkylating agents like methymethane sulfonate (MMS) introduces alkyl groups onto the bases at a number of location. For example after treatment with MMS, some guanines are methylated at 6 – oxygen to produce O6 – methyl guanine. The methylated guanine pairs with thymine rather than cytosine giving G C to AT transitions (Figure 12.12c).

Original base	Mutagen	Modified base Pairing partner	Predicted transition
Cytosine	Nitrous acid (H_2NO) ⟶	Uracil ═══ Adenine	CG TA
Cytosine	Hydroxylamine (NH_2OH) ⟶	Hydroxyl amino cystosine ═══ Adenine	CG TA
Guanine	Methylmethane sulfonate(MMS) (alkylating agent) ⟶	O-Methylguanine ═══ Thymine	CG TA

Figure 12.12: Action of three base modifying agents.

iii. Intercalating agents

Acridine, proflavin, ethidium bromide are examples few examples of intercalating agents. These insert (intercalate) themselves between adjacent bases in one or both strands of the DNA double helix. Due to this an extra base (chosen at random, G in the Figure 12.13a) must be inserted into the new DNA strand opposite the intercalating agent. Intercalating agents can cause either additions or deletions.

The Ames Test: A Screen for Potential Carcinogens

Everyday we are exposed to a wide variety of chemicals in our environment, such as drugs, cosmetics, food additives, pesticides, and industrial compounds. Many of these chemicals can have mutagenic effects,

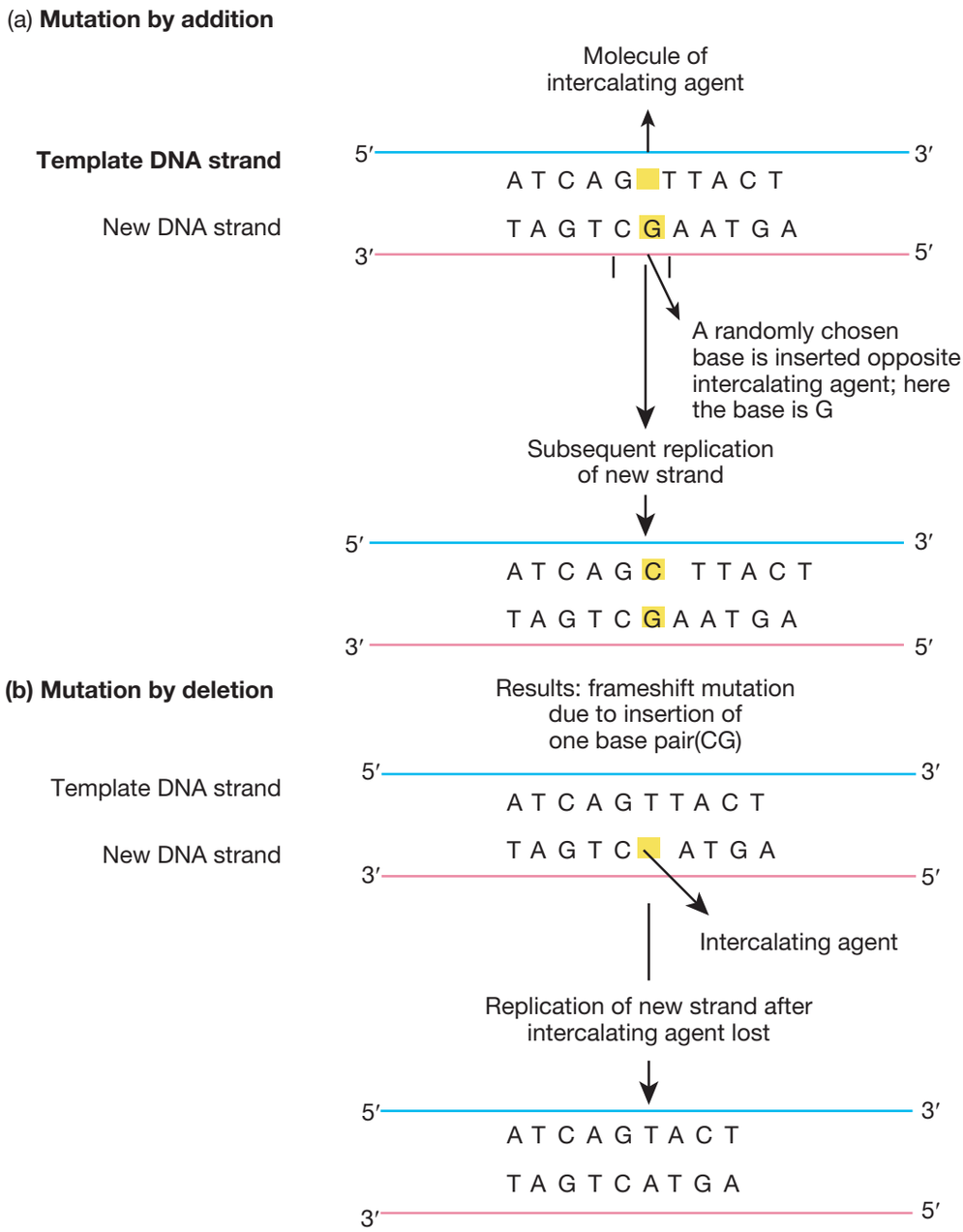


Figure 12.13: Mutations due to intercalating agents

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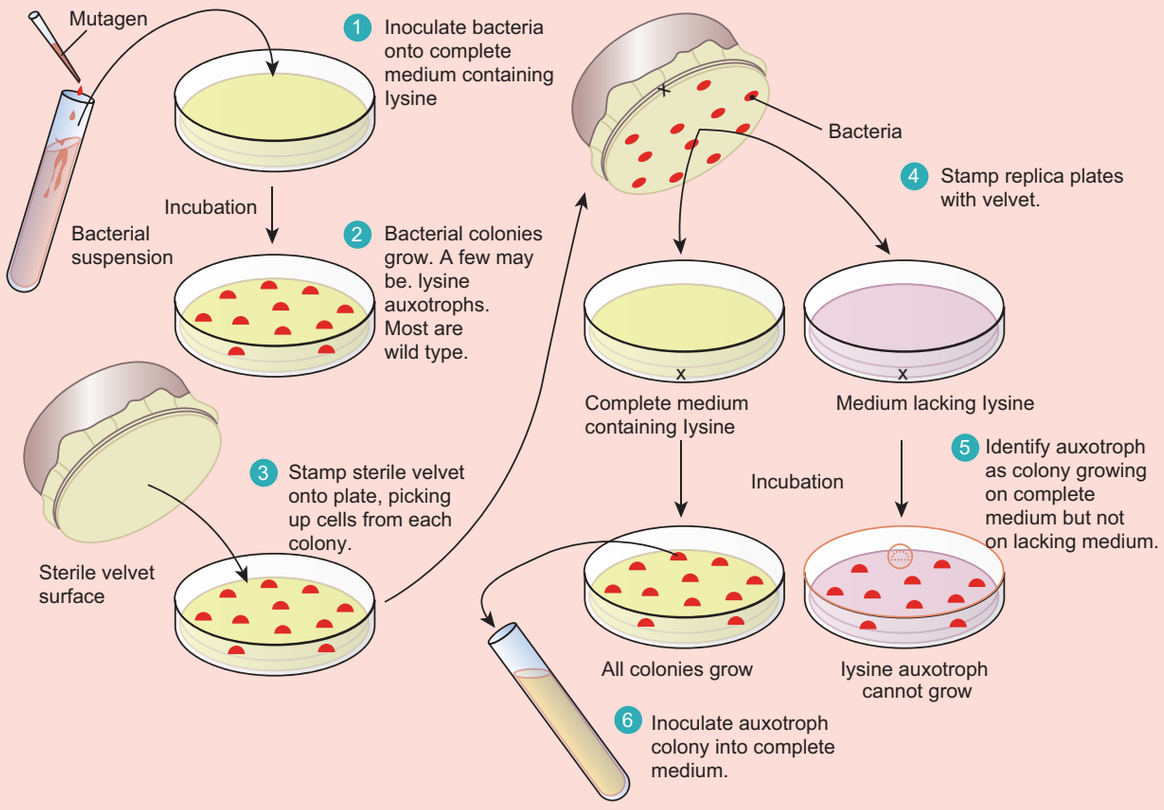
Isolation and detection of Mutants

Once mutations are induced, then, they must be detected if they are to be studied. Selection and screening procedures historically have helped geneticists isolate mutants of interest from a heterogenous mixture in a mutagenized population. When isolating mutants of a particular organism, one must know the normal or wild type characteristics so as to recognize an altered phenotype. A suitable detection system for the mutant phenotype under study also is needed. Detection systems in bacteria and other haploid organisms are straightforward because any new allele should be seen immediately, even if it is recessive mutation. The detection of mutants can be direct and complex. For example, the replica plating technique is used to detect auxotrophic mutants (mutants which are deficient in synthesizing a particular biochemical compound). **Replica plating technique** distinguishes between mutant and wild type strain based on their ability to grow in the absence of a particular biosynthetic end product Figure below. A lysine auxotroph, for instance, will grow on lysine supplemented media but not on a medium lacking an adequate supply of lysine because it cannot synthesize this amino acid.



including genetic diseases and cancer. Some banned chemical warfare agents (e.g. mustard gas) also are mutagens.

A number of chemicals (subclass of mutagens) induce mutations that result in tumorous or cancerous growth. These chemical agents are called chemical carcinogens. Directly testing the chemicals for their ability to cause tumors in animals is time consuming and expensive. However, the fact that most chemical carcinogens are mutagens led Bruce Ames to develop a simple, inexpensive, indirect assay for mutagens. In general Ames test is an indicator of whether the chemical is a mutagen. The Ames test assays the ability of chemicals to revert mutant strains of the bacterium *Salmonella typhimurium* to wild type. The mutant strain of *S.typhimurium* is auxotrophic to histidine (his^-), that is it requires histidine for its growth and cannot grow in the absence of histidine. The mutant strain is grown in a histidine deficient medium containing the chemical to be tested. A control plate is also set up which does not contain the chemical. After incubation the control plates may have few colonies resulting from spontaneous reversion of the his- strain. Compared to the control plates if there are increased number of colonies on test plate, it indicates that the chemical has reverted the mutant strain back to wild type. This chemical is likely to be a carcinogen. Figure 12.14 shows steps in Ames test.

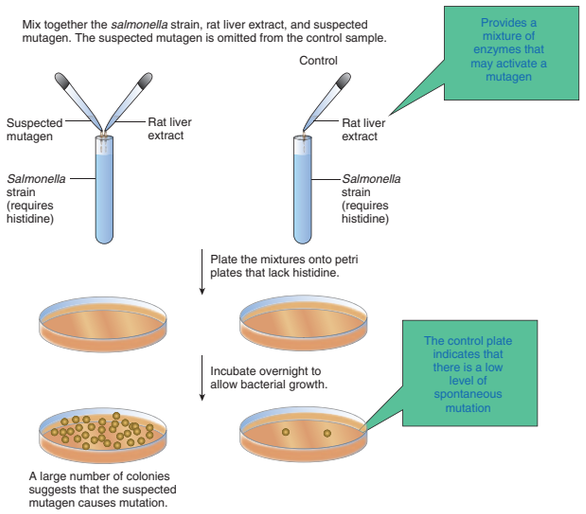


Figure 12.14: Steps in Ames test

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DNA Repair

Both prokaryotes and eukaryotes have a number of repair systems that deal with different kinds of DNA damage. All the systems use enzymes to make correction. Without this repair systems lesions would accumulate and be lethal to the cell or organism. Not all lesions are repaired, and mutations do appear, but at low frequencies. At high doses of mutagens, repair systems are unable to correct all of the damage, and cell death may result. We can group repair systems into different categories on the basis of the way they operate. Some systems correct damaged areas by reversing the damage. This type of repair is called direct correction or direct reversal. Other systems excise the damaged areas and then repair the gap by new DNA synthesis. Some of the DNA repair systems are

- Mismatch repair by DNA polymerase proofreading
- Repair of UV induced pyrimidine dimers- Photo reactivation or Light repair
- Base excision repair
- Nucleotide excision repair

With recombinant DNA technology it is possible to mutate a gene at specific positions in the test tube by SITE SPECIFIC MUTAGENESIS and then introduce the mutated gene back into the cell and investigate the phenotypic changes produced by the mutation in vivo. Such techniques enable geneticists to study, for example, genes with unknown function and specific sequences involved in regulating a gene's expression.

12.7 Transfer of Genetic Material

Normally, genes and the characteristics they code for are passed down from parent to progeny. This is called vertical gene transfer. Bacteria and some lower eukaryotes are unique in that they can pass DNA from one cell of the same generation to another. The exchange of genes between two cells of the same generation is referred to as horizontal gene transfer. Mechanisms like transformation, transduction and conjugation takes place naturally and may bring about genetic variation and genetic recombination. These gene transfer mechanisms are also employed in genetic engineering to introduce desired gene into the cells. Introducing a foreign gene or recombinant DNA into the cells is one of the techniques used in genetic engineering. The success of cloning depends on the efficiency of gene transfer process. The most commonly employed gene transfer methods are transformation, conjugation, transduction, electroporation, lipofection and direct transfer of DNA. The choice of the method depends on the type of host cell (bacteria, fungi, plant, animal). Figure 12.15 shows methods of DNA transfer.

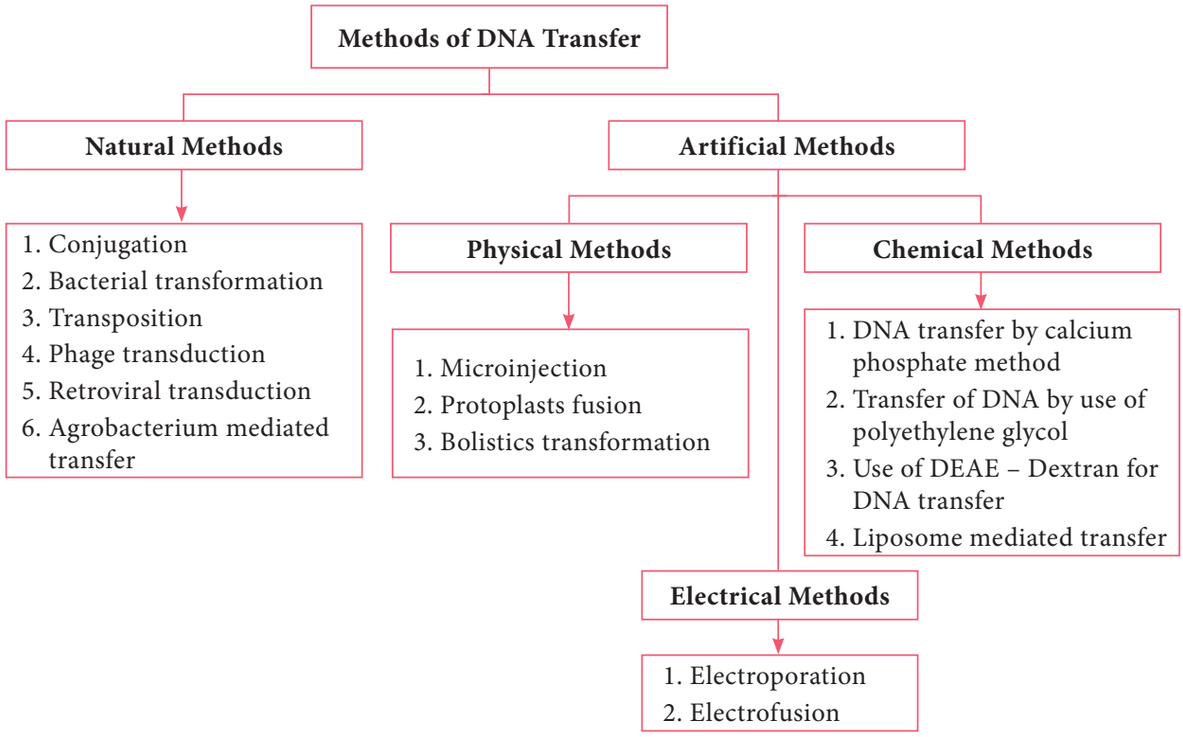

Figure 12.15: Methods of DNA transfer

Note: The term Transfection is used for the transfer of DNA into eukaryotic cells by various physical or chemical means.

12.7.1 Transformation

Transformation is genetic alteration of a cell resulting from the direct uptake, incorporation and expression of exogenous genetic material (exogenous DNA) from its surroundings. Transformation occurs naturally in some species of bacteria, but it can also take place by artificial means in other cells. For transformation to happen, bacteria must be in a state of competence. Competence refers to the state of being able to take up exogenous DNA from the environment. There are two forms of competence: natural and artificial. Transformation works best with DNA from closely-related species. The naturally-competent bacteria carry sets of genes that provide the protein machinery to bring DNA across the cell membrane(s).

There are some differences in the mechanisms of DNA uptake by gram positive and gram negative cells. However, they share some common features that involve related proteins. The DNA first binds to the surface of the competent cells on a DNA receptor, and passes through the cytoplasmic membrane via DNA translocase. Only single stranded DNA may pass through, one strand is therefore degraded by nucleases in the process, and the translocated single-stranded DNA may then be integrated into the bacterial chromosomes. Figure 12.16 shows mechanism of transformation.

Artificial competence can be induced in laboratory procedures that involve making the cell passively permeable to DNA. Typically, the cells are incubated in a solution containing divalent cations; most commonly, calcium chloride solution under cold condition, which is then exposed to a pulse of heat shock. Electroporation is another method of promoting competence. Using this method, the cells are briefly shocked with an electric field of 10–20 kV/cm which is thought to create holes in the cell membrane through which the plasmid DNA may enter. After the electric shock, the holes are rapidly closed by the cell's membrane-repair mechanisms.

