16MBU201 **VIROLOGY** **Semester – II (4H – 4C)**

Instruction Hours / week: L: 4 T: 0 P: 0 **Marks:** Internal: **40** External: **60** Total: **100**
End Semester Exam: 3 Hours

SCOPE

Virology is a potential arena in the medical research companies, Pharmaceutical companies, governmental agencies, laboratory testing companies, or cancer treatment or research companies depending upon the specialization.

OBJECTIVES

- To study general aspects of viral morphology and classification, replication, interactions and immunity to viruses
- To discuss the application of various immunological and molecular diagnostic tools.

Unit I – Nature and Properties of Viruses

Introduction: Discovery of viruses, nature and definition of viruses, general properties, concept of viroids, virusoids, satellite viruses and Prions. Structure of Viruses: Capsid symmetry, enveloped and non-enveloped viruses. Isolation, purification and cultivation of viruses. Viral assay, Viral taxonomy: Classification and nomenclature of different groups of viruses

Unit II – Bacteriophages

Diversity, classification, one step multiplication curve, lytic and lysogenic phages (lambda phage) concept of early and late proteins, regulation of transcription in lambda phage

Unit III – Viral Transmission, salient features of viral nucleic acids & replication

Modes of viral transmission: Persistent, non-persistent, vertical and horizontal. Salient features of viral Nucleic acid: Unusual bases (TMV,T4 phage), overlapping genes (φX174 , Hepatitis B virus), alternate splicing (HIV), terminal redundancy (T4 phage), terminal cohesive ends (lambda phage), partial double stranded genomes (Hepatitis B), long terminal repeats (retrovirus), segmented (Influenza virus), and non-segmented genomes (picornavirus), capping and tailing (TMV).

Unit IV – Viruses and Cancer, Prevention & control of viral diseases

Viral multiplication and replication strategies: Interaction of viruses with cellular receptors and entry of viruses. Replication strategies of viruses as per Baltimore classification (phi X 174, Retroviridae, Vaccinia, Picorna), Assembly, maturation and release of virions

Unit V – Applications of Virology

Introduction to oncogenic viruses. Types of oncogenic DNA and RNA viruses: Concepts of oncogenes and proto-oncogenes. Antiviral compounds and their mode of action. Interferon and their mode of action. General principles of viral vaccination. Immunization schedule. Use of viral vectors in cloning and expression, gene therapy and phage display

SUGGESTED READINGS

1. Dimmock, N.J., Easton., A.L., Leppard, K.N. (2007). Introduction to Modern Virology. 6th edition, Blackwell Publishing Ltd.
2. Carte,r J., and Saunders, V. (2007). Virology: Principles and Applications. John Wiley and Sons.
3. Flint, S.J., Enquist, L.W., Krug, R.M., Racaniello, V.R., Skalka, A.M. (2004). Principles of Virology, Molecular biology, Pathogenesis and Control. 2nd edition. ASM press Washington DC.
4. Levy, J.A., Conrat, H.F., Owens, R.A. (2000). Virology. 3rd edition. Prentice Hall publication, New Jersey.
5. Wagner, E.K., Hewlett, M.J. (2004). Basic Virology. 2nd edition. Blackwell Publishing.
6. Mathews. (2004). Plant Virology. Hull R. Academic Press, New York.
7. Nayud, M.V. (2008). Plant Viruses. Tata McGraw Hill, India.
8. Bos, L. (1999) Plant viruses-A text book of plant virology by. Backhuys Publishers.
9. Versteeg J. (1985). A Color Atlas of Virology. Wolfe Medical Publication.

KARPAGAM ACADEMY OF HIGHER EDUCATION

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Coimbatore – 641 021.

LECTURE PLAN
DEPARTMENT OF MICROBIOLOGY

STAFF NAME: Dr. RAMALAKSHMI. S
SUBJECT NAME: VIROLOGY SUB.CODE:17MBU201
SEMESTER: II CLASS: I B.Sc (MB)

S.No	Lecture Duration Period	Topics to be Covered	Support Material/Page Nos
		UNIT-I	
1	1	History of Viruses	T1: Virology - P.Saravanan.MSP publishers 1-4
2	1	Structure of viruses	T2: Textbook of Microbiology-Panicker-Orient dongman publishers. 421-430
3	1	Classification of viruses	T1: Virology - P.Saravanan.MSP publishers.49-54
4	1	Nomenclature of viruses	T1: Virology - P.Saravanan.MSP publishers.49-54
5	1	Taxonomy of viruses	R1: Medical Virology-White and Fenner-Academic
6	1	Isolation of viruses	www.Microbiology.com
7	1	Purification of viruses	Viruses
8	1	Cultivation of viruses	T2: Textbook of Microbiology-Panicker-Orient dongman publishers. 430-436

9	1	Viral Assay	R2: Medical Virology- Jawetz-McGraw Hill publishers and W1: www.microbiology.com
10	1	Concept- viroids,virusoids	T2: Textbook of Microbiology-Panicker-Orient dongman publishers 417-420
11	1	Satellite viruses,virophage, prions	T2: Textbook of Microbiology-Panicker-Orient dongman publishers 417-420
12	1	Unit Revision	
	Total No of Hours Planned For Unit 1=12		
		UNIT-II	
1	1	Diversity of viruses	T1: Virology - P.Saravanan.MSP publishers. 49-54
2	1	Classification of viruses	T1: Virology - P.Saravanan.MSP publishers. 49-54
3	1	One-step multiplication curve	T1: Virology - P.Saravanan.MSP publishers.66-68
4	1	Lytic phage	T1: Virology - P.Saravanan.MSP publishers. 80-82
5	1	Lysogenic phagees	T1: Virology - P.Saravanan.MSP publishers. 80-82
6	1	Concept of early proteins	W1:www.microbiology.com
7	1	Late proteins	W1:www.microbiology.com
8	1	Lysogenic phages genome organisation	J1:Viruses

9	1	Regulation of transcription	J1:Viruses
10	1	Lytic phage	J1:Viruses
11	1	Regulation of transcription-Lysogenic	J1:Viruses
12	1	Unit Revision	
	Total No of Hours Planned For Unit II=12		
		UNIT-III	
1	1	Viral transmission	T1: Virology - P.Saravanan.MSP publishers. 123-128
2	1	Viral Nucleic acid -features	R2: Medical Virology- Jawetz-McGraw Hill publishers.367-395
3	1	Unusual bases	R2: Medical Virology- Jawetz-McGraw Hill publishers. 367-395
4	1	Overlapping genes	R2: Medical Virology- Jawetz-McGraw Hill publishers. 367-395
5	1	Splicing genes	R2: Medical Virology- Jawetz-McGraw Hill publishers. 367-395
6	1	Terminal redundancy	J1: Viruses
7	1	Cohesive ends	J1: Viruses
8	1	Capping and Tailing	J1: Viruses
9	1	Viral genome organisation	J1: Viruses
10	1	Lambda phage- Genome organisation	J1: Viruses
11	1	Phi X 174 phage- genome organisation	R1: Medical Virology-White

			and Fenner-Academic. 531-541.
12	1	Unit revision	
	Total No of Hours Planned For Unit III=12		
		UNIT-IV	
1	1	Viral multiplication	T1: Virology - P.Saravanan.MSP publishers. 420-422
2	1	Entry of viruses	T2: Textbook of Microbiology-Panicker-Orient dongman publishers. 447-450.
3	1	Interaction of viruses	T2: Textbook of Microbiology-Panicker-Orient dongman publishers. 436-447.
4	1	Baltimore classification	www.microbiology.com
5	1	Baltimore classification	www.microbiology.com
6	1	PhiX 174 and HIV	R1: Medical Virology-White and Fenner-Academic. 531-561.
7	1	Vaccinia and Picorna viruses	T2: Textbook of Microbiology-Panicker-Orient dongman publishers. 470-476
8	1	Assembly,maturation and release of virions	www.microbiology.com
9	1	Oncogenic viruses and types	T2: Textbook of Microbiology-Panicker-Orient dongman publishers. 550-

			555
10	1	Mechanism of oncogenic viruses	T2: Textbook of Microbiology-Panicker-Orient dongman publishers. 555-557
11	1	Prevention and control of viral diseases	R1: Medical Virology-White and Fenner-Academic. 270-277, www.microbiology.com
12	1	Unit Revision and test	
	Total No of Hours Planned For Unit IV=12		
		UNIT-V	
1	1	Antiviral compounds and introduction	www.microbiology.com
2	1	Mode of action- antiviral compounds	www.microbiology.com
3	1	Interferons	R1: Medical Virology-White and Fenner-Academic. 270-274
4	1	Viral vaccination	R1: Medical Virology-White and Fenner-Academic. 275-277.
5	1	Immunization schedule	www.microbiology.com
6	1	Viral vectors	www.microbiology.com
7	1	General therapy	www.microbiology.com
8	1	Phage display	T1: Virology - P.Saravanan.MSP publishers. 54-57
9	1	Unit revision 1- 5	
10	1	Discussion of Previous ESE Question Papers.	

11	1	Discussion of Previous ESE Question Papers.	
12	1	Discussion of Previous ESE Question Papers.	
	Total No of Hours Planned for unit V=12		
Total Planned Hours	**60**		

UNIT 1

1.1. **Introduction**

- *Virology* is the study of viruses, complexes of nucleic acids and proteins that have the capacity for replication in animal, plant and bacterial cells.
- Many viruses have co-evolved with mammals and other animals over long periods of time. Examples of such viruses are herpes viruses, which have been traced back to fish and birds, as well as mammals.
- It is thought that herpes viruses have existed for two hundred million years or longer, and that they have infected humans since the early times of our speciation.
- Other viruses have entered human populations only recently, due to changes in agriculture (use of domestic animals), population dynamics (urbanization), migration of populations, commerce and changes in the environment. Examples of these agents include measles virus and HIV-1.

1.2.1. **Important definitions in Virology**

1. **Virus:** A capsid encoding organism that is composed of proteins and nucleic acid, self assemble in a nucleocapsid and uses a ribosome encoding organism (host) for the completion of its life cycle.
2. **Protein subunit:** It is the individual folded protein molecule in which the structural subunit is made of.
3. **Morphological subunit:** These are known as capsomeres. They are seen in electron microscope on the surface of virus particles. They represent cluster of polypeptides which then assembled to form a capsid.
4. **Capsid**: It is the regular shell like structure composed of aggregated protein subunits which surrounds the viral Nucleic acid. The protein subunits are called promoters, and these are aggregated to form capsomeres and are further aggregated to form capsid.
5. **Nucleocapsid:** The capsid together with the enclosed nucleic acid. The core of a virus particle consisting of the genome plus a complex of protein.
6. **Envelope:** It is the viral membrane composed of lipid bilayer containing viral glycoprotein spikes and the phospholipids derived from the host cell.
7. **Virion:** The viral particle with (Retrovirus) or without (Picornavirus) envelope is known as virion. This is the complete infective virus particle.
8. **Defective virus:** A virus particle that is functionally deficient in some aspect of replication is referred as defective virus.
9. **Pseudovirus:** During viral replication the capsid sometimes encloses host nucleic acid rather than viral nucleic acid. Such particles look like ordinary virus, particles when observed by electron microscopy, but they do not replicate. Pseudo virions contain the "wrong" nucleic acid.
10. **Spikes:** These are glycoprotein projections arising from envelope. They have enzymatic adsorption and haemagglutinin activity. They are highly antigenic in nature.

1.4. **A virus**

A capsid encoding organism that is composed of proteins and nucleic acid, self assemble in a nucleocapsid and uses a ribosome encoding organism (host) for the completion of its life cycle.

1.4.1 **General Properties of virus**

a. Obligate intracellular parasites of bacteria, protozoa, fungi, algae, plants, and animals.
b. Ultramicroscopic size, ranging from 20 nm up to 450 nm (diameter).
c. Not cellular in nature; structure is very compact and economical.
d. Do not independently fulfill the characteristics of life.
e. Inactive macromolecules outside the host cell and active only inside host cells.
f. Basic structure consists of protein shell (capsid) surrounding nucleic acid core.
g. Nucleic acid can be either DNA or RNA but not both.
h. Nucleic acid can be double-stranded DNA, single-stranded DNA, single-stranded RNA, or double-stranded RNA.
i. Molecules on virus surface impart high specificity for attachment to host cell.
j. Multiply by taking control of host cell's genetic material and regulating the synthesis and assembly of new viruses.
k. Lack enzymes for most metabolic processes.
l. Lack machinery for synthesizing proteins.

1.10.**Properties of Viroids**

1.10.1. **Introduction**

Potato spindle tuber and at least 15 other crop diseases are caused by Viroids, an entity that nobody had ever heard of before 1971, its official date of discovery. Theodor. O. Diener, the Agricultural Research Service plant pathologist who discovered the pathogen, named it the "viroid," because it is "like a virus."

1.10.2**. Definition**

Viroids are unencapsidated, small, circular, single-stranded RNAs which replicate autonomously when inoculated into host plants. Some are pathogenic, others replicate without eliciting symptoms.

1.10.3. **Structure**

- Viroids are infectious agents composed exclusively of a single piece of circular single stranded RNA which has some double-stranded regions.

- Because of their simplified structures both prions and Viroids are sometimes called sub viral particles. Viroids mainly cause plant diseases but have recently been reported to cause a human disease.

- Viroids are catalytic RNAs that cleave RNA to produce fragments containing a 5' hydroxyl and 2'3' cyclic phosphates.

1.5.

Fig: Structure of Viroids

1.10.4. **Replication**

Circular, pathogenic RNAs are replicated by a rolling circle mechanism *in vivo*. There are two variations of this rolling circle mechanism:

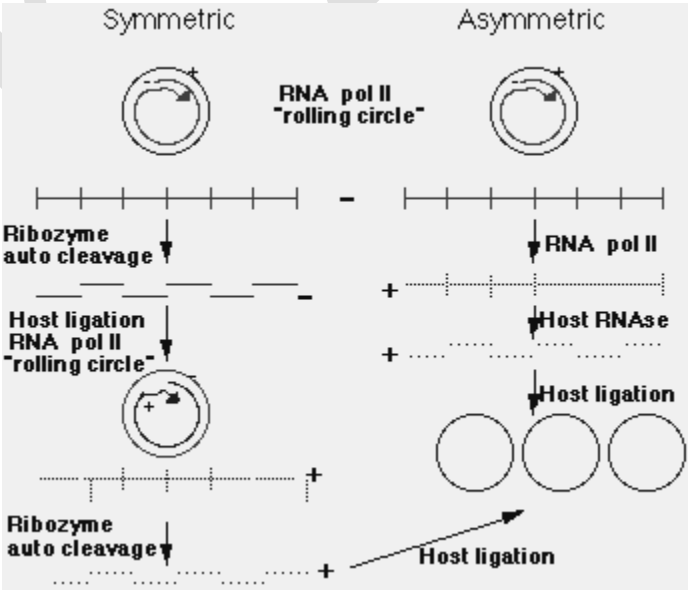


Fig: Replication of Viroid

In the symmetric variation, the circular plus strand is copied by viroid RNA-dependent RNA polymerase to form a concatameric minus strand. Site-specific cleavage (arrows) of this strand produces a monomer that is circularized by a host RNA ligase and then copied by the RNA polymerase to produce a concatameric plus strand. Cleavage of this strand produces monomer which, on circularization, produces the progeny circular, plus RNA, the dominant form i*n vivo*.

In the asymmetric variation, the concatameric minus strand of step 1 is not cleaved but is copied directly to give a concatameric plus strand, which is cleared specifically to monomers for ligation to the circular progeny. Those RNAs that self-cleave only in the plus strand *in vitro* are considered to follow this route.

The hepatitis D viroid genome is a minus strand that gives rise to two RNA species. One of these is a mRNA for the delta antigen and the other is a complete complimentary copy (plus strand or anti-genome). The anti-genome acts as a template to make more minus strands. The minus strand self-cleaves and self-ligates. HDV replication takes place in the nucleus but delta antigen is made in the cytoplasm. The delta antigen is the only protein made by the HDV mRNA.

1.10.5. **Pathogenesis of Viroids**

Probably there is more than one mechanism responsible for viroid pathogenesis. Recent evidence suggests that one pathway is due to viroid RNA activating a plant RNA activated protein kinase, or PKR (analogous to the PKR enzyme activated by viral RNAs in mammalian cells). Protein synthesis is reduced and this causes pathogenic effects. In the case of potato spindle tuber viroid, there is a good correlation between strains pathogenicity and its ability to activate PKR in vitro.

Human pathologies induced by viroids

The only human disease known to be caused by a viroid is hepatitis D. This disease was previously ascribed to a defective virus called the delta agent. However, it now is known that the delta agent is a viroid enclosed in a hepatitis B virus capsid.

For hepatitis D to occur there must be simultaneous infection of a cell with both the hepatitis B virus and the hepatitis D viroid. There is extensive sequence complementarily between the hepatitis D viroid RNA and human liver cell 7S RNA, a small cytoplasmic RNA that is a component of the signal recognition particle, the structure involved in the translocation of secretory and membrane-associated particles. The hepatitis D viroid causes liver cell death via sequestering this 7S RNA and/or cleaving it.

1.10.6. **Transmission**

The hepatitis D viroid can only enter a human liver cell if it is enclosed in a capsid that contains a binding protein. It obtains this from the hepatitis B virus. The delta agent then enters the blood stream and can be transmitted via blood or serum transfusions.

1.11.**Properties of Prions**

1.11.1. **Introduction**

Prions were first discovered in the 1960's by radiation biologist Tikvah Alper and the mathematician John Stanley Griffith who found that the spongiform encephalopathies in cattle were mainly caused by protein infective agents. In 1982, Stanley B. Prusiner purified the hypothetical infectious agent and coined the name "prion" for this protein molecule. All known prion diseases affect the structure of the brain or other neural tissue and all are currently untreatable and universally fatal. In 2013, a study revealed that 1 in 2,000 people in the United Kingdom might harbour the infectious prion protein that causes vCJD.

1.11.2. **Definition**

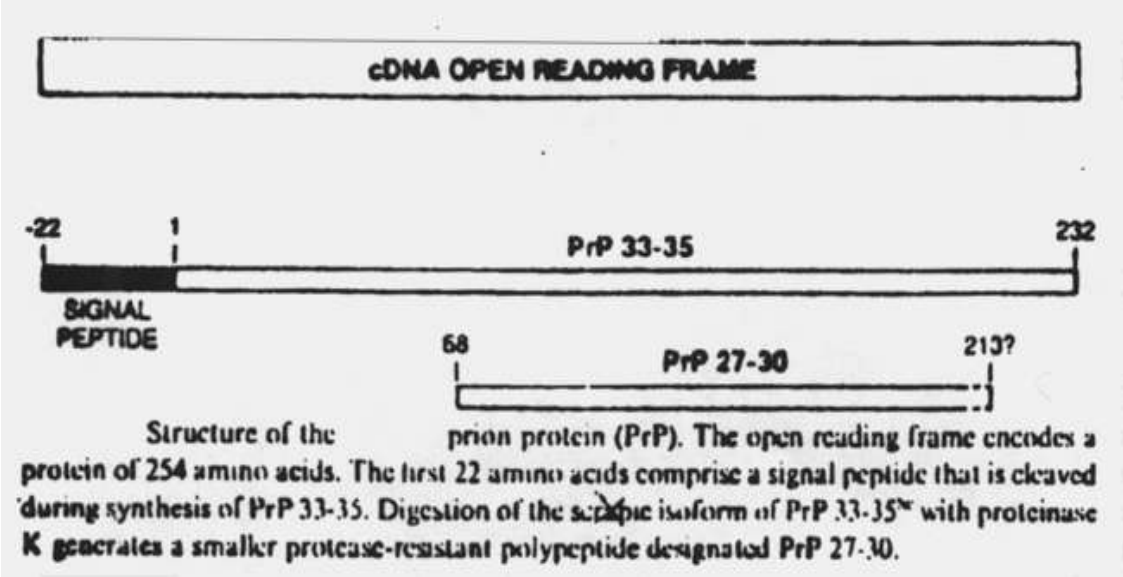
A prion is a small infectious particle composed of abnormally folded protein that causes progressive neurodegenerative conditions. These mis-folded proteins do not multiply in the host organism that they infect. Instead they affect the brain structure by acting as a template, inducing proteins with normal folding to convert to the abnormal prion form.

1.11.3. **Structure**

Prions are infectious agents composed exclusively of a single sialoglycoprotein called PrP 27-30. They contain no nucleic acid. PrP 27-30 has a mass of 27,000 - 30,000 daltons and is composed of 145 amino acids with glycosylation at or near amino acids 181 and 197. The carboxy terminus contains a phosphatidylinositol glycolipid whose components are ethanolamine, phosphate, myoinositol and stearic acid. This protein polymerizes into rods possessing the ultra structural and histochemical characteristics of amyloid.

Amyloid is a generic term referring to any optically homogenous, waxy, translucent glycoprotein; it is deposited intercellularly and/or intracellularly in many human diseases such as:

- Alzheimer's disease
- Creutzfeldt-Jakob disease
- Down's syndrome
- Fatal familial insomnia
- Gerstmann-Straussler syndrome
- Kuru Leprosy



Structure of the prion protein (PrP). The open reading frame encodes a protein of 254 amino acids. The first 22 amino acids comprise a signal peptide that is cleaved during synthesis of PrP 33-35. Digestion of the scrapie isoform of PrP 33-35^{sc} with proteinase K generates a smaller protease-resistant polypeptide designated PrP 27-30.

Fig: Structure of a Prion protein

1.11.4. **Replication**

The prion is a product of a human gene, termed the PrP gene, found on chromosome 20. This gene contains two exons separated by a single intron. Exon I and Exon II are transcribed and the two RNAs ligated into a single mRNA. This mRNA contains an open reading frame (ORF) or protein coding region which is translated into the PrP protein. The PrP protein is a precursor of the prion protein. It is termed PrP 33-35.

The PrP 33-35 undergoes several post-translational events to become the prion protein (PrP 27-30):

1. Glycosylation - at two sites.
2. Formation of a disulfide bond between two cysteine residues.

3. Removal of the N-terminal signal peptide.
4. Removal of the C-terminal hydrophobic segment.
5. Addition of a phosphatidylinositol glycolipid at the C-terminal.
6. Removal of the N-terminal first 57 amino acids.

In normal cells only the PrP 33-35 protein is synthesized. It is found in the neural cell membrane where its function is to sequester Cu++ ions. In abnormal ("infected") cells, the PrP 27-30 is produced from the PrP 33-35 protein. The PrP 27-30 triggers a series of reactions that produce more PrP 27-30 proteins, i.e., PrP 27-30 induces its own synthesis. In addition to the post translational modifications, the PrP 27-30 protein differs from the PrP 33-35 protein in a single amino acid residue. Residue 178 in the PrP 27-30 contains an asparagine residue whereas the PrP 33-35 protein has an aspartate residue at this position. This causes a conformational change in the PrP 27-30 protein from an a-helix to a b-sheet. This conformational change in the PrP 27-30 protein has three effects:

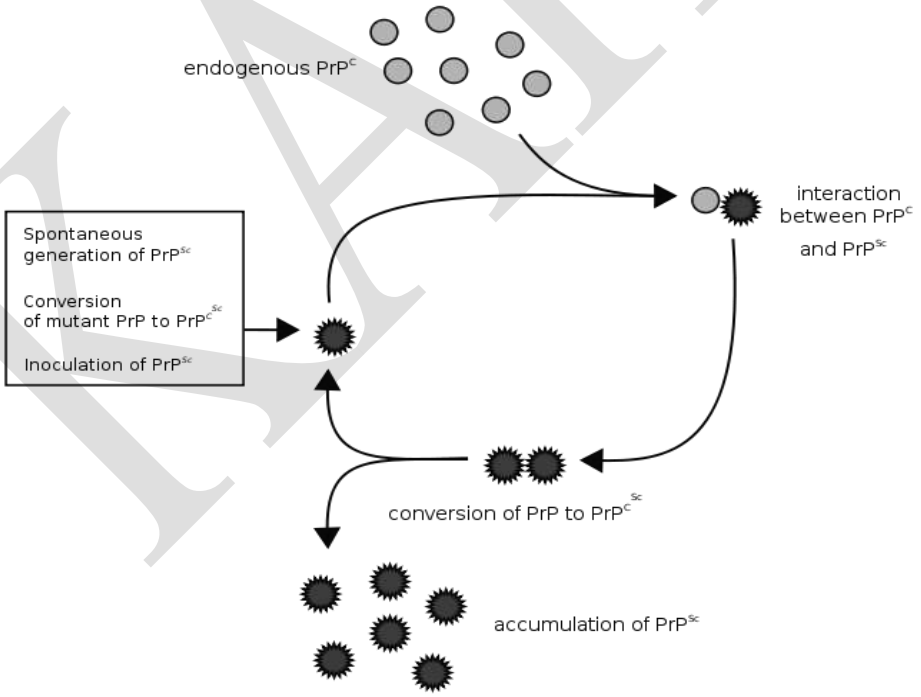


Fig: Replication of Prions

1. It imparts to the PrP 27-30 protein the ability to induce the same a-helix to b-sheet conformation in the PrP 33-35protein. This is a permanent conformational change. It thus induces its own "replication."

2. The b-sheet-forming peptides aggregate to form amyloid fibrils.

3. The amyloid fibrils kill thalamus neurons through apoptosis, a programmed series of events that leads to cell death.

1.11.5. **Human Pathologies induced by prions**

All diseases known to be of prion etiology, in animals and humans, are neurodegenerative diseases. In the human this includes:

- Creutzfeldt-Jakob disease (CJD)
- Fatal Familial Insomnia
- Gerstmann-Straussler syndrome
- Kuru

The pathological and clinical signs of these diseases suggest that they are closely related. In fact they may be variants of the same disorder. All pathological features are confined to the central nervous system. The prion protein accumulates selectively and abnormally in CNS nerve cells during the course of the disease. PrP 27-30 accumulates within the neuropil where it causes:

1. Astrocyte gliosis (an increase in the number of astrocytes).
2. Depletion of dendritic spines in neurons.
3. Formation of numerous vacuoles in the cerebellar cortex (spongiform encephalopathy).
4. Amyloidosis - deposition of amyloid in the cerebellar cortex, thalamus, brain stem and in the lumen of blood vessels within the brain.

1.11.6. **Transmission**

Spread of the disease is via horizontal transmission, i.e., transmission from one person to another, either directly or by fomites or by ingestion of contaminated meat.

1.11.7. **Diagnosis**

- Examination of brain biopsies taken from patients in advanced stages of the disease
- An immunofluorescence test on tonsilar biopsies.

- Based on a Western blot.
- Detect the prion protein in blood.

4.6.Plant RNA Viruses –Satellite viruses

4.6.1. **Introduction**

Kasinis in 1962, described the first satellite viruses. Satellite viruses are infectious particles that are capable of infecting bacteria, plants, fungi, and animals. They code for their own protein capsid, however they rely on a helper virus in order to replicate. Satellite viruses cause plant diseases by interfering with specific plant gene activity. In some instances, plant disease development is dependent upon the presence of both the helper virus and its satellite. While satellite viruses alter the infectious symptoms caused by their helper virus, they do not influence or disrupt viral replication.There are also viruses which depend for their replication on helper viruses: a good example is tobacco necrosis satellite virus (sTNV), which has a small piece of ssRNA which codes only for a capsid protein, and depends for its replication on the presence of TNV. Another good example is the hepatitis delta agent with its circular ssRNA genome. The adeno-associated viruses (AAVs) are also satellite viruses dependent on the linear dsDNA adenoviruses for replication, but which have linear ssDNA genomes and appear to be degenerate or defective parvovirus.

4.6.2.*Morphology*

Satellites are sub-viral agents composed of nucleic acid molecules that depend for their replication on co-infection of a host cell with a specific helper virus. Nucleotide sequences are substantially distinct from those of the genomes of the helper virus and of the host. Replication of the helper virus is often decreased and virus symptoms may be modified. Satellite viruses encode their own coat protein (unlike satellite nucleic acids) and all known members contain ssRNA. In all cases the satellite virus particles are antigenically, and usually morphologically, distinct from those of the helper virus.Virions are isometric, 17 nm in diameter, associated with the helper virus.

The infection is transmitted by

- Mechanical transmission through sap by plants touching one another, through root grafts, and manhandling.
- Vegetative propagation and grafting.
- Seed, pollen, mites, nematodes, dodder, fungi (carried by zoospores and mycelium) and insects (aphids, leafhoppers, scale insects, thrips, grasshoppers, beetles, whiteflies). For example, cucumber mosaic virus and barley yellow dwarf virus moved by aphids.

4.6.3.*Genome*

Satellite viruses are small viruses with either ssRNA or ssDNA as their genomic material that require another virus to replicate. There are two types of DNA satellite viruses – the alpha satellites and the

beta satellites – both of which are dependent on begomaviruses. At present satellite viruses are not classified into genera or higher taxa.

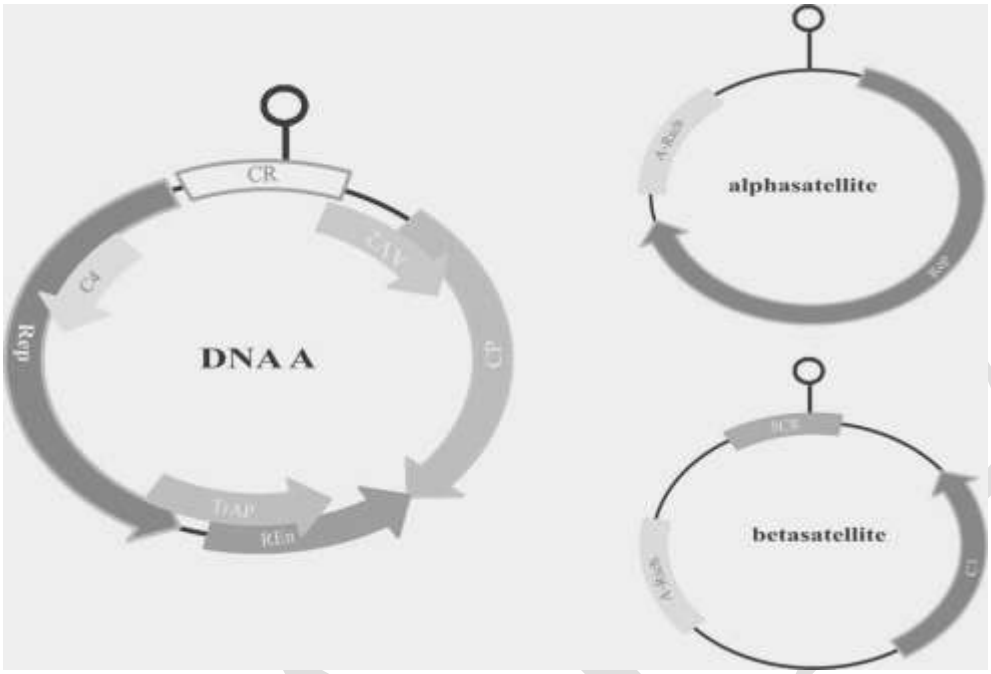


Fig: Genome organization of monopartite begomoviruses-satellite complex.