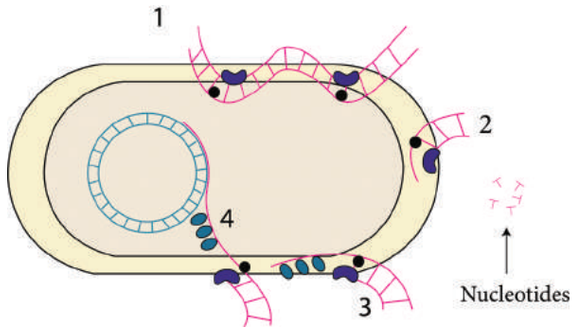

Figure 12.16: Mechanism of transformation **1.** Binding of DNA; **2.** Degradation of one strand; **3.** Entry of ssDNA; **4.** Integration into host chromosome.

12.7.2 Conjugation

The initial evidence for bacterial conjugation, came from an experiment performed by Joshua Lederberg and Edward L Tatum in 1946. Later in 1950, Bernard Davis gave evidence that physical contact of the cells was necessary for conjugation. During conjugation, two live bacteria (a donor and a recipient) come together, join by cytoplasmic bridges (e.g. pilus) and transfer single stranded DNA (from donor to recipient).

Inside the recipient cell, the new DNA may integrate with the chromosome (rather rare) or may remain free (as is the case with plasmids). Conjugation can occur among the cells from different genera of bacteria, while transformation takes place among the cells of a bacterial genus.

A plasmid called the fertility or F factor plays a major role in conjugation. The F factor is about 100 kilobases long and bears genes responsible for cell attachment and plasmid transfer between specific bacterial strains during conjugation. F factor is made up of

a. tra region (tra operon / transfer genes): genes coding the F pilus and DNA transfer,

b. Insertion sequence: genes assisting plasmid integration into host cell chromosome.

Thus, the F factor is an **episome** - a genetic material that can exist outside the bacterial chromosome or be integrated into it.

During $F^+ \times F^-$ mating or conjugation (Figure 12.17a) the F factor replicates by the rolling circle mechanism and a copy moves to the recipient. The channel for DNA transfer could be either the hollow F pilus or a special conjugation bridge formed upon contact. The entering strand is copied to produce double – stranded DNA.

F factor can integrate into the bacterial chromosome at several different locations by recombination between homologous insertion sequences present on both the plasmid and host chromosomes. The integration of F factor into bacterial chromosome results in formation of HFR (High Frequency Recombination) cell. When integrated, the Fplasmid's tra operon is still functional; the plasmid can direct the synthesis of pili, carry out rolling circle replication, and transfer genetic material to an F^- recipient cell. An HFR cell is so called because it exhibits a very high efficiency of chromosomal gene transfer in comparison with F^+ cells. In F^+ cells the independent F factor rarely transfer chromosomal genes hence the recombination frequency is low. Figure 12.17b shows formation of HFR cell. When an HFR cell is mated with F^- cell the F^- recipient does not become F^+ unless the whole chromosome is transferred as explained in Figure 12.17c. The connection usually breaks before this process is finished. Thus, complete F factor usually is not transferred, and the recipient remains F^-.

Because the F plasmid is an episome, it can leave (deintegrate) the bacterial chromosome. Sometimes during this process, the plasmid makes an error in excision and picks up a portion of the chromosomal material to form an F′ plasmid. Figure 12.17d shows formation of F′. During $F'XF^-$ conjugation (Figure 12.17e) the recipient becomes F′ and is a partially diploid since it has two set of the genes carried by the plasmid.

The natural phenomenon of conjugation is now exploited for gene transfer and Recombinant DNA technology. In general, the plasmids lack conjugative functions and therefore, they are not as such capable of transferring DNA to the recipient cells. However, some plasmids with conjugative properties can be prepared and used.

12.7.3 Transduction

Transduction is the transfer of bacterial genes from one bacteria to other by viruses. Example: Bacteriophage (Bacterial viruses). To understand the

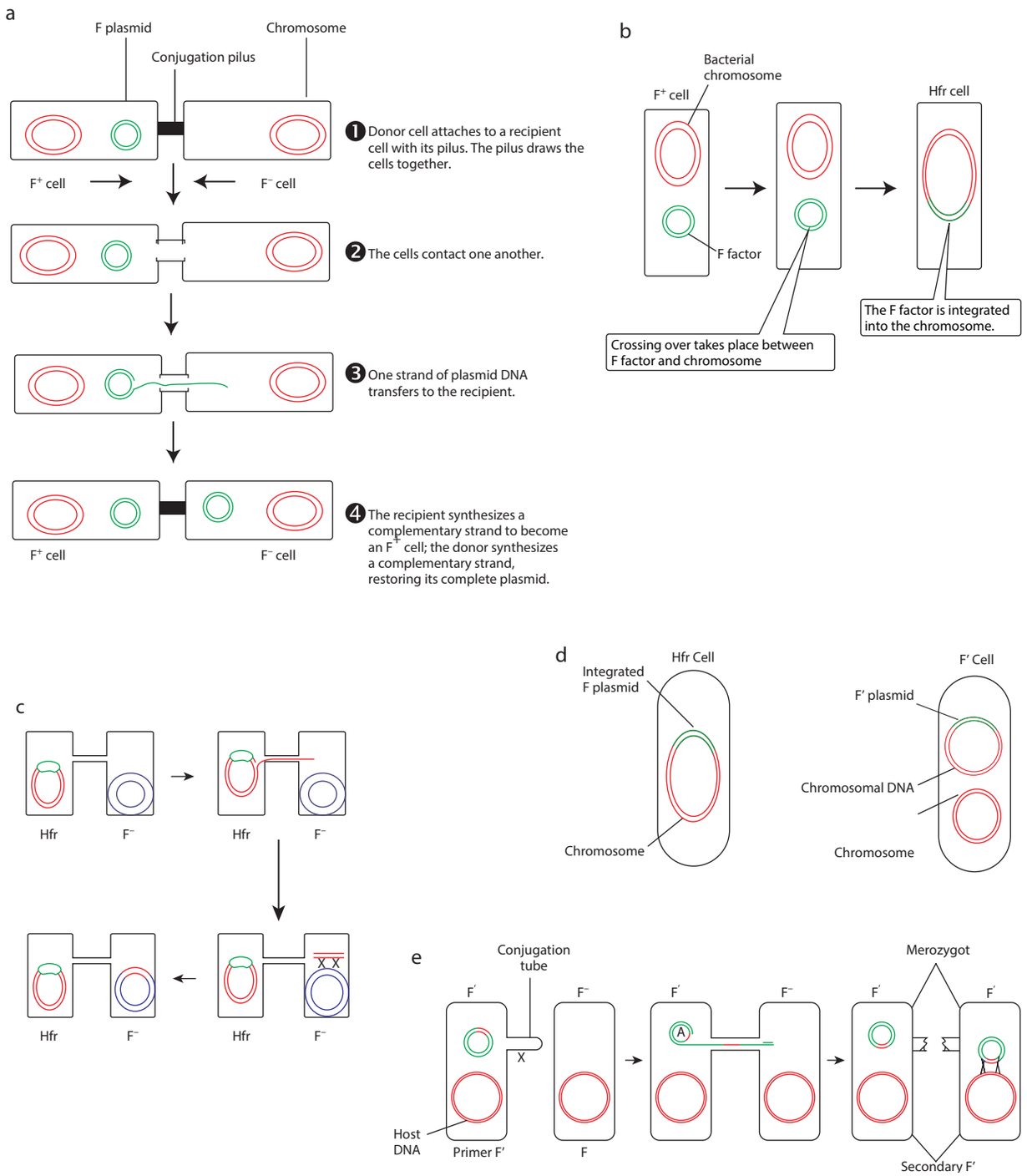

Figure 12.17: Mechanism of conjugation **(a)** $F^+ \times F^-$ **(b)** HFR cell Formation **(c)** HFR $\times$ F^- **(d)** F′ formation **(e)** F′ $\times$ F^-

role of bacteriophage in gene transfer, the lifecycle of bacteriophage is described below briefly.

After infecting the host cell, a bacteriophage (phage for short) often takes control and forces the host to make many copies of the virus. Eventually the host bacterium bursts or lyses and releases new phages. This reproductive cycle is called a lytic cycle because it ends in lysis of the host.

The lytic cycle (Figure 12.18) has four phases.

1. Attachment - Virus particle attaches to a specific receptor site on the bacterial surface.
2. Penetration - the genetic material, which is often double stranded DNA, then enters the cell.
3. Biosynthesis - After adsorption and penetration, the virus chromosome forces the bacterium to make viral components- viral nucleic acids and proteins.
4. Assembly - Phages are assembled from the virus components. Phage nucleic acid is packed within the virus's protein coat.
5. Release - mature viruses are released by cell lysis.

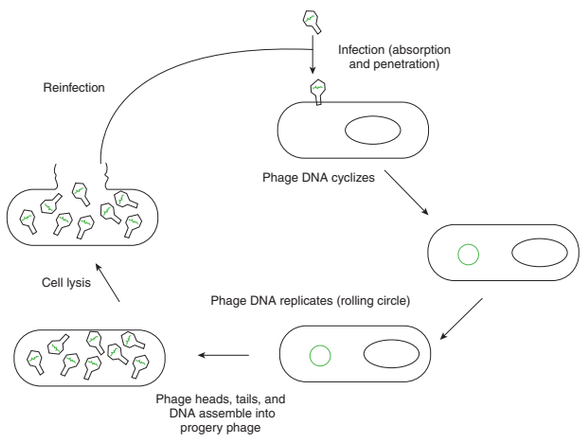

Figure 12.18: Lytic cycle

Bacterial viruses that reproduce using a lytic cycle often are called **virulent bacteriophages** (e.g. T phages) because they destroy the host cell. The genome of many DNA phages such as the lambda phage, after adsorption and penetration do not take control of its host and does not destroy the host. Instead the viral genome remains within the host cell and is reproduced along with the bacterial chromosome. The infected bacteria may multiply for long periods while appearing perfectly normal. Each of these infected bacteria can produce phages and lyses under appropriate environmental conditions. This relationship between phage and its host is called lysogeny (Figure 12.19).

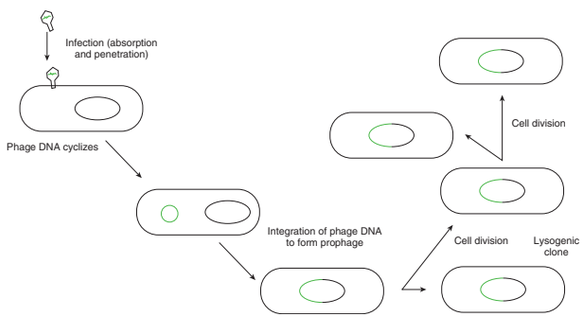

Figure 12.19: Lysogeny

Bacteria that can produce phage particles under some conditions are said to be **lysogens** or **lysogenic bacteria**. Phages which are able to establish lysogeny are called **temperate phages**.

The latent form of virus genome that remains within the host without destroying the host is called the prophage.

The prophage usually is integrated into the bacterial genome. Sometimes phage reproduction is triggered in a lysogenized culture by exposure to UV radiation or other factors. The lysosomes are then destroyed and new phages released – This phenomenon is called induction (Figure 12.20).

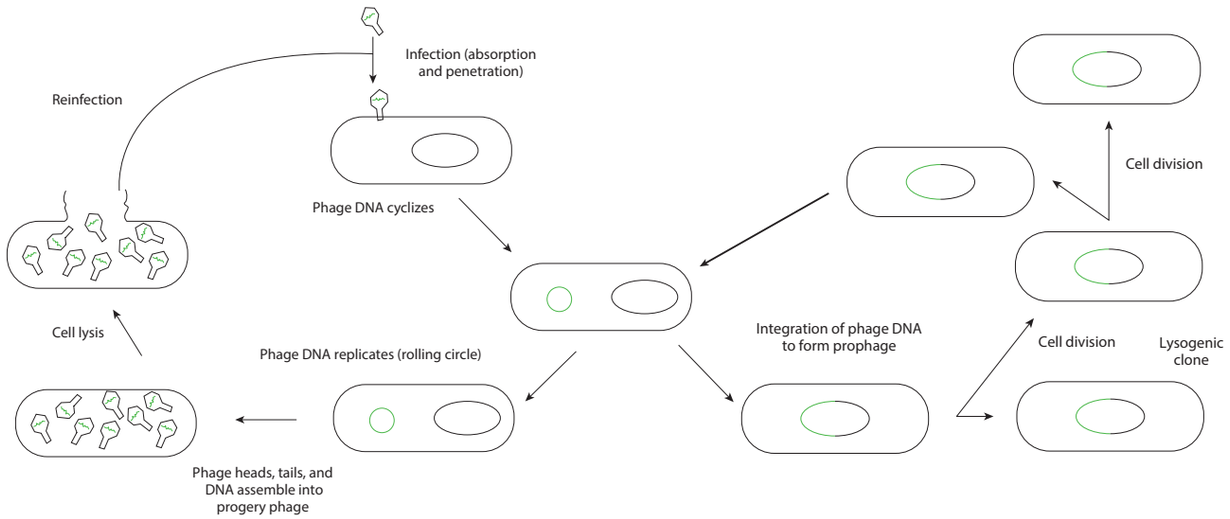

Figure 12.20: Induction of lysogen

Sometimes, bacterial genes are incorporated into a phage capsid because of errors made during the virus life cycle. The virus containing these genes then infects them into another bacterium, resulting in the transfer of genes from one bacterium to the other. Transduction may be the most common mechanism for gene exchange and recombination in bacteria.

There are two very different kinds of transduction.

1. Generalized transduction
2. Specialized transduction

Generalized transduction (Figure 12.21a) occurs during the lytic cycle of virulent and temperate phages. During the assembly stage, when the viral chromosomes are packaged into protein capsids, random fragments of the partially degraded bacterial chromosome also may be packaged by mistake. The resulting virus particles often injects the DNA into another bacterial cell but does not initiate a lytic cycle. Thus in generalized transduction any part of the bacterial chromosome can be transferred. Once the DNA has been injected it may integrate into the recipient cell's chromosome to preserve the transferred genes. About 70 to 90% of the transferred DNA is not integrated but is often able to survive and express itself. However, if the transferred DNA is degraded gene transfer is unsuccessful.

Specialized Transduction (Figure 12.21b) is also called restricted transduction in which only specific portions of the bacterial genome is carried by the phage. When a prophage is induced to leave the host chromosome, exicision is sometimes carried out improperly. The resulting phage genome contains portions of the bacterial chromosome next to the integration site. When this phage infects another bacterium, it transfers the bacterial genes from the donor bacterium along with phage DNA. Here only the bacterial genes that are close to the site of prophage are transferred. So, this transduction is called specialized.

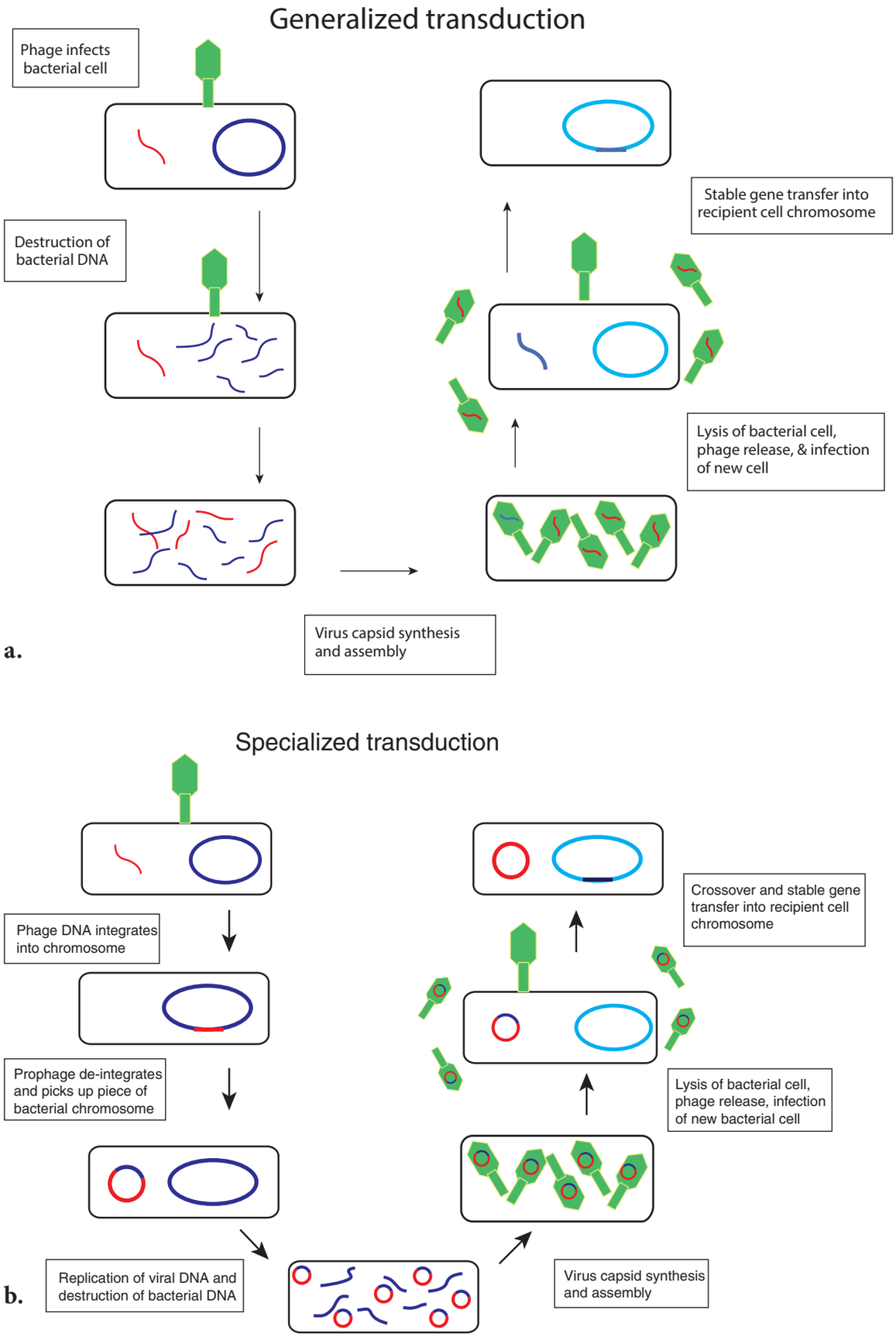

Figure 12.21: **(a)** Generalized Transduction **(b)** Specialized Transduction

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The chromosomes of bacteria, viruses, and eukaryotic cells contain pieces of DNA that move around the genome. Such movement is called transposition. DNA segments that carry the genes required for this process and consequently move about chromosomes are transposable elements or transposons. Transposons are also called jumping genes because they can jump from one DNA to another, resulting in mutation of the cell. They were first discovered in 1951 by Barbara McClintock whose significant discovery was ignored by scientific community for many years. She was awarded the Nobel Prize in 1983.

12.8 Recombinant DNA Technology

One of the practical applications of microbial genetics and the technology arising from it is the recombinant DNA technology. The deliberate modification of an organism's genetic information by directly changing its nucleic acid genome is called genetic engineering and is accomplished by a collection of methods known as recombinant DNA technology. Recombinant DNA technology opens up totally new areas of research and applied biology. Thus, it is an essential part of biotechnology, which is now experiencing a stage of exceptionally rapid growth and development. In general sense, **recombination** is the process in which one or more nucleic acids molecules are rearranged or combined to produce a new nucleotide sequence. Usually genetic material from two parents is combined to produce a recombinant chromosome with a new, different genotype. Recombination results in a new arrangement of genes or parts of genes and normally is accompanied by a phenotypic change.

There are many diverse and complex techniques involved in gene manipulation. However, the basic principles of recombinant DNA technology are reasonably simple, and broadly involve the following stages (Figure 12.22).

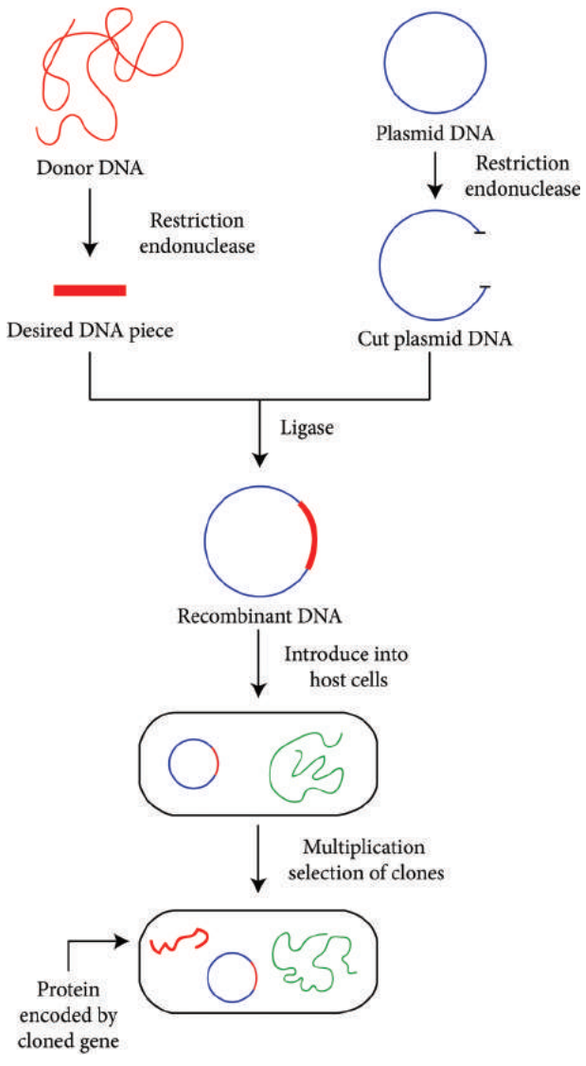

Figure 12.22: Basic principles of recombinant DNA technology

1. Isolation of DNA from the source (Donor)
2. Generation of DNA fragments and selection of the desired piece of DNA
3. Insertion of the selected DNA into a cloning vector (Example: a plasmid) to

create a recombinant DNA or chimeric DNA.

4. Introduction of the recombinant vectors into host cells (Example: bacteria)
5. Multiplication and selection of clones containing the recombinant molecules
6. Expression of the gene to produce the desired product.

Cloning in the molecular biology sense (as opposed to cloning whole organisms) is the making of many copies of a segment of DNA, such as a gene. Cloning makes it possible to generate large amounts of pure DNA, such as genes, which can then be manipulated in various ways, including mapping, sequencing, mutating and transforming cells. An overview of cloning strategies in recombinant DNA technology is shown in Figure 12.23.

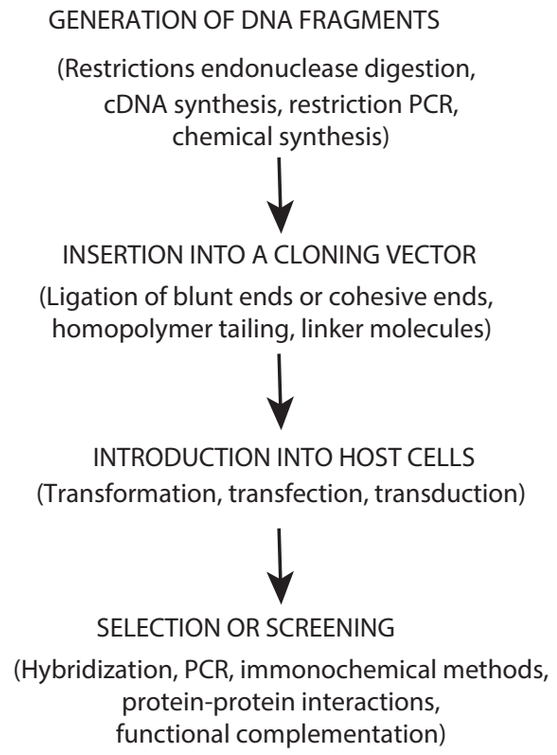

Figure 12.23: An overview of cloning strategies in recombinant DNA technology

12.9 Vectors, Types and Characteristics

Vectors are the DNA molecules, which carry a foreign DNA fragment to be cloned. They are cloning vehicles, examples of which are Plasmids, Bacteriophages, cosmids, phagemids and artificial chromosomes. The vector types differ in the molecular properties they have and in the maximum size of DNA that can be cloned into each.

Characteristics of an ideal vector.

1. Should be small in size
2. Should contain one or more restriction site
3. Should be self replicating
4. Should containing an origin of replication sequence (ori)
5. Should possess genetic markers (to detect the presence of vectors in recipient cells)

Plasmid Cloning Vectors

Bacterial plasmids are extra chromosomal elements that replicate autonomously in cells. Their DNA is circular and double stranded and carries sequences required for plasmid replication (ori sequence) and for the plasmid's other functions. (Note: A few bacteria contain linear plasmids. Example: *Streptomyces species, Borellia burgdorferi*). The size of plasmids varies from 1to 500 kb. Plasmids were the first cloning vectors. DNA fragments of about 570kb are efficiently cloned in plasmid cloning vectors. Plasmids are the easiest to work with. They are easy to isolate and purify, and they can be reintroduced into a bacteria by transformation. Naturally occurring plasmid vectors rarely possess all the characteristics of an ideal vector. Hence plasmid cloning vectors are

derivatives of natural plasmids and are "engineered" to have features useful for cloning DNA.

Examples of plasmid cloning vectors : pBR 322 (plasmid discovered by Bolivar and Rodriguez 322) and pUC 19 (plasmid from University of California). Herbert Boyer and Stanley Cohen in 1973 showed it was possible to transplant DNA segments from a frog into a strain of *Escherichia coli* using pSC101, a genetically modified plasmid, as the vector. The work laid the foundation for the birth of Genetech, the first company dedicated to commercialization of recombinant DNA.

Figure 12.24a and 12.24b shows genetic maps of plasmid cloning vectors PUC19 and PBR322 respectively.

Plasmid cloning vector PUC 19 has 2,686 –bp and has following features:

1. It has a high copy number; so many copies of a cloned piece of DNA can be generated readily.
2. It has amp R (ampicillin resistant) selective marker
3. It has a number of unique restriction sites clustered in one region, called a multiple cloning site (MCS) or polylinker

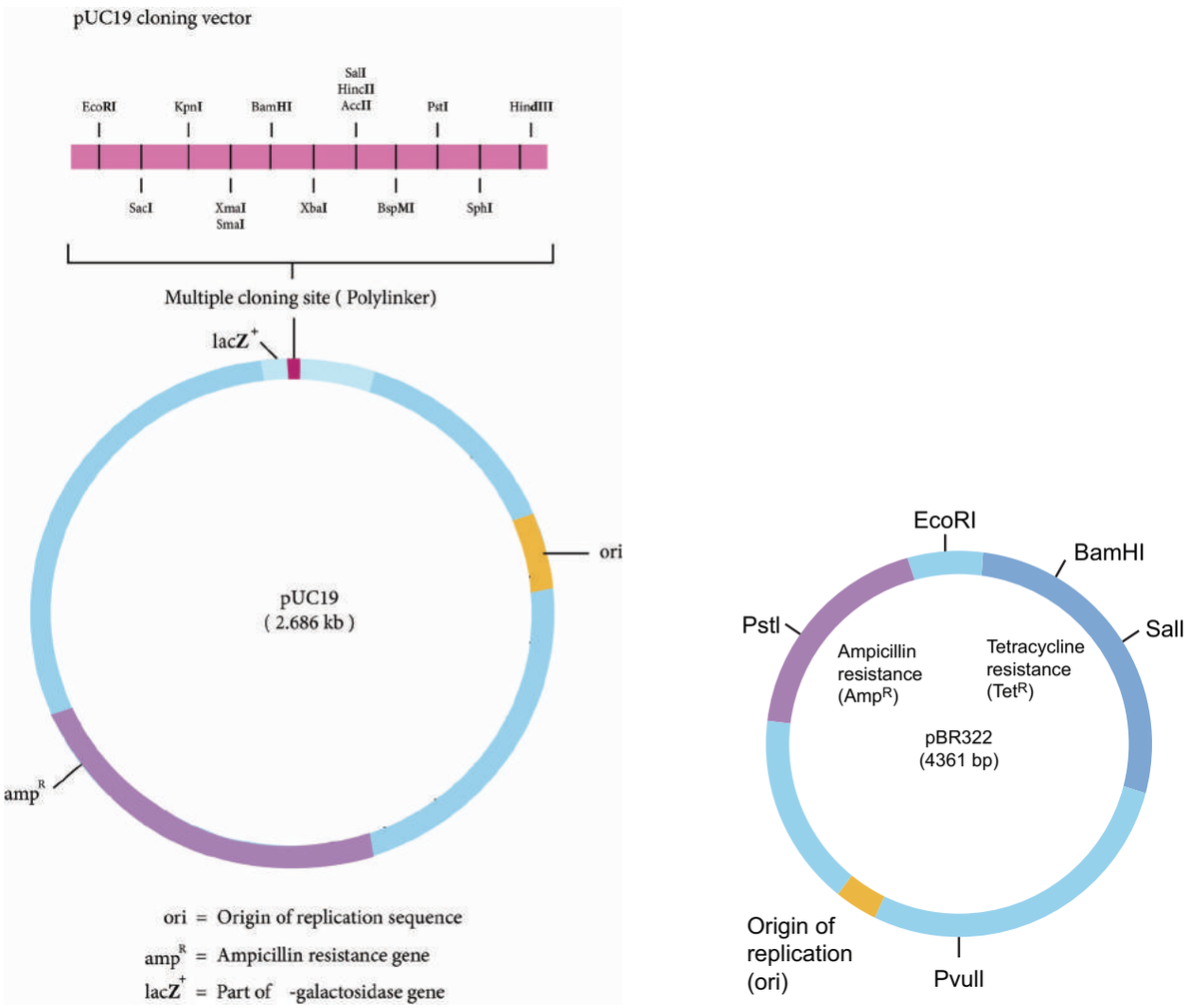

Figure 12.24: (a) PUC19 **(b)** PBR322

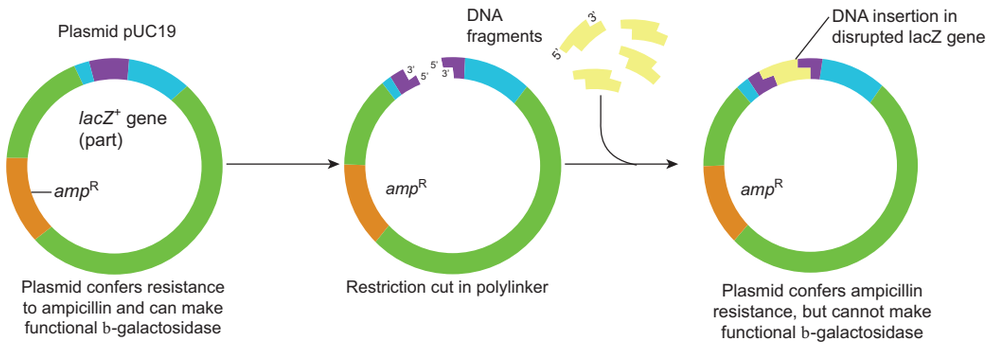

Figure 12.25: Insertion of a piece of DNA into the plasmid cloning vector pUC19 to produce a recombinant DNA molecule

4. The MCS is inserted into part of the E.coli β – galactosidase (lac Z^+) gene. Figure 12.25 illustrates how a piece of DNA can be inserted into a plasmid cloning vector such as pUC19

Bacteriophage as Cloning Vectors

They are viruses that replicate within the bacteria. A phage can be employed as vector since a foreign DNA can be spliced into phage DNA, without causing harm to phage genes. The phage will reproduce (replicate the foreign DNA) when it infects bacterial cell. Both single and double stranded phage vectors have been employed in recombinant DNA technology. Derivatives of phage can carry fragments up to about 45 kb in length. PI bacteriophage can carry fragments up to 95 kb

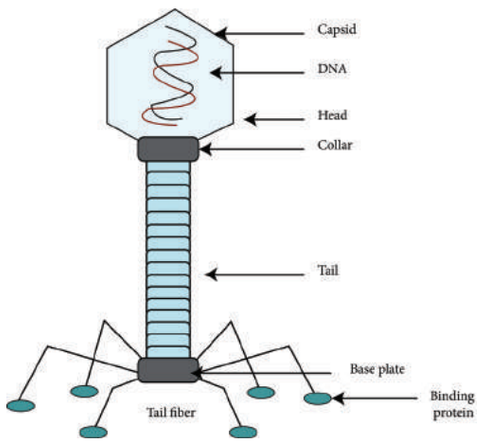

Figure 12.26: Structure of phage λ

Phage λ (Figure 12.26) consists of a head and a tail (both proteins). The DNA, located in the head, is a linear molecule of about 50 kb. At each end of the DNA, there are single stranded extensions called

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Terms associated with plasmids:

1. Low copy number plasmids are plasmids that occur low in number in each cell.
2. High copy number are plasmids that occur high in number in each cell.
3. Conjugative plasmids carry a set of transfer genes (tra genes) that facilities bacterial conjugation.
4. Non – conjugative plasmidsare plasmids that do not possess transfer genes.
5. Stringent plasmids are plasmids that are present in a limited number (1–2 per cell).
6. Relaxed plasmids are plasmids that occur in large number in each cell.
7. F plasmids possess genes for their own transfer from one cell to another
8. R plasmids carry genes resistance to antibiotics.

cohesive (cos) ends. On attachment with tail to E.coli, phage injects its DNA into the cell. Inside E.coli the phage linear DNA cyclizes and gets ligated through cos ends to form a circular DNA .The phage DNA has two fates – lytic cycle and lysogenic cycle (Figure 12.27).

Only about 50% of phage λ DNA is necessary for its multiplication and other functions. Thus, as much as 50% (i.e. up to 20 kb) of the phage DNA can be replaced by a donor DNA for use in cloning experiments. However, several restriction sites are present on phage which is not by itself a suitable vector. The λ based phage vectors are modifications of the natural phage with much reduced number of restriction sites.

The main advantage of using phage vectors is that foreign DNA can be packed into the phage (invitro packaging), the latter in turn can be injected into the host cell very effectively (Note: no transformation is required). Figure 12.28 shows how a λ phage is used for cloning.

Cosmids: Cosmids are the vectors possessing the characteristics of both plasmid and bacteriophage. Cosmids can be constructed by adding a fragment of phage λ DNA including cos site, to plasmids. Once inside the host cell, cosmids behave just like plasmids and replicate. The advantage with cosmids is that they carry larger fragments of foreign DNA (35–45 kb) compared to plasmids.