DNA-A (encoding replication-associated protein [Rep], coat protein [CP], replication enhancer protein [REn], transcriptional activator protein [TrAP] and proteins possibly involved in virus movement [AV2], pathogenicity determinant and a suppressor of RNA silencing [AC4], viral genome replication [AC5]) Alpha satellites are self-replicating molecules encoding their own Rep. Beta satellites are dependent on their helper viruses for their replication and encode a single protein, βC1, which up regulate replication of helper virus and suppress host defense. Both satellites have an A-rich region and in addition to this beta satellites have a region of sequence conserved between all examples known as the satellite conserved region (SCR).

4.6.4.**Replication**

The result of a complex interaction among satRNAs, helper viruses, RNA silencing mechanisms, and the physiologically important genes of the host plants which could be regarded as a competition for the use, or targeting, of the RNA silencing systems of the host plants. Example scenarios depicting the consequences of the trilateral competition. If the host plants activate the RNA silencing system against the invading virus and satRNAs, the system can be considered an antiviral and anti-satRNA defense system. However, if the RNA silencing systems are programmed by satRNAs against mRNAs of host plants, the system can be considered as using the pathogenic factors of the satRNA to target the mRNAs of the physiologically important gene for degradation. In rare cases, the satRNAs may assist the helper viruses in counter-defending the RNA silencing pathways in plants.

A

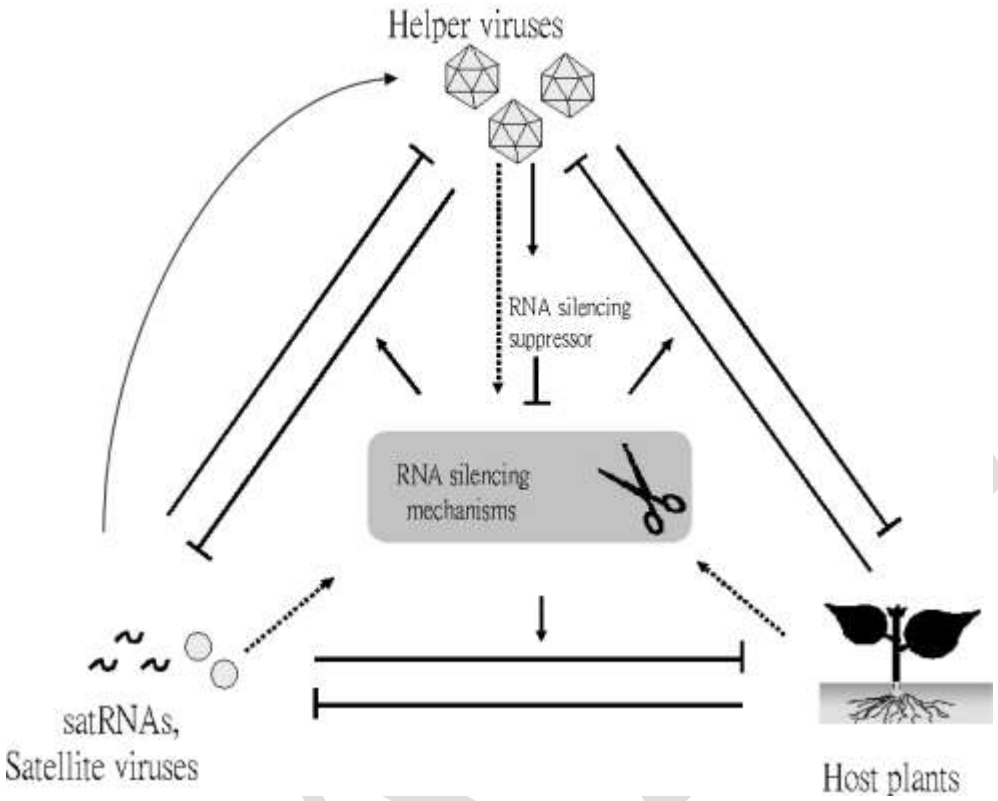


Fig: A. Replication of Satellite virus

4.6.5. **Symptoms**

In affected leaves, it shows yellow or green colour marking with reduction in its leave size.

4.6.6.**Pathogenesis**

There is a peculiar set of viruses, some of which infect bacteria and others infect higher organisms, termed satellite viruses. These viruses are unable to infect and replicate in a host by themselves, but rather depend on coinfection of the host with another related virus, the helper virus, for their multiplication. They enter the cell along with the helper virus causing a dual infection. However, satellites have lost the ability to produce some critical gene products, and the co infecting virus provides these proteins during its own infectious cycle. The satellites are literally parasites of the helper virus.

4.6.7. **Control**

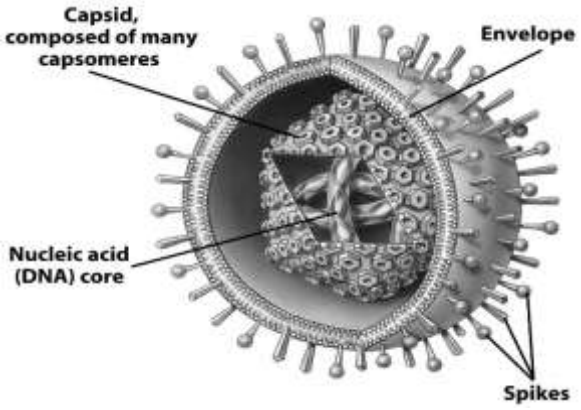
1. Milk inactivates many viruses - use milk to wash tools/hands. "Milk does a plant body good!" Soap and water work well too!
2. Removing diseased plants, killing and removing potential virus vectors (primarily weeds and insects).
3. Disease-resistant cultivars.
4. Disease or virus free seed, roots or tubers.
5. Cross protection (inoculation with a less-virulent strain of a virus protects the plant from a more virulent strain later when exposed to it).
6. Heat (some viruses are killed at temperatures that will not kill host). For example, dormant propagative organs dipped in hot water (35 C) for few minutes or hours, or by growing plants in greenhouse at 35-40 C for several days, weeks or months may inactivate virus.

Structure of Viruses

When a single virus is in its complete form and has reached full infectivity outside of the cell, it is known as a virion. Virion size range is ~10-400 nm. All virions contain a nucleocapsid which is composed of nucleic acid (DNA or RNA) and a protein coat (capsid). Some viruses consist only of a nucleocapsid, others have additional components

Viral components

- Viral Nucleic acids
- Viral Capsid
- Viral Envelope
- Viral Spikes



1.5.2. **Viral Capsid**:

It is the regular shell like structure composed of aggregated protein subunits which surrounds the viral Nucleic acid. The protein subunits are called protomers, and these are aggregated to form capsomeres and are further aggregated to form capsid.

1.5.2.1 ***Functions***:

It is the outermost shell of the virus and protects the fragile nucleic acid genome from three different damages:

- *Physical damage*: E.g. mechanical forces
- *Chemical damage*: E.g. Chemical and UV irradiation (from sunlight) leading to chemical modification.
- *Enzymatic damage*: E.g. Nucleases derived from dead or leaky cells or deliberately secreted by vertebrates as defence against infection.
- Furthermore, the outer surface of the virus is responsible for recognition of the host cell. Initially, this takes the form of binding of a specific virus-attachment prote into a cellular receptor molecule. However, the capsid also has a role to play in initiating infection by delivering the genome from its protective shell in a form in which it can interact with the host cell.

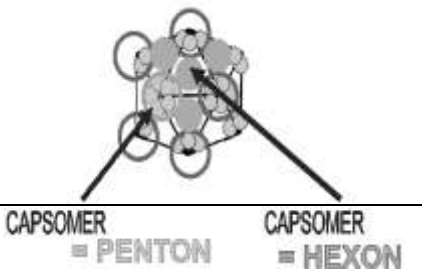
1.5.2.2.***Nucleocapsid***

The core of a virus particle consisting of the genome plus a complex of proteins

Symmetry of Nucleocapsid

1. Icosahedral symmetry
2. Helical symmetry-
3. Complex structureS

1. ***Icosahedral symmetry***

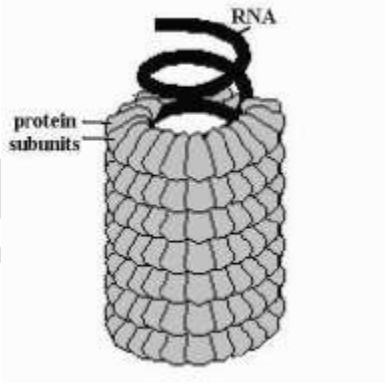


Composed of 12 vertices, has 20 faces (each an equilateral triangle) with the approximate outline of a sphere and two types of capsomer namely,

i. *Pentagonal capsomer*– They are present at vertices, touching 5 neighbours and are known as pentons.

ii. *Hexagonal capsomer*- They are present at facets, touching 6 neighbours and are known as hexons

- Naked icosahedral E.g. Poliovirus, Adenovirus, Hepatitis A virus
- Naked helical E.g. Tobacco mosaic virus, so far no human viruses with this structure known
- Enveloped icosahedra E.g. Herpes virus, Yellow fever virus, Rubella virus



2. *Helical symmetry*-

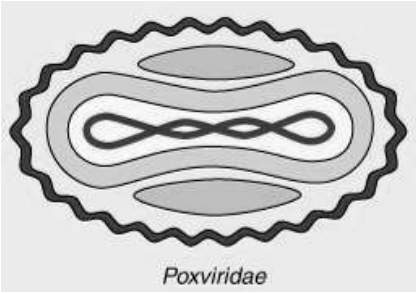
In which the capsomeres are arranged in a hollow coil that appears rod shaped. The helix can be either rigid or flexible.

- Enveloped helical. E.g. Rabies virus, Influenza virus, Para influenza virus, Mumps, Measles

3. *Complex structures*-

They never resemble icosahedral or helical capsid because of their structural complexity

E.g. Poxviruses



1.5.3. **Viral envelope**

It lies outside of the capsid and is made up of lipids bilayer, proteins and carbohydrates. It is also known as Peplomer in some viruses it is present as spikes.

1.5.3.1***Functions of Envelope***:

- It promotes interaction with nucleocapsid protein during assembly.
- It acts as viral attachment protein (VAP) during infection. This VAP binds to erythrocytes and is referred as haemagglutinin which is a major antigen.

Envelope Proteins:

Even though lipid envelope gives the protection to the nucleocapsid; it would not permit recognition of receptor molecules on the host cell. Therefore, viruses modify their lipid envelopes by the synthesis of several classes of proteins includes Matrix protein and glycoprotein which are associated in one of three ways with the envelope:

Matrix Proteins: These are internal virion proteins whose function is effectively to link the internal nucleocapsid assembly

Glycoproteins: These are transmembrane proteins, anchored to the membrane by a hydrophobic domain & can be subdivided into two types, by their function:

a. External Glycoproteins - Anchored in the envelope by a single transmembrane domain. Most of the structure of the protein is on the outside of the membrane, with a relatively short internal tail. Often individual monomers associate to form the 'spikes' visible on the surface of many enveloped viruses in the electron microscope. Such proteins are the major antigens of enveloped viruses.

b. Transport Channels - This class of proteins contains multiple hydrophobic transmembrane domains, forming a protein-lined channel through the envelope, which enables the virus to alter the permeability of the membrane, e.g. ion-channels.

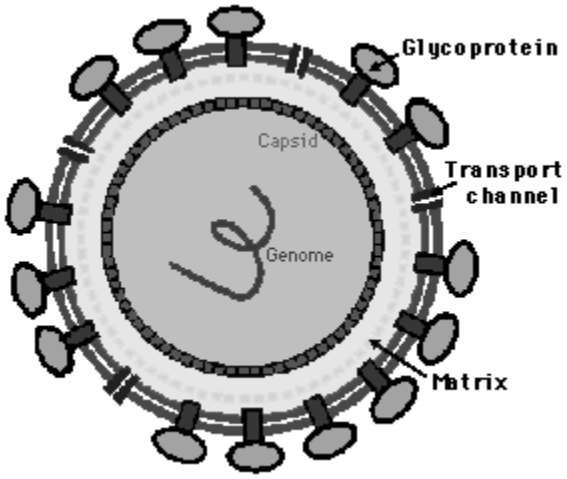


Fig: Viral Envelope

1.5.4. **Viral Spikes**

The glycoprotein projections arising from envelope are referred as Spikes

1.5.4.1.***Functions of Spikes***

- They have enzymatic adsorption and haem agglutinating activity.
- They are highly antigenic in nature.

1.7.Cultivation of viruses

As the viruses do not reproduce independent of living host cells, they cannot be cultured in the same as bacteria and eukaryotic microorganisms. However, the cultivation of viruses can be done in different ways

- Cultivation of animal viruses
- Cultivation of plant viruses
- Cultivation of bacteriophages.

1.7.1. Cultivation of Animal Viruses

(i) In Animal Cells

a. Human volunteers, Reed and colleagues- yellow fever.
b. Monkeys- Landsteiner & Popper (viruses.(1909) - polio viruses.

c. White mice- Theiler (1903).
d. Animals still in use
    1. Suckling mice
    2. Rabbits

Suitable living mammals (such as sheep or calves or rabbits) are selected for cultivation of viruses. The selected animals should be healthy and free from any communicable diseases. The specific virus is introduced into the healthy animals. The site of administration varies according to the type of virus is allowed to grow in the living animal. At the end of incubation period, the animals are slaughtered and washed thoroughly and viruses are obtained from them.

Advantages

a. Isolation of those viruses which do not grow in cell lines/eggslines/eggs
b. Understanding pathogenesis
c. immune responseimmune response
d. Test efficacy of vaccine/drugs

Disadvantages

a. Expensive
b. Difficult to handle
c. Maintenance-difficult
d. Show biological diversity
e. Presence of latent viruses
f. Pressure from animal friends groups

(ii) In Chick-Embryo

The animal viruses can be successfully cultivated using chick-embryo technique. In this method fertile hen eggs are selected. Eggs must not be more then 12 days old. To prepare the egg for virus cultivation, the shell surface is first disinfected with iodine and penetrated with a small sterile drill. After inoculation, the drill hole is sealed with gelatin and the egg is then incubated.

Several sites of inoculation

1. Amniotic cavity -primary isolation of Influenza and Mumps virus
2. Yolk sac - Rickettsiae , Chlamydia and Herpes Simplex Virus
3. Allantoic cavity- Influenza and Mumps virus
4. Chorioallantoic membrane (CAM)- Herpes Simplex Virus

Major Advantages

The major advantages of embryonated eggs over other systems are;

i) Easily available, economical and convenient to handle.

ii) Relatively free from bacterial infections.

iii) This system is the most suitable for production of viral vaccines.

Consequences of virus growth

The presence of virus may be regarded by;

i. Death of the embryo
ii. Dwarf growth
iii. Hemorrhages
iv. Oedema and pock lesions on CAM
v. Intracytoplasmic inclusion bodies

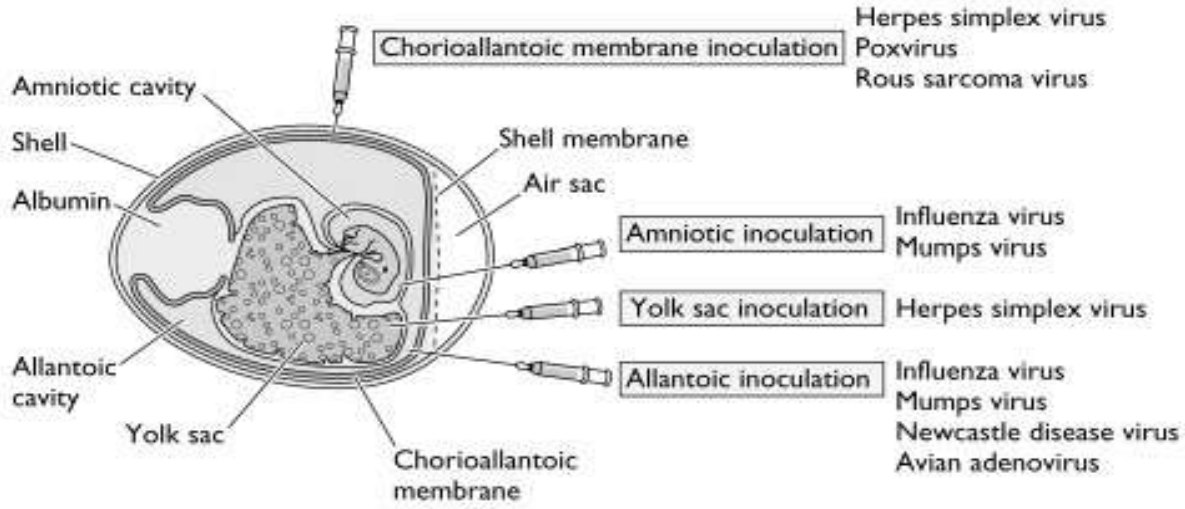


Fig: Several sites of inoculation in embryonated egg

(iii) In Cell Cultures

Organ Culture

Small bits of organs maintained *in vitro*, preserving their original architecture & function.

E.g. Tracheal ring organ culture - Corona virus.

Explant Culture

Fragments of minced tissue grown as explants embedded in plasma clots.

E.g. Adenoid tissue explant cultures - Adeno viruses.

Tissue Culture

Tissues are dissociated into component cells. Cells washed, counted, suspended in growth medium.

Constituents of growth medium:

a. Essential amino acids, vitamins, salt, glucose, a buffering system, fetal calf serum, antibiotics and Phenol red as indicator.
b. Most cell types multiply in this medium.
c. Cell suspension dispensed in bottles, tubes and Petri dishes.
d. Cells adhere to glass on incubation form a confluent monolayer sheet of cells - 1 week.

Based on origin, chromosomal characters & no. of generations through which they can be maintained,

Tissue cultures - 3 types.

a. Primary cell culture
b. Diploid cell culture
c. Continuous cell lines

a. *Primary cell cultures*

These are obtained directly from animal or human tissue by breaking the intercellular substance with proteolytic enzymes (trypsin, collagenase, pronase). Dissociated (dispersed) cells placed in a culture medium are capable of adhering to the surface of a culture vessel and of proliferating there. Since cells of most primary cultures remain viable for several generations, they may be repeatedly subcultured (passaged).

b. *Diploid cell strains*

Several passages may produce a diploid culture, i.e., a population of fibroblast-like cells which can be rapidly reproduced and endure 30 to 60 passages still retaining their initial sets of chromosomes.

c. *Continuous cell cultures*

This can be subcultured endlessly. They are derived from the primary cultures of cells due to their genetic variability during the growing process, rapidly become dominant in the cell population, and have chromosomal sets typical of all continuous cell lines. E.g., cervical carcinoma (HeLa) Continuous cell lines are stored in liquid nitrogen and thawed before use.

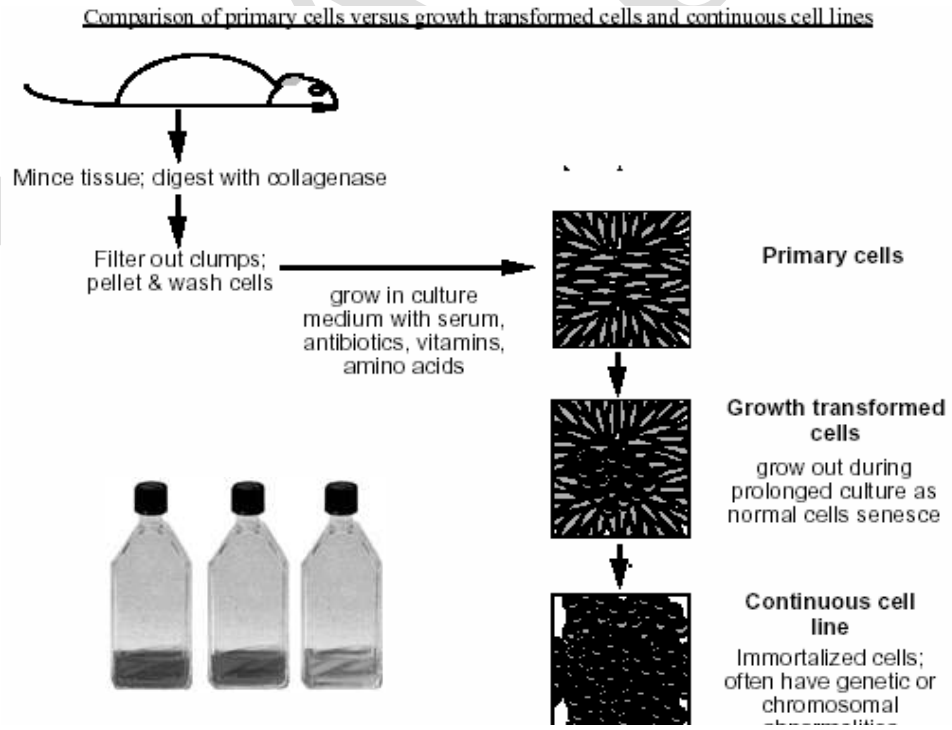


Fig: Cell cultures

1.7.2. Cultivation of Plant Viruses

There are several methods of cultivation of viruses such as plant tissue cultures, cultures of separated cells, or cultures of protoplasts, etc. Viruses also can be grown in whole plants. Leaves are mechanically inoculated by rubbing with a mixture of viruses and an abrasive such as carborundum. When the cell wall is broken by the abrasive, the viruses directly contact the plasma membrane and infect the exposed host cells. (The role of the abrasive is frequently filled by insects that suck of crush plant leaves and thus transmit viruses.) A localized necrotic lesion often develops due to the rapid death of cells in the infected area. Even when lesions do not arise, the infected plant may show symptoms such as change in pigmentation or leaf shape. Some plant viruses can be transmitted only if a diseased part is grafted onto a healthy plant.

1.7.3. Cultivation of Bacteriophages

Bacterial viruses of bacteriophages (phages for short) are cultivated in either broth or agar cultures of young, actively growing bacterial cells. Several host cells are destroyed that turbid bacterial cultures may clear rapidly because of cell lysis: Cultures are prepared by mixing the bacteriophage sample with cool, liquid agar and a suitable bacterial culture medium. The mixture is quickly poured into a petri dish containing a bottom layer of sterile agar. After hardening, bacteria in the layer of top agar grow and reproduce, forming a continuous, opaque layer or lawn. Wherever a virion comes to rest in the top agar, the virus infects an adjacent cell and reproduces. Finally, bacterial lysis generates a plaque or clearing in the lawn.

1.8. Detection of Viral growth in cell cultures

Detection of viruses in the cell culture is based on their cytopathic effect, electron microscopic identification of intracellular inclusions, immunofluorescence, and haemadsorption and also on interference and the "plaque" formation phenomena. A positive haemagglutination test indicates the presence of a virus in the culture fluid.

1. A cytopathic effect (CPE) represents degenerative cell alterations resulting from intracellular virus reproduction. It is manifested within the first days after cell culture inoculation with some viruses '(variola, polio, etc.) and much later (in 1-2 weeks) when others (adenoviruses, parainfluenza viruses, etc.) are used. The nature of CPE primarily depends on a virus species.

Monolayer cell degeneration is subdivided into total and partial.

Total degeneration due to such viruses as polio, Coxsackie and ECHO significantly affects monolayer cells, with great numbers of them sloughing off the slide.

The remaining separate cells are shrunken (nuclear and cytoplasmic pyknosis) and characterized by double refraction, i.e., strong fluorescence on microscopy.

Partial degeneration of cultured cells falls into several types:

a. *Racemose formation*: rounding, enlargement, and partial confluence of cells producing characteristic racemose aggregates. Degeneration of this type is caused by adenoviruses.

b. *Focal degeneration*: local cell injuries (microplaques) appearing against the background of a largely intact monolayer. This type of degeneration is induced by certain strains of variola and influenza viruses.

c. *Symplast formation*: virus-induced cell aggregation resulting in the formation of giant multinuclear cells (symplasts or syncytia). Symplast formation is caused by measles, mumps, parainfluenza, respiratory-syncytial, and herpes viruses Certainoncogenic viruses cause malignant transformation of cells provoking their intense proliferation, in other words *changes of a proliferative type.*

2. Intracellular inclusions occur when certain viruses are reproduced in cell nuclei and cytoplasm (variola, rabies, influenza, herpes viruses, etc.). They are detected by light microscopy after staining a monolayer-carrying slide with the Romanowsky-Giemsa solution or with other dyes, or by the luminescent microscopy, using acridine orange (1:20000).

Depending on a virus type, solitary virions or their crystalloid clusters can. be visualized in cell nuclei and cytoplasm with the electron microscope. A specific virus antigen can be detected in virus-infected cell cultures. using the direct immunofluorescence test.

3. Haemagglutination test is based on the ability of certain viruses to clump (agglutinate) red blood cells obtained from animals of definite species. Influenza andsome other viruses with supercapsid membrane contain the surface antigen haemagglutinin responsible for the erythrocyte agglutination.

4. Haemadsorption test makes it possible to reveal the virus before the onset of CPE due to the appearance of the virus-specific antigen (haemagglutinin) on the surface of an infected cell. After a period of incubation appropriate for a virus, 0.2 ml of 0.5 per cent erythrocyte suspension is added to the cell culture (both control and virus-infected) so that the monolayer is covered, and the culture is stored for 15-20 min at 4°, 20° or 37 °C (depending on virus properties). Then, the test tubes are shaken in order to remove unadsorbed erythrocytes, and erythrocyte clusters are counted on single cells or throughout the monolayer by low-magnification microscopy. Uninfected cells should carry no erythrocytes.

5. Plaque formation. Plaques, or negative virus colonies, are sites of virus-destroyed cells in the agar-coated monolayer. Infective virus activity is quantified by counting these colonies.

To obtain the plaques, different dilutions of virus suspension are streaked onto one-layer tissue cultures in Hat vials or Petri dishes and overlaid with a layer of nutrient agar; virus reproduction and CPE are thus confined to initially infected and adjacent cells. Sites of cell degeneration, i.e., plaques, are identified by staining the culture with neutral red which is either included in the composition of the agar layer or added immediately before reading the results. Consisting of dead cells, the plaques are not stained with neutral red and, therefore, are recognized as light spots on a pink-red cell monolayer.Other techniques of detecting virus plaques in cell cultures are also available, e.g., demonstration of plaque formation under a *bentonite layer.* Finely dispersed purified bentonite is added to a fluid nutrient medium, and the infected cell monolayer is immersed with this mixture. Because of adsorption of bentonite particles on cell surfaces, the monolayer becomes milk-coloured. At sites of virus reproduction (plaques), cells are not covered with bentonite, and are partially or completely stripped off the slide.

1.9. **Viral Assays**

1.9.1. **Different Assay method:**

Basically three methods are used in viral assays:

1. By counting the total virus particles

a. *Enumeration by Electron Microscope* - Direct particle count. Electron microscope. Mix virus with known number of latex particles, spray into EM grid, and count. This method counts total (including defective) particles. The Efficiency of Plating (EOP) is defined as the ratio of the number of viral particles (determined by electron microscopy) to the number of infectious units.

b. Hemagglutination titers - The most common indirect method of measuring the number of virus particles is the hemagglutination assay. Many animal viruses adsorb to the red blood cells of various animal species. Each virus particle is multivalent in this regard; that is, it can adsorb to more than one cell at a time.

2. By referring to the infectious virions only

a. **Quantal assay** - Quantal assays are less quantitative but more sensitive than plaque as-says. In plaque assays each plaque-forming unit (PFU) is counted as developing from a single infectious virus. In quantal assays a positive response may be caused by one or more infectious virus particles and thus quantitation is accomplished through inoculation of replicate samples and the use of most probable number statistics. These assays don't count the number of infectious virus particles present in an inoculum. To perform the assay;

Step1: Serial dilution of a virus inoculum is made and is inoculated into tubes containing cell monolayer.

Step2: After incubation, the incubated tubes are examined for virus infection i.e. by looking to the changes (e.g. CPE) in the inoculated cells, the titre of the inoculum is determined.

This method of **Reed** and **Muench** is widely used to calculate the 50% end point. The **titre** can be defined as the reciprocal of the dilution of the inoculum that infects 50% of the inoculated cells and the titre is expressed as the 50% tissue culture infective dose/TCID50. The titre expressed as the 50% lethal dose/LD50 tells us the infectivity of the inoculum that kills the 50% inoculated cells.

b. Quantitative assay - These assays quantify the number of virus particles in an inoculum.

a. Plaque assay

b. Pock assay

Plaque assay: Titer-concentration of a virus in a sample

Plaque: circular zone of infected cells that results from a single infectious particle. Plaques due to cell death

Assay method

a. Serial dilution of sample
b. Add diluted virus to cell monolayer
c. Incubate cultures at 37oC to allow adsorption of virus
d. Remove inoculum
e. Add overlay to culture (Culture media +agar or agarose)
f. Plaque results from a single virus

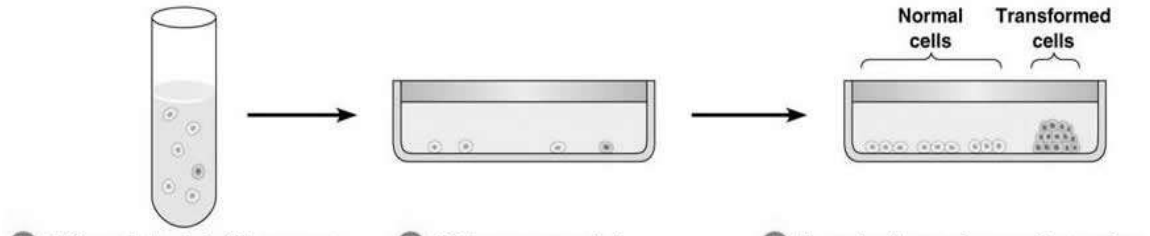


Fig: Viral assays

Pock Assay: Macroscopically recognizable foci or lesions on chorioallantoic membrane of developing chick embryo. Basically similar to a plaque assay. The principal of the assay is the detection of an infection resulting from a single virus particle, which will result in foci of infection due to the limitation in the sped of the progeny. First developed for bacterial viruses or phage the assay has been extended to include, with modification, both plant and animal viruses.

1.3. Classification of viruses

Classical virus classification schemes have been based on the consideration of four major properties of viruses:

i. The type of nucleic acid which is found in the virion (RNA or DNA)
ii. The symmetry and shape of the capsid
iii. The presence or absence of an envelope
iv. The size of the virus particle.