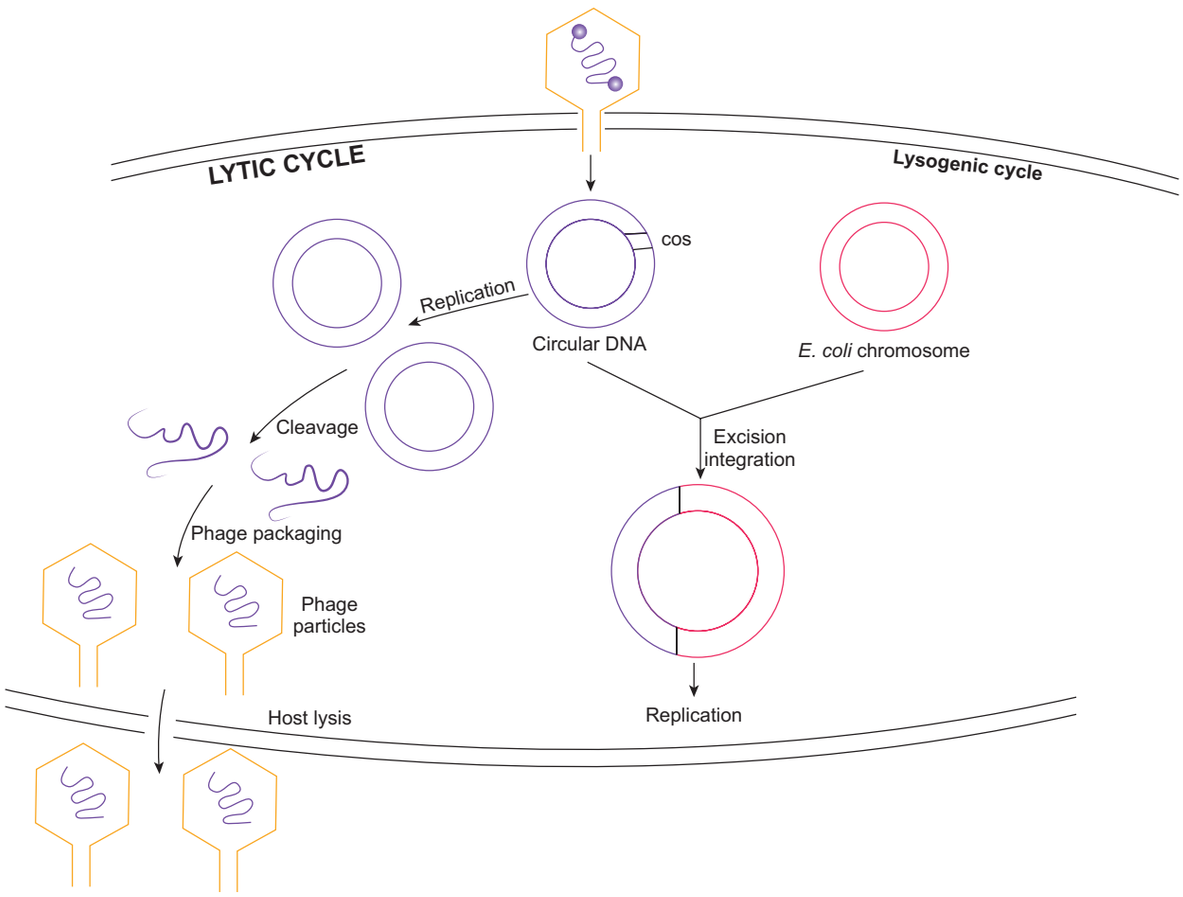

Figure 12.27: Life cycle of phage λ

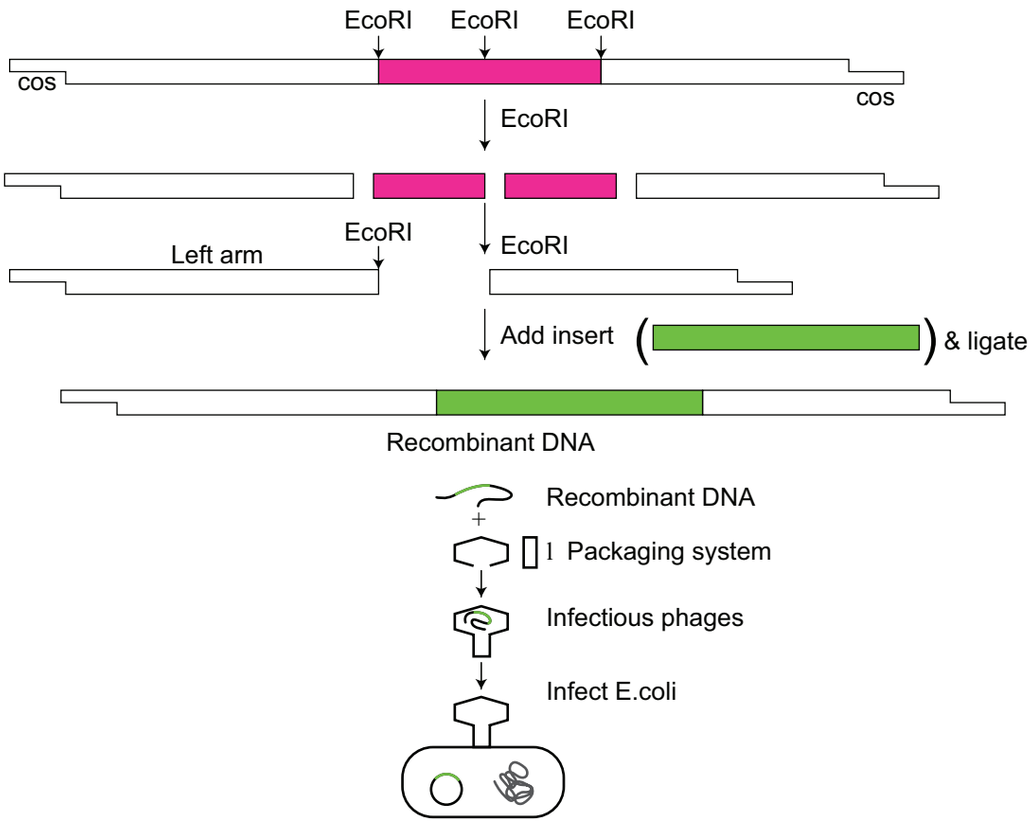

Figure 12.28: Cloning using a λ phage

Phagemids: Phagemids are the combination of plasmid and phage and can function as either plasmid or phage. Since they posses functional origins of replication of both plasmid and phage λ they can be propagated (as plasmid or phage) in appropriate E.coli.

Artificial chromosome Vectors: Artificial chromosomes are cloning vectors that can accommodate very large pieces of DNA, producing recombinant DNA molecules resembling small chromosomes.

1. Yeast artificial chromosome YAC is a synthetic DNA that can accept large fragments of foreign DNA between 0.2 to 2.0 Mb (particularly human DNA). In addition to origin of replication sequence and selectable markers they possess centromeric and telomeric regions, and therefore the recombinant DNA can be maintained like a yeast chromosome.
2. (BACs) Bacterial Artificial chromosomes: BACS can accept DNA inserts around 300 kb.

A major part of the sequencing of human genome has been accomplished by using a library of BAC recombinant. BACS are vectors containing the origin replication of a natural plasmid called the F factor, a MCS, a selectable marker and often some other features.

Plasmid shuttle Vectors: The plasmid vectors that are specifically designed to replicate in two or more different host organisms(say in E.coli and yeast) are referred to as shuttle vectors. The origins of replication for two hosts are combined in one plasmid.

Expression vectors: An expression vector is a cloning vector containing the regulatory sequences (promoter sequence) necessary to allow the transcription and translation of a cloned gene or genes. Expression vectors are essentially derivatives of the plasmid cloning vectors used in the host. They are used to produce the protein encoded by a cloned gene in the transformed host. For example, the biotechnology industry produces pharmaceutically active proteins with the use of expression vectors and the appropriate host.

A genomic library is a collection of clones that contains at least one copy of every DNA sequence in an organism's genome. Like libraries with books, genomic libraries are a great source of information; in this case, the information is about the genome. Specific sequences in cDNA libraries and genomic libraries can be identified via a number of approaches, including the use of specific antibodies, cDNA probes and oligonucleotide probes

Human artificial chromosome (HAC)-based vectors offer a promising system for delivery and expression of full-length human genes of any size into human cells, and a tool for determining human chromosome function. It does not have the problem of limited cloning capacity of other vectors, and it also avoids possible insertional mutagenesis caused by integration into host chromosomes by viral vector.

12.10 Restriction Enzymes

In 1960s Swiss microbiologist Werner Arber and American microbiologists Hamilthon Othanel Smith and Daniel Nathans discovered restriction enzymes. The discovery, for which the three men shared the 1978 Nobel Prize for Physiology or Medicine. Restriction enzymes or restriction endonucleases are one of the most important groups of enzymes for the manipulation of DNA. It is one of the important molecular tools used by a genetic engineer. These are the bacterial enzymes that recognize a specific base sequence in a DNA molecule (from any source) and make two cuts one in each strand generating 3′ – OH and 5′ – P termini. They were first discovered in E.*coli*. E.*coli* produces the restriction enzyme to cut the viral DNA and protect itself. The host E.coli DNA is protected by its own restriction enzyme since its methylated. Since these enzymes restrict the viral replication the word restriction is added to these enzymes. Hind II was the first discovered restriction endonuclease.

The site where the DNA is cut by a restriction enzyme is called recognition sequence. Restriction endonucleases can specifically recognize DNA with a particular sequence of 4-8 nucleotides and cleave. Each recognition sequence has two fold rotational symmetry i.e. the same nucleotide sequence occurs on both strands of DNA which run in opposite direction. Such sequences are referred to as palindromes, since they read similar in both directions (forwards and backwards). Majority of restriction endonucleases (particularly type II) cut DNA at defined sites within recognition sequence. Type II restriction enzymes make

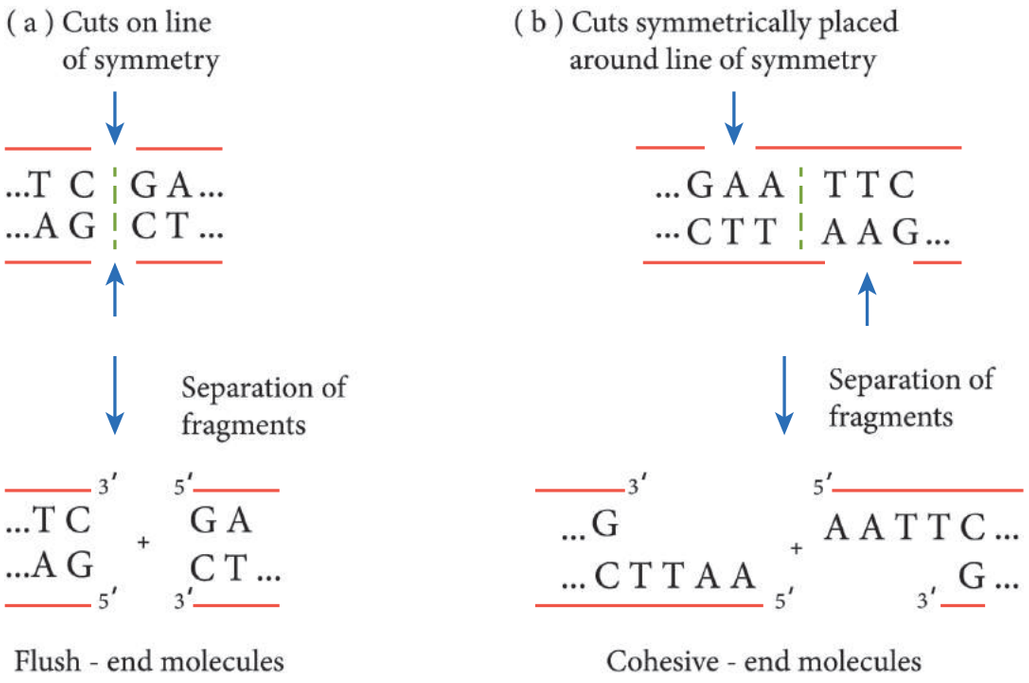

Figure 12.29: Two types of cuts made by restriction enzymes

two single – stand breaks) one break in each strand. There are two distinct arrangements of these breaks 1. both breaks at the center of symmetry (generating flush or blunt ends) or 2. breaks that are symmetrically placed around the line of symmetry generating cohesive ends. Figure 12.29 shows two types of cuts made by restriction enzymes. The arrow indicates the cleavage site. The dashed line is the center of symmetry of the sequence (Table 12.2).

Application of Recombinant DNA Technology

a. Production of medically useful proteins such as somatostain, insulin, human growth hormone and interferon. It decreases the dependency on human tissues and solves problem of limited production.

b. Development of synthetic vaccines for instance, vaccines for malaria and rabies a recombinant hepatitis vaccine is already commercially available.

Type	No of Enzyme and sub units	Cleavage site	Examples	Bacterial source
I	One with 3 sub units for recognition cleavage and methylation	1000 bp from recognition site	EcoK1 Cfr A1	*Escherichia coli* *Citrobacter fruendii*
II	Two different enzymes to cleave or modify the recognition sequence	Same as recognition or close to recognition site	Eco R1 Alu I	*Escherichia coli* *Arthrobacter luteus*
III	One with 2 subunits	24- 26 bp from recognition site	Hinf III Pst II	*Haemophilus influenzae* *Providencia stuarti*

Table 12.2: Types and features of restriction enzyme

c. Gene therapy

d. Diagnosis of infection diseases.

e. To manufacture industrially important products like enzymes using bacteria, fungi and cultured mammalian cells.

12.11 Techniques in Genetic Engineering

There are several techniques used in recombinant DNA technology or gene manipulation. The most frequently used methods are agarose gel electrophoresis, isolation and purification of nucleic acids, nucleic acid blotting techniques, DNA sequencing, chemical synthesis of DNA, gene transfer methods, polymerase chain reaction, construction of gene library, radiolabeling of nucleic acids etc, few of them are discussed here.

12.11.1 Agarose Gel Electrophoresis

Electrophoresis refers to the movement of charged molecules in an electric field. The negatively charged molecules move towards the positive electrode while the positively charged molecules migrate towards the negative electrode. Gel electrophoresis is a routinely used analytical technique for the separation and purification of specific DNA fragments. The gel is composed of either polyacrylamide or agarose. Polyacrylamide gel electrophoresis (PAGE) is used for the separation of smaller DNA fragments while agarose electrophoresis is convenient for the separation of DNA fragments ranging in size from 100 base pairs to 20 kilobase pairs. Gel electrophoresis can also be used for the separation of RNA molecules. A diagramatic view of the agarose gel electrophoresis unit is shown in Figure 12.30a.

Steps

1. Gel is set with wells on one end.
2. The gel is placed in an electrophoresis apparatus and covered with buffer solution.
3. The DNA samples along with tracer dye are placed in the wells of gel.

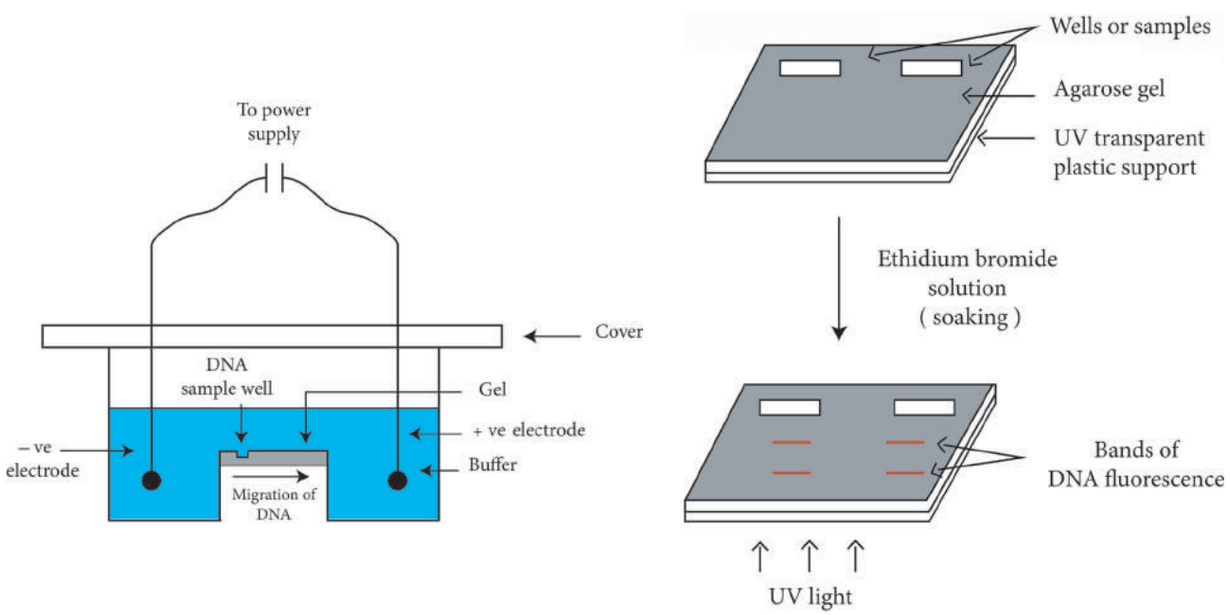

Figure 12.30: **(a)** agarose gel electrophoresis unit **(b)** DNA electrophoresis gel

4. Power supply is switched on and gel is run till the tracer dye reaches the end of the gel.

As the DNA is negatively charged, DNA fragments move through the gel towards the positive electrode. The rate of migration of DNA is dependent on the size and shape. In general, smaller linear fragments move faster than the larger ones. Hence, gel electrophoresis can be conveniently used for the separation of a mixture of DNA fragments, based on their size. The bands of the DNA can be detected by soaking the gel in ethidium bromide solution (Ethidium bromide can also be added to molten agarose prior to setting the gel). When activated by ultraviolet radiation, DNA base pairs in association with ethidium bromide, emit orange fluorescence. And in this way the DNA fragments separated in agarose electrophoresis can be identified (Figure 12.30b).

PAGE is composed of chains of acryl amide monomers crosslinked with methylene bisacryalmide units. The pore size of the gel is dependent on the total concentration of monomers and the cross links. PAGE is used for the separation of single stranded DNA molecules that differ in length by just one nucleotide. Agarose gels cannot be used for this purpose. This is because polyacrylamide gels have smaller pore sizes than agarose gels and allow precise separation of DNA molecule from 10–1500 bp.

HOTS

1. Explain how gel electrophoresis can be used to determine the size of a PCR product.

12.11.2 Polymerase Chain Reaction (PCR)

The PCR technique has already proven exceptionally valuable in many areas of molecular biology, medicine, and biotechnology. PCR technique has great practical importance and impact on biotechnology. Between 1983 and 1985 American biochemist Kary Mullis developed PCR technique that made it possible to synthesize large quantities of a DNA fragment without cloning it. Mullis received the 1993 Nobel Prize for Chemistry for his invention. PCR is a cell free amplification technique.