More recent classification systems adopted by the International Committee on Viral Taxonomy (ICTV) have really emphasized the viral genome as the primary determinant for viral taxonomy. Furthermore, there is a drift towards the use of genomics for virus classification – that is sequence analysis of the viral genome, and comparison to other known viral sequences.

1.3.1. Baltimore System for Virus Classification

The Baltimore system for virus classification is a system of classification which complements the ICTV classification system.

Based on genetic contents and replication strategies of viruses. According to the Baltimore classification, viruses are divided into the following seven classes:

i. dsDNA viruses
ii. ssDNA viruses
iii. dsRNA viruses
iv. (+) sense ssRNA viruses (codes directly for protein)

v. (-) sense ssRNA viruses
vi. RNA reverse transcribing viruses
vii. DNA reverse transcribing viruses

1.3.1.1. **Genetic Content of Viruses**

DNA viruses:

Almost all DNA viruses which infect animals contain double-stranded DNA. Exceptions include the *Parvoviridae* (e.g., parvovirus B19, adeno-associated virus) and the *Circoviridae* (these include the recently discovered TT virus, which may be related to the development of some cases of hepatitis).

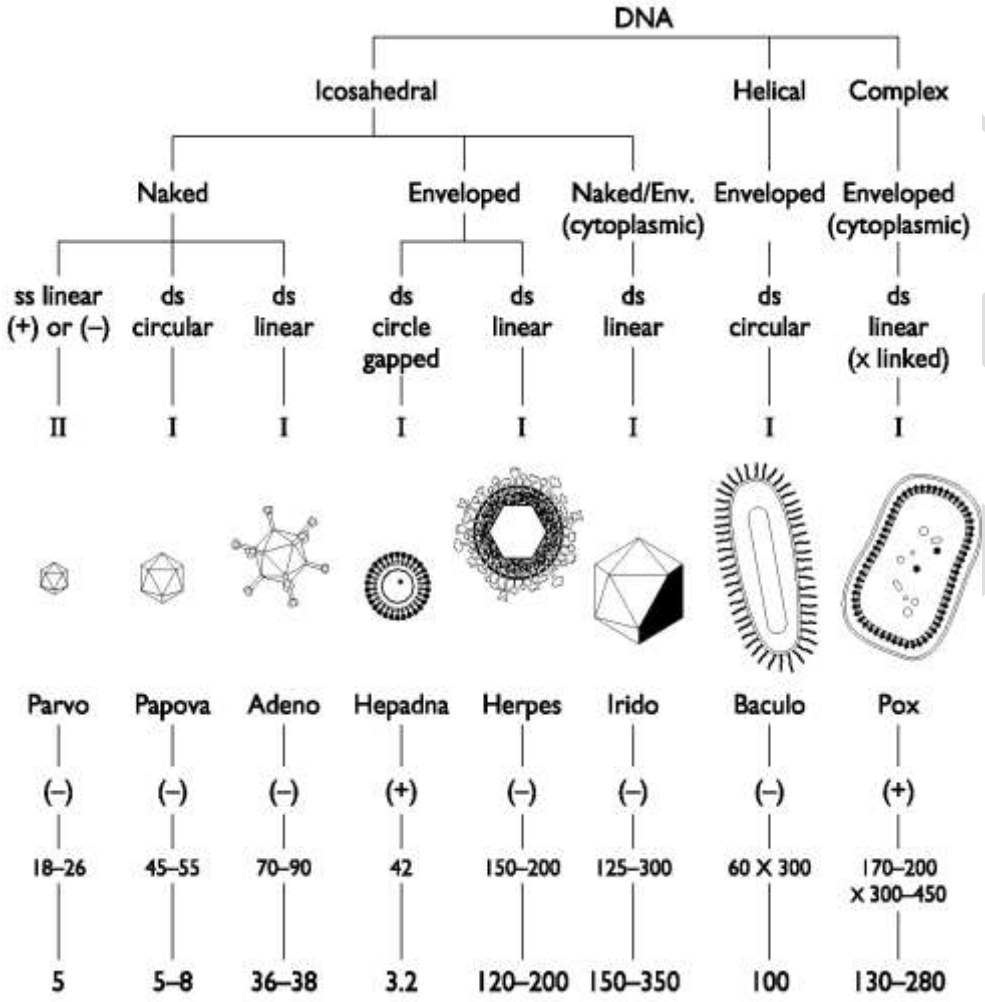
RNA viruses:

Almost all RNA viruses contain single-stranded RNA. Exceptions include the *Reoviridae* (e.g., rotaviruses) which contain double-stranded RNA. Other RNA viruses can be broadly subdivided as follows:

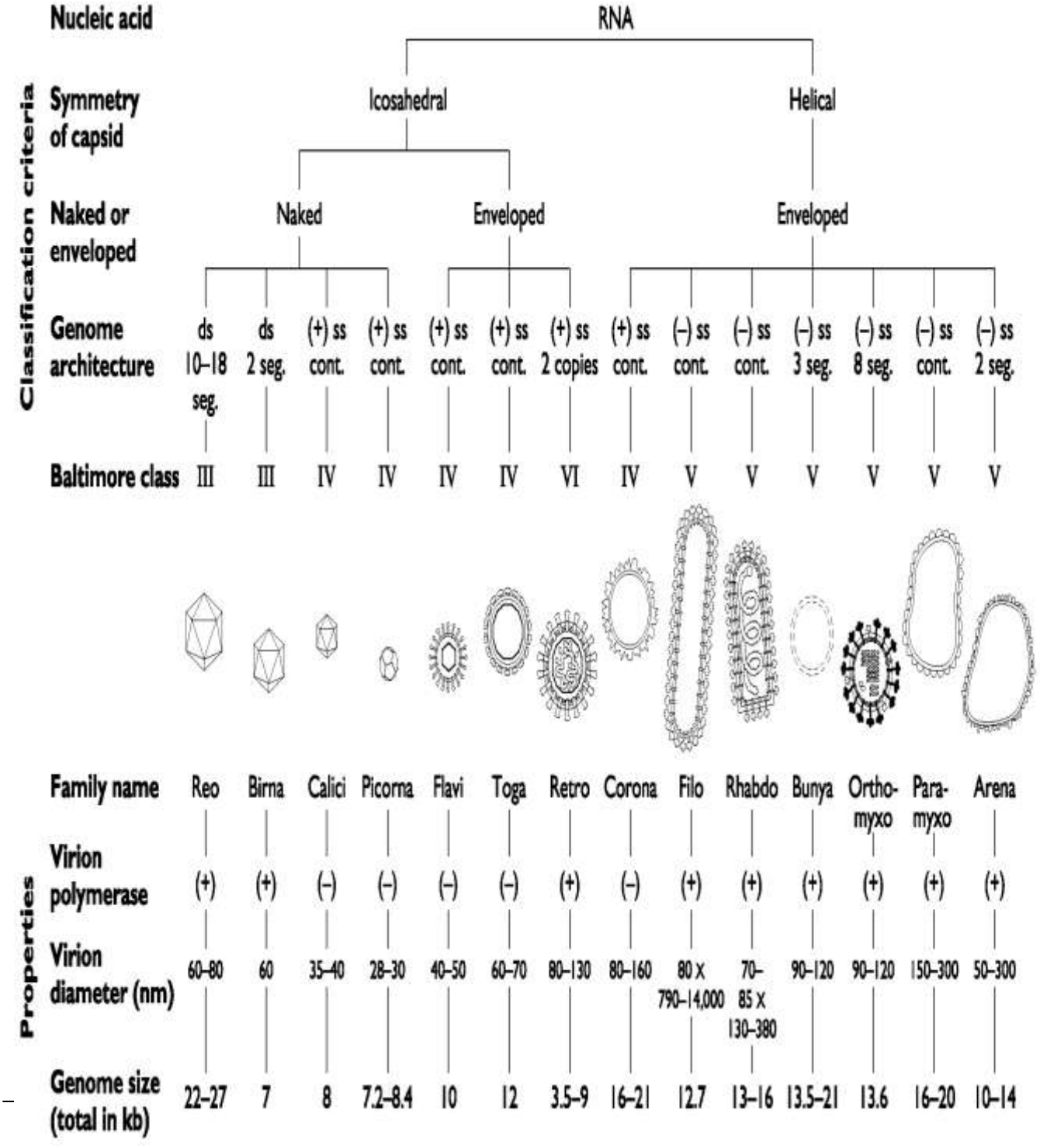
• *Viruses with positive strand (+) RNA genomes*– i.e., genomes of the same polarity as mRNA. Viruses in this category include picornaviruses and caliciviruses. In addition, retroviruses contain two copies of +RNA, although they replicate by a unique mechanism.

• *Viruses with negative strand (-) RNA genomes*– i.e., genomes of opposite polarity to mRNA. Viruses in this category all have helical capsids. Three members of the class are sufficiently closely related to comprise a distinct taxonomic order – the Mononegavirales (rhadboviruses, paramyxoviruses and filoviruses). The other (-) strand RNA viruses have segmented genomes (orthomyxoviruses have 8 segments while arenaviruses and bunyaviruses have either two or three segments, respectively.

DNA Viruses


DNA
Icosahedral
Helical
Complex
Naked
Enveloped
Naked/Env. (cytoplasmic)
Enveloped
Enveloped (cytoplasmic)
ss linear (+) or (–)
ds circular
ds linear
ds circle gapped
ds linear
ds linear
ds circular
ds linear (x linked)
II
I
I
I
I
I
I
I
Parvo
Papova
Adeno
Hepadna
Herpes
Irido
Baculo
Pox
(–)
(–)
(–)
(+)
(–)
(–)
(–)
(+)
18–26
45–55
70–90
42
150–200
125–300
60 X 300
170–200 x 300–450
5
5–8
36–38
3.2
120–200
150–350
100
130–280

RNA VIRUSE


Classification criteria
Nucleic acid
RNA
Symmetry of capsid
Icosahedral
Helical
Naked or enveloped
Naked
Enveloped
Enveloped
Genome architecture
ds 10–18 seg.
ds 2 seg.
(+) ss cont.
(+) ss cont.
(+) ss cont.
(+) ss cont.
(+) ss 2 copies
(+) ss cont.
(–) ss cont.
(–) ss cont.
(–) ss 3 seg.
(–) ss 8 seg.
(–) ss cont.
(–) ss 2 seg.
Baltimore class
III III IV IV IV IV VI IV V V V V V V
Properties
Family name
Reo Birna Calici Picorna Flavi Toga Retro Corona Filo Rhabdo Bunya Ortho-myxo Para-myxo Arena
Virion polymerase
(+) (+) (–) (–) (–) (–) (+) (–) (+) (+) (+) (+) (+) (+)
Virion diameter (nm)
60–80 60 35–40 28–30 40–50 60–70 80–130 80–160 80 x 790–14,000 70–85 x 130–380 90–120 90–120 150–300 50–300
Genome size (total in kb)
22–27 7 8 7.2–8.4 10 12 3.5–9 16–21 12.7 13–16 13.5–21 13.6 16–20 10–14

Unit I Question	Opt 1
Viruses are_____intracellular parasites.	Obligate
Who discovered TMV.	Ivanosky and Beijerinck
Which year HIV was discovered	1985
Virus means____	pellet
The complete protein-nucleic acid complex is called as_____	capsid
Virus which requires second virus for its replication is called as_____	defective virus
Infectious virus particle	virion
Virus is classified based on _____-	DNA
_____ classified virus into seven classes	David Baltimore
Virus replicates by _____ mechanism	host
Virus is sensitive to _____	Interleukins
_____mice is used for viral inoculation	young
_____ is primary detection of virus	CFE
Yolk sac inoculation is used for which virus cultivation.	HIV
_____ ring culture is done for isolation of Corona virus	lung
At which stage, burst time is calculated	attachment
Advantage of Lytic cycle is/are	replication is fast
_____ cells are used for traditional viral culture	human cells
How viruses are purified	centrifugation
Which assay is used for HIV detection	Transformation assay
What RBCs are used in haemagglutination assay?	human
Which metal is chelated in BCA	Cs
_____blotting is used for detection of RNA	western
Which enzyme plays a key role in PCR.	ligase
Real time PCR analysis id performed with help of process called____	RIA
The upcoming powerful technology is _____	Biotechnology
Plaque formation can be seen from _____	5 to 10 days
_____ membrane is used in blotting	cellulose
qPCR means_____	quantification PCR
TCID 50 means_____	time consumed infective do
For propagation, viruses depend on_____cells	Host
Monopartite genomes means	one nucleic acid
Proteins associated with nucleic acid is called as	Proteins
Envelope comes from____	virus
Single type of capsomeres stacked around a central axis form a _____	capsid
Virus which requires second virus for its replication is called as_____	defective virus
Size of Filo virus is	80 & 400 nm
Virus is classified based on _____	DNA
_____ symmetry is seen in animal virus	Helical
Capsomers o the triangular faces are surrounded by six others are call	tetrads
Minimum number of identical capsomeres required is_____	15
Which virus is isolated from Chile and Australia	Mega
_____ is size of ss linear DNA	4 - 6 kb
How many non- structural proteins codes for functional in virus trans	5-8

How many structural proteins will a make up a capsid	3 or 2
What is another name of antigenic shift	conjuction
What type of mutation occurs in viral genomes	reverse
Viruses can enter cells via_____.	penetration
During Uncoating stage cellular _____enzymes digest the ca	lytic
_____ may be produced during viral replication when the ho	Prions
What is the most common cause of aseptic meningitis of viral etiolog	Entero viruses
Protection against influenza A virus in a non immune individual can l	Viral endonuclease activity
Which one of the following immunizations should be administered in	Diphtheria-pertusis-tetanus
Which one of the following infection routes is most often involved in	Blood transfusion
The finding of large, multinucleated, clumps of cells in the bronchial	Bordetella pertusis
All of the following picornaviruses are resistant to the acidity of the s	Coxsackievirus A
A divorced mother of four tests positive for HIV-1 Infection during in	Treatment with zidovudine (
In a chronic carrier of hepatitis B virus (HBV), which positive test is	Hepatitis B Surface Antigen
A retrovirus is found in a high proportion of laboratory animals of a g	gag
A sexually active 22 year old college student presents to the local clin	A prolonged period of viren
Viruses range in size from:	1-100 nm

Opt 2	Opt 3	Opt 4
aerobic	anaerobic	Facultative
Twort and Felix	Edward Jenner	Louis Pasteur
1965	1997	1983
poison	protein	incomplete
protein coat	nucleocapsid	nucleic acid
direct virus	temperate virus	Provirus
viriod	prion	capsid
RNA	DNA & RNA	Host
Edward jenner	Montangier	Felix
own	cell	Direct
Interferons	antivirals	Antitumours
trickling	suckling	Old
CPE	CDE	CHE
Pox	HSV	Influenza
liver	trachea	Kidney
biosynthesis	uncoating	release
replication is slow	host remains live	Generation passes on
kidney cells	human cells & kidney cell	Plant cells
sedimentation	concentration	Dilution
Endpoint dilution assay	MAGI assay	ELISA
monkey	sheep	Dog
Mg	Cu	Ag
southern	northern	eastern
protease	polymerase	helicase
HIA	FRET	SRID
molecular technology	Microarray technology	immuno technology
3 to 10 days	3 to 5 days	3 to 14 days
nitrocellulose	sulfocellulose	ferricellulose
qualifying PCR	quantitation pCR	quality PCR
Tissue culture infectious dose	Tissue culture infective do	time consumed infectious
other	own	neighbour
two nucleic acid	multiple nucleic acid	no nucleic acid
Nucleous	Nucleoproteins	Capsid
host	protein	nucleic acid
protein coat	nucleocapsid	nucleic acid
helical structure	complex virus	Provirus
18 & 40 nm	80 & 40 nm	18 & 400 nm
RNA	DNA & RNA	Host
Icosahedral	radical	spiral
trions	hexons	pentons
5	12	10
Mimi	Pandora	Alien
1.7 - 2.3 kb	5.1 - 7.8 kb	6 - 7.8 kb
5-6	5-9	5-7

3 or 1	3 or 4	3 or 5
Assortment	recombination	reassortment
frame shift	point	active
adsoption	Entry	absorption
lipolytic	proteolytic	digestive
Virions	Pseudovirions	viriods
Herpes viruses	Arbo viruses	Retroviruses
binding of host messenger RN	Synthesis of viral progeny	Uncoating of nucleic acid
Haemophilus influenzae type	Hepatitis B vaccine	HIV Vaccine
Fetal contact with infected blo	Ingestion of the virus via	Transmission of the virus
Epstein-Barr virus	Mycoplasma hominis	Respiratory syncytial virus
Coxsackievirus B	Echo virus	Rhinovirus
All the patients close contacts	A western blot (immunob	The patient should be reass
Hepatitis B Core Antigen (Hb	Hepatitis B e Antigen (Hb	Anti-HBs Ag
pol	env	onc
A second infection with a sim	failure of the patient to co	reactivation of a latent infe
25-300 nm	10-100 μm	400-1000 nm

Opt 5	Answer
	Obligate
	Twort and Felix
	1965
	poison
	nucleocapsid
	defective virus
	virion
	DNA & RNA
	David Baltimore
	host
	Interferons
	suckling
	CPE
	Pox
	trachea
	release
	replication is fast
	human cells & kidney cells
	centrifugation
	MAGI assay
	sheep
	Cu
	northern
	polymerase
	FRET
	Microarray technology
	3 to 14 days
	nitrocellulose
	quantitation pCR
dose	Tissue culture infectious dose
	Host
	one nucleic acid
	Nucleoproteins
	host
	capsid
	defective virus
	80 & 400 nm
	DNA & RNA
	Icosahedral
	hexons
	12
	Pandora
	4 - 6 kb
	5-6

	3 or 2
	reassortment
	point
	adsoption
	proteolytic
	Pseudovirions
	Herpes viruses
	Viral endonuclease activity
	Diphtheria-pertusis-tetanus (DPT) vaccine
from hospital personnel during	Fetal contact with infected blood during childbirth
(RSV)	Respiratory syncytial virus (RSV)
	Echo virus
sured and told that her disease	Treatment with zidovudine (azidothymidine, AZT)
	Hepatitis B Surface Antigen (Hbs Ag)
	gag
ection.	A prolonged period of viremia following the initial infection
	25-300 nm

Unit 2

Structure and Properties of Bacteriophage T4

Structure and Properties

Bacteriophage (phage) are obligate intracellular parasites that multiply inside bacteria by making use of some or all of the host biosynthetic machinery (i.e., viruses that infect bacteria.).

The basic structural features of Bacteriophage T4 include,

1. *Size* - T4 is among the largest phages; it is approximately 200 nm long and 80-`100 nm wide. Other phages are smaller. Most phages range in size from 24-200 nm in length.

2. *Head or Capsid* - All phages contain a head structure which can vary in size and shape. Some are icosahedral (20 sides) others are filamentous. The head or capsid is composed of many copies of one or more different proteins. Inside the head is found the nucleic acid. The head acts as the protective covering for the nucleic acid.

3. *Tail* - Many but not all phages have tails attached to the phage head. The tail is a hollow tube through which the nucleic acid passes during infection. The size of the tail can vary and some phages do not even have a tail structure. In the more complex phages like T4 the tail is surrounded by a contractile sheath which contracts during infection of the bacterium. At the end of the tail the more complex phages like T4 have a base plate and one or more tail fibers attached to it. The base plate and tail fibers are involved in the binding of the phage to the bacterial cell. Not all phages have base plates and tail fibers. In these instances other structures are involved in binding of the phage particle to the bacterium.

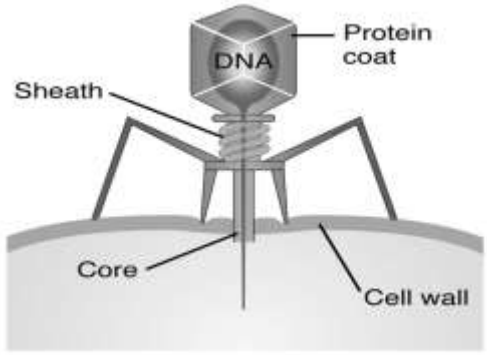


Fig: Structure of Bacteriophage T4

4.9.2. **Replication**

1. Adsorption – T4 adheres with tail fibers to lipopolysaccharide and tryptophan receptors on the bacterial host. The phage particle undergoes a chance collision at a chemically complementary site on the bacterial surface, then adheres to that site by means of its tail fibers.

2. Penetration – the phage injects its DNA into the bacterial cell. The tail sheath contracts and the core is driven through the wall to the membrane. This process is called penetration and it may be both mechanical and enzymatic. Phage T4 packages a bit of lysozyme in the base of its tail from a previous infection and then uses the lysozyme to degrade a portion of the bacterial cell wall for insertion of the tail core. The DNA is injected into the periplasm of the bacterium, and generally it is not known how the DNA penetrates the membrane.

3. Replication – Immediately after injection of the viral DNA there is a process initiated called **synthesis of early proteins**. This refers to the transcription and translation of a section of the phage DNA to make a set of proteins that are needed to replicate the phage DNA. Among the early proteins produced are a repair enzyme to repair the hole in the bacterial cell wall, a DNAase enzyme that degrades the host DNA into precursors of phage DNA, and a virus specific DNA polymerase that will copy and replicate phage DNA. During this period the cell's energy-generating and protein-synthesizing abilities are maintained, but they have been subverted by the virus. The result is the synthesis of several copies of the phage DNA.

   The next step is the synthesis of late proteins. Each of the several replicated copies of the phage DNA can now be used for transcription and translation of a second set of proteins called the **late proteins**. The late proteins are mainly structural proteins that make up the capsomeres and the various components of the tail assembly. Lysozyme is also a late protein that will be packaged in the tail of the phage and be used to escape from the host cell during the last step of the replication process.

4. Assembly – Once late genes are expressed, the (1) viral base plate is first assembled, this then attaches to the (2) tail and (3) tail fiber proteins. These three different protein pathways combine to form a mature T4 phage capsid. DNA is packaged into the mature capsid protein by packaging and cutting the concatemers using a terminase complex found at the end of the concatemerized DNA strand. The terminase complex binds to the capsid head and moves DNA into the empty capsid head. The capsid also encases necessary enzymes for future infections such as virally encoded DNA polymerase.

5. Release – The viral encoded enzyme holin (gpt) creates the holes in the in membrane of the bacterial host-cell to allow lysozymes to exit and degrade the peptidoglycan cell wall. Cell lysis subsequently follows releasing a shower of bacteriophage progeny into the extracellular space.

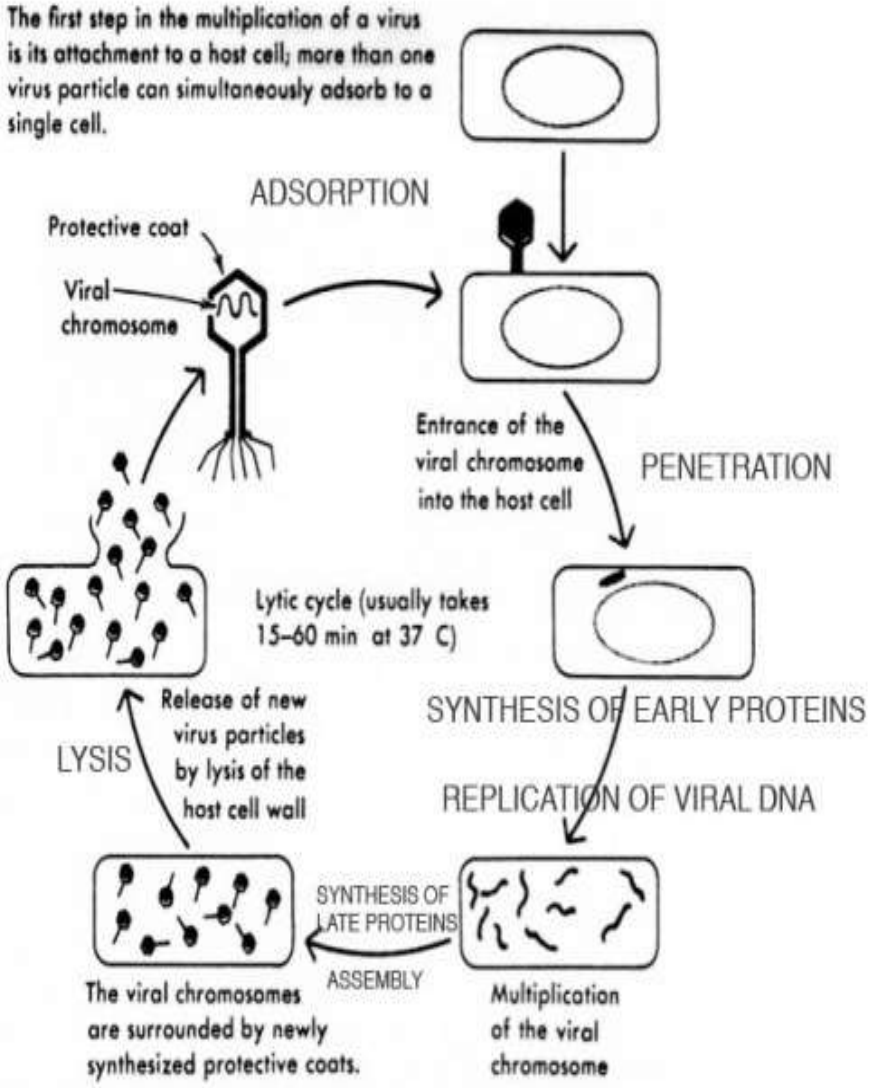


Fig: Replication of Bacteriophage T4

Unit II Question
A structural component that is found in all viruses is:
A chemical component that is found in all viruses is:
A common polyhedral capsid shape of viruses is a :
Enteroviruses differ from rhinoviruses mainly in their:
Viruses that can remain latent (usually in neurons) for many years are
What types of viruses contain the enzyme lysozyme to aid in their inf
Bacteriophage is readily counted by the process of:
A type of cell culture that can reproduce for an extended number of g
Which of the following is not an RNA virus?
Which of the following disinfectant is effective against viruses?
Viruses largely lack metabolic machinery of their own to generate en
Viruses require _____ for growth.
Reverse transcriptase is a useful enzyme to have when
The sequence of nucleic acid in a variety of viruses and viral host, wi
When a virus enters a cell but does not replicate immediately, the situ
Viruses are separated into several large groups based primarily on
The first step in infection of a host bacterial cells by a phage is
Which of the following viruses has not been associated with human c
The viral nucleocapsid is the combination of
Edward Jenner began inoculating humans with material from _____
The viruses in an attenuated vaccine
Enveloped viruses have a _____ shape.
The envelope of which of the following viruses is derived from the h
Which of the following is semi-continuous (diploid) cell line?
Plant viruses may be cultivated in
The oncogene theory refers to
In cell culture, measles virus may lead to
A change from lysogeny to lysis is generally not induced by
The viral DNA is removed from the host's chromosomes and the lytic
The lysogenic state is governed by the activity of the regulatory regio
The capsomeres consist of a number of proteins subunits or molecule
In order for a virus to replicate
Which of the following viruses belong to family Flaviviridae?
Which of the following viruses show/s transformation of infected ce
Which of the following may affect proteins and nucleic acids, but not
The viral DNA of the temperate phage, instead of taking over the fun
Which of the following statements is not true of viruses?
Which of the following viruses belong/s to family caliciviridae?
In the simplest capsid, there is a capsomere at each of the 12 vertices
The size of viruses is usually measured in
The temperate phage that have no site specificity for insertion and ma
Enzyme neuraminidase is carried by which of the following viruses?
Lysozyme (an endolysin) which will lyse the bacterial cell, releasing
Which of the following is continuous cell line?

The repressor protein, since the cell is resistant to lysis from external
Which of the following virus is susceptible to chloroform?
Group E phages have
The temperate phage possesses a gene that codes for a repressor prote
The bacterial viruses having head made up of large capsomeres, but n
The process by which phage reproduction is initiated in lysogenized c
Area of lysis on a bacterial lawn culture produced by a phage is know
The procapsid is assembled with the aid of _____ proteins.
Which of the following is/are synthesized from late mRNA?
Which capsid symmetry is exhibited by most of the phages?
Contractile sheath of the tail is present in which of the following pha
One of the first enzymes synthesized by many bacteriophage is _____
In viruses with envelopes
Which of the following bacteria can be typed by phage typing metho
_____ protein keeps the prophage dormant and prevents virus repr

Opt 1	Opt 2
The envelope	DNA
Protein	Lipid
Pentagon	Cube
Type of nucleic acid	Size
. Toga viruses	Herpes viruses
Bacteriophage	Animal Viruses
Immunoassays	ELISA
Primary cell culture	Continuous cell line
Retrovirus	Entero virus
Hydrogen peroxide	Hypochlorite
protein	carbohydrate
bacteria	plants
an RNA virus converts its RNA to D	there are no host cells present
among different viruses than betwee	among different viral hosts than among di
lysogeny	fermentation
nature of the host	nucleic acid characteristics
adsorption	absorption
Hepatitis C virus	Hepatitis B virus
genome and Capsid	capsid and spikes
Smallpox	Avianpox
have no genome	continue to replicate
icosahedral	helical
Paramyxo viruses	Retro viruses
HeLa	HEp-2
tissue culture	cultures of separated cells
how chemicals inactivate viruses wh	how viruses replicate in host cells
nuclear pyknosis	transformation of cells
ultraviolet light	chemicals
spontaneous induction	inductive infection
immunity repressor	immunity operon
protomers	caproprotein
the capsid must enter the host cell cy	the host cell must be undergoing mitosis
Rubella virus	Yellow fever virus
Hepatitis B virus	T cell lymphotronic virus type I
Denaturation	Enzyme treatment
lysogeny	spontaneous induction
Viruses have been successfully grow	All viruses are obligatory intracellular par
Hepatitis B virus	Hepatitis D virus
penton	polyhedra
centimeters	micrometers
λ phage enzyme	λ DNA
Human immunodeficiency virus	Epstein-Barr virus
immediate early phage gene	late genes
HeLa	HEp-2

immunity repressor	immunity operon
Herpes	Influenza
single stranded DNA	double stranded DNA
phage destruction	lytic infection by other viruses
A	B
infection	integration
pock	plaque
ladder	framing
Phage structural proteins	Proteins that help with phage assembly wi
Helical	Icosahedral
T3	T2
RNA transcriptase	RNA polymerase
the envelope and the embedded prote	the envelope is derived from the host but i
Staphylococcus aureus	Salmonella typhi
Operator	Promotor

Opt 3	Opt 4
Capsid	Tail fibers
DNA	RNA
Icosahedron	Pyramid
Capsid shape	Ability to survive acidic conditions
Entero viruses	Rhinoviruses
Plant Viruses	Fungal Viruses
Plaque assays	Tissue cell culture
Cell strain	Diploid fibroblast
Rhabado virus	Adenovirus
Formaldehyde	chlorine
alcohol	lipids
animals	living cells
nutrients are scarce	spikes are forming in the new virus
among different viral hosts than betv	between viruses and their hosts than
symbiosis	synergism
capsid symmetry	diameter of the viroin or nucleocapsi
penetration	replication
Varicella-Zoster virus	Herpes simplex virus type 2
envelope and Capsid	capsomere and genome
Cowpox	Chickenpox
are usually larger than bacteria	is altered with chemicals
roughly spherical	complex
Orthomyxo viruses	Herpes viruses
WI-38	KB
whole plants	human cells
how viruses transform normal cells i	no change
syncytium formation	rounding and aggregation of cells
irradiation	alcohol
resultant induction	spontaneous infection
operon repressor	Lac operon
bprocapsid	capsomers
the genome must be released in the c	the host cell must lack a cell membra
Hepatitis C virus	Dengue
Epstein-Barr virus	CAMV
Pressure	Sedimentation
lytic phase	Induced induction
All viruses have either DNA or RNA	Viruses probably arose from small fr
Hepatitis E virus	Hepatitis A virus
icosahedral	helical
nanometers	millimeters
Phage Mu	Phage Mn
Influenza virus	Adenovirus
delayed early genes	Early genes
KB	All of these

operon repressor	Operon deppressor
Measles	HIV
single stranded RNA	double stranded RNA
Both (a) and (b)	lysogenic
C	D
repression	induction
pox	Colony
scaffolding	form
Proteins involved in cell lysis and ph	Complex protein
Complex	Cylinder
P22	P322
RNA ligase	RNA replicase
the envelope is coded by the viral nu	the envelope and its imbedded protei
Vibrio cholerae	E coli
Repressor	Enhancer

Opt 5	Answer
	Capsid
	Protein
	Icosahedron
	Capsid shape
	Herpes viruses
	Animal Viruses
	Plaque assays
	Continuous cell line
	Entero virus
	Formaldehyde
	protein
	living cells
	an RNA virus converts its RNA to DNA
among different viruses	among different viruses than between vi
	lysogeny
id	nature of the host
	adsorption
	Varicella-Zoster virus
	genome and Capsid
	Cowpox
	have no genome
	complex
	Retro viruses
	HeLa
	tissue culture
	how viruses transform normal cells into
	syncytium formation
	alcohol
	spontaneous infection
	immunity repressor
	caproprotein
ane	the genome must be released in the cyto
	Dengue
	T cell lymphotronic virus type I
	Denaturation
	lysogeny
agments of cellular chromosomes	Viruses have been successfully grown i
	Hepatitis E virus
	penton
	nanometers
	Phage Mn
	Influenza virus
	late genes
	HeLa

	immunity repressor
	Measles
	double stranded DNA
	lytic infection by other viruses
	D
	infection
	plaque
	ladder
	Proteins involved in cell lysis and phage
	Icosahedral
	T2
	RNA polymerase

ins are derived from the host's membra the envelope is coded by the viral nuclei

	Salmonella typhi
	Promotor

ruses and their hosts

tumor cells

plasm

n pure cultures in test tubes

ɛ release

c acids, but the proteins come from the host's membrane proteins

UNIT – 3

Viral Genome

The core of the virus is made up of nucleic acids, which then make up the genetic material in the form of RNA or DNA. Nucleic acid of the virus that encodes its genetic information and is classified into five types.

1. Single Stranded genome (Present in all RNA viruses except Reovirus)
2. Double stranded genome (present in all DNA viruses except Parvovirus)
3. Linear genome
4. Circular genome
5. Segmented genome

Further viral genome is classified into two types:

1. RNA virus genome
2. DNA virus genome

RNA virus genome

This is of three types namely Positive sense RNA genome, Negative sense RNA genome and Ambisense RNA genome

Positive sense RNA genome

- Purified (+) sense viral RNA is directly infectious when applied to susceptible host cells in the absence of any virus proteins.
- There is an untranslated region (UTR) at the 5' end of the genome which does not encode any proteins & a shorter UTR at the 3' end.
- These regions are functionally important in virus replication & are thus conserved in spite of the pressure to reduce genome size.
- Terminal Structures: Both ends of (+)stranded eukaryotic virus genomes are often modified, the 5' end by a small, covalently attached protein or a methylated nucleotide 'cap' structure and the 3' end by polyadenylation.
- These signals allow viral RNA to be recognized by host cells and to function as mRNA.

Negative sense RNA genome

- Genomes are not infectious as purified RNA. This is because such virus particles all contain a virus-specific polymerase.
- The first event when the virus genome enters the cell is that the (-) sense genome is copied by the polymerase, forming either (+)sense transcripts which are used directly as mRNA, or a double-stranded molecule known

either as the replicative intermediate (RI) or replicative form (RF), which serves as a template for further rounds of mRNA synthesis.

- Therefore, since purified negative-sense genomes cannot be directly translated & are not replicated in the absence of the virus polymerase, these genomes are inherently non-infectious.

Ambisense RNA genome

- Some RNA viruses are not strictly 'negative-sense' but ambisense, since they are part (-)sense and part (+)sense

DNA virus genome

This is of three types namely Small DNA genomes and Large DNA genomes

Small DNA genomes

- Bacteriophage - DNA replication and formation of products using host cell machinery
- M 13 Phage - Form replicative intermediate.
- Phage lambda - Concatemers formed and packaged into phage head.
- Phage T4 - Terminal redundant molecule of genomes are formed and get packaged into phage head.

Large DNA genomes

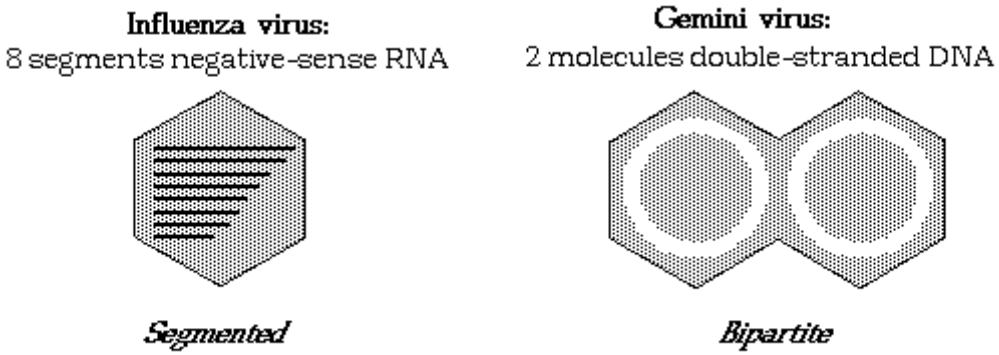
- They are very complex resembling the host cell.
  E.g. Herpes virus and Adenovirus

Segmented and Multipartite Virus Genomes

Segmented virus genomes are those which are divided into two or more physically separate molecules of nucleicacid,all of which are then packaged into a single virus particle.

Multi partite genomes are those which are segmented and where each genome segment is packaged into a separate virus particle. These discrete particles are structurally similar and may contain the same component proteins, but often differ in size depending on the length of the genome segment packaged.

Examples



4.3. **Plant RNA Viruses – Tobacco Mosaic Virus**

I**ntroduction**

- Mosaic Virus (TMV) worldwide distribution primarily infects tobacco and tomato, but more than 350 species are susceptible.
- Tobacco leaves become mottled with light and dark green areas; leave become distorted, puckering or blistering, especially areas of new growth.
- Stunting of plant growth. in tomato, mottling of leaves occurs and leaflets become long and pointed.
- Difficult to inactivate, and can survive for 5 years in dead, dried tissues and many months in living plant tissues.
- Many strains, those vary in virulence from severe to mild symptoms. Virus is spread from plant to plant through injuries caused by crop worker, contaminated equipment and chewing insects.
- Virus over winters in dead plant tissues and debris, on contaminated equipment, in contaminated soil, greenhouse containers, bedding, tools, and in living hosts, including weeds like horsenettle, *Solanum carolinense*, and other crop plants (tomato, pepper, and eggplant).

Morphology

- TMV is rod-shaped particles which are 300 nm long by 15-18 nm in diameter. It possesses ssRNA and a protein coat.
- A virus particle, when viewed under the electron microscope, appears as a cylinder that is 16 nm wide and about 300 nm long. It has a mass of 40 x 10^6 daltons and contains a single strand of RNA. This is its genetic material, in which 6500 nucleotides hold the codes necessary for producing more virus particles.
- In an intact particle, the viral RNA is wound into a helix that has a radius of 4 nm, and stretches all the way from one end of the cylinder to the other.

- Associated with this polynucleotide helix are 2130 identical polypeptides, folded into 2130 identical globular protein shapes. These protein molecules bind and complex with three adjacent nucleotides along RNA molecule, and with other proteins on either side of them.

Fig: Structure of TMV

Genome

The genomic RNA is shown on top and the 3' coterminal subgenomic (sg) RNAs are shown underneath. The predicted molecular weights of proteins encoded by the open reading frames (boxes) are given in kilodaltons,

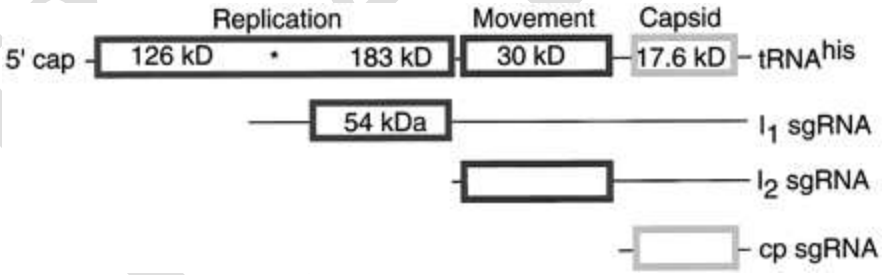


Fig: Genomic structure of TMV

Replication

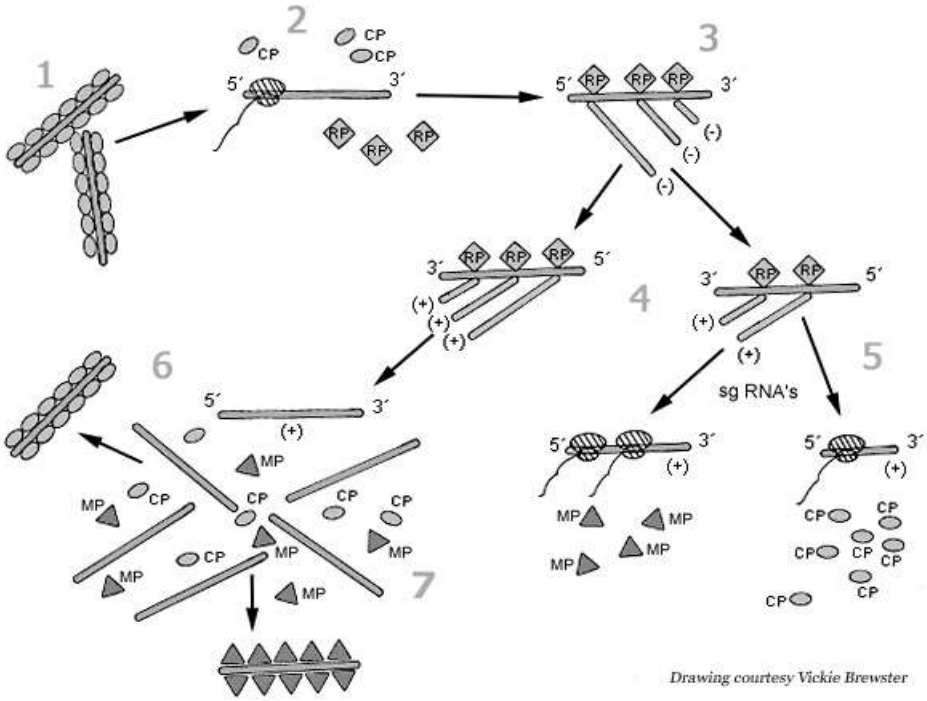


Fig: Replication of Tobacco Mosaic Virus

a. TMV enters a wounded plant cell to begin the replication cycle [1].
b. As the coat protein (CP) molecules are stripped away from the RNA [2], host ribosomes begin to translate the two replicase-associated proteins.
c. The replicase proteins (RP) are used to generate a negative-sense (- sense) RNA template from the virus RNA [3].
d. This - sense RNA is, in turn, used to generate both full-length positive-sense (+ sense) TMV RNA [4] and
e. the + sense subgenomic RNAs (sgRNAs) [5] that are used to express the movement protein (MP) and CP.
f. The + sense TMV RNA is either encapsidated by the CP to form new TMV particles [6] or
g. wrapped with MP [7] to allow it to move to an adjacent cell for another round of replication

Symptoms

The symptoms of TMV first appear about 10 days after plants become infected. Symptoms appear as light and dark green mottled areas on leaves. Leaves on infected plants are often small, curled, and puckered. Plants infected early in their development are stunted and have a yellowish cast. Symptoms may vary depending on virus strain, time of infection, variety, and environmental conditions. In hot weather, symptoms may not be as obvious although plants remain infected.

Certain strains of TMV can cause dark, longitudinal streaks of varying lengths on stems. Affected stems are brittle and appear brown internally. TMV can reduce size and number of fruit produced. The earlier a plant becomes infected, the greater the loss. Fruit usually do not show any malformation. Occasionally, mottling, bronzing, and internal browning of fruit occur. Internal browning is evident on mature but un ripened fruit.

Pathogenesis

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4.3.6. **Pathogenesis**

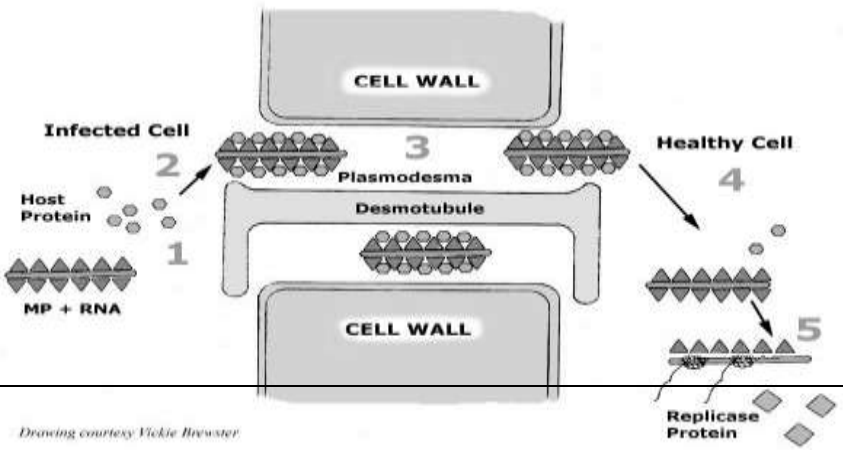

Fig: Cell-to-cell movement of *Tobacco mosaic virus* (TMV).

a. The movement protein (MP) binds to the viral RNA [1].
b. Host proteins and/or other virus-encoded proteins may be included in the MP-complex [2].
c. The MP-complex then moves from cell-to-cell through the plasmodesmata [3].
d. When the complex is localized to a new cell, the MP (and any host proteins) are presumably released from the TMV RNA [4], allowing for translation of the genomic RNA to express the replicase proteins and
e. To initiate a new round of replication [5].

4.3.7. **Control**

- Use virus-free seed (tomato seed can by treated with acid or bleach)
- Transplant in non infested soil
- Fumigation with methyl bromide or heated.
- No chewing of tobacco or smoking around seedbeds or in greenhouses.
- To eliminate spreading of virus wash hand with soap and water or milk.
- Spraying plants with milk (whole or skim) seems to help reduce Infections.
- Crop rotation with non host crops (corn, rice, and other cereal grains).
- Resistant cultivars

4.10. **Structure, properties and replication of Filamentous Phage ØX174**

4.10.1. **Structure and Properties**

The **phi X 174** (or **ΦX174**) bacteriophage was the first DNA-based genome to be sequenced. This bacteriophage has a [+] circular single-stranded DNA genome of 5386 nucleotides encoding 11 proteins. Of these 11 genes, only 8 are essential to viral morphogenesis. The GC-content is 44% and 95% of nucleotides belong to coding genes.

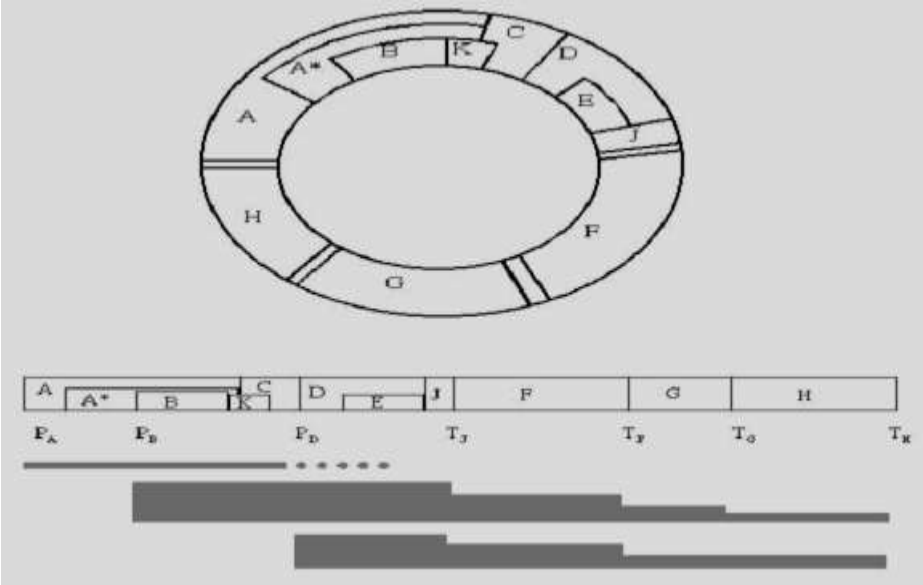

Fig:Genome of Filamentous Phage ØX174

Protein	Function
A	Stage II and Stage III DNA replication.
A*	An unessential protein for viral propagation. It may play a role in the inhibition of host cell DNA replication and superinfection exclusion
B	Internal scaffolding protein, required for procapsid morphogenesis and the assembly of early morphogenetic intermediates. 60 copies present in the procapsid.

C Facilitates the switch from Stage II to Stage III DNA replication. Required for Stage III DNA synthesis.

D External scaffolding protein, required for procapsid morphogenesis. 240 copies present in the procapsid.

E Host cell lysis.

F Major coat protein. 60 copies present in the virion and procapsid.

G Major spike protein. 60 copies present in the virion and procapsid.

H DNA pilot protein need for DNA injection, also called the minor spike protein. 12 copies in the procapsid and virion.

J DNA binding protein, needed for DNA packaging, 60 copies present in the virion.

K An unessential protein for viral propagation. It may play a role optimizing burst sizes in various hosts.

The steps in the formation of the ØX primosome involve:

- Coating of the single-stranded ØX174 DNA with *Escherichia coli* SSB DNA binding protein
- Binding of three proteins (PriA, priB and priC) to the primer assembly sequence.
- Formation of a complex of six subunits of dnaB protein coupled with six subunits of dnaC protein.

- Transfer of the complex of dnaB·dnaC to the priA-B-C complex at the primer assembly site via the dnaT gene product. dnaC dissociates at this step and the resulting complex is known as the preprimosome.

- Binding of primase (dnaG) to the Preprimosome complex to form the primosome.

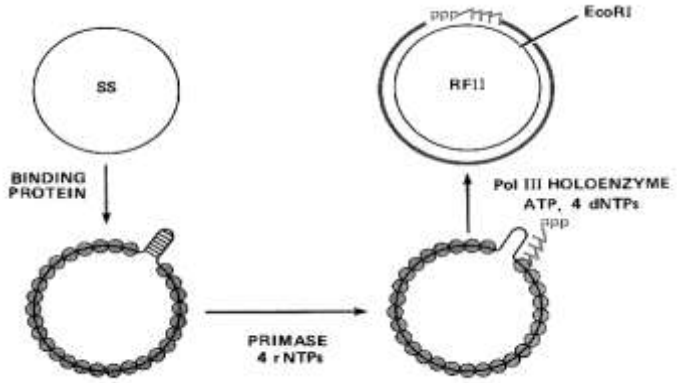

Fig: **Formation of the ØX primosome**

The mature primosome can then proceed in an ATP dependent fashion to traverse the DNA. The primosome can apparently be driven by either the dnaB protein in a 3'-5' direction or by the priA protein in the 5'-> 3' direction. Both the dnaB protein and the priA protein in the primosome can serve as DNA helicase activities. The priA protein can also displace SSB from in front of the moving primosome. While dnaB cannot and can only move on naked DNA template. During either of these motions, the primase activity can synthesize primers 11 nucleotides in length at various sites along the template in a reaction requiring the four rNTPs. Once these primers are extended by DNA polymerase III, the SSB protein is permanently displaced from the single-stranded DNA template. Removal of the RNA primers and ultimate sealing of the nicks in the DNA require the combined action of 5' exonuclease of DNA polymerase I and DNA ligase. It is believed that similar primosome complexes are present on the lagging strand of DNA synthesis because of the sensitivity of DNA synthesis to disruption by temperature sensitive mutants in dnaB, dnaC, dnaT and dnaG.

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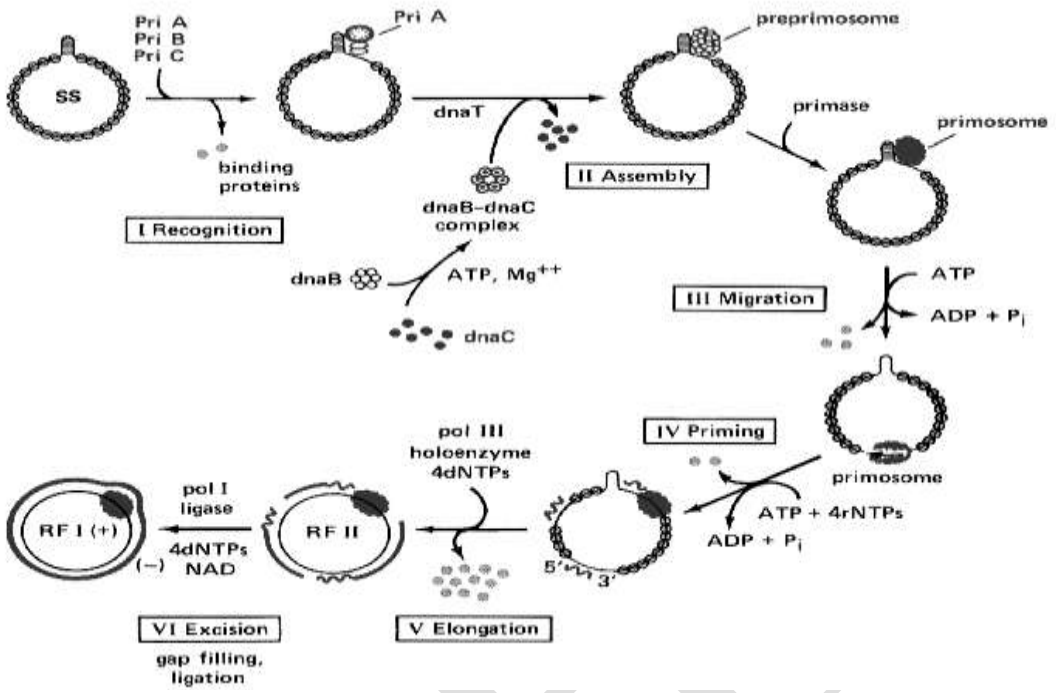


Fig: **Replication of Filamentous phage ØX174**

3.3.**Hepatitis B Virus**

3.3.1. **Introduction**

Hepatitis B virus (HBV), a member of the hepadnavirus group, double-stranded DNA viruses which replicate, unusually, by reverse transcription. Hepatitis B virus is endemic in the human population and hyper endemic in many parts of the world.

3.3.2. **Morphology**

a. Spherical, enveloped (lipid-containing, detergent disrupted) particles 42-47nm diameter containing partially d/s DNA plus an RNA-dependent DNA polymerase (i.e. reverse transcriptase)
b. The genome: a small, circular, partly double-stranded DNA of 3200 base

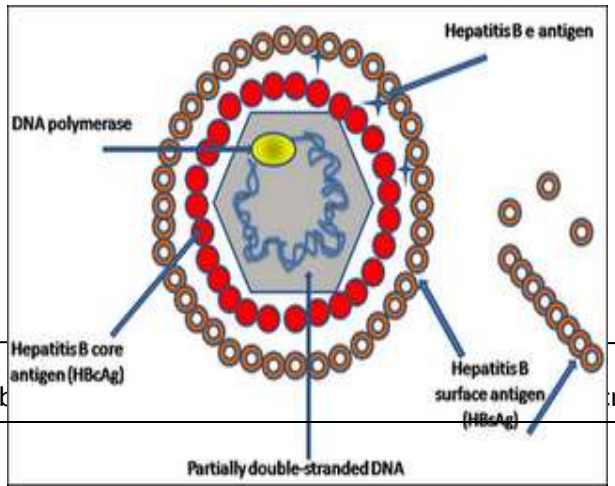
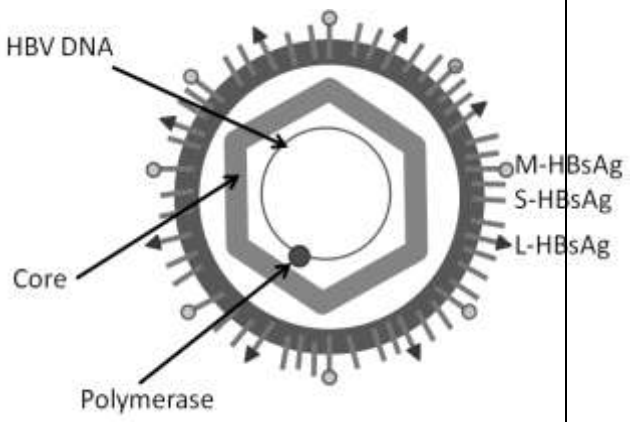


Fig: Structure of the Hepatitis B virus



Hepatitis B Antigens: There are three different types of hepatitis b antigens encoded by the HBV genome.

a. Hepatitis B Surface antigen (HBsAg)- There are three different types of hepatitis B surface antigens; small hepatitis B surface antigen (HBsAg or SHBsAg), middle hepatitis B surface antigen (MHBsAg), and large hepatitis B surface Antigen (LHBsAg).

b. Hepatitis B Core Antigen (HBcAg)- The only HBV antigen that can not be detected directly by blood test, this antigen can only be isolated by analyzing an infected hepatocyte. A 185 amino acid protein is expressed in the cytoplasm of infected cells; they are highly associated with nucleocapsid assembly.

Hepatitis B e Antigen (HBeAg)- The e antigen is named due to its "early" appearance during an acute HBV infection. Thought to be located in the core structure of the virus molecule, this antigen can be detected by blood test. If found its usually indicative of complete virus particles in circulation.

3.3.3.**Genome**

The HBV virion genome is circular and approximately 3.2 kb in size and consists of DNA that is mostly double stranded. It has compact organization, with four overlapping reading frames running in one direction and no noncoding regions. The minus strand is unit length and has a protein covalently attached to the 5' end. The other strand, the plus strand, is variable in length, but has less than unit length, and has an RNA oligonulceotide at its 5' end. Thus neither DNA strand is closed nor is circularity maintained by cohesive ends (Strauss, 2002). The four overlapping open reading frames (ORFs) in the genome are responsible for the transcription and expression of seven different hepatitis B proteins. The transcription and translation of these proteins is through the used of multiple in-frame start codons. The HBV genome also contains parts that regulate transcription, determine the site of polyadenylation and a specific transcript

for encapsidation into the nucleocapsid. The genomic arrangement of the hepatitis B virus family makes it unique among viruses. The unusually packaged may indicate that the method of replication employed by HBV is not of conserved DNAreplication

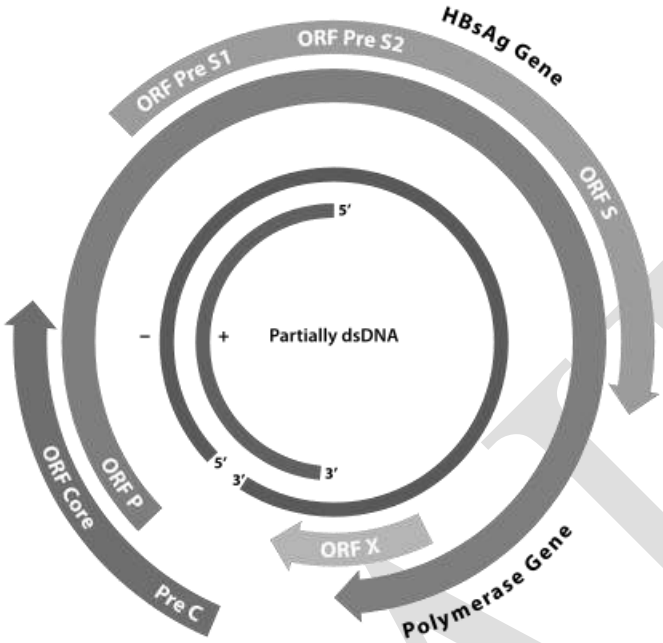
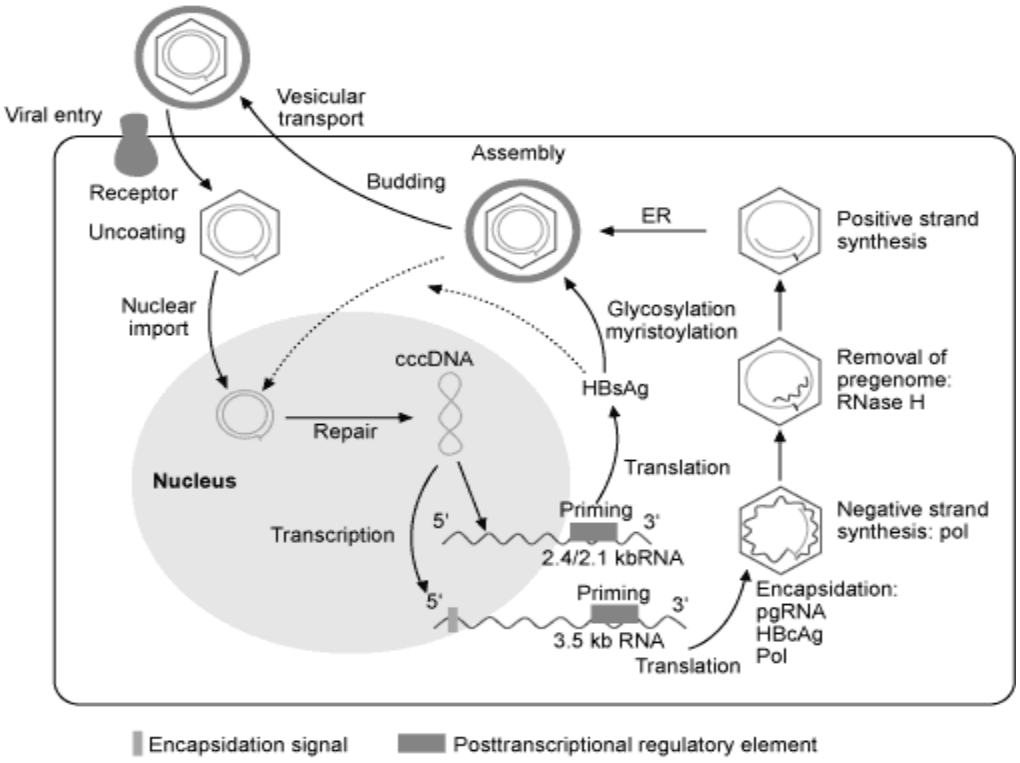


Fig: Genomic structure of Hepatitis B virus

3.3.4. **Replication**

HBV enters the hepatocyte mediated by receptor binding followed by internalization.Uncoating of virion DNA and delivery to the nucleus.The virion DNA is repaired and converted to circular covalently closed (ccc) HBV DNA from which HBV RNA is transcribed in several forms for translation(HBcAG,HBsAG) and polymerase (pol I) and for viral replication through encapsidation of the RNA pregenome pg(RNA) mediated by the encapsidation signal.Within core particles, negative followed by positive strand viral DNA is synthesized directly by the HBV polymerase while pregenome RNA

is degraded by the polymerase RNase H activity.The core particle with double stranded HBV DNA is transferred to the endoplasmic reticulum(ER) where it is coated with glycosylated and myristoylated HBsAg producing intact virions that exit the cell by buddibg and vesicular transport.