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Cloned genes and other DNA sequences often are analyzed to determine the arrangement and specific locations of restriction sites. The analytical process involves cleavage of the DNA with restriction enzymes, followed by separation of the resulting DNA fragments by agarose gel electrophoresis. The sizes of the DNA fragments are calculated, enabling restriction maps to be constructed. The many DNA fragments produced by cleaving genomic DNA show a wide range of sizes, resulting in a continuous smear of DNA fragments in the gel. In this case, specific gene fragments can be visualized only by transferring them to membrane filter by southern blotting, hybridizing a specific labelled probe with the DNA fragments, and detecting the hybrids. A similar procedure, Northern blotting is used to analyze the sizes and quantities of RNAs isolated from cell.

Figure 12.31 outlines how PCR technique works. To amplify (make large quantities) a particular DNA sequence by PCR a reaction mixture (often 100ml or less in volume) containing the following are required.

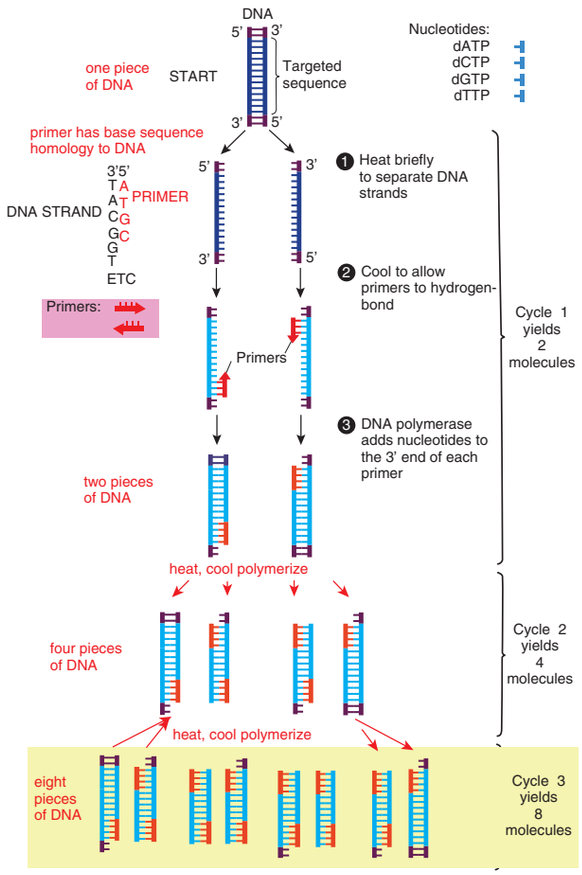


Figure 12.31: Steps in PCR

1. **Target DNA**
2. **Two primers**–These are synthetic oligonucleotides, usually about 20 nucleotides long. These are fragments with sequences identical to those flanking the targeted sequence.
3. **Thermostable DNA polymerase**– Two popular enzymes employed in the PCR technique are Taq polymerase from the thermophilic bacterium *Thermus aquaticus* and the vent polymerase from *Theromococcus litoralis*. These polymerases employed in PCR technique are able to function at high temperatures.
4. **Four deoxyribonucleoside triphosphates** (dNTPs)- dCTP, dATP, dCTTP, dTTP

Taq polymerase lacks proof reading exonuclease (3′–5′) activity which might contribute to errors in the products of PCR. Some other thermostable DNA polymerases with proof reading activity have been identified. Example: Tma DNA polymerase from *Thermotoga maritana*.

Steps in PCR

1. **Denaturation:** The target DNA containing the sequence to be amplified is heat denatured to separate its complementary strands at temperature 94 °C–95 °C.
2. **Annealing:** The temperature is lowered to 37 °C–55 °C so that the primers can hydrogen bond or anneal to the DNA on both sides of the target sequence. Because the primes are present in excess the targeted DNA strands normally anneal to the primers rather than to each other.
3. **Extension:** Heat resistant DNA polymerase extends the primers and synthesizes copies of the target DNA sequence using the deoxyribonucleoside triphosphate's at 70 °C–75 °C.

The three – step cycle (Figure 12.32) is repeated to obtain copies of target DNA in large numbers. At the end of one

cylcle, the targeted sequences on both strands have been copied. When the three – step cycle is repeated, the four strands from the first cycle are copied to produce eight fragments. The third cycle yields 16 products. Theoretically, 20 cycles will produce about one million copies of the target DNA sequence. Each cycle of PCR takes about 3 – 5 minutes.

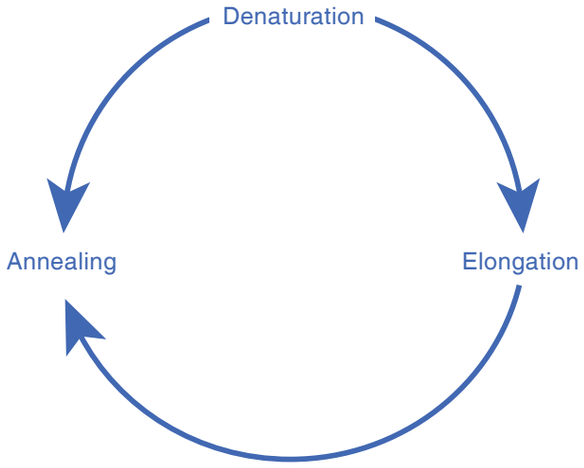


Figure 12.32: Three steps PCR cycle

The PCR technique has now been automated and is carried out by a specially designed machine (Figure 12.33) PCR machines are now fully automated and microprocessor controlled. They can process up to 96 samples at a time. PCR machines can carry out 25 cycles and amplify DNA 10^5 times in as little as 57 minutes.

The PCR has many applications in research and in commercial arena, including generating specific DNA segments for cloning or sequencing, amplifying DNA to detect specific genetic defects, and amplifying DNA for fingerprinting in crime scene investigation.

PCR technology is improving continually. Various forms of PCR are available. RNA too can be efficiently used in PCR procedures. Cellular RNAs and RNA viruses may be studied even when the RNA is present in very small amounts (as few as 100 copies can be transcribed and amplified). Quantitative PCR is quite valuable in virology and gene impression studies. PCR is modified as per the specific demands of the situation. Thus there are many variations in the original PCR Examples nested PCR, inverse PCR, reverse transcription PCR, time quantitative PCR, RAPD, RFLP, AFLP.

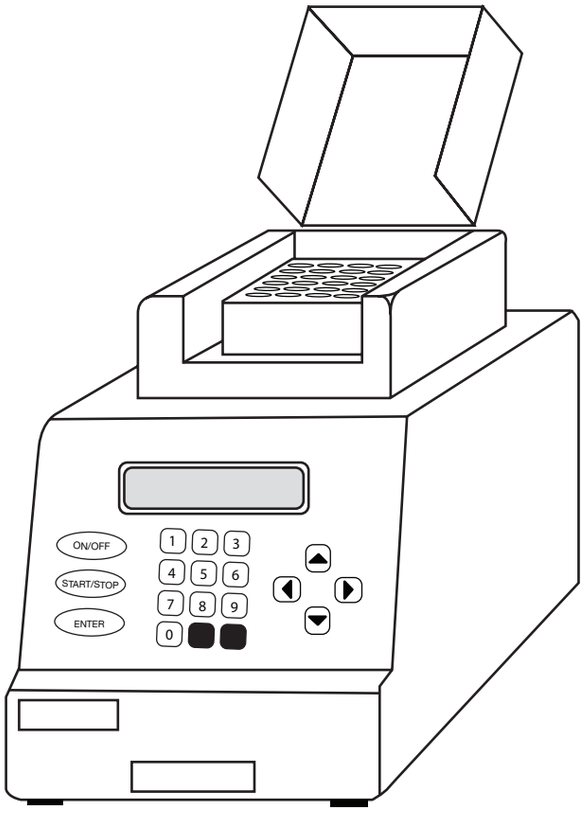


Figure 12.33: PCR machine

HOTS

Both PCR and Cloning allow for the production of many copies of a DNA sequence. What are the advantages of using PCR instead of cloning to amplify a DNA template?

What advantages are there to using a DNA polymerase for PCR that has proofreading activity?

12.11.3 Molecular Markers – RFLP, RAPD

A molecular marker is a DNA sequence in the genome which can be located and identified. As a result of genetic alterations (mutations, insertions, deletions), the base composition at a particular location of the genome may be different in different cells. These differences, collectively called as polymorphisms can be mapped and identified. The DNA markers are highly useful for genetic mapping of genomes. RFLPS (Restriction Fragment Length Polymorphisms), VNTRs (mini satellites or Variable Number Tandem Repeats), STRs (Microsatellites or Simple Random Repeats), SNPs (Single Nucleotide Polymorphisms) are types of DNA sequences (stretch of DNA) which can be used as markers. These markers are used in disease diagnosis and DNA fingerprinting.

Restriction fragment length polymorphism (RFLP) was the very first technology employed for the detection of polymorphism, based on the DNA sequence differences. RFLP is mainly based on the altered restriction enzyme sites, as a result of mutations and recombinations of genomic DNA. An outline of the RFLP analysis is given

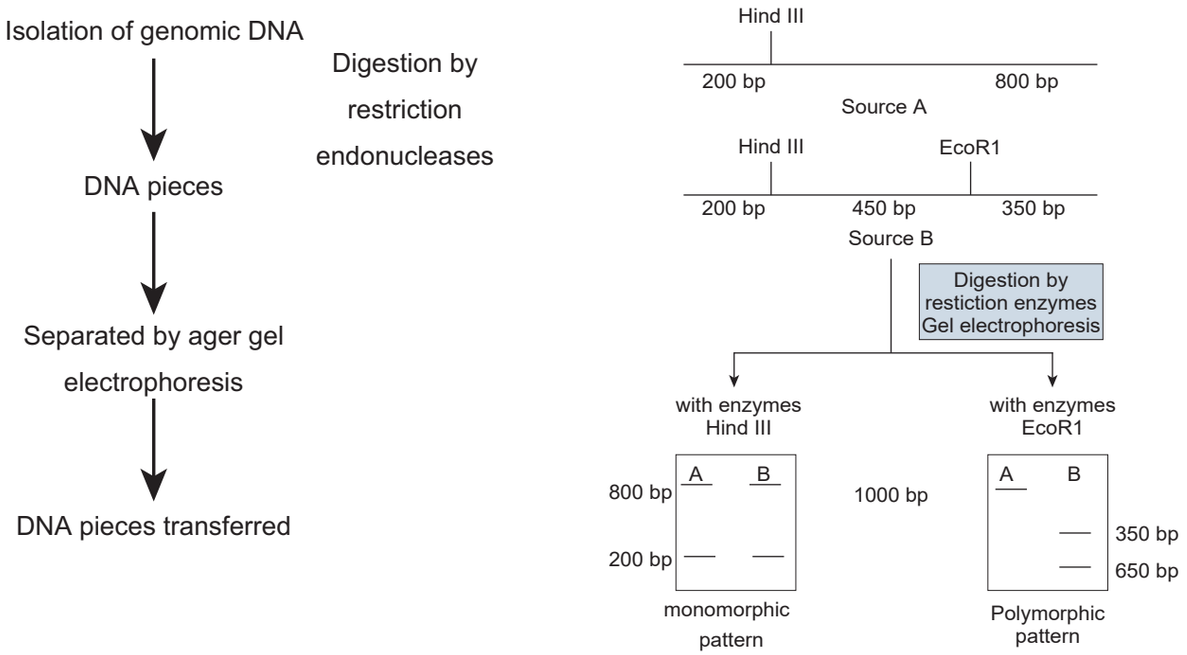


Figure 12.34: **(a)** An outline of RFLP analysis as a molecular marker **(b)** A schematic representation of restriction fragment length polymorphism (RFLP) analysis as a molecular marker

in Figure 34 a. The procedure basically involves the isolation of genomic DNA, its digestion by restriction enzymes, separation by electrophoresis, and finally hybridization by incubating with cloned and labeled probes (Figure 12.34 b).

Based on the presence of restriction sites, DNA fragments of different lengths can be generated by using different restriction enzymes. In the Figure 12.34 b. two DNA molecules from two sources (A and B) are shown. In source A, a mutation has

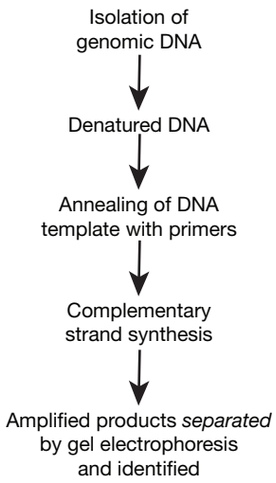


Figure 12.35: An outline of RAPD analysis as a molecular marker

Infobits

DNA Fingerprinting Or DNA Profiling:

DNA fingerprinting is the present day genetic detective in the practice of modern medical forensics. The underlying principles of DNA fingerprinting are briefly described.

The structure of each person's genome is unique. The only exception being monozygotic identical twins (twins developed from a single fertilized ovum). The unique nature of genome structure provides a good opportunity for the specific identification of an individual. The DNA fingerprint is an analysis of the nitrogenous base sequence in the DNA of an individual.

The original DNA fingerprinting technique was developed by Alec Jaffreys in 1985. Although the DNA fingerprinting is commonly used, a more general term DNA profiling is preferred. This is due to the fact that a wide range of tests can be carried out by DNA sequencing with improved technology.

Applications of DNA fingerprinting:

The amount of DNA required for DNA fingerprint is remarkably small. The minute quantities of DNA from blood strains, body fluids, hair fiber or skin fragments are enough. Polymerase chain reaction is used to amplify this DNA for use in fingerprinting. DNA profiling has wide range of applications – most of them related to medical forensics. Some important ones are listed below.

- Identification of criminals, rapists, thieves etc.
- Settlement of paternity disputes.
- Use in immigration test cases and disputes.

In general, the fingerprinting technique is carried out by collecting the DNA from a suspect (or a person in a paternity of immigration dispute) and matching it with that of a reference sample (from the victim of a crime, or a close relative in a civil case).

occurred leading to the loss of restriction site that can be digested by EcoRI.

The result is that when the DNA molecules are digested by the enzyme Hind III, there is no difference in the DNA fragments separated. However, with the enzyme EcoRI, source A DNA molecules is not digested while source B DNA molecule is digested. This results in a polymorphic pattern of separation.

Random amplified polymorphic DNA (RAPD) is a molecular marker based on PCR amplification. An outline of RAPD is depicted in Figure 12.35. The DNA isolated from the genome is denatured. The template molecules are annealed with primers, and amplified by PCR. Single short oligonucleotide primers (usually a 10-base primer) can be arbitrarily selected and used for the amplification of DNA segments of the genome (which may be distributed throughout the genome). The amplified products are separated on electrophoresis and identified.

Based on the nucleotide alterations in the genome, the polymorphisms of amplified DNA sequences differ which can be identified as bands on gel electrophoresis. Genomic DNA from two different sources often results in different amplification patterns i.e. RAPDs.

Infobits

In 1970s American molecular biologists Allan M. Maxam and Walter Gilbert and English biochemist Frederick Sanger developed some of the first techniques for DNA sequencing. Gilbert and Sanger shared the 1980 Nobel Prize for Chemistry for their work. Dideoxy procedure is one of the procedure used to sequence DNA.

Summary

The fundamental unit of information in living systems is the gene. Genome is the set of all genes and genetic signals of a cell. Gene is expressed through a sequence of events. The central dogma of molecular biology, comprises the three major processes replication, transcription and translation. The genetic message encoded in messenger RNA (mRNA) is translated on the ribosomes into a polypeptide with a particular sequence of amino acids. An RNA base sequence (a set of 3 bases) corresponding to a particular amino acid is called a codon. The genetic code is the set of all codons. The genetic code is a triplet code, and all 64 possible codons carry information of some sort. The code is highly redundant. Polypeptides are synthesized from the amino terminus toward the carboxyl terminus, by adding amino acids one by one to the carboxyl end. An important feature of intiation of polypeptide synthesis in both prokaryotes and eukaryotes is the use of a specific initiating tRNA molecule.

Mutation is the process by which the sequence of base pairs in a DNA molecule is altered. Mutations can be divided into

base pair substitution mutation and base pair insertion or deletions.

Frame shift mutation usually results in a nonfunctional protein. An addition or deletion of one base pair, for example, shifts the mRNA's downstream reading frame by one base, so that incorrect amino acids are added to the polypeptide chain after the mutation site. Point mutations are single base changes, that do not affect the reading frame, that is, the mutation only makes a single change in a single codon,

Mutations can also be defined according to their effects on amino acid sequences in proteins. They are missense mutation, silent mutation, nonsense mutation. Forward mutations change the genotype from wild type to mutant and reverse mutations (or reversions or back mutations) change the genotype from mutant to wild type or to partially wild type.