ER, endoplasmic reticulum; pgRNA, pregenomic RNA; Pol, polymerase.

Fig:Replication cycle of HBV

3.3.5.**Pathogenesis**

Hepatitis B virus primarily interferes with the functions of the liver by replicating in liver cells, known as hepatocytes. The receptor is not yet known, though there is evidence that the receptor in the closely related duck hepatitis B virus is carboxypeptidase D. HBV virions (DANE particle) bind to the host cell via the preS domain of the viral surface antigen and are subsequently internalized by endocytosis. PreS and IgA receptors are accused of this interaction. HBV-preS-specific receptors are expressed primarily on hepatocytes; however, viral DNA and proteins have also been detected in extrahepatic sites, suggesting that cellular receptors for HBV may also exist on extrahepatic cells.

During HBV infection, the host immune response causes both hepatocellular damage and viral clearance. Although the innate immune response does not play a significant role in these processes, the adaptive immune response, in particular virus-specific cytotoxic T lymphocytes (CTLs), contributes to most of the liver injury associated with HBV infection. CTLs eliminate HBV infection by killing infected cells and producing antiviral cytokines, which are then used to purge HBV from viable hepatocytes. Although liver damage is initiated and mediated by the CTLs, antigen-nonspecific inflammatory cells can worsen CTL-induced immunopathology, and platelets activated at the site of infection may facilitate the accumulation of CTLs in the liver.

3.3.6.**Clinical Features**

a. Persistant infection: Following acute infection, some individuals fail to eliminate the virus completely and become persistantly infected. The likelihood of this happening varies with age of exposure.
b. Chronic infection may take one of two forms:
    i. Chronic persistent hepatitis - the virus persists, but there is minimal liver damage, developing hepatocellular carcinoma.
    ii. Chronic active hepatitis - there is aggressive destruction of liver tissue and rapid progression to cirrhosis or liver failure.
c. Fulminant hepatitis: This is a complication of acute infection. Rare; accounts for 1% of infections.

3.3.7. **Lab diagnosis**

The following tests are done to identify and monitor liver damage from hepatitis B:

a. Albumin level
b. Liver function tests
c. Prothrombin time

The following tests are done to help diagnose and monitor people with hepatitis B:

a. Antibody to HBsAg (Anti-HBs)
b. Antibody to hepatitis B core antigen (Anti-HBc)
c. Hepatitis B surface antigen (HBsAg)
d. Hepatitis E surface antigen (HBeAg)

3.3.8. Treatment

a. Alpha-IFN is used for therapy of chronic HBV infection. 30-40% chronic carriers respond to this (expensive) treatment, c.f. 10-20% spontaneous loss of virus markers in untreated control groups.

b. Lamivudine (3TC - 2'deoxy, 3'thiacytidine - a reverse transcriptase inhibitor) is currently being investigated for therapy of chronic HBV infection.
c. Early results suggest this drug may be effective in patients who have previously failed to clear the virus with alpha-IFN.

3.3.9. Prevention

a. Avoid sexual contact with a person who has acute or chronic hepatitis B.

Use a condom and practice safe sex

a. Avoid sharing personal items, such as razors or toothbrushes.
b. Do not share drug needles or other drug equipment (such as straws for snorting drugs).
c. Clean blood spills with a solution containing 1 part household bleach to 10 parts water.

2.6. Human Immuno Deficiency Virus

2.6.1. **Introduction**

Human immunodeficiency virus (HIV) is a lentivirus (a member of the retrovirus family) that causes *acquired immunodeficiency syndrome* (AIDS).

Sexual intercourse:

This is the most common route of transmission world wide. The receptive partner is at greatest risk. There is an increased risk of transmission if partners have other sexually transmitted diseases and during primary HIV infection.

Vertical Transmission:

This is the second most common route of transmission world wide. Infection may occur in utero during birth (commonest) postnatally, through breast feeding.

Exposure to blood:

Intra-venous drug abusers - sharing of needles. Needle stick injuries – risk approximately 0.3% (depends on extent of the injury) mucocutaneous exposure - risk approximately 0.1%

2.6.2. **Morphology**

They are enveloped viruses with an RNA genome. The name is derived from the fact that the virus particle contains an RNA dependent DNA polymerase (**Reverse transcriptase**). This enzyme converts the RNA genome into DNA, which then integrates into the host chromosomal DNA. The reverse transcriptase is highly error prone and rapid genetic variation is a feature of this group of viruses.

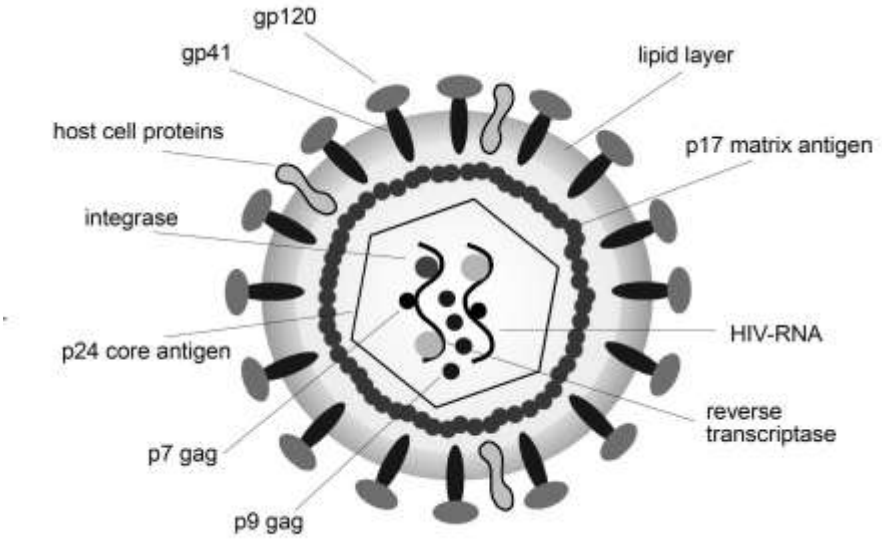


Fig:Structure of the HIV virion

2.6.3. Genome

Retroviruses have a diploid genome (2 copies of RNA genome per virus particle). The genome codes for at least three genes: *gag*, *pol* and *env which* encode the nucleocapsid, polymerase, and envelope proteins, respectively. The LTR (long terminal repeat) regions represent the two end parts of the viral genome, that are connected to the cellular DNA of the host cell after integration and do not encode for viral proteins. The *gag* and *env* genes code for the nucleocapsid and the glycoproteins of the viral membrane; the *pol* gene codes for the reverse transcriptase and other enzymes.

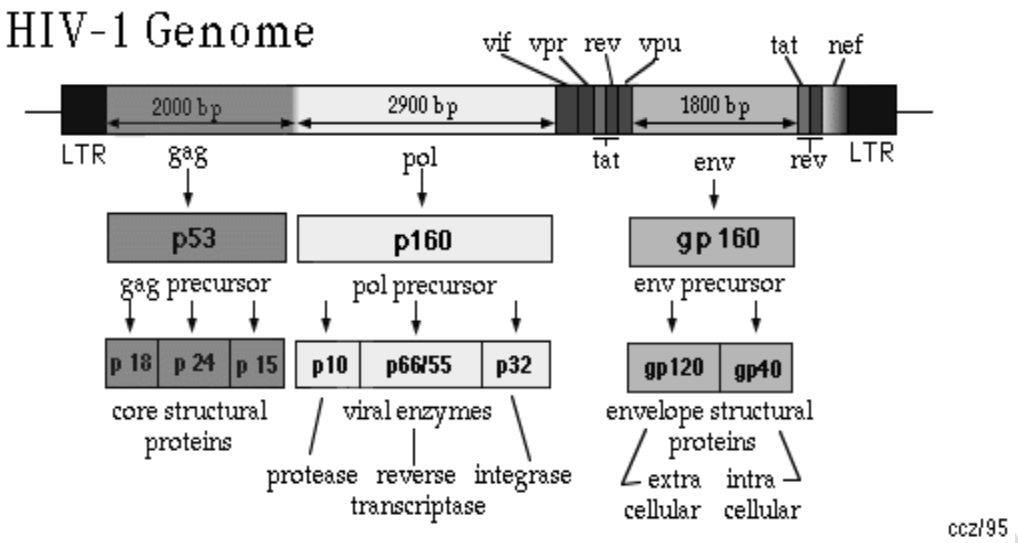


Fig: Genome of HIV

2.6.4.**Replication**

HIV attaches via its gp 120 envelope glycoprotein (surface spike) to the CD4 antigen complex which is the primary HIV receptor on CD4+ (helper/inducer) T lymphocytes and cells of the macrophage lineage. The CD4 molecule has binding avidity for gp 120 of HIV. Then by pinocytosis, the nucleocapsid of the virion enters into host cell. The envelope of the same does not enter into the cell. After entry into the cell, the nucleocapsid releases its RNA into the cytoplasm. The viral reverse transcriptase, acting as an RNA dependent DNA polymerase, makes a DNA copy of the genomic RNA. The ssDNA is made double stranded by the same enzyme, now acting as a DNA-dependent DNA- polymerase. This ds DNA moves to the nucleus and several such molecules become integrated as provirus at random sites in the host cell chromosome causing a latent infection. The integrated provirus is transcribed by cellular RNA polymerase II either for the production of mRNAs which are translated into proteins or for the production of genomic RNA for insertion into progeny virions. HIV establishes a latent infection with or without an initial productive phase.

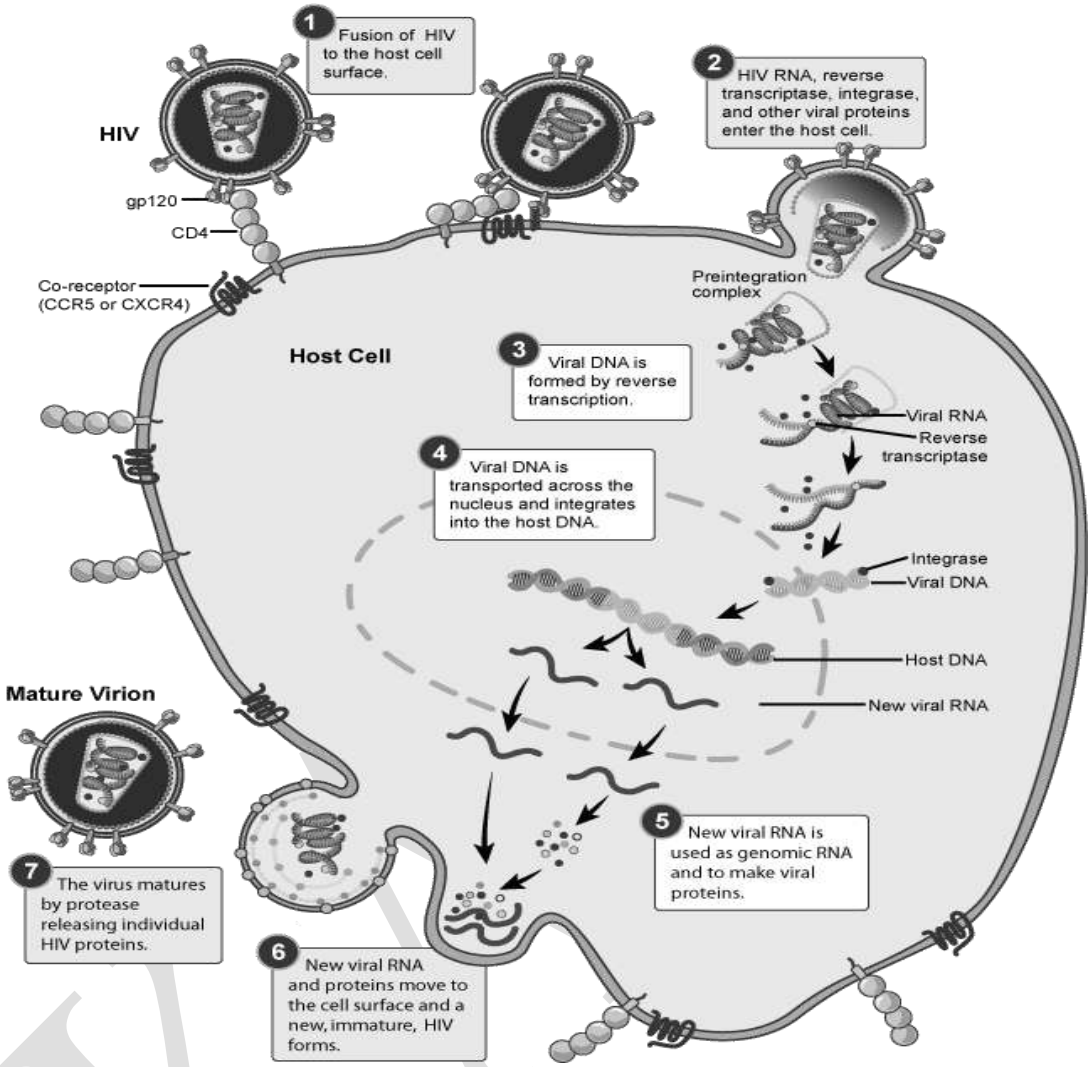


Fig: Replication of HIV

2.6.5.Pathogenesis

When a new infection is established, the first cells to be exposed are the dendritic cells. These cells are resident in the skin and genital mucosa. It is their job to take

up antigen in the tissues and to transport it to regional lymph nodes where they present it to T cells. Dendritic cells express a receptor called DC SIGN to which HIV can attach. HIV particles remain attached to the surface of the cell and are passively transported to the very cells that HIV most likes to infect, namely CD4+ T cells. Cycles of infection are set up in the CD4 cells in the lymphoid tissue. Helper T cells are the primary target of HIV. They are cytokine secreting cells that provide the signals to control the immune response. Without them the immune response cannot function.

In the early days after infection, HIV is able to replicate to very high levels while the immune system learns to deal with it. CD4 + levels in the blood fall and virus levels peak at approximately 21 days post infection. The CD4 cell population in the gut is particularly severely affected early on. However, an immune response to the virus does develop after a while and virus levels in the blood fall to a steady state level. Unfortunately, the immune response is not able to control the infection completely and virus replication continues in the lymphoid tissue. As time passes, the antiviral immunity begins to fail and virus levels begin to rise again and the person succumbs to the infection.

2.6.6. Clinical Features

HIV establishes a persistent infection in its host and only causes death many years later.

Primary infection

Most individuals experience a febrile illness about 2-4 weeks after exposure. This illness coincides with seroconversion (development of antibodies) and so is often referred to as the seroconversion illness. The symptoms are similar to those of glandular fever, namely fever, sore throat, night sweats, lymphadenopathy, diarrhoea. The illness is self limiting.

Asymptomatic phase

Following the primary infection, the patient enters a stage of clinical latency. During this time the patient feels fine, but they are infectious as they have on-going viral replication. They also have HIV antibodies in their blood (and will test positive in HIV tests). This healthy state may last many years.

Prodromal phase

As the CD4 counts drop, there is a gradual onset of a variety of prodromal disorders, such as weight loss, fever, persistant lymphadenopathy, oral candidiasis and diarrhoea. These symptoms precede the progression to AIDS.

Acquired Immunodeficiency Syndrome (AIDS)

Syndrome with the following features:

***Constitutional disease*:** fever, diarrhoea, weight loss, skin rashes
Neurocognitave defects: dementia, myelopathy, peripheral neuropathy
***Immunodeficiency*:** Increased susceptibility to opportunistic infections:
Rare malignancies: Kaposi sarcoma, oral hairy leukoplakia, lymphomas.

2.6.7. Laboratory Diagnosis

Serology

The main stay of diagnosis is the detection of HIV specific antibody. IgG develops 4-6 weeks post exposure and remains detectable for life.

There are two situations where further tests may be necessary to confirm a diagnosis:

(a) **Early infection** - the period after exposure before antibody becomes detectable, (sometimes termed the "window" period).

(b) **Infants of HIV positive mothers:** all have passively acquired HIV-specific antibody, but only 10-40% are infected. This antibody may take 12 to 18 months to disappear.

Direct detection of virus

1. Viral **p24 antigen** in serum - This is a useful marker of early infection. It appears in the blood 3-5 weeks post exposure and becomes detectable approximately 6 days before antibody (during the so called window period.) Once antibody appears, the p24 antigen is usually cleared.

2. Detection of **viral genome** (proviral DNA or viral RNA) by **PCR:** This is a very sensitive indicator of infection. PCR becomes positive about 2 weeks after infection and remains positive throughout the course of the infection. This is the test of choice for confirming infection in infants of HIV positive mothers

3. Culture of virus from peripheral blood mononuclear cells (PBMCs). This is difficult and not routinely done.

2.6.8. Treatment

There is no cure for HIV. However, a number of anti-HIV drugs have been developed in recent years that interfere with specific steps in the virus replication cycle. Used in combination, they halt viral replication and can prolong the life of infected individuals. A regimen of at least three drugs (HAART) has to be given simultaneously to suppress HIV replication. This is because drug resistance develops very rapidly if they

are used alone. Unfortunately these drugs need to be taken for life to maintain viral suppression. They also have toxic side effects and response to therapy has to be carefully monitored. Three classes of anti-retroviral drugs are used in South African public sector treatment programmes:

a. Nucleoside and nucleotide reverse transcriptase inhibitors
b. Non nucleoside reverse transcriptase inhibitors
c. Protease inhibitors.

Vaccine

The development of an effective vaccine for HIV is probably still some years away. The difficulty is that traditional approaches do not work. This is because the presence of HIV-specific **antibody** in the blood **does not prevent infection**. There are various reasons for this:

1. There is extensive **variability of the envelope antigens** in the many subtypes of HIV that are prevalent around the world.
2. Specific antibody may, in fact, **enhance** infection because antibody coated virus can bind to Fc receptors on the surface of susceptible cells.
3. The envelope glycoprotein gp120 is heavily glycosylated and this masks the protein so that antibodies can't bind to it.
4. 4. Critical epitopes on gp120 are hidden and are only exposed when the protein changes shape at the time of fusion with

2.6.8. Prevention

- Correct and consistent use of **condoms** can protect against the spread of HIV.
- Antiretroviral drugs taken by the HIV-negative partner can be effective in preventing acquisition from an infected partner. This is called **pre-exposure prophylaxis (PrEP)**.
- Health care workers with needle prick injuries in the workplace are recommended **post-exposure prophylaxis (PEP)**.
- Antiretroviral drugs are used within 72 hours of exposure to HIV in order to prevent infection. Counseling, first aid care and HIV testing are also done.
- A 28-day course of antiretroviral drugs with follow-up care is advised depending on the level of risk.
- People exposed to any of the risk factors are strongly advised to **test for HIV and other STIs**.
- **Mother-to-child transmission (MTCT)** during pregnancy, delivery or breastfeeding can be fully prevented if both the mother and the child are provided with antiretroviral drugs

- **Male circumcision** reduces the risk of heterosexually acquired HIV infection in men by approximately 60%.
- Use **sterile needles and syringes** for each injection.

Antiretroviral therapy decreases the HIV concentration (viral load) in the blood and in genital secretions. According to a new trial the risk of transmitting the virus through sexual contact can be reduced by 96% if an HIV-positive person follows an effective antiretroviral therapy regimen

3.6.Influenza Virus

3.6.1.Introduction

Influenza or "flu" is a respiratory tract infection caused by influenza viruses. Contrary to common belief flu is not is a trivial illness. According to WHO statistics, influenza infection results in 250,000-500,000 deaths annually in industrialised countries alone. In addition, the influenza virus is able to mutate and cause pandemic with significant morbidity and mortality across the world. The most infamous 1918 "Spanish flu" pandemic was estimated to cause 50 million deaths worldwide.

Influenza viruses are classified into three groups, include,

- **Influenza A viruses** infect a wide variety of mammals, including man, horses, pigs, ferrets **and** birds. The main human pathogen, associated with epidemics and pandemics. There are 15 known haemagglutinin (H) serotypes and 9 known neuraminidase (N) serotypes. Pigs and birds are believed to be particularly important reservoirs, generating pools of genetically/antigenically diverse viruses which get transferred back to the human population via close contact between humans and animals.

- **Influenza B viruses** infect mammals only and cause disease, but generally not as severe as A types. Unlike influenza A viruses, influenza B viruses do not have distinguishable serotypes.

Influenza C viruses also infect mammals only, but rarely cause disease. They are genetically and morphologically distinct from A and B types

3.6.2.Morphology

- Family: Orthomyxovirus Genera: influenzavirus A,B,C
- spherical or filamentous enveloped particles 80 to 120 nm in diameter
- Single strand RNA virus with segmented genome

- enveloped with two surface glycoprotein:
  - H - Haemagglutinin - responsible for viral attachment
  - N - Neuraminidase - responsible for viral exit from infected cell
- Influenza A is the type that is responsible for pandemics. It can be further subtyped according to its H and N group. There are 16 H types and 9 N types of influenza A that exist in nature, mainly found in the natural reservoir wild aquatic birds. Only H1, 2 and 3 and N1 and 2 subtypes circulate widely in human.
- Both Haemagglutinin and Neuraminidase are important antigens that confer subtype specific immunity and are therefore used in the vaccine formulations

The influenza virion (as the infectious particle is called) is roughly spherical. It is an enveloped virus – that is, the outer layer is a lipid membrane which is taken from the host cell in which the virus multiplies. Inserted into the lipid membrane are 'spikes', which are proteins – actually glycoproteins, because they consist of protein linked to sugars – known as HA (hemagglutinin) and NA (neuraminidase). Beneath the lipid membrane is a viral protein called M1, or matrix protein. This protein, which forms a shell, gives strength and rigidity to the lipid envelope. Within the interior of the virion are the viral RNAs – 8 of them for influenza A viruses. These are the genetic material of the virus; they code for one or two proteins. Each RNA segment, as they are called, consists of RNA joined with several proteins B1, PB2, PA, NP. These RNA segments are the genes of influenza virus. The interior of the virion also contains another protein called NEP.

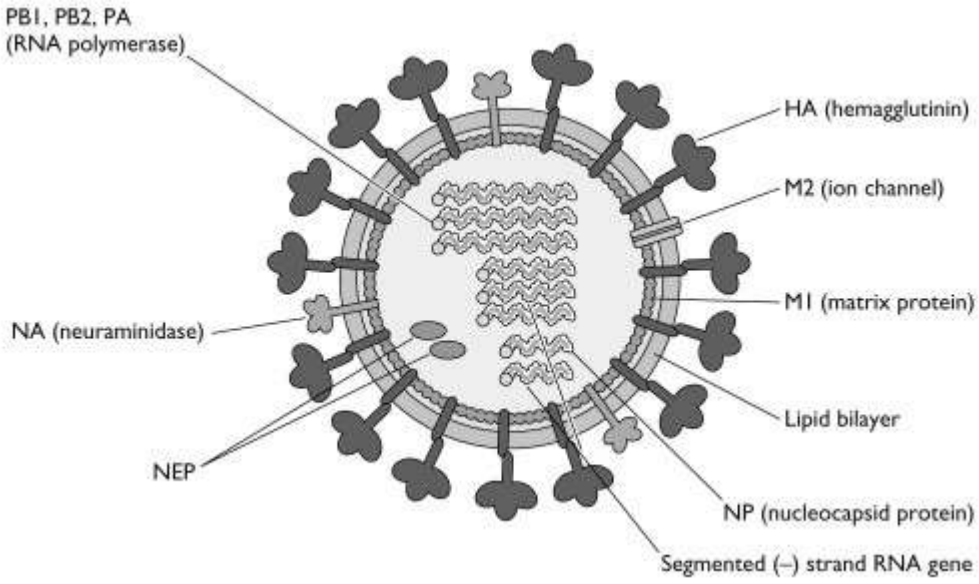
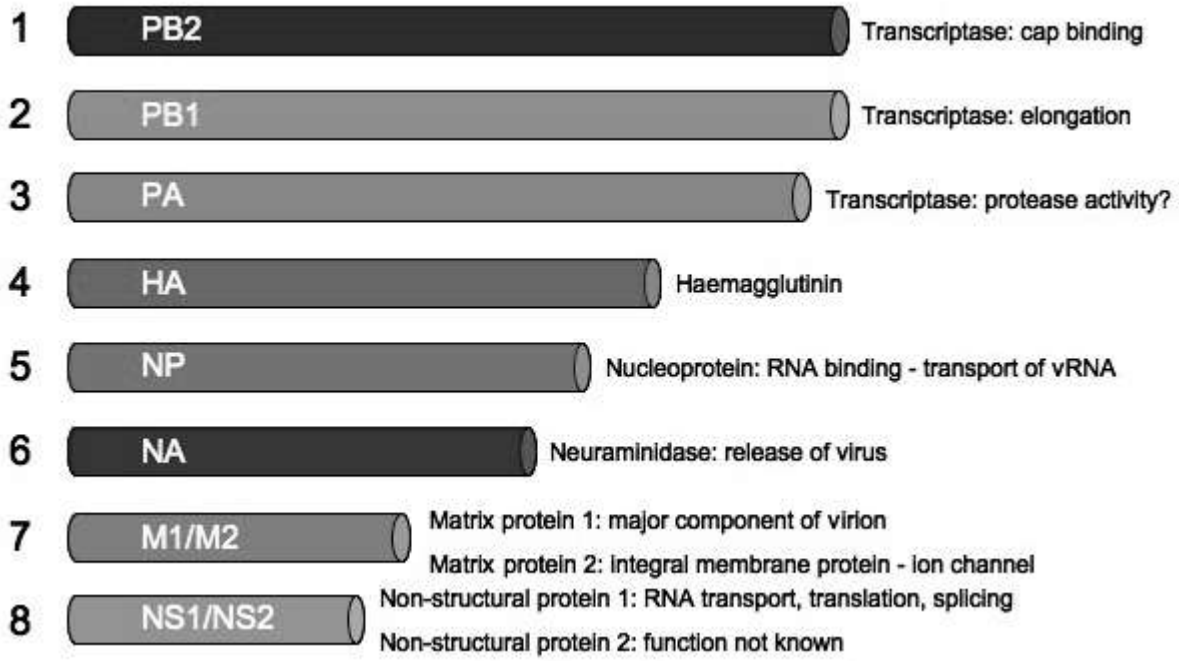


Fig: Structure of Influenza virus

3.6.3. **Genome**

Fig: Genome of Influenza virus



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d B viruses contain eight RNA segments (genes), whereas influenza C viruses contain only seven RNA segments. Influenza C viruses contain a single surface glycoprotein (the haemagglutinin-esterase-fusion, or HEF, glycoprotein), which functionally replaces the two surface glycoproteins that are found in influenza A and B viruses, namely haemagglutinin and neuraminidase [HA and NA].

3.6.4.Epidemiology

In temperate regions, influenza causes seasonal epidemics in the winter months (between April and September for the southern hemisphere). In tropical climates, influenza tends to occur all year round. Every few decades, a new influenza strain

emerges that sweeps across the world infecting millions of people. This is called a pandemic.

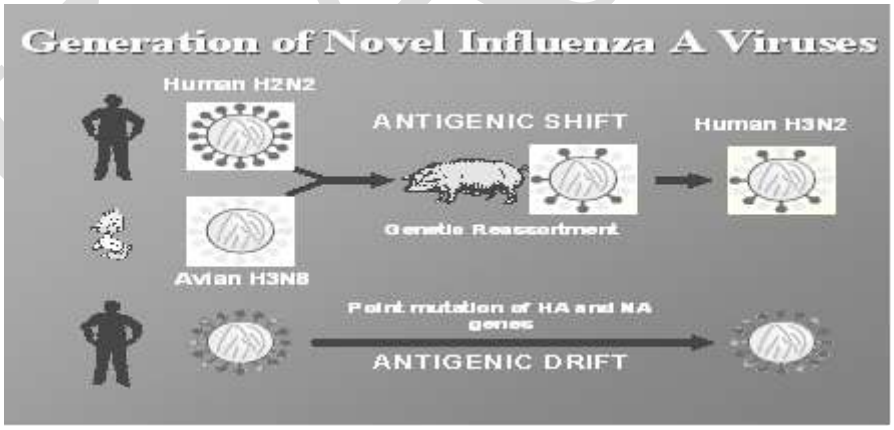
Individuals can suffer repeated attacks of influenza because the antigenic structure of the virus is highly variable. Two viral evolutionary mechanisms account for antigenic change, namely **drift** (both influenza A and B) and **shift** (only for influenza A).

Drift (minor antigenic change)

The envelope glycoproteins (HA and NA) of influenza virus change their antigenic character gradually over time. This is due to random **point mutations** introduced during replication of the viral genome. The viral RNA polymerase has no proof-reading function and is therefore highly error prone. Drift results in annual epidemics of influenza A and B in humans.

Shift (major antigenic change)

Influenza viruses have segmented genomes (each gene is on a separate gene segment). If a single cell is simultaneously infected with 2 different influenza viruses, gene swapping can occur during the formation of new virus particles. This genetic change process is called **reassortment.** Antigenic shift occurs when progeny viruses acquire a new HA or NA gene usually from an animal influenza virus. The new virus will have very different antigenic characteristics from the parent virus.



3.6.5.Replication

Orthomyxovirus replication takes about 6 hours and kills the host cell. The viruses attach to permissive cells via the hemagglutinin subunit, which binds to cell membrane glycolipids or glycoproteins containing N-acetylneuraminic acid, the receptor for virus adsorption. The virus is then engulfed by pinocytosis into endosomes. The acid environment of the endosome causes the virus envelope to fuse with the plasma membrane of the endosome, uncoating the nucleocapsid

and releasing it into the cytoplasm. A transmembrane protein derived from the matrix gene (M2) forms an ion channel for protons to enter the virion and destabilize protein binding allowing the nucleocapsid to be transported to the nucleus, where the genome is transcribed by viral enzymes to yield viral mRNA. Unlike replication of other RNA viruses, orthomyxovirus replication depends on the presence of active host cell DNA. The virus scavenges cap sequences from the nascent mRNA generated in the nucleus by transcription of the host DNA and attaches them to its own mRNA. These cap sequences allow the viral mRNA to be transported to the cytoplasm, where it is translated by host ribosomes. The nucleocapsid is assembled in the nucleus

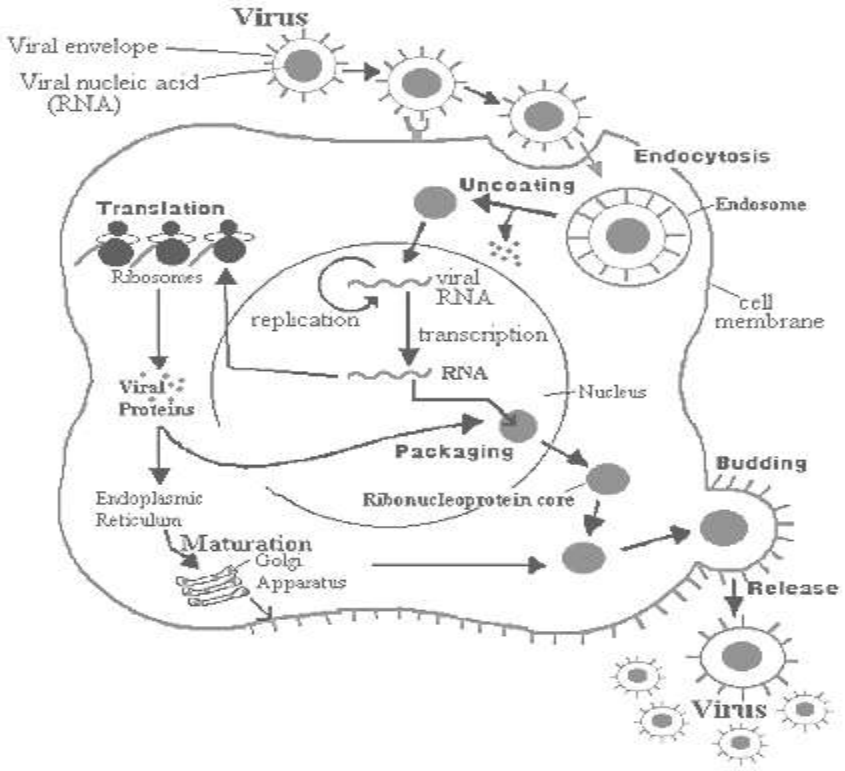


Fig: Replication of Influenza virus

Virions acquire an envelope and undergo maturation as they bud through the host cell membrane. During budding, the viral envelope hemagglutinin is subjected to proteolytic cleavage by host enzymes. This process is necessary for the released particles to be infectious. Newly synthesized virions have surface glycoproteins that contain N acetylneuraminic acid as a part of their carbohydrate structure, and thus are vulnerable to self-agglutination by the hemagglutinin. A major function of the viral neuraminidase is to remove these residues.

3.6.6. Pathogenesis

Influenza virus is transmitted from person to person primarily in droplets released by sneezing and coughing. Some of the inhaled virus lands in the lower respiratory tract, and the primary site of disease is the tracheobronchial tree, although the nasopharynx is also involved. The neuraminidase of the viral envelope may act on the N-acetylneuraminic acid residues in mucus to produce liquefaction. In concert with mucociliary transport, this liquified mucus may help spread the virus through the respiratory tract. Infection of mucosal cells results in cellular destruction and desquamation of the superficial mucosa. The resulting edema and mononuclear cell infiltration of the involved areas are accompanied by such symptoms as nonproductive cough, sore throat, and nasal discharge. Although the cough may be striking, the most prominent symptoms of influenza are systemic: fever, muscle aches, and general prostration. Viremia is rare, so these systemic symptoms are not caused directly by the virus. Circulating interferon is a possible cause: administration of therapeutic interferon causes systemic symptoms resembling those of influenza.

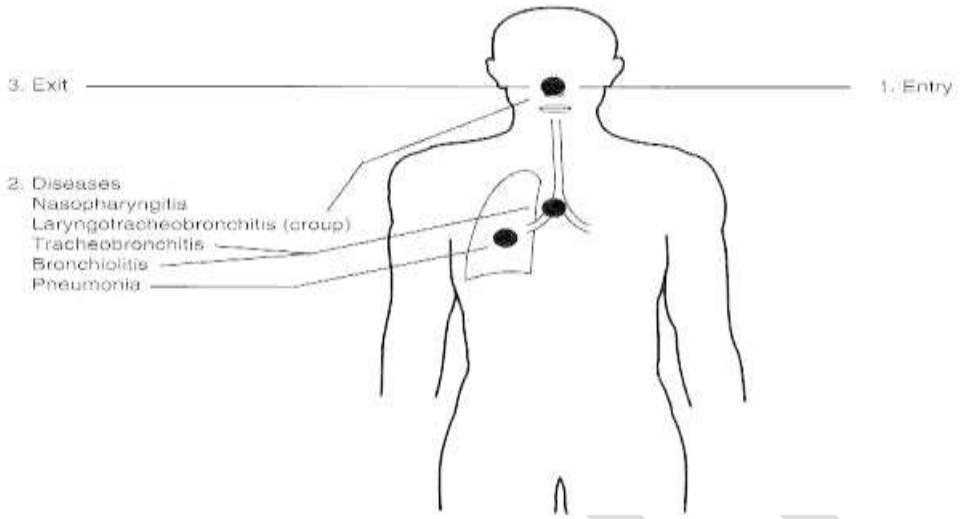


Fig : Pathogenesis of Influenza

3.6.7. Clinical features

Influenza virus is transmitted by respiratory droplets and has an incubation time of 2-3 days. Flu is a primarily respiratory tract infection but systemic symptoms are common.

Illness	Influenza	Common cold
Clinical spectrum	Often systemic	Mostly local
Speed of onset	Abrupt	Gradual
Fever	Usually high	Usually low-grade
Presentation	Chills, myalgia, malaise, sore throat	Sneezing, sore throat, nasal congestion
Fatigue	Marked	Mild
Course	Unwell for 1-2 weeks, chest problems common, severe malaise	Rapid recovery
Complications	More common and often severe - pneumonia	Uncommon
Occurrence	Seasonal	All year round

Reye's syndrome is a rare but severe complication seen in children with influenza (and other viral infections). This fatal condition causes encephalitis and liver disease in children treated with Aspirin. Therefore children with influenza infection should not be given aspirin.

Risk factors

Risk factors for severe influenza disease include pregnancy, chronic pulmonary, cardiac or renal disease, diabetes mellitus, individuals over 65 years and children less than 5 years.

3.6.8.Diagnosis

The virus replicates in the cells lining the respiratory tract. Therefore an appropriate sample for influenza testing is a nasopharyngeal aspirate in children and throat swab in adult. Influenza virus can be detected by direct antigen detection (immunofluorescence) in exfoliated cells, viral culture, or by means of molecular techniques such as polymerase chain reaction (PCR).

3.6.9.Treatment

There are two classes of antivirals against influenza viruses.

M2 channel inhibitors - E.g. Amatidine and Rimantidine

- prevent the entry of the virus to the cells

Neuraminidase inhibitors – E.g. Zanamivir and Oseltamivir

- prevent the budding off of the newly formed virions. This class of antivirals is only effective if given early (first 48 hours of infection.

3.6.10.Prevention

1. ***Vaccine*** - There are two types of vaccine

a. ***Trivalent Inactivated Vaccine (TIV)***
b. Live, Attenuated Influenza Virus vaccine (LAIV)

2. ***Wash your hands*** **-** Thorough and frequent hand-washing is the best way to prevent many common infections. Scrub your hands vigorously for at least 15 seconds.
3. ***Contain your coughs and sneezes*** **-** Cover your mouth and nose when you sneeze or cough.
4. ***Avoid crowds*** **-** Flu spreads easily wherever people congregate — in child care centers, schools, office buildings, auditoriums and public transportation.

PIVORNAVIRUS

3.1.Poliovirus

3.1.1. Introduction

a. 3 serotypes of poliovirus (1, 2, and3) but no common antigen.
b. Have identical physical properties but only share 36-52% nucleotide homology.
c. Humans are the only susceptible hosts.
d. Poliovirus is on course of being eradicated worldwide by the end of 2000 or 2001.
e. Poliovirus has three serotypes; PV1, PV2, and PV3. There are many strains of each serotype. The three serotypes are very infectious, but differ in the protein capsid. PV1 is the most common and harmful serotype. Poliovirus is commonly known as the most important and basic virus because of its short genome and simple structure

3.1.2. Morphology

a. Poliovirus is made up of a RNA genome and a protein capsid.
b. The RNA genome is a linear, single-stranded, positive-strand RNA. It is about 7,500 nucleotides long.

c. The viral component is about 300 Ångström wide with icosahdral symmetry.
d. Poliovirus builds its RNA genes and its protective protein capsid from these elements.

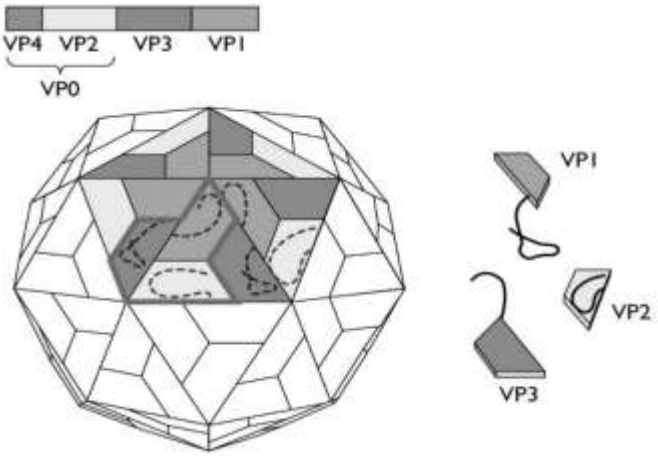


Fig: Structure of Poliovirus

3.1.3. **Genome**

Genomic structure of poliovirus and processing of the P3 domain of the polyprotein. The plus strand RNA genome of poliovirus with the terminal protein VPg covalently linked to the 5' end of the RNA. The 5' nontranslated region (NTR) and 3' NTR are shown with single lines. The genome is terminated with a poly(A) tail. The polyproteins contains structural (P1) and nonstructural (P2 and P3 domains) that are processed into precursor and mature proteins.

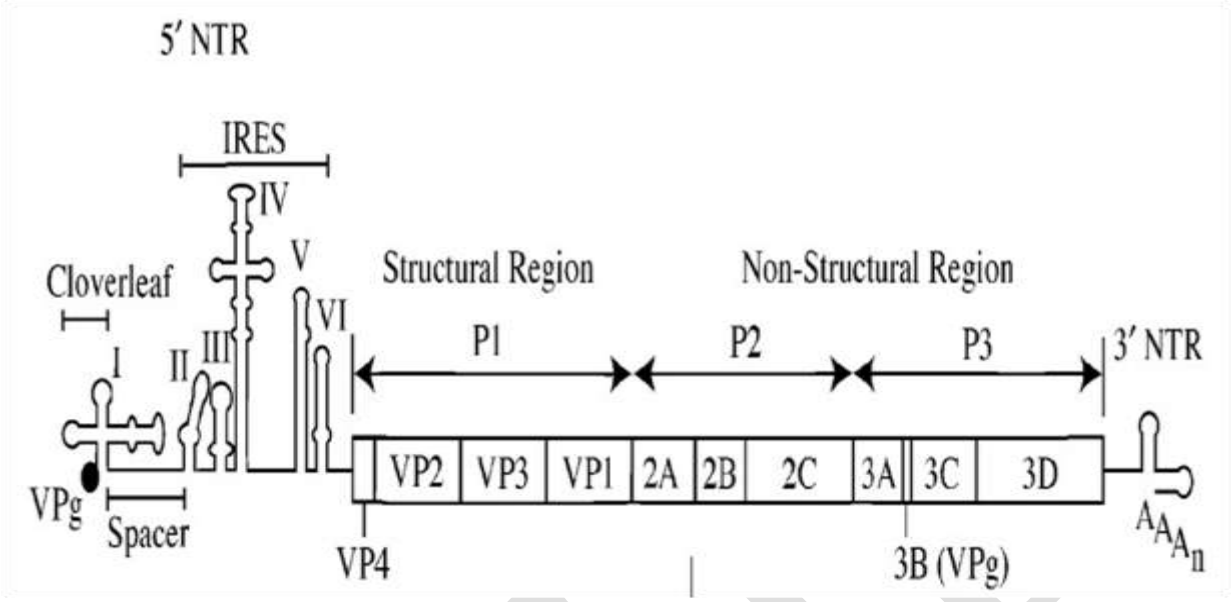
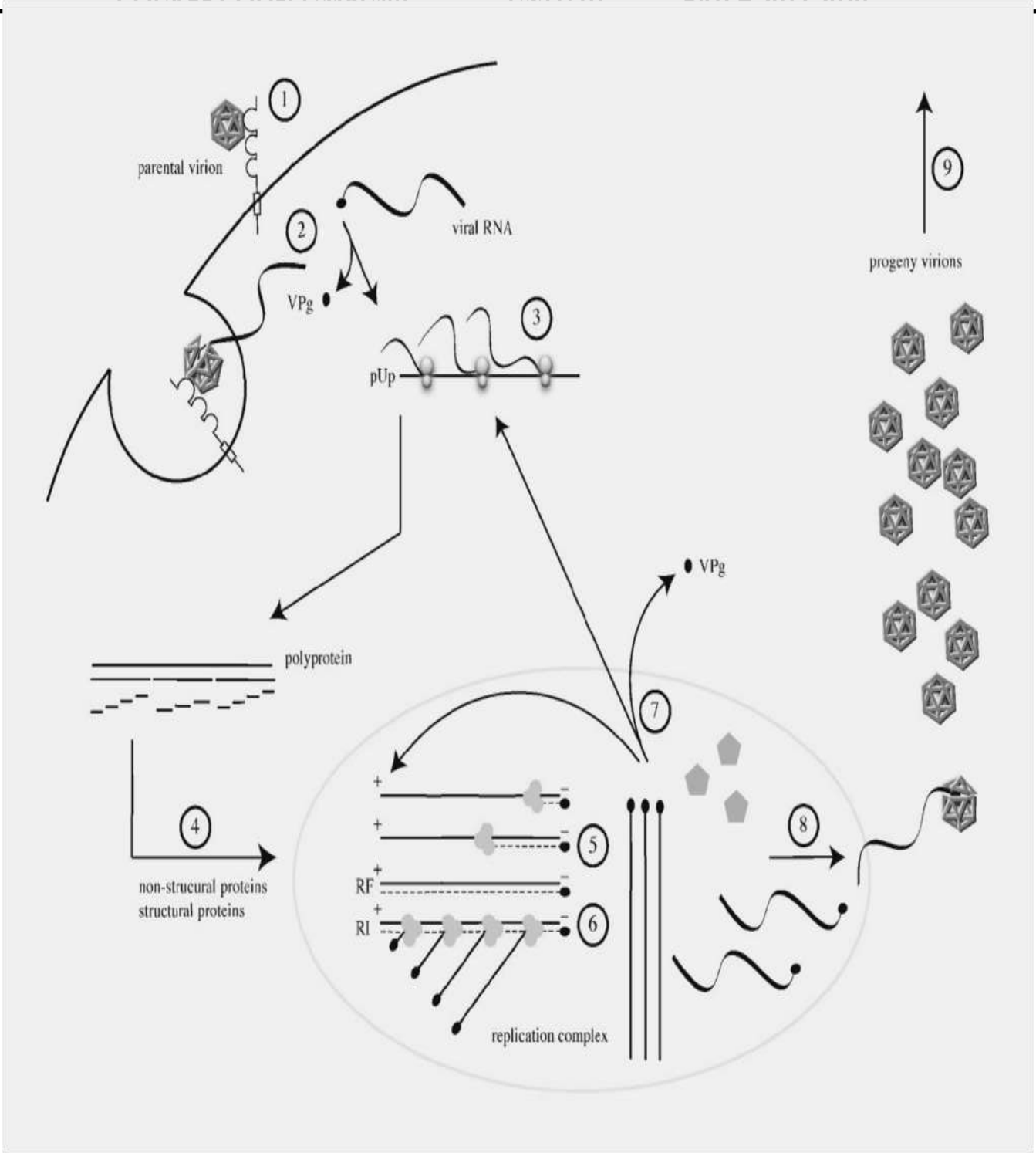


Fig:Genomic structure of poliovirus

3.1.4. **Replication**

The cellular life cycle of poliovirus begins by binding of a poliovirion to the host cell surface receptor, CD155 (1). Destabilization of the virus capsid which is receptor dependent uncoats the viral RNA (2). Cellular phosphodiesterase cleaves the viral protein VPg and viral RNA is translated by a cap-independent (IRES-mediated) process (3). The viral polyprotein generates mature structural and non-structural proteins by proteolytic processing (4). The positive-sense RNA acts as template for complementary negative-strand synthesis, which constructs a double-stranded RNA (replicative form, RF) (5). Initiation of many positive strands from a single negative strand produces the partially single-stranded replicative intermediate (RI)



(6). The synthesized positive-sense RNA molecules can act as templates for translation (7) or associate with capsid precursors to undergo encapsidation and the maturation cleavage of VP0 (8), which produces virions. The infected cell bursts and releases infectious viruses into the bloodstream (9).

Fig: Replication of Poliovirus

3.1.5. **Pathogenesis**

The poliovirus can take over the nervous system and can cause paralysis in hours. Poliovirus enters the body through the mouth and multiplies in the throat and intestine. Once the virus remains stable in the intestine, it can enter the blood stream and pass onto the central nervous system. As poliovirus replicates, it damages motor neurons which affect the muscles for swallowing, circulation, respiration, as well as the trunk, arms, and legs. The damage of the motor neurons are irreparable and can cause acute flaccid paralysis (AFP) which affects the limbs. Paralysis of the trunk and muscles of the thorax and abdomen can result in quadriplegia.

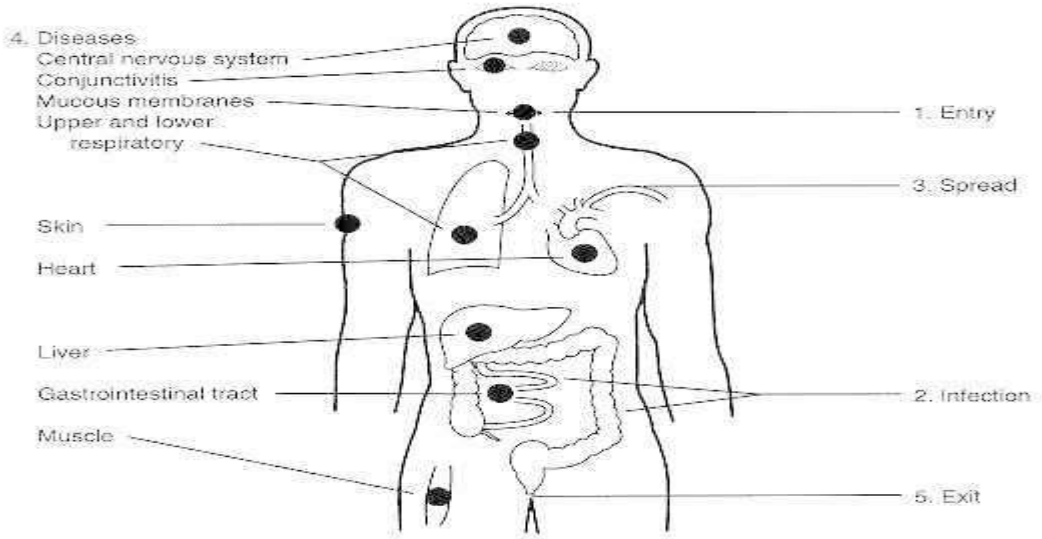


Fig: Pathogenesis of Poliovirus infections

Poliovirus causes the viral disease, poliomyelitis. Poliomyelitis is a very infectious disease. Humans are the exclusive natural host for poliovirus. It cannot naturally infect other species. There are two types of poliomyelitis;

a. ***Nonparalytic polio*** – It is the main type of polio that doesn't lead to paralysis (abortive poliomyelitis). About 5-10% of people are affected
b. ***Paralytic polio*** - About 1% of people are affected by paralytic polio. There are three types of paralytic poliomyelitis; spinal polio, bulbar polio, and bulbospinal polio.

- *Spinal polio* is the most common paralytic polio. The poliovirus invades motor neurons in the spinal cord which may cause paralysis of the muscles that control breathing as well as the arms and legs.
- *Bulbar polio* is the more severe case of poliomyelitis. The poliovirus attacks the motor neurons of the brain stem and causes difficulty in swallowing and speaking. Without respiratory support, bulbar polio can lead to death.
- *Bulbospinal polio* is a combination of bulbar and spinal polio. Bulbospinal polio can lead to paralysis of the arms and legs. It may also affect breathing, swallowing and heart function.

3.1.6. **Clinical Features**

There are 3 possible outcomes of infection:

a. ***Subclinical infection*** (90 - 95%) - inapparent subclinical infection account for the vast majority of poliovirus infections.
b. ***Abortive infection*** (4 - 8%) - a minor influenza-like illness occurs, recovery occurs within a few days and the diagnosis can only be made by the laboratory. The minor illness may be accompanied by aseptic meningitis
c. ***Major illness*** (1 - 2%) - the major illness may present 2 - 3 days following the minor illness or without any preceding minor illness. Signs of aseptic meningitis are common. Involvement of the anterior horn cells lead to flaccid paralysis. Involvement of the medulla may lead to respiratory paralysis and death.

3.1.7. **Lab diagnosis**

a. Virus Isolation
   i. Mainstay of diagnosis of poliovirus infection
   ii. Poliovirus can be readily isolated from throat swabs, faeces and rectal swabs. It is rarely isolated from the CSF
   iii. Can be readily grown and identified in cell culture
   iv. Requires molecular techniques to differentiate between the wild type and the vaccine type.

b. Serology
   i. Very rarely used for diagnosis since cell culture is efficient. Occasionally used for immune status screening for immunocompromised individuals.
   ii.

3.1.8. Treatment

No specific antiviral therapy is available. However the disease may be prevented through vaccination. There are two vaccines available.

a. ***Intramuscular Poliovirus Vaccine*** (IPV)
   i. Consists of formalin inactivated virus of all 3 poliovirus serotypes.
   ii. Produces serum antibodies only: does not induce local immunity and thus will not prevent local infection of the gut.
   iii. However, it will prevent paralytic poliomyelitis since viraemia is essential for the pathogenesis of the disease.

b. ***Oral Poliovirus Vaccine*** (OPV)
   i. Consists of live attenuated virus of all 3 serotypes.
   ii. Produces local immunity through the induction of an IgA response as well as systemic immunity.
   iii. Rarely causes paralytic poliomyelitis, around 1 in 3 million doses.

3.1.9. Prevention

Control of picornavirus diseases depends largely on mass education of the public on the mode of virus transmission, stressing the importance of good personal hygiene, and on provision of a good sewage disposal system and uncontaminated water supply. Fecal and pharyngeal discharges are infectious; hence, they must be handled with care and disposed of safely**.**

Unit III Question
_____ is specifically able to have a l
Which of the following properties is due t
T-even phage binding to E. coli probably
The filamentous bacteriopohage infect ma
A bacterial defense mechanism against ba
Intracellular structures formed during ma
Viral RNA is replicated in the host cell
Which of the following virus is always de
Viroids are composed of
Which of the following is the agent assoc
_____is probably the most important
The _____ of the influenza-envelop
Negative strand ssRNA viruses need to ha
Which of the following reflects the correc
What can be coated to the plastic dish if a
The nucleocapsid is covered by an outer r
Which type of interferon is produced by v
Fluorescence microscopy can be used for
Viral matrix proteins are
The microbes which are most likely to be
The two major components of viruses are
The transcription of the viral nucleic acid
What does the positive strand in double st
Which of the following characteristics wo
The reovirus and influenza virus contain
Which type of interferon is produced by T
Transfer of infection from cell to cell occ
Intracytoplasmic inclusion bodies are fou
What is the function of positive strand in
Which of the following virus is transmitte
The predominant lipid substance found in
Supercoiling refers to the extra turns in th
The tissue structure deteriorates as the vir
The structural abnormality of cells is term
The penetration of animal viruses into atta
Viropexis takes place in the phagocytic va
Negri bodies are found in cells infected w
The uncontrolled proliferation of cells is t
The RNA sarcoma viruses, the most stron
Plant viruses penetrate host cells through
The disease(s) caused by genus Orthopox
The virus(es) which can cause aseptic me
Which of the following genera can cause
Which of the coxsackieviruses can cause

Which of the following genera is included
Transmission of Molluscum contagiosum
The symmetry of nucleocapsid of poxviru
Which of the following hepatitis viruses i
Which of the following nucleic acid is pre
The agent representing an isolate of hepati
Hepatitis G virus belongs to the family
Transmission of hepatitis G virus is
The most serious infection is
Which of the following specimens contai
Vertical transmission may be seen in
Which of the following viral infections ca
Most reliable test for detection of hepatiti
Which of the following viral infections is
Which of the following viruses is star-sha
What is the shape of rabies virus?

Opt 1	Opt 2
Temperate virus	Adsorbed virus
Toxin production in Corynebacteri	Toxin production in Clostridium botulinum
electrostatic interaction	hydrophobic interaction
lipopolysaccharide	the cell wall
Cconcatamerization	polymerization
prokaryotes	chromosomal disruptions
cytoplasmic matrix	nucleus
Hepatitis B virus	Herpes simplex virus
single-stranded DNA	double-stranded DNA
Prions	Viroids
Host preference	Morphology
fimbriae	flagellae
use it to terminate transcripts when	have to make a positive strand copy that can
Attachment, penetration, maturatio	Penetration, attachment, biosynthesis, matur
Patient serum	Anti-polio antibody
envelope	covering
alpha	beta
Subacute sclerosing panencephaliti	Herpes simplex encephalitis
exposed on the surface of the virus	found mainly on naked viruses
viruses	bacteria
fat and protein	nucleic acid and protein
RNA viruses	ds DNA viruses
rRNA	tRNA
Type of cell wall structure	Type of nucleic acid
10 different segments of dsRNA ar	8 different segments of dsRNA and 10 differ
α	β
plasmodesmata	cytodesmata
Rabies virus	Vaccinia virus
Synthesis of protein	Production of ribosomes
Rhinovirus	Coronavirus
phospholipids	glycolipids
DNA gyrase	DNA pyrase
aneuploidy	protopathic effect
hyperplasia	anaplasia
vitropexis	viropexis
DNA gyrase	lysosomal protease
Paramyxoviruses	Vaccinia virus
hyperplasia	anaplasia
fibroblasts	myoblasts
ectodesmata	endodesmata
Vaccinia	Smallpox
Polioviruses	Coxsackieviruses
Orthopoxvirus	Parapoxvirus
Group A	Group C

Enterovirus	Rhinovirus
direct contact	sexual contact
complex	icosahedral
Hepatitis A virus	Hepatitis B virus
dsDNA	ssRNA
GBA-A	GBV-B
Caliciviridae	Flaviviridae
Blood	Sexually
superinfection of an HBsAg carrier	infection with HBV alone
Blood	Semen
Hepatitis B virus	Hepatitis C virus
HBV	HCV
ELISA test for IgM anti-HEV	Western blot assay for IgM anti-HEV
Rabies	Influenza
Astrovirus	Calicivirus
Spherical	Polygonal

Opt 3	Opt 4
RNA phage	DNA phage
Antigenic variation in Salmonella a	toxic chemical shift
covalent bonds	bivalent bonds
the tip of the pilus	the cell membrane
restriction	lysogeny
inclusion bodies	cytocidal bodies
mitochondria	lysozomes
Varicella-zoster virus	Cytomegalovirus
single-stranded RNA	double-stranded RNA
virions	Virinos
Physical nature of virion constituen	Chemical nature of virion constituents
hemagluttinin	neuraminidase
use it to modify host enzymes that a	none of the above
Attachment, penetration, biosynthe	Attachment, release, biosynthesis, maturation, per
Polio capsid protein	Colored substrate
Membronocapsid	capsid
Both (a) and (b)	gamma
Rabies	hepatitis
anchor the envelope of enveloped v	part of the nucleoprotein core of viruses
fungi	algae
carbohydrate and nucleic acid	fat and carbohydrate
ss DNA viruses	ss RNA viruses
mRNA	RNA
Presence of an envelope	Symmetry
5 different segments of dsRNA and	7 different segments of dsRNA and 5different seg
γ	γ and β
protodesmata	cytomorpha
Fowlpox virus	HIV
Both (a) and (b)	biosynthesis
Measles virus	CAMV
neutral fat	Lipds
RNA gyrase	RNA pyrase
cytopathic effect	CFE
metastasis	cytostasis
ectodesmata	vivopexis
lysosomal lipase	DNA lipase
Fowlpox virus	Rabies virus
metastasis	cytostasis
iris epithelial	protoplasts
cytodesmata	protodesmata
Cowpox	Chicken pox
Echo viruses	HIV
Molluscipoxvirus	Mesopox
Group D	Group E

Hepatovirus	HIV
Indirect	Mechanical
helical	Cylinderical
Hepatitis E virus	
ssDNA	dsRNA
GBV-C	GBV
Hepadnaviridae	Coronaviridae
Saliva	neonatal
coinfection of HBVand HDV	re infection
Saliva	pus
Hepatitis D virus	Hepatitis E Virus
HDV	All of these
Polymerase chain reaction for detec	ELISA test for IgG anti-HEV
Polio	Hepatitis
Hepatitis E virus	Hepatitis D virus
Bullet-shaped	Tubular

Opt 5	Answer
	Temperate virus
	Antigenic variation in Salmonella anatum
	electrostatic interaction
	the cell wall
	restriction
	cytocidal bodies
	cytoplasmic matrix
	Hepatitis B virus
	double-stranded DNA
	Prions
	Chemical nature of virion constituents
	neuraminidase
	use it to terminate transcripts when they copy host cell mRNA
ıetration	Attachment, penetration, biosynthesis, maturation, release
	Anti-polio antibody
	envelope
	Both (a) and (b)
	Herpes simplex encephalitis
	exposed on the surface of the virus
	viruses
	nucleic acid and protein
	ds DNA viruses
	mRNA
	Presence of an envelope
ments of ssRNA respectively	8 different segments of dsRNA and 10 different segments of s
	γ and β
	plasmodesmata
	Fowlpox virus
	Synthesis of protein
	Rhinovirus
	glycolipids
	DNA pyrase
	cytopathic effect
	anaplasia
	viropexis
	lysosomal protease
	Paramyxoviruses
	metastasis
	fibroblasts
	cytodesmata
	Smallpox
	Coxsackieviruses
	Orthopoxvirus
	Group A

	Enterovirus
	sexual contact
	complex
	Hepatitis E virus
	dsDNA
	GBA-A
	Flaviviridae
	Sexually
	infection with HBV alone
	Blood
	Hepatitis B virus
	HCV
	Western blot assay for IgM anti-HEV
	Rabies
	Astrovirus
	Bullet-shaped

ssRNA respectively

UNIT – 4

1.6. Viral Replication

It is the process of synthesis of new virus particles

The primary purposes of viral cultivation are:

1. To isolate and identify viruses in clinical specimens;
2. To prepare viruses for vaccines; and
3. To do detailed research on viral structure, multiplication cycles, genetics, and effects on host cells.