The term mutant refers to an organism in which either the base sequence of DNA or the phenotype has been changed. The process of formation of mutant organism is called mutagenesis. Ames test is an indicator of whether the chemical is a mutagen. The Ames test assays the ability of chemicals to revert mutant strains of the bacterium Salmonella typhimurium to wild type. The most commonly employed gene transfer methods are transformation, conjugation, transduction, electroporation, lipofection and direct transfer of DNA. Transformation is genetic alteration of a cell resulting from the direct uptake, incorporation and expression of exogenous genetic material (exogenous DNA) from its surroundings. Competence refers to the state of being able to take up exogenous DNA from the environment. During conjugation, two live bacteria (a donor and a recipient) come together, join by cytoplasmic bridges (e.g. pilus) and transfer single stranded DNA (from donor to recipient). Transduction is the transfer of bacterial genes from one bacteria to other by viruses, e.g. Bacteriophage (Bacterial viruses). **Recombination** is the process in which one or more nucleic acids molecules are rearranged or combined to produce a new nucleotide sequence. Cloning in the molecular biology sense (as opposed to cloning whole organisms) is the making of many copies of a segment of DNA, such as a gene. Cloning makes it possible to generate large amounts of pure DNA, such as genes, which can then be manipulated in various ways

Vectors are the DNA molecules, which carry a foreign DNA fragment to be cloned. They are cloning vehicles, examples of which are Plasmids, Bacteriophages, cosmids, phagemids and artificial chromosomes. Bacterial plasmids are extra chromosomal elements that replicate autonomously in cells. They are viruses that replicate within the bacteria. Cosmids are the vectors possessing the characteristics of both plasmid and bacteriophage. Phagemids are the combination of plasmid and phage, and can function as either plasmid or phage. The plasmid vectors that are specifically designed to replicate in two or more different host organisms (say in E. *coli* and yeast) are referred to as shuttle vectors. An expression vector is a cloning vector containing the regulatory sequences (promoter sequence) necessary to allow the transcription and translation of a cloned gene or genes. Restriction enzymes are the

bacterial enzymes that recognize a specific base sequence in a DNA molecule (from any source) and make two cuts one in each strand generating 3′ – OH and 5′ – P termini.

There are several techniques used in recombinant DNA technology or gene manipulation. The most frequently used methods are agarose gel electrophoresis, isolation and purification of nucleic acids, nucleic acid blotting techniques, DNA sequencing, chemical synthesis of DNA, gene transfer methods, polymerase chain reaction, construction of gene library, radiolabeling of nucleic acids etc. Gel electrophoresis is a routinely used analytical technique for the separation and purification of specific DNA fragments. PCR is a cell free amplification technique. The three – step cycle is repeated to obtain copies of target DNA in large numbers.

The DNA markers are highly useful for genetic mapping of genomes. RFLPS (Restriction Fragment Length Polymorphisms), VNTRs (mini satellites or Variable Number Tandem Repeats), STRs (Microsatellites or Simple Random Repeats), SNPs (Single Nucleotide Polymorphisms) are types of DNA sequences (stretch of DNA) which can be used as markers. These markers are used in disease diagnosis and DNA fingerprinting.

Evaluation

Multiple choice questions

1. Which of the following properties is essential for the function of a tRNA molecule?

   a. Recognition of a codon
   b. Recognition of an anticodon
   c. Ability to distinguish one amino acid from another
   d. Recognition of DNA molecule

2. Which chain termination codon could be formed by a single base change from UCG, UGG and UAU?
   a. UAA b. UAG
   c. UGA d. AUG

3. Which of the following base-pair changes are transitions?
   a. AT → TA b. AT → GC
   c. Both a and b d. GC → AT

4. UV light usually causes mutations by a mechanism involving
   a. One-strand breakage in DNA
   b. Deletion of DNA segments
   c. Induction of thymine dimers and their persistence
   d. Inversion of DNA segments

5. The form of genetic information used directly in protein synthesis is
   a. DNA b. mRNA
   c. rRNA d. tRNA

6. _____ display one anticodon each
   a. eukaryotic mRNAs
   b. transfer RNAs
   c. ribosomal RNAs
   d. mRNAs

7. _____ contains exons and introns.
   a. Eukaryotic mRNAs b. rRNA
   c. tRNAs d. primers

8. The symbol lac^+ refer to

a. genotype b. phenotype
c. both a & b d. none

9. _____ sequence terminates protein synthesis
   a. UAA b. UAG
   c. UGA d. All the above
10. The principal start codon corresponds to which amino acid?
    a. Valine
    b. arginine
    c. Methionine
    d. Isoleucine
11. Number of nucleoprotein subunit in a prokaryotic ribosome
    a. 2 b. 4
    c. 5 d. 6
12. A deletion occurs that eliminates a single amino acid in a protein. How many base pairs were deleted?
    a. 1 b. 2
    c. 3 d. 4
13. During conjugation plasmids undergo
    a. Theta replication
    b. rolling circle replication
    c. sigma replication
    d. gamma replication
14. If a plasmid is mobilizable but nonconjugative, what function does it lack?
    a. Antibiotic resistance
    b. Fertility
    c. Colicinogenic
    d. Restriction sequences
15. The uptake of naked DNA from the surrounding is known as
    a. Transduction
    b. Conjugation
    c. Transformation
    d. Lysis
16. The F plasmid makes a cell
    a. Donor b. Recipient
    c. Resistant d. None
17. Which of the following is more efficient in transferring chromosomal DNA
    a. F^+cell b. F^-cell
    c. Hfr cell d. R^+cell
18. Which of the following statement is true
    a. Protein is the only gene product
    b. A functional gene product is protein or might also be one of several classes of RNA molecules
    c. Carbohydrate is the only gene product
    d. Lipids are the only gene product
19. DNA is transcribed into
    a. mRNA b. tRNA
    c. sRNA d. hnRNA
20. Which of the following is found as part of all prokaryotic promoters
    a. Pribnow box
    b. Shine dalgarno sequence
    c. AUG sequence
    d. UAG sequence

Answer the following

1. What is the direction of synthesis of RNA?
2. Define coding strand.
3. What parts of a mRNA molecule not translated? Ans. Leader & Introns
4. How many codons could be contained in a four-letter code? Ans 4^4=256

5. What is the principal start codon and to what amino acid does it correspond?
6. Restriction endonucleases are naturally found in bacteria. What purpose do they serve?
7. There are many varieties of cloning vectors that are used to propagate cloned DNA. One type of cloning vector used in E.coli is a plasmid vector. What features does a plasmid vector have that makes it useful for constructing and cloning recombinant DNA molecules?
8. What is shuttle vector and why is it used?
9. What information and materials are needed to amplify a segment of DNA using PCR?
10. In most PCR reactions, a DNA polymerase that can withstand short periods of very high (near boiling) temperatures is used. why?
11. The sequence of nucleotides in an mRNA is 5′-AUG-ACCCAUU-CAUUGGUCUCGUUAG-3′. Assuming that ribosomes could translate this mRNA, how many amino acids long would you expect the resulting polypeptide chain to be?
12. The N-terminus of a protein has the sequence Met-His-Arg-Lys-Val-His-Cys-Gly. A molecular Biologist wants to synthesize a DNA chain that can encode this portion of the protein. How many DNA sequences can encode this polypeptide?
13. Explain the process by which an infected bacterium releases progeny phage.
14. Define coding strand.
15. Distinguish a missense and a nonsense mutation.
16. By what mechanism does 5-bromouracil induce mutations?
17. Define the term conjugative.
18. How does an Hfr cell differ from F^+ cell?
19. How are F' plasmids produced?
20. Define a lysogen.
21. Restriction enzymes generate two types of termini. What are they?
22. Explain cosmids and the advantages resulting from the use of a cosmid?
23. Explain the use of bacteriophage in cloning DNA fragment.
24. What are expression vectors?
25. Diagramatically describe the plasmid cloning vector PUC19.
26. How is the natural phenomenon of conjugation used to transfer foreign gene?
27. List the stages involved in Recombinant DNA technology.
28. Discuss RAPD and RFLP.

MICROBIOLOGY

PRACTICAL

Std XII Microbiology Practical Manual

Higher Secondary – First Year Practical Examination

Microbiology	
Marking Scheme	
Allotment of Marks	
Internal Assessment	05 marks
External Assesment	15 marks
Total	**20 marks**
Internal Assessment (Practicals) Marks Break Up	
1. Record Note Book	03 marks
2. Skill of performing Experiments	02 marks
Total	**05 marks**
External Assessment Mark Break Up	
1. Major Practical	09 marks
2. Spotters	06 marks
Total	**15 marks**
I. Major Practical (Any one out of 5 questions) 9×1 = 9 marks	
• Aim	01 mark
• Principle	02 marks
• Procedure	03 marks
• Diagram	01 marks
• Observation	01 marks
• Results	01 marks
Total	**09 marks**
II. Spotters (Any three – one from each category) 2×3 = 6 marks	
• Identification	½ marks
• Two salient points	1 mark
• Diagram	½ mark
Total	**02 marks × 3 spotters = 6marks**

Key for Practical Examination

I. Major Practical (Any one) 9×1 = 9 marks

1. Determine the gram nature of bateria present in the given sample (curd/idly batter/yeast)
2. Identify whether the given fungus is Aspergillus or Mucor or Rhizopus based on its microscopic characteristics.
3. Determine the blood group of the given blood sample.
4. Carry out blood staining using field's stain and observe the erythrocytes and leucocytes.
5. Identify whether the given culture is catalase positive.

II. Spotters

A. Specimen	2 marks
B. Slide	2 marks
C. Spotter	2 marks

1. Gram's staining of curd/idly batter/yeast

Aim: To determine the gram nature of bacteria present in the given sample(curd/idly batter/yeast) by Gram's staining technique.

Theory and Principle:

Based on gram staining reaction bacteria can be divided into two large groups called gram positive and gram negative. Gram positive bacteria have thicker peptidoglycan compared to the gram negative bacteria. The lipid content of the cell wall of gram negative bacteria is higher than that of gram positive bacteria. Both gram positive and gram negative bacteria take up the primary stain crystal violet and stain red. When decolorized the porosity and permeability of the gram negative bacteria increases due to which the crystal violet iodine complex is given out by the gram negative bacteria. Further the gram negative bacteria takes up the counterstain safranin and stains red. Hence when bacteria are observed under microscope after gram staining the gram positive cells appear violet and gram negative cells appear red.

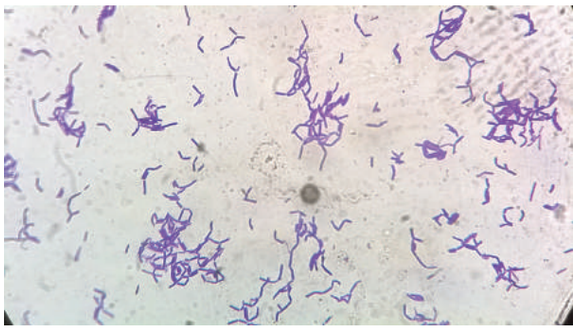
Requirements:

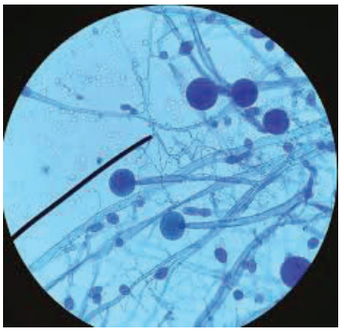
- Clean grease free slide
- Nichrome loop
- Given culture
- Crystal violet
- Grams iodine
- Decolorizer(Acetone Alcohol)
- Safranin
- D/W

Procedure:

1. Take a loopful of the given culture and place on the slide.
2. Prepare a smear and heat fix it.
3. Cover the smear with Crystal Violet for one minute.
4. Wash gently
5. Add Grams iodine for one minute
6. Decolorise with acetone alcohol
7. Wash the slide immediately
8. Cover the smear with safranin for a minute
9. Wash and Air dry.
10. Observe the slide under high power and oil immersion objectives.
11. Record your observations.

Diagram: (Any one Diagram)





Observation Table: (any one shape and stain)

Sr. no	Morphology	Arrangement	Colour of Cytoplasm	Colour of Background	Inference
1.	Rod (bacilli)	Singles, chains	Violet	colourless	Gram positive
2.	Oval yeast cells	Singles, budded	Violet	colourless	Gram positive

Results: Gram staining of the given culture revealed gram positive violet colored rod-shaped bacteria in chains.

2. Identification of the fungus (Aspergillus/Mucor/Rhizopus) by wet mount using LPCB.

Aim: To identify whether the given fungus is Aspergillus or Mucor or Rhizopus based on microscopic characteristics by wet mount method using lactophenol cotton blue stain.

Theory and Principle :

Filamentous fungi are reliably identified by their characteristics microscopic morphology such as shape, size and arrangement of spores and hyphae. Fungi are eukaryotic and range from unicellular yeast to multicellular molds. They reproduce by producing spores.

Common fungi are Aspergillus, Mucor and Rhizopus. They are filamentous and collectively form mycelium. The morphology of the hyphae and spores can be identified using a simple wet mount technique using lactophenol cotton blue stain.

The organism suspended in the stain are killed due to the presence of phenol. Lactic acid preserves fungal structures and cotton blue stains the fungal cell wall.

Fungi	Characteristics of Hyphae	Spores borne in
Aspergillus sp.	Septate	Conidiophore bear conidia
Mucor and Rhizopus sp.	Aseptate	Sporangiosphore bear sporangium containing sporangiospore.

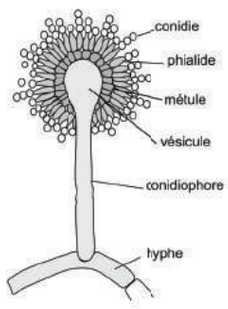
Requirements :

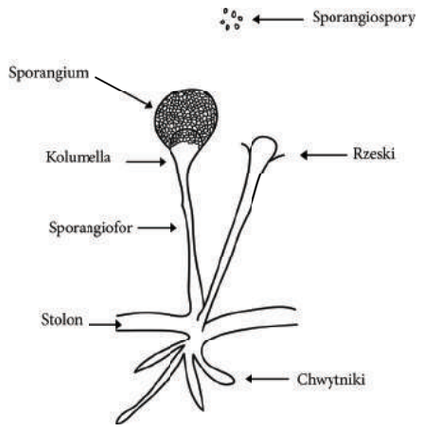
- Clean grease free slide
- Coverslip
- Forcep
- Teasing needle
- Distilled water
- Lactophenol Cotton Blue

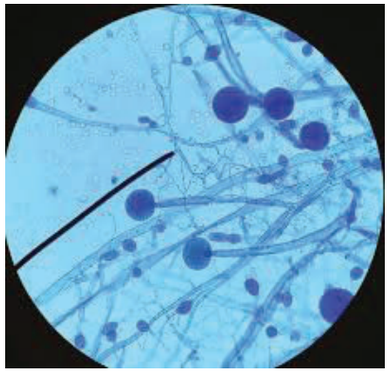
Procedure:

1. Take a clean slide.
2. Place a drop of water on the slide.
3. With the help of forceps transfer the fungal mycelium.
4. Tease it with needle to separate the filaments (hyphae).
5. Add a drop of lactophenolcotton blue.
6. Gently place a coverslip avoiding air bubble formation.
7. Observe under low power and high power objective lens.
8. Read the observations and interpret.

Diagram:







OBSERVATION;

Filamentous hyphae bearing sporangia were observed.

Results:

Wet mount using lactophenol cotton blue was carried to identify the fungus sample. Hyphae with sporangium bearing sporangiospores were observed. It is likely to be of mucor species.

3. Blood Grouping

Aim: To determine the blood group of the blood sample by the slide agglutination test.

Theory and Principle:

Blood grouping is an essential requirement before blood is transfused from one person to another.It is also useful in settling paternity disputes and medicolegal problems.

Red blood cells contain blood group antigens. Antibodies to the blood group antigens are present in the blood plasma.The antigens are generally determined and are responsible for blood types. When RBCs of a person are mixed with corresponding antiserum, agglutination occurs due to antigen-antibody reactions.

Materials Required

- Blood sample (anticoagulated)
- Sterile cotton
- Sterile lancet
- Clean dry grease free slides or white tile
- Toothpicks
- Marker pen
- Commercially available Anti A sera, Anti B sera and Anti D sera

Procedure

1. Prick the finger under aseptic conditions
2. Place a drop of blood on the slide on each side marked as A, B and D.
3. Add a drop of antiserum A , B and D on A, B and D side respectively.
4. Mix with toothpick using separate toothpicks for each mixture.
5. Wait for 2 mins and observe for clumping reaction if any confirm it by observing under microscope.
6. Interpret the results and report.

Interpretation

If agglutination on A side the blood group is A

If agglutination seen on B side the blood group is B

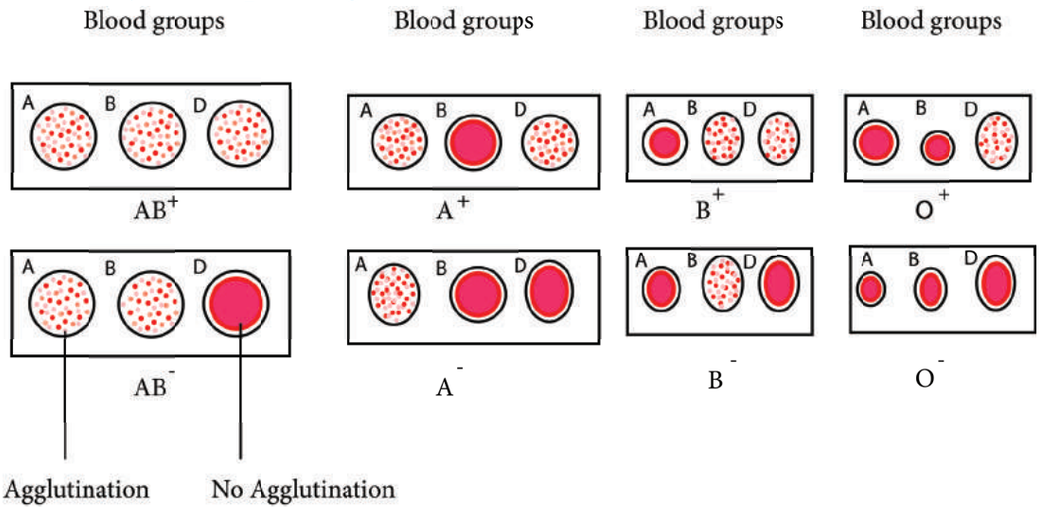
If Agglutination on both A and B side the blood group is AB

If No agglutination on A and B side the blood group is O

If agglutination is seen on D side the blood group is Rh(D) positive

If No agglutination on D side the blood group is Rh(D) negative.