1.6.1. *Steps in Viral Replication*

Attachment or Adsorption

This is the first step in viral replication. Surface proteins of the virus interact with specific receptors on the target cell surface. These may be specialized proteins with limited distribution or molecules that are more widely distributed on tissues throughout the body. The presence of a virus-specific receptor is necessary but not sufficient for viruses to infect cells and complete the replicative cycle.

Penetration

Enveloped viruses (e.g., HIV, influenza virus) penetrate cells through fusion of the viral envelope with the host cell membrane. Non-enveloped viruses penetrate cells by translocation of the virion across the host cell membrane or receptor mediated endocytosis of the virion in clathrin coated pits with accumulation of viruses in cytoplasmic vesicles.

Uncoating (disassembly)

A complex process which differs by taxonomic class and is not fully understood for many agents. This process makes the nucleic acid available for transcription to permit multiplication of the virus.

Biosynthesis

The key to understanding the genomic expression of viruses is noting the fact that viruses must use host cellular machinery to replicate and make functional and structural proteins.

Assembly and Release

The process of virion assembly involves bringing together newly formed viral nucleic acid and the structural proteins to form the nucleocapsid of the virus. There are basically three strategies that viruses employ:

- Nonenveloped viruses exhibit full maturation in the cytoplasm (e.g., picornaviruses) or the nucleus (e.g., adenoviruses) with disintegration of the cell and release of virions.

- For enveloped viruses, including the (-) strand RNA viruses, the (+) strand togaviruses and the retroviruses, final maturation of the virion takes place as the virion exits the cell. Viral proteins are inserted into the host cell membrane. Nucleocapsids bind to the regions of the host cell membranes with these inserted proteins and bud into the extracellular space. Further cleavage and maturation of proteins may occur after viral extrusion to impart full infectivity on the virion. Viruses in this group differ in their degree of cytolytic activity.

- Herpesviruses, which are enveloped viruses, assemble their nucleocapsids in the nuclei of infected cells and mature at the inner lamella of the nuclear membrane. Virions accumulate in this region, in the endoplasmic reticulum and in vesicles protected from the cytoplasm. Release of virions from the cell surface is associated with cytolysis.

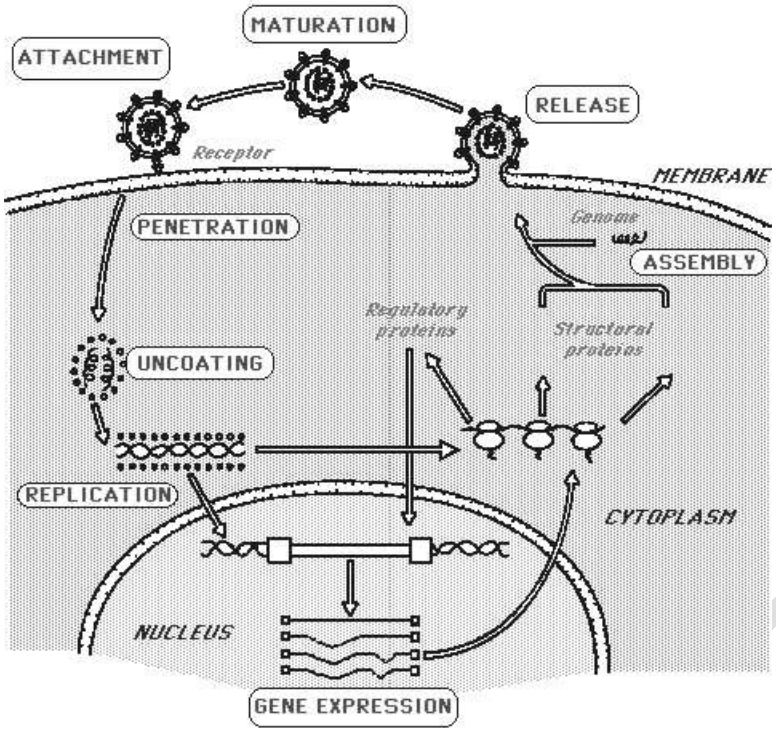


Fig: Replication cycle of viruses

2.1.**Pox viruses**

2.1.1. **Introduction**

Poxviruses (members of the family ***Poxviridae***) are viruses that can, as a family, infect both vertebrate and invertebrate animals. Four genera of poxviruses may infect humans: orthopox, parapox, yatapox, molluscipox.

Orthopox: smallpox virus (variola), vaccinia virus, cowpox virus, monkey pox virus; **Parapox**: orf virus, pseudo cowpox, bovine papular stomatitis virus;

Yatapox: tanapox virus, yaba monkey tumor virus;

Molluscipox: molluscum contagiosum virus (MCV).

2.1.2. **Morphology**

Oval or "brick-shaped" particles 200-400nm long - can be visualized by the best light microscopes (just). The external surface is ridged in parallel rows, sometimes arranged helically. The particles are extremely complex, containing many proteins (more than 100) and detailed structure is not known. The extracellular forms contain 2 membranes (**EEV** - extracellular enveloped virions), intracellular particles only have an inner membrane (**IMV** - intracellular mature virions). The outer surface is composed of lipid and protein which surrounds the core, which is biconcave (dumbbell-shaped), with 2 "lateral bodies" (function unknown). The core is composed of a tightly compressed nucleoprotein.

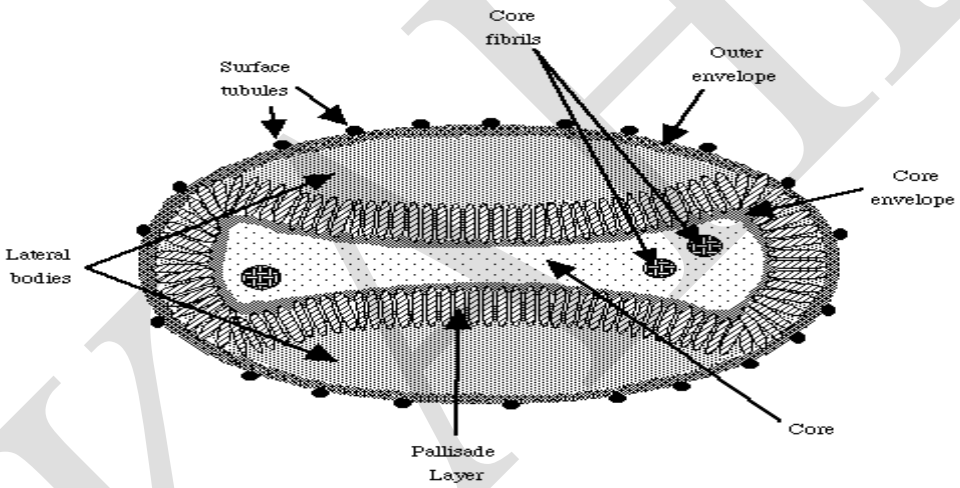


Fig: Structure of Pox virus

2.1.3. Genome

Linear, d/s DNA of 130-300kbp. Ends of genome consist of a terminal hairpin loop (no free ends) with several tandem (i.e. direct) repeat sequences (this arrangement is found at the ends of chromosomes from a number of different organisms). The ends of the genome form direct repeats called inverted terminal repeats (ITRs). Several

poxvirus genomes have been sequenced. Most of the essential genes are located in the central part of the genome, while non-essential (in tissue culture) genes are located at the ends. There are ~200 genes in the genome.

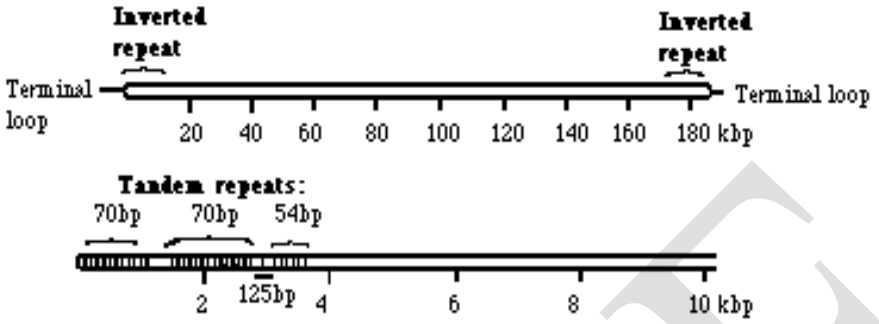


Fig: Genome of Pox virus

2.1.4. **Replication**

Occurs in the **cytoplasm** - the virus is sufficiently complex to have acquired all the functions necessary for genome replication. There is some contribution from the cell but it is not clear what this is - poxvirus gene expression and genome replication occur in enucleated cells, but maturation is blocked.

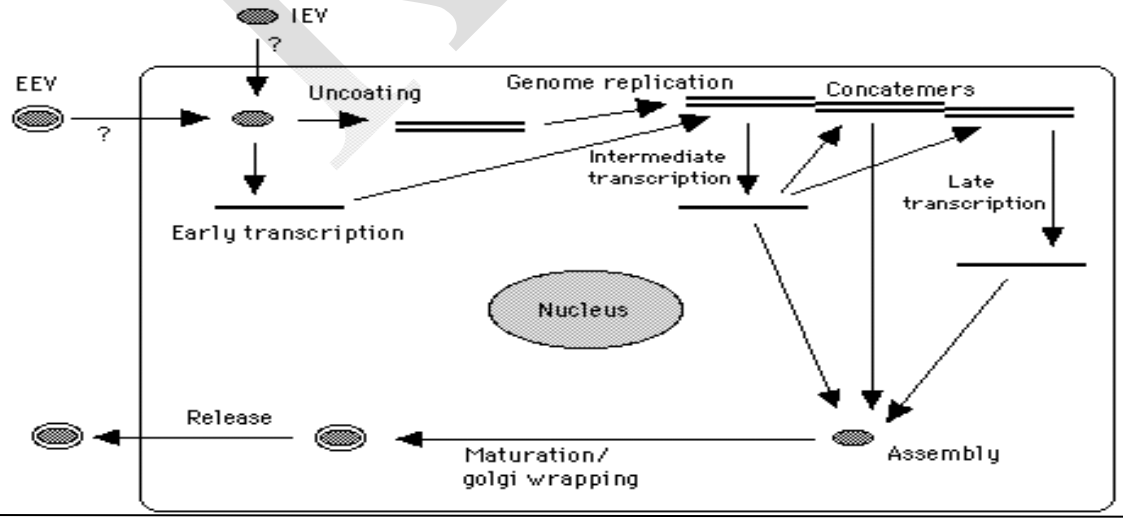


Fig: Replication cycle of Pox virus

Receptorsare not known, but probably >1 on different cell types. For Vaccinia, one of these is probably the EGF receptor (epidermal growth factor).

Penetration is complex and may also involve >1 mechanism.

Uncoating occurs in two stages, removal of the outer membrane as the particle enters the cell and in the cytoplasm, the particle (minus its outer membrane) is further uncoated and the core passes into the cytoplasm.

Gene expression is carried out (exclusively?) by viral enzymes associated with the core and is divided into 2 phases:

Early genes: ~50% genome, expressed before genome replication

Late genes: expressed after genome replication; late promoters are dependent on DNA replication for activity.

Genome replication is believed to involve self-priming, leading to the formation of high m.w. concatemers (isolated from infected cells) which are subsequently cleaved and repaired to make virus genomes.

Assembly occurs in the cytoskeleton; the events involved in putting together such a complex particle are not understood, but probably involve interactions with the cytoskeleton (e.g. actin-binding proteins). Inclusions are formed in the cytoplasm which matures into virus particles.

Overall, replication of this large, complex virus is rather quick ~12h.

2.1.5. **Pathogenesis**

The pathogenesis of localized poxvirus infections is simple. Virus invades through broken skin, replicates at the site of inoculation, and causes dermal hyperplasia and leukocyte infiltration. With cowpox, and to a lesser extent with parapox, there is limited lymphatic spread; this causes lymph

adenopathy and elicits an immune response. The lesion of molluscum is circumscribed by a connective tissue capsule, and the dermis, although distorted, is not usually broken. Some poxviruses express an epidermal growth factor and host range genes which play a role in pathogenesis and cell tropism.

Human monkeypox is usually acquired via the respiratory tract, and during a 12-day incubation period viremia distributes infection to internal organs, which are damaged by virus infection. Spread to the skin initiates the clinical phase, and the lesions progress through the classic stages of macule to papule to vesicle to pustule to crust. Lymphadenopathy, usually involving the cervical and inguinal areas, is often marked.

2.1.6. **Clinical Features**

Poxvirus infections are characterized by the production of skin lesions. With most poxviruses there is typically just a primary lesion, but generalized lesions develop with human monkeypox and molluscum .In human cowpox and parapox infections the lesion develops at the site of inoculation (usually the hand), and infection may be spread to other sites such as the face and/or genitals by scratching. When seen by the physician, cowpox and parapox lesions are usually hemorrhagic crusting ulcers, but early in infection the former are usually vesicular and the latter nodular. The lesions of molluscum, usually multiple, are firm, pearly, flesh-colored nodules.

Parapox and molluscum infections are relatively painless and cause very little constitutional disturbance. Human cowpox is very painful, particularly in young children, usually causes pyrexia and marked lymphadenopathy; patients often require hospitalization. Rare encephalitic complications of cowpox have been reported, and erythema multiforme is a complication of parapox infections. Infection in immunocompromised or eczematous individuals is more severe and usually results in generalized illness, and in cowpox has caused deaths.

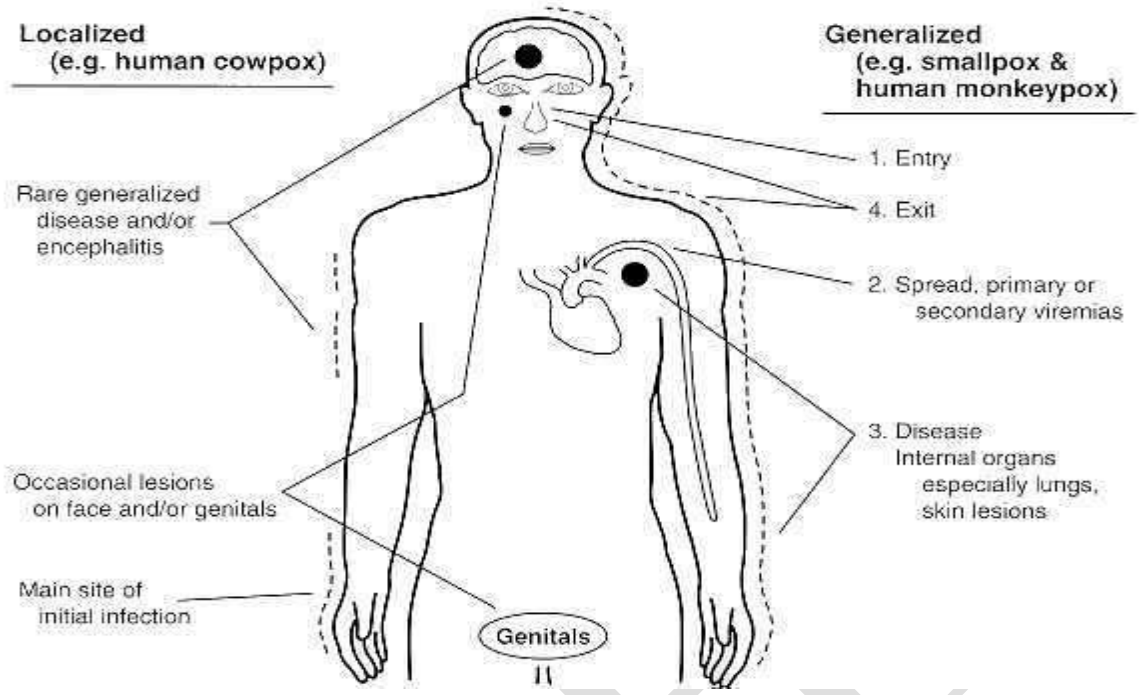


Fig: Localized and generalized poxvirus infections.
Note: Numbers indicate progression of infection

2.1.7. **Lab diagnosis**

The clinical definition of smallpox is an illness with acute onset of fever greater than 101 °F (38.3 °C) followed by a rash characterized by firm, deep seated vesicles or pustules in the same stage of development without other apparent cause. If a clinical case is observed, smallpox is confirmed using laboratory tests.

Microscopically, poxviruses produce characteristic cytoplasmic inclusions, the most important of which are known as Guarnieri bodies, and are the sites of viral replication. Guarnieri bodies are readily identified in skin biopsies stained with hematoxylin and eosin, and appear as pink blobs. They are found in virtually all poxvirus infections but the absence of Guarnieri bodies cannot be used to rule out smallpox. The diagnosis of an orthopoxvirus infection can also be made rapidly by electron microscopic examination of pustular fluid or scabs. However, all orthopoxviruses exhibit identical brick-shaped virions by electron microscopy.

Definitive laboratory identification of variola virus involves growing the virus on chorioallantoic membrane (part of a chicken embryo) and examining the resulting pock lesions under defined temperature conditions. Strains may be characterized by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis. Serologic tests and enzyme linked immunosorbent assays (ELISA), which measure variola virus-specific immunoglobulin and antigen have also been developed to assist in the diagnosis of infection.

2.1.8. **Treatment**

a. If a the smallpox vaccine is given within 1-4 days after a person is exposed to the disease, it may prevent illness or make the illness less severe. Once symptoms have started, treatment is limited.
b. There is no drug specifically for treating smallpox. Sometimes antibiotics are given for infections that may occur in people who have smallpox. Taking antibodies against a disease similar to smallpox (vaccinia immune globulin) may help shorten the duration of the disease.

2.1.9. **Prevention**

a. People who have been diagnosed with smallpox and everyone they have come into close contact with need to be isolated immediately. They need to receive the vaccine and be monitored.

Emergency measures would need to be taken immediately to protect the general population. Health officials would follow the recommended guidelines from the CDC and other federal and local health agencies.

Unit IVQuestion
The species of animals which is most susceptible to rabies infection is
Which of the following clinical specimens can be used for the demonstration of rab
Hepatocellular carcinoma (Hepatoma) may be caused by
The defective satellite virus is
All of the following viruses are enveloped except
The diagnosis of hepatitis A virus infection is carried out from the method based on
Which of the following is not correct for hepatitis A virus?
The viruses, which is/are transmitted by parenteral and sexual routes is/are
Which of the following viruses can be transmitted by the parenteral route?
Which of the following antigenic types of hepatitis B virus is present in the envelope
The development of hepatocellular carcinoma is associated with
Which of the following antigenic types of hepatitis B virus is prevalent in India?
Which of the following viral agents can cause diarrhea?
The virus(es) which has club-shaped peplomers and infects the respiratory tract is/a
Which of the following is the necessary step for cultivating the microorganisms?
Which of the following characteristic of the Rotavirus was important for the constru
The nature of the poliovirus gives for oral vaccination (satin vaccine) as part of the
The agent responsible for causing mad cow disease is thought to be a
Coxsackie virus B3, a subgroup of enteroviruses, commonly causes
The influenza vaccine is administered each year because
Which of the following disease is caused by DNA viruses?
What can be coated to the plastic dish if an ELISA is performed to directly detect p
Influenza virus undergoes antigenic shift resulting in major antigenic changes by wh
The nucleocapsid is covered by an outer membrane like structure called
Which type of interferon is produced by virus-infected cells?
Fluorescence microscopy can be used for the diagnosis of
Viral matrix proteins are
Viruses range in size from:
A structural component that is found in all viruses is:
A chemical component that is found in all viruses is:
A common polyhedral capsid shape of viruses is a :
Enteroviruses differ from rhinoviruses mainly in their:
Viruses that can remain latent (usually in neurons) for many years are most likely:
What types of viruses contain the enzyme lysozyme to aid in their infection?
Bacteriophage are readily counted by the process of:
A type of cell culture that can reproduce for an extended number of generations and
Which of the following is not an RNA virus?
What is the most common cause of aseptic meningitis of viral etiology?
Protection against influenza A virus in a non immune individual can be achieved th
Which one of the following immunizations should be administered immediately aft
Which one of the following infection routes is most often involved in the neonatal t
The finding of large, multinucleated, clumps of cells in the bronchial secretions of a
All of the following picornaviruses are resistant to the acidity of the stomach except
A divorced mother of four tests positive for HIV-1 Infection during investigation of

In a chronic carrier of hepatitis B virus (HBV), which positive test is most indicativ
A retrovirus is found in a high proportion of laboratory animals of a given species. I
When a virus enters a cell but does not replicate immediately, the situation is called
Usually viruses are separated into several large groups based primarily on
The first step in infection of a host bacterial cells by a phage is
Which of the following viruses has not been associated with human cancer?
The viral nucleocapsid is the combination of
Which of the following is the agent associated with the development of neurodegen
_____is probably the most important characteristic for classification of viruse
The _____ of the influenza-enveloped virus appear to be involved in attachm
Intracellular structures formed during many viral infections, called _____ , wi
Viral RNA is replicated in the host cell
Which family has received most interest in their development as a biological contro
In cancer, the reversion of cells to a more primitive or less differentiated state is cal
Which of the following has been linked to Kaposi's sarcoma?
Virulent and nonvirulent viruses may not

Opt 1	Opt 2
dog	cat
Salivary smears	Corneal smears
Hepatitis A virus	Hepatitis C virus
hepatitis B	hepatitis C
Hepatitis A virus	Hepatitis B virus
aminotransferase levels	detection of faecal HAV by immunoelectron
It can be grown in cell cultures of primate and	It is one of the most stable viruses infecting l
Hepatitis B virus	Hepatitis C virus
HBV	HCV
HbsAg	HBcAg
ground squirrel hepatitis virus	hepatitis B virus
Adw	Adr
Rotaviruses	Norwalk virus
Coronavirus	Astroviruses
Preparing a culture medium for the growth of r	Sterilizing in order to eliminate all living mic
The possession of a segmented RNA genome	A limited number of capsule types
heat killed virus	live attenuated strains of all three immunolog
fungus	protozoan
acute hemorrhagic conjunctivitis	muscular dystrophy
mutations in the viral hemagglutinin may allov	it is a polysaccharide vaccine that does not c
Poliomyelitis	Yellow fever
Patient serum	Anti-polio antibody
Somatic recombination of heavy and light chai	Expression of several different capsule types
envelope	covering
α	β
Subacute sclerosing panencephalitis	Herpes simplex encephalitis
exposed on the surface of the virus	found mainly on naked viruses
1-100 nm	25-300 nm
The envelope	DNA
Protein	Lipid
Pentagon	Cube
Type of nucleic acid	Size
Togaviruses	Herpesviruses
Bacteriophage	Animal Viruses
Immunoassays	ELISA
Primary cell culture	Continuous cell line
Retrovirus	Enterovirus
Enteroviruses	Herpesviruses
Viral endonuclease activity	binding of host messenger RNA (mRNA) ca
Diphtheria-pertusis-tetanus (DPT) vaccine	Haemophilus influenzae type b vaccine
Blood transfusion	Fetal contact with infected blood during chil
Bordetella pertusis	Respiratory syncytial virus (RSV)
Coxsackievirus A	Rhinovirus
Treatment with zidovudine (azidothymidine, A	All the patients close contacts would be teste

Hepatitis B Surface Antigen (HbsAg)	Hepatitis B Core Antigen (HbcAg)
gag	pol
lysogeny	fermentation
nature of the host	nucleic acid characteristics
adsorption	absorption
Hepatitis C virus	Hepatitis B virus
genome and capsid	capsid and spikes
Prions	Viroids
Host preference	Morphology
fimbriae	flagellae
prokaryotes	chromosomal disruptions
cytoplasmic matrix	nucleus
Reoviridae	Baculoviridae
neoplasia	anaplasia
Epstein-Barr virus	Human T-cell lymphotropic virus
inhibit host cell DNA synthesis	inhibit host cell RNA synthesis

Opt 3	Opt 4
fowl	cow
Conjunctival smears	blood smears
HSV	HIV
hepatitis D	hepatitis A
Hepatitis C virus	Hepatitis D virus
both (a) and (b)	detection of IgM anti-HAV by ELISA
It may cause hepatocellular carcinoma	nothing change
Hepatitis G virus	Hepatitis E virus
HDV	HEV
HBeAg	HBxAg
woodchuck hepatitis virus	HIV
Ayw	Ayr
Astroviruses	Rhinovirus
Norwalk agent	Rhinovirus
Inoculating the microorganisms in the pr	Incubating
The ability of monkey Rotavirus strains t	The ability of the Rotavirus to be transmitte
small dosage of wild-type live viruses	formalin-inactivated viruses
prion	virus
myocarditis	gastroenteritis
the vaccine is sufficiently toxic to make i	none of the above
Measles	Small pox
Polio capsid protein	Colored substrate
Changing the receptor binding canyon th	Reassortment of RNA segments from differ
membronocapsid	capsid
Both (a) and (b)	Nothing will be produced
Rabies	AIDS
anchor the envelope of enveloped viruses	part of the nucleoprotein core of viruses
10-100 μm	400-1000 nm
Capsid	Tail fibers
DNA	RNA
Icosahedron	Pyramid
Capsid shape	Ability to survive acidic conditions
Enteroviruses	Rhinoviruses
Plant Viruses	Fungal Viruses
Plaque assays	Tissue cell culture
Cell strain	Diploid fibroblast cell
Rhabadovirus	Adenovirus
Arboviruses	Retroviruses
Synthesis of viral progeny RNA	Uncoating of nucleic acid
Hepatitis B vaccine	HIV Vaccine
Ingestion of the virus via maternal breast	Transmission of the virus from hospital pers
Mycoplasma hominis	Epstein-Barr virus
Echo virus	Coxsackievirus B
The public health authorities should be n	A western blot (immunoblot) test should be

Hepatitis B e Antigen (HbeAg)	Anti-HBsAg
env	onc
symbiosis	synergism
capsid symmetry	diameter of the viroin or nucleocapsid
penetration	replication
Varicella-Zoster virus	Herpes simplex virus type 2
envelope and capsid	capsomere and genome
virions	Virinos
Physical nature of virion constituents	Chemical nature of virion constituents
hemagglutinin	neuraminidase
inclusion bodies	cytocidal bodies
mitochondria	lysozomes
Iridoviridae	Poxviridae
metastasis	oncogenic
Human papilloma virus	Human herpes virus 8
stimulate host cell macromolecule synthe	degrade host cell DNA

Opt 5	Answer
	dog
	Salivary smears
	Hepatitis A virus
	hepatitis D
	Hepatitis D virus
	detection of IgM anti-HAV by ELISA
	It can be grown in cell cultures of primate and humai
	Hepatitis B virus
	HBV
	HbsAg
	hepatitis B virus
	Adw
	Rotaviruses
	Coronavirus
	Preparing a culture medium for the growth of microc
d faster	A limited number of capsule types
	formalin-inactivated viruses
	prion
	acute hemorrhagic conjunctivitis
	mutations in the viral hemagglutinin may allow the v
	Poliomyelitis
	Anti-polio antibody
ent influenza viruses	Reassortment of RNA segments from different influ
	envelope
	α
	Subacute sclerosing panencephalitis
	found mainly on naked viruses
	25-300 nm
	Capsid
	Protein
	Icosahedron
	Type of nucleic acid
	Togaviruses
	Bacteriophage
	Plaque assays
	Continuous cell line
	Rhabadovirus
	Arboviruses
	Viral endonuclease activity
	Diphtheria-pertusis-tetanus (DPT) vaccine
onnel during childbirth	Fetal contact with infected blood during childbirth
	Respiratory syncytial virus (RSV)
	Coxsackievirus A
ordered	Treatment with zidovudine (azidothymidine, AZT)

	Hepatitis B Surface Antigen (HbsAg)
	gag
	lysogeny
	capsid symmetry
	adsorption
	Varicella-Zoster virus
	genome and capsid
	Prions
	Host preference
	neuraminidase
	cytocidal bodies
	cytoplasmic matrix
	Baculoviridae
	neoplasia
	Epstein-Barr virus
	stimulate host cell macromolecule synthesis

n cells

ɔrganisms

/irus to evade the immune response elicited by prev

ıenza viruses

vious vaccines

UNIT – 5 (complete content)

2.7.Oncogenic Viruses

2.7.1. **Definition**

A virus capable of inducing the formation of tumors is known as oncogenic viruses. Also called tumor virus.

2.7.2. **Classes of Tumor Viruses**

There are two classes of tumor viruses:

- DNA tumor viruses
- RNA tumor viruses, the latter also being referred to as Retroviruses.

These two classes have very different ways of reproducing themselves but they often have one aspect of their life cycle in common: the ability to integrate their own genome into that of the host cell.