Diagram: (any one depending on the results)



Observation: (will vary with the type of blood group an example is given below)

Agglutination is seen on A, B and D side

Result: The blood group of the blood sample was determined by slide agglutination test and was found to be AB Rh positive.

1. BLOOD STAINING

AIM

To make a blood smear ,stain it using Field's stain and observe the erythrocytes and leucocytes.

Theory and Principle:

Blood smears are used to determine leukocyte differentials, to evaluate erythrocyte, platelet and leukocyte morphology, and, if necessary, to estimate platelet and leukocyte counts. It is also used for diagnosis of parasites like plasmodium in the blood.

Field's Stain is a romanowsky stain, used for rapid processing of blood specimens and is used to stain thick and thin films. It consists of two differential stain.**Field stain A**

which is methylene blue and Azure dissolved in a phosphate buffer solution.It is the basic component of the stain and **Field stain B** made up of Eosin Y in a buffer solution which is the acidic component of the stain.These basic and acidic dyes induce several colours when applied to cells.The fixator, methanol, does not allow any additional changes to the slide. The basic component of peripheral white blood cell(cytoplasm) is stained with acid dye and the acid component that is nucleic acid of the nucleus takes on the basic dye and is stained blue to violet. The neutral components of the cells are stained by both dyes(Field's stain A and B solution).

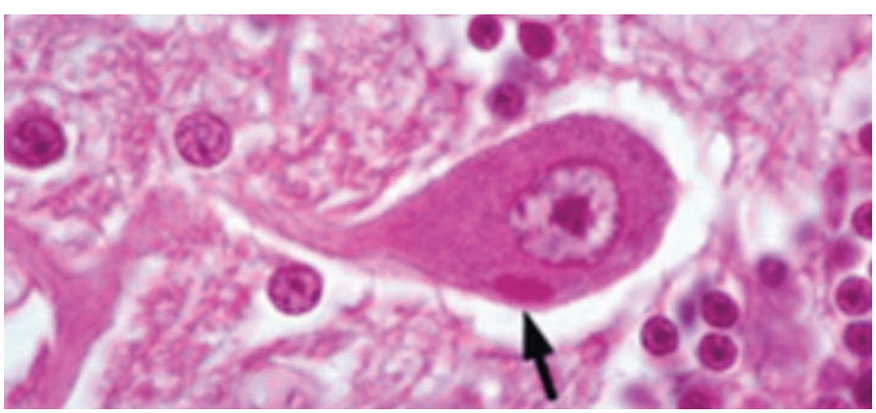
REQUIREMENTS

- Cotton
- Spirit
- Blood sample
- Clean grease free slides
- Methanol fixative
- Field's stain A and Field's stain B.

PROCEDURE

1. Finger Prick under aseptic condition.
2. Place a small drop of blood, on one side about 1-2 cm from one end of a slide.
3. Without delay place another slide at an angle of 45° to make contact with the drop.
4. Spread it over an area of about 2 cm^2(The film should be distributed so thinly that it appears transparent.
5. After air drying the thin blood film,immerse or fix the smear in methanol for 1 minutes.
6. Flood or dip the slide in Field's Stain A for 2-3 seconds.
7. Wash it with distilled water,
8. Flood or dip the slide in Field's Stain B for 2-3 seconds and wash with distilled water.
9. Now air dry the smear and observe under microscope.

DIAGRAM



OBSERVATION

TYPE OF CELL	COLOUR OF CYTOPLASM	COLOUR OF NUCLEUS	COLOUR OF GRANULES
RBC	pink	-	-
WBCs(leucocytes)			
Neutrophil	pink	blue	lilac
Eosinophil	pink	blue	orange
Basophil	pink	blue	Dark blue black
lymphocyte	blue	violet	-

RESULTS

The blood smear was stained using field's stain and erythrocytes and leucocytes were observed under microscope.

5. Test for Catalase

AIM

To test whether the given culture is catalase positive by the catalase test

THEORY AND PRINCIPLE

Catalase test demonstrates the presence of catalase, an enzyme that catalyses the release of oxygen from hydrogen peroxide (H_2O_2). It is used to differentiate those bacteria that produces an enzyme catalase, such as *staphylococci*, from non-catalase producing bacteria such as *streptococci*.

The enzyme catalase mediates the breakdown of hydrogen peroxide into oxygen and water. The presence of the enzyme in a bacterial isolate is evident when a small inoculum is introduced into hydrogen peroxide, and the rapid elaboration of oxygen bubbles occurs. The lack of catalase is evident by a lack of or weak bubble production. The culture should not be more than 24 hours old.

REQUIREMENTS

- Slides
- Nichrome loop or toothpick
- 24hour old culture
- 3%hydrogen peroxide

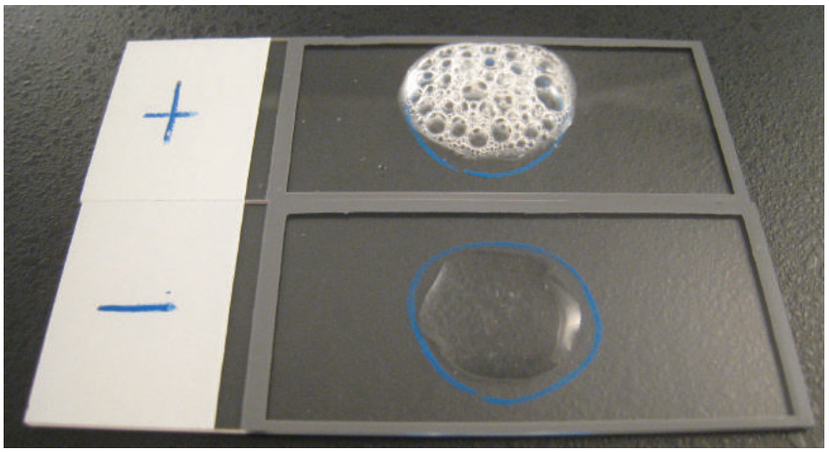
- Dropper

PROCEDURE

Slide Method

1. Use a loop or sterile wooden stick to transfer a small amount of colony growth in the surface of a clean, dry glass slide.
2. Place a drop of 3% H_2O_2 in the glass slide.
3. Observe for the evolution of oxygen bubbles.

DIAGRAM



$2H_2O_2 \xrightarrow[\text{catalase}]{} 2H_2O + O_2$ (gas bubbles)

OBSERVATION (any one to be reported depending on the culture)

Positive: Copious bubbles produced, active bubbling

Examples: *Staphylococci, E. coli, Enterobacter, Klebsiella, Shigella, Yersinia, Pseudomonas.*

Negative: No or very few bubbles produced.

Examples: *Streptococcus and Enterococcus* sps.

Result

The given culture was found to be catalase positive as determined by the catalase slide test.

6. WIDAL TEST (slide test)

AIM

To carry out the widal test for the given blood sample and to determine the presence of antibodies against salmonella antigens.

THEORY AND PRINCIPLE

Widal test is a serological test which is used for the diagnosis of enteric fever or typhoid fever. Typhoid or enteric fever is caused by a gram negative bacteria *Salmonella enterica* (*Salmonella* Typhi or *Salmonella* Paratyphi). *Salmonella* possess O antigen on their cell wall and H antigen on their flagella. On infection, these antigen stimulates the body to produce specific antibodies which are released in the blood. The Widal test is used to detect these specific antibodies in the serum sample of patients suffering from typhoid using antigen-antibody interactions. These specific antibodies can be detected in the patient's serum after 6 days of infection (fever).

Salmonella Typhi possesses O antigen on the cell wall and H antigen on flagella. *Salmonella* Paratyphi A and *S.* Paratyphi B also possess O antigen on their cell wall and but have AH and BH antigen on their flagella respectively.

Widal test is an agglutination test in which specific typhoid fever antibodies are detected by mixing the patient's serum with killed bacterial suspension of Salmonella carrying specific O, H, AH and BH antigens and observed for clumping ie. Antigen-antibody reaction. The main principle of Widal test is that if homologous antibody is present in patient's serum, it will react with respective antigen in the suspension and gives visible clumping on the test slide.

Requirements

Fresh serum

The complete kit containing five vials containing stained Salmonella antigen

- *S.* Typhi ⟶ O antigen
- *S.* Tyhhi ⟶ H antigen
- *S.* Paratyphi ⟶ AH antigen
- *S.* Paratyphi ⟶ BH antigen

Widal positive control

Widal test card or slide

v) Applicator stick

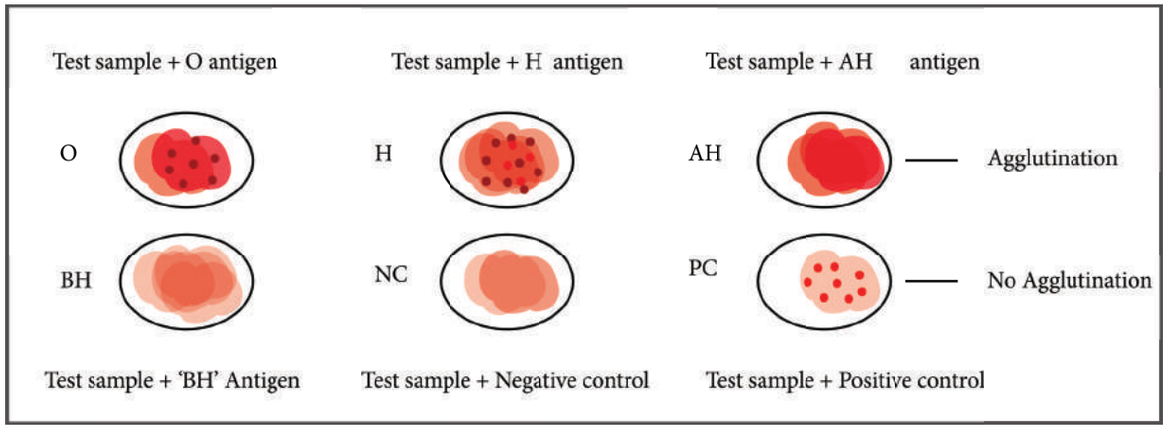
Procedure

- Widal test can be done in two ways-one is rapid test on slide and another is tube test in which result may be obtained after one night of incubation.

Rapid slide test:

1. Clean the glass slide or test card supplied in the kit well and make it dry.
2. Label the circles (1, 2, 3, 4, 5 and 6) in the test card as O, H, AH, BH, Negative control and Positive control
3. Place a drop of undiluted test serum in each of the four labelled circle (1, 2, 3 and 4) ie O, H, AH and BH and place a drop of Negative control serum in circle 5 and Positive control in circle 6.
4. Place a drop of antigen O, H, AH and BH in circle 1, 2, 3, and 4 respectively and no antigen in circle 5 and O/H antigen in circle 6.
5. Mix the content of each circle with a separate wooden applicator stick and spread to fill the whole area of the individual circle.
6. Rock the test card for a minute and observe for agglutination.

DIAGRAM



OBSERVATION

Agglutination was observed in O and H side within a minute which indicates the presence of antibodies in the serum sample against Salmonella typhi antigens.

Proceed for quantitative slide test or tube test for the quantitative estimation of the titre of the antibody.

Result

Qualtative widal test was carried out using rapid slide agglutination method. Antibodies against O and H antigens of Salmonella typhi were detected in the serum.

7. Demonstration of rhizobium from root nodules and its isolation

Aim:

To demonstrate the presence of rhizobium in root nodules by gram staining and isolate them on a nutrient medium.

Theory and Principle:

Leguminous plants like cowpea, red gram , black gram contain root nodules formed by rhizobium.Rhizobium in the soil enter into the roots of leguminous plant and form nodules and establish symbiotic association. Bacteria derive nutrients from the plants. The rhizobacteria fix nitrogen which is beneficial to the plant. Rhizobium is a symbiotic N_2 fixer found to occur as bacteroids in the root nodules of leguminous plants. They can be easily isolated and cultured in vitro.

Based on gram staining reaction bacteria can be divided into two large groups called gram positive and gram negative. Gram positive bacteria have thicker peptidoglycan compared to the gram negative bacteria. The lipid content of the cell wall of gram negative bacteria is higher than that of gram positive bacteria. Both gram positive and gram negative bacteria take up the primary stain crystal violet and stain red. When decolorized the porosity and permeability of the gram negative bacteria increases due to which the crystal violet iodine complex is given out by the gram negative bacteria. Further the gram negative bacteria takes up the counterstain safranin and stains red. Hence when bacteria are observed under microscope after gram staining the gram positive cells appear violet and gram negative cells appear red. **Rhizobia are Gram- negative rods which are motile with bi-polar, sub-polar and peritrichous flagella**.

Rhizobium grows well on Yeast Extract Mannitol Agar (YEMA). Congo red added to the medium differentiates rhizobia that stand out as white, translucent, glistening elevated, small colonies with entire margin, in contrast to the red stained colonies of Agrobacterium and other bacteria.

Requirements:

1. Root nodules (pink) of any leguminous plant
2. **Congo red, Yeast Extract, Mannitol Agar (pH 6.8 – 7.0):**

Mannitol	10.0 g
K_2HPO_4	0.5 g
$MgSO_4.7H_2O$	0.2 g
NaCl	0.1 g
Yeast extract	1.0 g
$CaCO_3$	3.0 g
Agar	25.0 g

Congo red (1% aqueous)

2.5 ml (1.0 g in 100 ml)

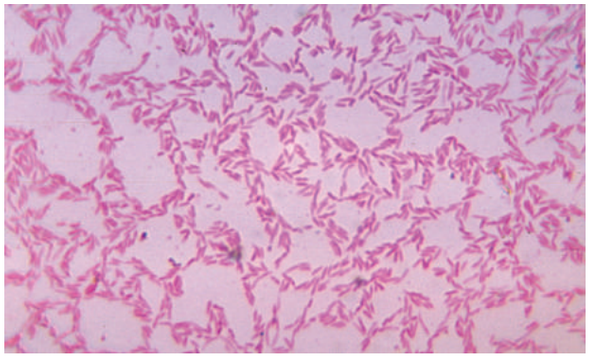
Distilled water 1000.0 ml

3. Inoculation loop
4. Bunsen burner/laminar clean air flow hood.
5. Slides and glass rod.
6. Petri plates with YEMACR medium.
7. Sterile distilled water.
8. 95% alcohol and 0.1% $HgCl_2$.

Procedure:

1. Wash the root system under a slow stream of running tap water, taking care to see that the nodules are intact.
2. Select pink nodules and remove them
3. Wash and keep the nodules in 95% ethanol for a minute, wash and transfer them to 0.1% $HgCl_2$.
4. Remove after five minutes and wash the nodules about four to five times with sterile distilled water.
5. Place the nodule on a sterile slide in a drop of sterile distilled water and crush it either with a sterile glass rod or a flat tipped forceps.
6. Remove a loopful of this cloudy suspension and streak inoculate on YEMACR plates and label.
7. Incubate in dark at 28°-30°C for 2-3 days and observe the colonies.
8. Make a smear of the remaining crushed material and gram stain and observe the gram negative bacilli. Even samples from the colonies can be gram stained.

Diagram:



Observation

Gram's stain

Organism	Morphology	Arrangement	Colour of cytoplasm	Colour of Background	Inference
Rhizobium from root nodule.	Rod (bacilli)	Singles	red	colourless	Gram negative

Colony characteristics of rhizobium on YEMA after incubation for 2-3 days at room temperature

Size – 2-4 mm

Shape- circular

Colour – White

Margin - entire

Elevation – convex, raised

Opacity - semitranslucent

Texture – creamy

Consistency – mucilaginous

Gram nature – gram negative

Motility – actively motile

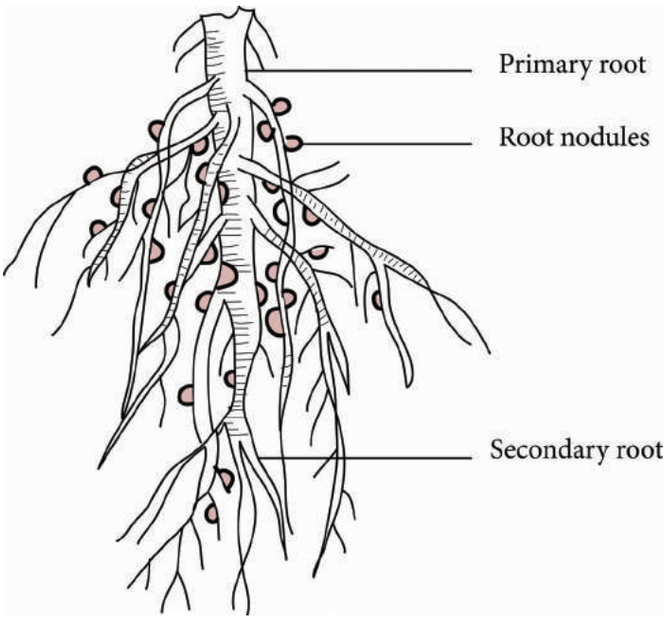
Results: Gram staining of the root nodule exudate revealed the presence of gram negative rods.

The colony characteristics of rhizobia were studied after isolation on YEMA medium. White, creamy, mucoid colonies were obtained.

Spotters

II A) SPECIMEN

1. Root nodules of leguminous plant

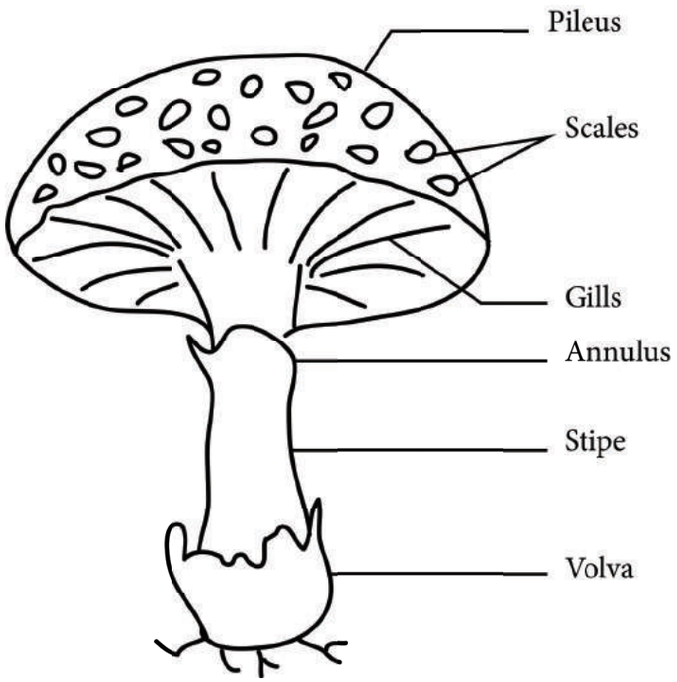


- Leguminous plants like cowpea, red gram contain root nodules formed by rhizobium.
- Rhizobium in the soil enter into the roots of leguminous plant and form nodules and establish symbiotic association.
- Bacteria derive nutrients from the plants.
- The rhizobacteria fix nitrogen which is beneficial to the plant.

2. Tikka leaf spot of groundnut plant

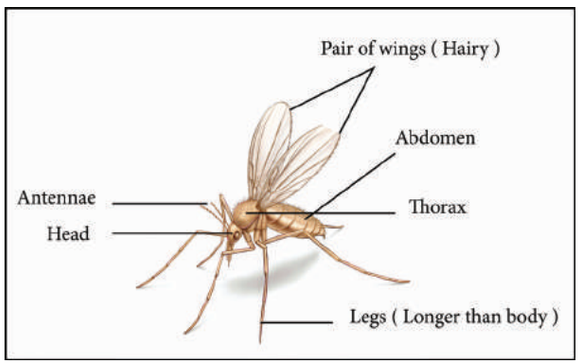
- Tikka leaf spot disease is a kind of fungal disease seen in groundnut leaf.
- This disease is caused by Cercaspora personata.
- Brown spots surrounded by a yellow halo appear on the upper surface of the leaf.
- The fungal spores can be demonstrated if the leaf is processed and observed under microscope.

3. Mushroom



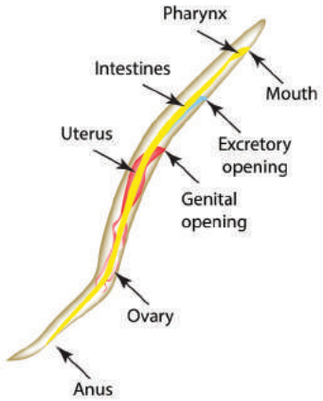
- Mushroom is a saprophytic fungus.
- Primary mycelium grows from basidiospores.
- It has high protein content and edible mushrooms are used as food.
- Example: Agaricus species and Pleurotus species.

4. Sand fly



- Bite of an infected sandfly transmits leishmania donovani infection.
- Female sandfly during a blood meal ingest free as well as intracellular amastigotes in the blood.
- In the midgut these are transformed to flagellated promastigote.

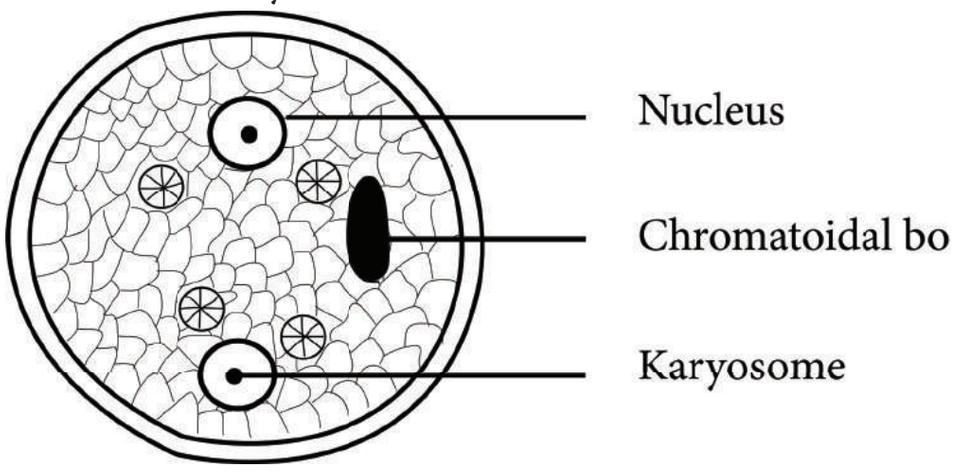
5. Ascaris



- The adult worm of ascaris lives in the small intestine of humans
- They are large cylindrical worms with tapering ends, the anterior end being thinner than the posterior end
- The adult male worm is smaller than female worms.

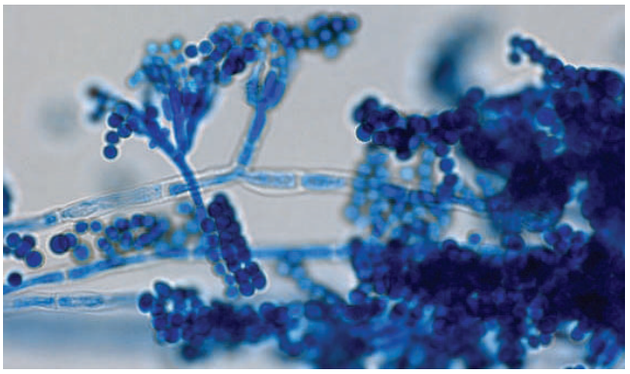
IIB) Slide

6. Cyst of Entamoeba histolytica



- Cyst is one of the three forms of entamoeba histolytica
- A mature cyst is a quadrinucleate spherical body.
- Mature cysts are passed in the stool of infected person
- Direct examination of wet mount of stool for cysts is diagnostic of intestinal amoebiasis

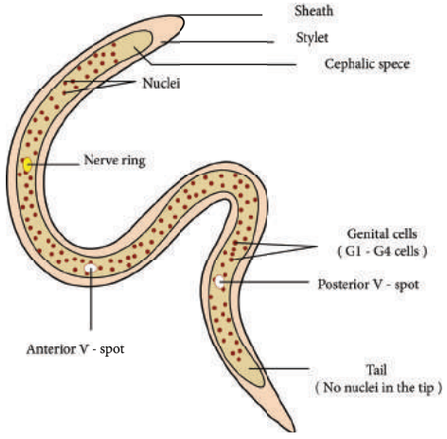
7. Penicillium species



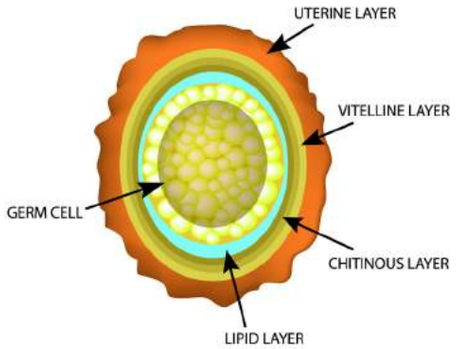
- Colony of penicillium are initially white and fluffy and later produce pigmented spores and turn into shades of green or blue green
- Hyphae are hyaline and septate
- Condiophores are long, give rise to branching phialids
- Phialids branch and give the appearance of brush or penicillins
- They produce sterigmata bearing chain of conidia (spores) which are oval or spherical and measure 1-2micrometer.

8. Microfilariae

- Filariasis is caused by nematodes (roundworms) like *Wuchereria bancrofti* that inhabit the lymphatics and subcutaneous tissues.
- The female worms release the first stage larvae called microfilariae, which are detected in the peripheral blood.
- Identification of microfilariae by microscopic examination is the most practical diagnostic procedure.
- The blood sample can be a thick smear, stained with Giemsa.
- The larva measures about 290microns in length and 6-7micron in breath.



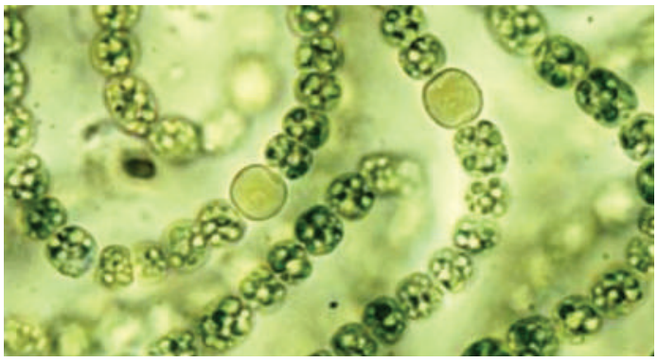
9. Egg of Ascaris lumbricoides



EGG OF ASCARID FERTILIZED

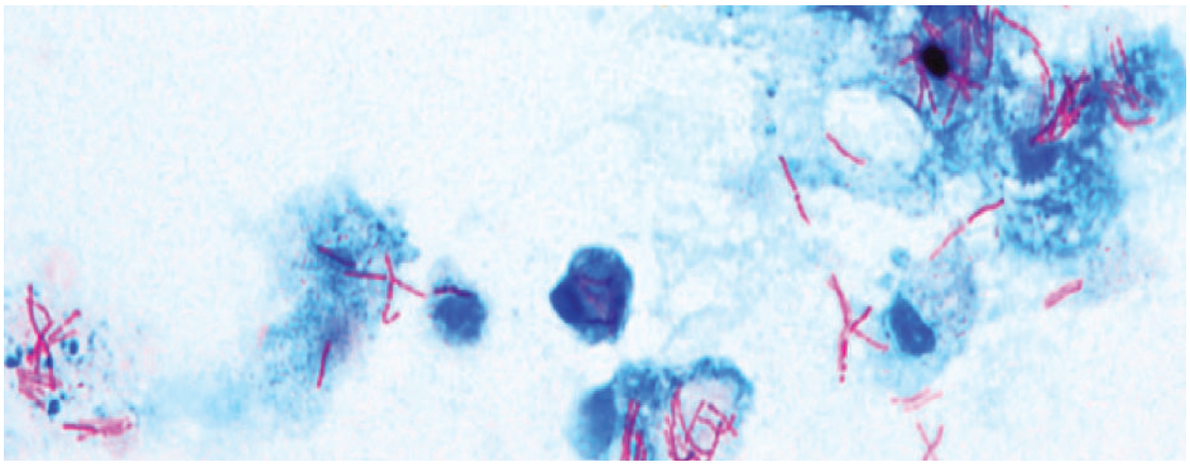
- These are passed in stool of the infected host.
- Brownish due to bile pigment.
- Fertilised eggs are rounded and have a thick shell (chitinous).
- Unfertilised eggs are elongated and larger than fertile eggs.
- When ingested through water or contaminated food by human it causes Ascariasis.
- Microscopic identification of eggs in the stool is the most common method for diagnosing intestinal ascariasis.

10. Heterocysts of Nostoc



- Heterocysts are specialized structures having thick cell wall formed in some filamentous blue green algae like Nostoc, Anabena.
- They may be terminal or found in between the vegetative cells attached to it by means of pores.
- They are sites of atmospheric nitrogen fixation.
- They serve as a store house of food material.

11. Acid fast bacilli

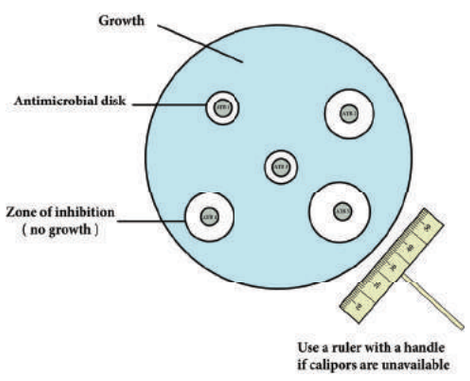


- Acid fast bacilli contains mycolic acid in their cell walls hence do not get stained easily, however once stained cannot be decolourised easily.
- Special method like Ziehl- Neelson's Carbol fuchsin is used to stain acid fast bacilli.
- The acid- fast bacilli are stained red in colour while the non acid fast cells appear blue when counterstained with methylene blue.
- Mycobacterium tuberculosis is and acid fast bacilli.

IIC) SPOTTER

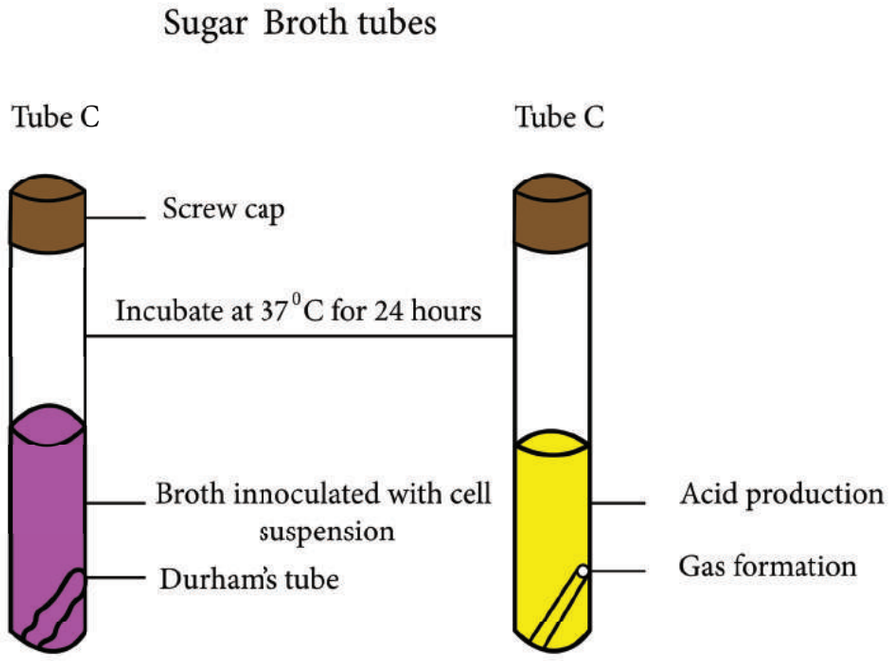
12. Antibiotic sensitivity plate set up by Kirby Bauer technique

- Kirby Bauer technique is used to determine the susceptibility of the organism to various antimicrobial agents.
- Standard suspensions of rapidly growing test bacterium is inoculated on the surface of muller hinton agar plates.
- Antibiotic discs are pressed on the surface of the seeded plates.
- The zone of inhibition or the zone of growth determines the degree of susceptibility of the organism towards antibiotic.



13. Sugar fermentation tube showing acid and gas production

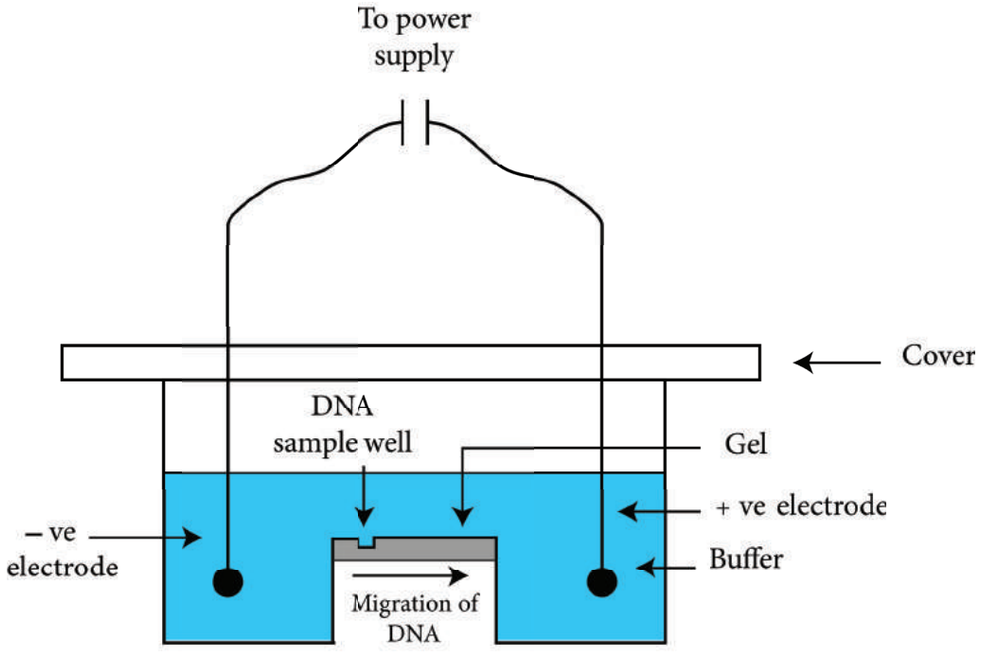
- Carbohydrate broth with bromocresol purple as indicator is used for testing the ability of pure bacterial culture to ferment a specific sugar like lactose, xylose, mannitol and other sugars.
- Acid production is indicated by colour change of the indicator from purple to yellow
- Gas production is indicated by an air bubble in the durham's tube.
- Escherichia coli ferments lactose producing acid and gas.



In the above image Test tube C was innoculated with cell suspension and incubated at 37 C for 24 hours and after innoculation the purple colour of broth was changed to yellow colour with gas bubble formation in durham's tube.

14. Agarose gel electrophoresis apparatus.

- Electrophoresis refers to the movement of charged molecules in an electric field.
- The negatively charged molecules move towards the positive electrode while the positively charged molecules migrate towards the negative electrode.
- Gel electrophoresis is a routinely used analytical technique for the separation and purification of specific DNA fragments.
- As the DNA is negatively charged, DNA fragments move through the gel towards the positive electrode. The rate of migration of DNA is dependent on the size and shape.



15. Spoiled food

- Spoilage is a process in which food deteriorates such that its quality of edibility is reduced.
- Food poisoning may result on eating contaminated or spoiled food.
- Foods spoil due to attacks from enzymes, oxidation and microorganisms.
- These include bacteria, mold, yeast, moisture, temperature and chemical reaction.

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