DNA Tumor Viruses

DNA tumor viruses have two life-styles:

a. In permissive cells, all parts of the viral genome are expressed. This leads to viral replication, cell lysis and cell death
b. In cells that are non-permissive for replication, viral DNA is usually, but not always, integrated into the cell chromosomes at random sites. Only part of the viral genome is expressed. This is the early, control functions (e.g. T antigens) of the virus. Viral structural proteins are not made and no progeny virus is released.

Small DNA Tumor Viruses

Papovaviruses

The papovaviridae are small non-enveloped icosahedral DNA viruses. The major capsid protein, VP1, is present as 72 pentamers. Each pentamer is associated with one molecule of another minor capsid protein, either VP2 or VP3. The DNA is complexed with histone proteins encoded by the host cell.

Papillomaviruses

Papilloma viruses have a genome size about 8 kilo bases. They cause warts and also human and animal cancers. Warts are usually benign but can convert to malignant carcinomas. This occurs in patients with epidermo dysplasia verruciformis. This results in the growth of scaly macules and papules on many parts of the body but especially on the hands and feet.

Polyoma Viruses

These are small viruses with genomes of about 5 kilo bases

Murine Polyoma virus

Polyoma virus was so named because it causes a wide range of tumors in a number of animal species at many different sites.

Simian virus 40

SV40 virus was initially discovered in the rhesus monkey kidney cells that were used to make inactivated Salk polio vaccine virus. It was found that when the inactivated polio virus made in these cells was added to African Green Money Kidney cells, the vaccine gave a cytopathic effect indicative of the presence of a live virus.

Human polyoma viruses

The first two human polyoma isolates, known as BK and JC were discovered in 1971. Neither came from a tumor. BK came from the urine of a kidney transplant patient and JC came from the brain of a Hodgkin's lymphoma patient who progressed to progressive multifocal leukoencephalopathy (PML); however, they cause tumors when injected into animals.

Adenoviruses

These viruses are somewhat larger than polyoma and papilloma viruses with a genome size of about 35 kilobases. They were originally isolated from human tonsils and adenoids, are highly oncogenic in animals and only a portion of the virus is integrated into the host genome.

Complex Tumor Viruses

Herpes viruses

Herpes viruses are much larger than the DNA viruses described above and have a genome size of 100 to 200 kilobases. Because of their large size, a lot remains to be discovered concerning the way in which these viruses transform cells. The herpes virus genome integrates into the host cell at specific sites and may cause chromosomal breakage or other damage.

Epstein-Barr virus (Human herpes virus 4)

EBV is the herpes virus that is most strongly associated with cancer. It infects primarily lymphocytes and epithelial cells.

Human Herpes Virus 8 (HHV-8, Kaposi's sarcoma Herpes Virus)

HHV-8 infects lymphocytes and epithelial/endothelial cells and is the causative agent of Kaposi's sarcoma.

Human cytomegalovirus (Human Herpes Virus 5)

This herpes virus is frequently associated with Kaposi's sarcoma but this disease is now thought probably to be caused by human herpes virus 8.

Hepatitis B Virus

Hepatitis B is a vast public health problem and hepatocellular carcinoma (HCC), which is one of world's most common cancers, may well be caused by HBV. There is a very strong correlation between HBsAg (hepatitis B virus surface antigen) chronic carriers and the incidence of HCC.

RNA Tumor Viruses (Retroviruses)

Retroviruses are different from DNA tumor viruses in that their genome is RNA but they are similar to many DNA tumor viruses in that the genome is integrated into host genome. Since RNA makes up the genome of the mature virus particle, it must be copied to DNA prior to integration into the host cell chromosome. Viruses in this group that cause tumors in humans are:

HTLV-1 (human T-cell lymph tropic virus-1) which causes Adult T-cell Leukemia (Sezary T-cell Leukemia). HTLV-1 is sexually transmitted.

2.7.3. **Oncogenesis**

If a virus takes up residence in a cell and alters the properties of that cell, the cell is said to be transformed. Transformation by a virus is the change in the biological properties of a cell that results from the regulation of the cell by viral genes and that confer on the infected cells.Transformation often includes loss of growth control, exhibit chromosomal aberrations etc. The process of development of cancer is referred as oncogenesis.

Cancers are the result of a disruption of the normal restraints on cellular proliferation. It is apparent that the number of ways in which such disruption can occur is strictly limited and there may be as few as forty cellular genes in which mutation or some other disruption of their expression leads to unrestrained cell growth.

There are two classes of these genes in which altered expression can lead to loss of growth control:

(a) Those genes those are stimulatory for growth and which cause cancer when hyperactive. Mutations in these genes will be dominant. These genes are called oncogenes.

(b) Those genes that inhibit cell growth and which cause cancer when they are turned off. Mutations in these genes will be recessive. These are the anti-oncogenes or tumor-suppressor genes

2.7.4. **Oncogenes**

An oncogene is a gene that codes for a protein that potentially can transform a normal cell into a malignant cell. It may be transmitted by a virus in which case we refer to it as a viral oncogene.

Function of oncogenes

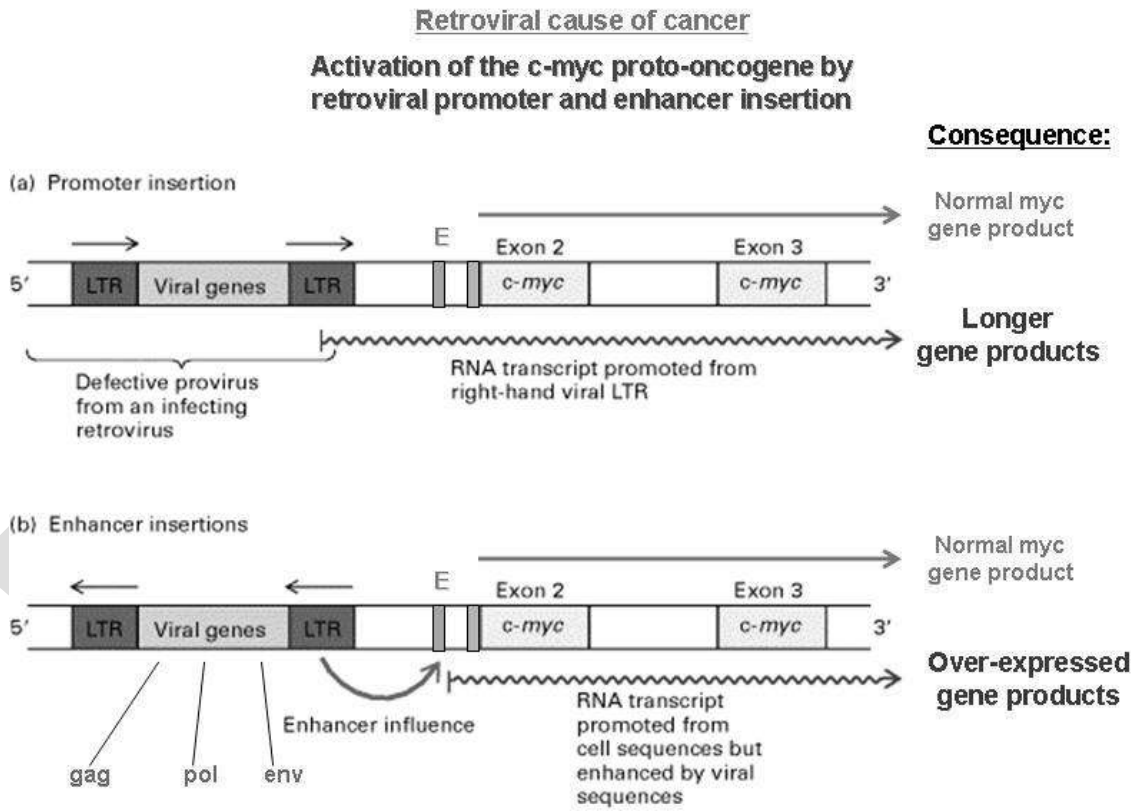
c-oncs are normal cellular genes that are expressed and function at some stage of the life of the cell. More than 40 oncogenes have been identified and there are probably a few undiscovered ones.

The cellular oncogenes can be divided into those that encode nuclear proteins and those that encode extra-nuclear proteins. The latter are mostly associated with the plasma membrane of the cell.

Products of oncogenes that are nuclear proteins: e.g. *myc*, *myb*. These are involved in control of gene expression (that is the regulation of transcription - they are transcription factors) or the control of DNA replication. Neoplasia is associated with elevated transcription of the oncogene but strong expression is not always necessary, rather there is a need to make the gene constitutively active rather than under control of normal regulatory processes.

Products of oncogenes that are cytoplasmic or membrane-associated proteins*:* e.g. *abl, src, ras.* This type does not exhibit altered expression but seems to convert from proto-oncogene to oncogene by mutation. Thus, in src-induced tumors, strong over expression of the oncogene has no effect.

Fig: Oncogenes



2.7.5. Anti-oncogenes(Tumor Suppressor Genes)

Tumor suppressor gene, or **antioncogene**, is a gene that protects a cell from one step on the path to cancer. When this gene is mutated to cause a loss or reduction in its function, the cell can progress to cancer, usually in combination with other genetic changes. The loss of these genes may be even more important than proto-oncogene/oncogene activation for the formation of many kinds of human cancer cells.

5. Antiviral vaccines and drugs

5.1. Introduction to vaccines

A vaccine is a preparation of killed microorganisms, living attenuated organisms, or living fully virulent organisms that is administered to produce or artificially increase immunity to a particular disease. The goal is to present the virus in as natural a way as possible so as to accurately mimic the stimulation obtained by natural infection, yet avoid the illness normally experienced in conjunction with the pathogen. Once presented with the pathogen at low concentrations, the body's immune system will in the future recognize the substance as harmful and will attack any viral particles that may enter the body before they have a chance to replicate.

Each vaccine is administered by injection, introducing either modified or killed versions of pathogens to the body. The immune system recognizes the germs as harmful and proceeds to manufacture special proteins called antibodies. These antibodies will "remember" that specific germs and viruses are bad, and will be prepared and able to fight the real disease should your pet be exposed to it again. This state of having memory cells capable of fighting of viral diseases is known as immunity.

5.2. Viral Vaccines

Measles Vaccines

- Live attenuated virus grown in chick embryo fibroblasts, first introduced in the 1960's. Its extensive use has led to the virtual eradication of measles in the first world. In developed countries, the vaccine is administered to all children in the second year of life (at about 15 months). However, in developing countries, where measles is still widespread, children tend to become infected early (in the first year), which frequently results in severe disease. It is therefore important to administer the vaccine as early as possible (between six months and a year). If the vaccine is administered too early, however, there is a poor take rate due to the interference by maternal antibody. For this reason, when vaccine is administered before the age of one year, a booster dose is recommended at 15 months.

Mumps Vaccines

- Live attenuated virus developed in the 1960's. In first world countries it is administered together with measles and rubella at 15 months in the MMR vaccine.
- The current "Jeryl Lynn" strain of the mumps vaccine was developed by Dr. Maurice Hillman from the mumps virus that infected his 5-year-old daughter (whose name was Jeryl Lynn). This vaccine, combined with rubella or both rubella and measles vaccines (MMR), has been widely used worldwide (300 million doses given) since it was approved by the FDA in 1967.

Rubella Vaccines

- Live attenuated virus. Rubella causes a mild febrile illness in children, but if infection occurs during pregnancy, the fetus may develop severe congenital abnormalities. Two vaccination policies have been adopted in the first world. In the USA, the vaccine is administered to all children in their second year of life (in an attempt to eradicate infection), while in Britain, until recently, only post pubertal girls were vaccinated. It was feared that if the prevalence of rubella in the community fell, then infection in the unimmunized might occur later - thus increasing the likelihood of infection occurring in the child-bearing years. This programme has since been abandoned in Britain and immunization of all children is the current practice.

- MMR — live measles virus, live mumps virus, live rubella virus, chick embryo, human foetal cells, neomycin, sorbitol, gelatine.

Polio Vaccines

- Two highly effective vaccines containing all 3 strains of poliovirus are in general use:

The **killed virus vaccine** (Salk, 1954) is used mainly in Sweden, Finland, Holland and Iceland.

The **live attenuated oral polio vaccine** (Sabin, 1957) has been adopted in most parts of the world; its chief advantages being: low cost, the fact that it induces mucosal immunity and the possibility that, in poorly immunized communities, vaccine strains might replace circulating wild strains and improve herd immunity. Against this is the risk of reversion to virulence (especially of types 2 and 3) and the fact that the vaccine is sensitive to storage under adverse conditions. - Orimune®

- The inactivated Salk vaccine is recommended for children who are immunosuppressed.

- 3 types of live polio virus, magnesium chloride, amino acid, polysorbate 80, purified water, neomycin, sulphate, streptomycin, penicillin and monkey kidney cell cultures.

Rabies Vaccines

- No safe attenuated strain of rabies virus has yet been developed for humans. Vaccines in current use include:
- The neurotissue vaccine - here the virus is grown in the spinal cords of rabbits, and then inactivated with beta-propiolactone. There is a high incidence of neurological complications following administration of this vaccine due to a hypersensitivity reaction to the myelin in the preparation and largely it has been replaced by
- A human diploid cell culture-derived vaccine (also **inactivated**) which is much safer.

There are two situations where vaccine is given:

a) Post-exposure prophylaxis, following the bite of a rabid animal: A course of 5-6 intramuscular injections, starting on the day of exposure. Hyper immune rabies globulin may also administer on the day of exposure.

b) Pre-exposure prophylaxis is used for protection of those whose occupation puts them at risk of infection with rabies; for example, vets abattoir and laboratory workers. This schedule is 2 doses one month apart and a booster dose one year later. (Further boosters every 2-3 years should be given if risk of exposure continues).

Hepatitis B Vaccines

- Two vaccines are in current use: a serum derived vaccine and a recombinant vaccine. Both contain purified preparations of the hepatitis B surface protein.

- The serum derived vaccine is prepared from hepatitis B surface protein, purified from the serum of hepatitis B carriers. This protein is synthesised in vast excess by infected hepatocytes and secreted into the blood of infected individuals. A vaccine trial performed on homosexual men in the USA has shown that, following three intra-muscular doses at 0, 1 and 6 months, the vaccine is at least 95% protective.

- A second vaccine, produced by recombinant DNA technology, has since become available. Previously, vaccine administration was restricted to individuals who were at high risk of exposure to hepatitis B, namely: infants of hepatitis B carrier mothers, health care workers, homosexual men and intravenous drug abusers. However, hepatitis B has been targeted for eradication, and since 1995 the vaccine has been included in the universal childhood immunization schedule. Three doses are given; at 6, 10, and 14 weeks of age. As with any killed viral vaccines, a booster will be required at some interval (not yet determined, but about 5 years) to provide protection in later life from hepatitis B infection as a venereal disease.

- HEPATITIS B — Hepatitis B virus gene, aluminium hydroxide, mercury, formaldehyde. For the genetically engineered vaccine: aluminium hydrochloride, sodium chloride and mercury.

Hepatitis A Vaccines

- A vaccine for hepatitis A has been developed from formalin-inactivated, cell culture-derived virus. Two doses, administered one month apart, appear to induce high levels of neutralizing antibodies. The vaccine is recommended for travelers to third world countries, and indeed all adults who are not immune to hepatitis A.

Influenza Vaccines

- Repeated infections with influenza virus are common due to rapid antigenic variation of the viral envelope glycoproteins. Antibodies to the viral neuraminidase and haemagglutinin proteins protect the host from infection. However, because of the rapid antigenic variation, new vaccines, containing antigens derived from influenza strains currently circulating in the community, are produced every year. Surveillance of influenza strains now allows the inclusion of appropriate antigens for each season. The vaccines consist of partially purified envelope proteins of inactivated current influenza A and B strains.
- Individuals who are at risk of developing severe, life threatening disease if infected with influenza should receive vaccine. People at risk include the elderly, immunocompromised individuals, and patients with cardiac disease. In these patients, protection from disease is only partial, but the severity of infection is reduced.

Varicella-Zoster virusVaccines

- A live attenuated strain of Varicella zoster virus has been developed. It is not licensed in South Africa for general use, but is used in some oncology units to protect immuno-compromised children who have not been exposed to wild-type Varicella zoster virus. Such patients may develop severe, life threatening infections if infected with the wild type virus.

Yellow Fever Vaccines

- The 17D strain is a live attenuated vaccine developed in 1937. It is a highly effective vaccine which is administered to residents in the tropics and travelers to endemic areas. A single dose induces protective immunity to travelers and booster doses, every 10 years, are recommended for residents in endemic areas

.

TABLE 1. *Viral vaccines licensed in the United States*[a]

Virus vaccine	Number of serotypes covered by vaccine	Type of vaccine: Live	Type of vaccine: Nonliving	Target population	Comments
Adenovirus	2 (types 4 and 7)	+		Military recruits	Wild-type virus in enteric coated capsules for oral administration to selectively infect the gut; lapse in manufacturing
Hepatitis A	1		+	Travelers, health care workers	Parenteral immunization with whole inactivated virus vaccine, 2 doses
Hepatitis B	1		+	Universal childhood	Parenteral immunization with recombinant VLP, 3 doses
Influenza A and B	3 (H1N1, H3N2, and type B)		+	Elderly, patients with cardiopulmonary disease, others	Repeat annual parenteral immunization with disrupted virus vaccine
Japanese encephalitis virus	1		+	Travelers to endemic region	Parenteral immunization with whole inactivated virus vaccine
Measles	1	+		Universal childhood	Parenteral immunization; booster dose recommended at 4–6 years of age
Mumps	1	+		Universal childhood	Parenteral immunization; booster dose recommended at 4–6 years of age
Poliovirus	3	+	+	Universal childhood	Parenteral immunization with nonliving vaccine only is now recommended.
Rabies	1		+	High-risk persons	Prophylactic and therapeutic uses
Rotavirus	4	+		Universal childhood	Oral vaccine, three doses
Rubella	1	+		Universal childhood	Parenteral immunization
Smallpox	1	+		No longer used	Intradermal vaccine used to eradicate smallpox
Varicella	1	+		Universal childhood	Parenteral immunization
Yellow Fever	1	+		Travelers to endemic region	Parenteral immunization
Total number of viruses covered	22	16	10		

[a]See individual chapters for specifics on target populations and on schedules for immunization for individual agents, which can be more varied than presented in this overview. Rotavirus vaccine has been withdrawn from the market because of a concern over its possible association with intussusception.
VLP, virus-like particle

5.3.

Interferons (IFNs) **as Antiviral Agent**

1. These are natural p

roteins produced by the cells of the immune systems in response to challenges by foreign agents such as viruses, bacteria, parasites and tumor cells.

2. Interferons are proteins, immunologist prefer to call them cytokines
    - They are glycosylated
3. The name originates from the fact that they interfere with viral infection
4. Three classes of interferons – **α , β, γ**
    - **α and β interferons** are produced by all the cells in response to *viral infections*
    - **γ interferons** are produced only by T lymphocyte and NK cells in response to cytokines – *immune regulating effects*
    - γ has less anti-viral activity compared to α and β interferons

5. Cells producing IFNs

    - Plasmacytoid DCs (major producers of IFN- α and IFN- β)
    - Fibroblasts and epithelial cells
    - Macrophages and Th1 Cells (predominantly IFN- γ)

5.3.1. **Mechanism of action of Interferons**

1. **Induction** of the following enzymes:
    - *a protein kinase which inhibits protein synthesis*
    - *an oligo-adenylate synthase which leads to degradation of viral mRNA*
    - *a phosphodiesterase which inhibit t-RNA*

   The action of these enzymes leads toan **inhibition of translation**

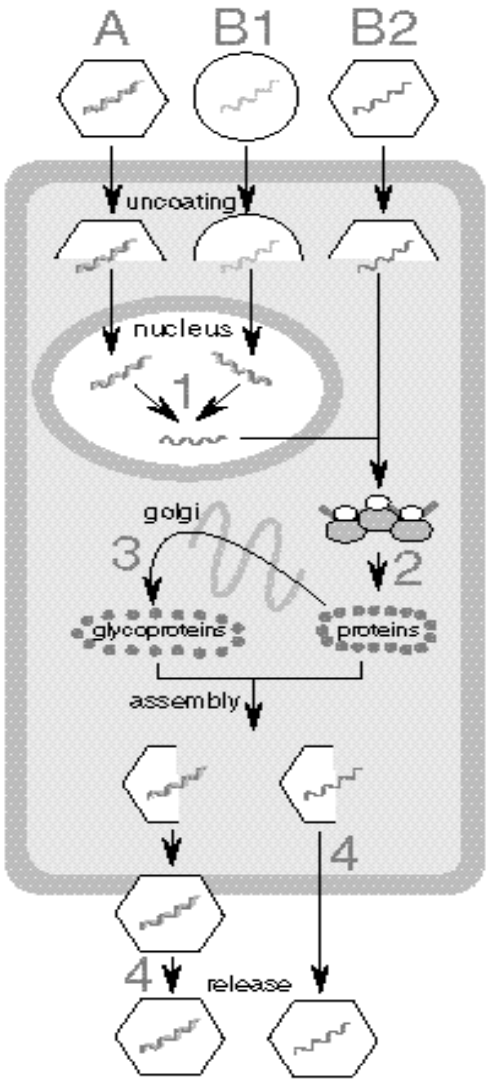
2. **Antiviral spectrum : Interferon α**
    - Includes HBV, HCV and HPV.
    - Anti-proliferative actions may inhibit the growth of certain cancers - like Kaposi sarcoma and hairy cell leukemia.

3. **Pharmacokinetics : Interferons**
    - Oral bioavailability: < 1%
    - Administered Intralesionally, S.C, and I.V
    - Distribution in all body tissues, except CNS and eye.
    - Half lives: 1-4 hours

4. **Adverse effects of Interferons**
    - Acute flu-like syndrome (fever, headache)
    - Bone marrow suppression (granulocytopenia, thrombocytopenia)
    - Neurotoxicity (confusion, seizures)

- Cardiotoxicity - arrhythmia
- Impairment of fertility

5. **Therapeutic uses Interferons**
   - Chronic hepatitis B and C (complete disappearance is seen in 30%).
   - HZV infection in cancer patients (to prevent the dissemination of the infection)
   - CMV infections in renal transplant patients
   - Condylomata acuminata (given by intralesional injection). Complete clearance is seen ~ 50%.
   - Hairy cell leukemia (in combination with zidovudine)
   - AIDS related Kaposi's sarcoma.



Viruses
A. DNA
B. RNA
1. orthomyxoviruses and retroviruses
2. picornaviruses and most RNA viruses

IFN Effects
1. transcription inhibition
activates Mx protein
blocks mRNA synthesis

2. translation inhibition
activates methylase —>
blocks mRNA cap methylation

activates 2'5' oligoadenylate synthetase —> 2'5'A —> inhibits mRNA splicing and activates RNaseL —> cleaves viral RNA

activates protein kinase P1 —> blocks eIF-2α function —> inhibits initiation of mRNA translation

activates phosphodiesterase —> blocks tRNA function

3. protein processing inhibition
glycosyltransferase —> blocks protein glycosylation

4. virus maturation inhibition
glycosyltransferase —>blocks glycoprotein maturation

causes membrane changes —> blocks budding

Fig: Mechanism of action of interferons

5.4. **Anti-viral drugs**

1. Many antiviral drugs arePurine or Pyrimidine analogs.
2. Many antiviral drugs are Prodrugs. They must be phosphorylated by viral or cellular enzymes in order to become active.
3. Anti-viral agents inhibits active replication so the viral growth resumes after drug removal.
4. Current anti-viral agents do not eliminate non-replicating or latent virus
5. Effective host immune response remains essential for the recovery from the viral infection
6. Clinical efficacy depends on achieving inhibitory concentration at the site of infection within the infected cells

5.4.1. **Stages of viral replication**

- Cell entry – attachment
- penetration
- Uncoating
- Transcription of viral genome
- Translation
- Assembly of virion components
- Release.

The life cycle of a virus comprises several stages such as binding to the cell surface, replication, protein synthesis etc. and all of these stages may be the target of anti-viral drugs.

5.4.2. **Targets of antiviral drugs**

Among the life cycle stages that have been targeted by potential therapeutic agents are:

- Attachment of the virus to the cell surface, perhaps as a result of competition with a specific viral receptor and Uptake into intracellular vesicles (endosomes). E.g. *Antiviral antibodies (gamma globulin), Arilodone.*
- Uncoating of virus (loss of protein coat, fusion of lipid membrane with endosome/lysosome). Note: the endosome/lysosome compartment is acidic and inhibition of acidification of this compartment might be a good target.E.g.*Amantadine, rimantadine*.

- Integration of the viral DNA into chromosomal DNA of the host cell (where this occurs).
- Transcription of genome to new RNA or DNA (polymerases are the target).E.g. *Acyclovir, Gancyclovir*
- mRNA transcriptionE.g.*fomivirsenAcyclovir, Gancyclovir*
- mRNA processing (poly adenylation, methylation, capping, splicing)and translation to proteinE.g. *Ribavirin, Interferons*
- Post-translational modification of proteins (glycosylation, phosphorylation, fatty acylation, proteolysis). Some of these are essential for functional, infective viral progeny. E.g.*Protease inhibitors- Saquinavir, Indinavir , Ritonavir*
- Assembly of the components into the whole virus. E.g. *Interferons, Rifampin*
- Release of the progeny virion is released from cell. E.g. *Antiviral antibodies, Cytotoxic T lymphocytes.*

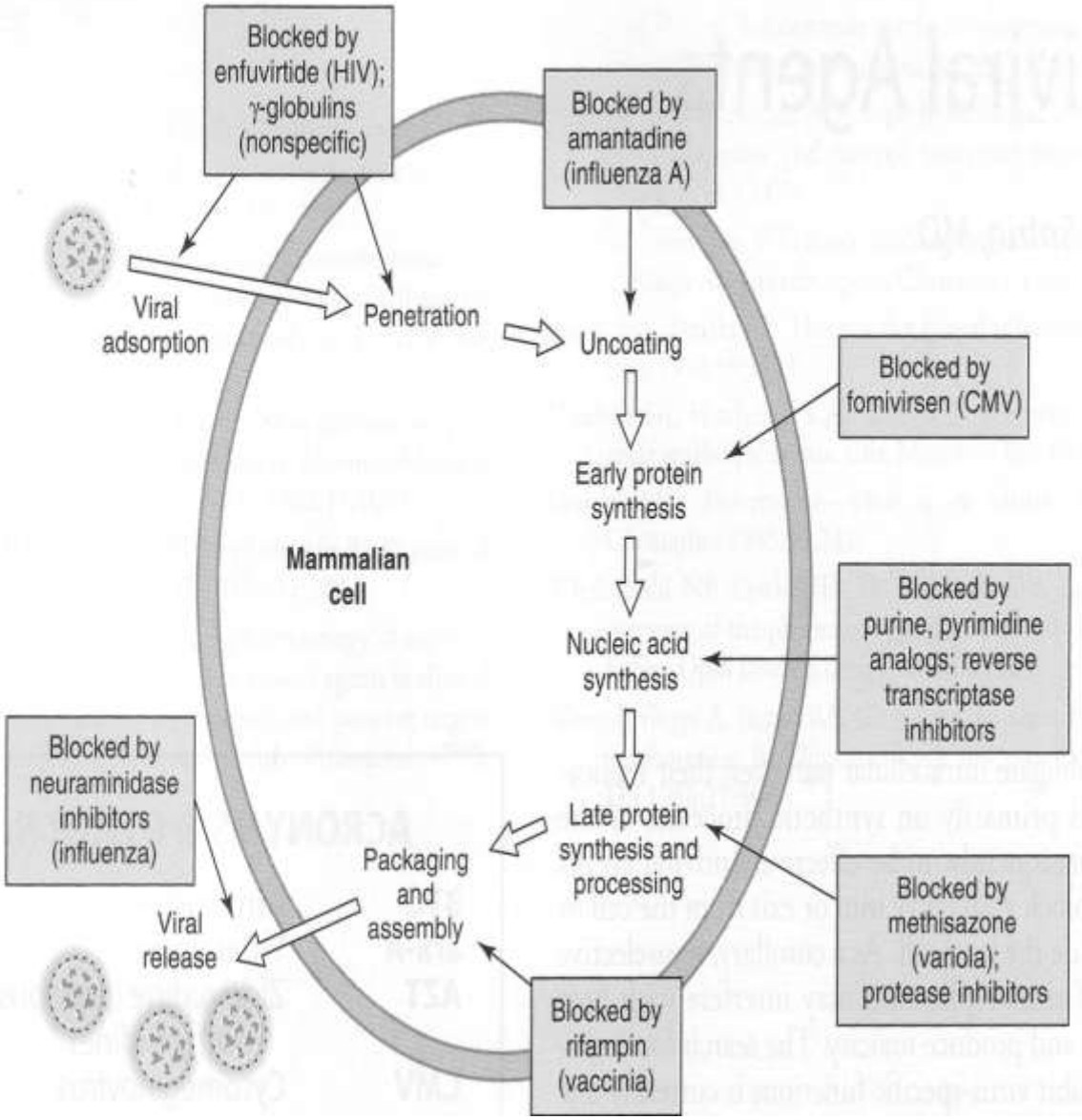


Fig: Targets of antiviral drugs

5.4.3. **Mechanism of action of antiviral comp**

ounds

Drug	Mode of action	Spectrum of action	Resistance
Attachment antagonists			
Arilodone	Block attachment molecule on host cell.	Picornaviruses	Nil
Inhibit viral uncoating			
Amantadine	Neutralize acid environment within phagolysosomes that is necessary for viral uncoating	Influenza A virus	Nil
Rimantadine			
Inhibit Nucleic acid synthesis			
Acyclovir	Phosphorylation of viral coded kinase enzyme activates the drug: Inhibits DNA and RNA synthesis	Herpes viruses, EBV,CMV	Mutation in genes of drug activation
Gancyclovir			
Ribavirin	Phosphorylation of viral coded kinase enzyme activates the drug: Inhibits	RSV, Hepatitis C, Influenza A, Measles viruses	Nil

	DNA and RNA synthesis		
Adenosine arabinoside	Phosphorylation of cell coded kinase enzyme activates the drug: inhibits DNA synthesis, viral DNA polymerase incorporate the drugs than human DNA polymerase	Herpes viruses	Mutation of viral polymerase
Nucleotide analogs	Phosphorylation of cell coded kinase enzyme activates the drug: used along with protease inhibitor.	HIV	Mutation of viral reverse transcriptase
Inhibit viral proteins			
Protease inhibitors	Blocks the active site given along with drugs active against nucleic acid synthesis	HIV	Mutation of protease gene

Development of AIDS vaccine

5.6.1. **Introduction**

HIV offers a uniquely difficult target for vaccine development. The HIV isolates that infect humans and cause AIDS include a genetically diverse population of viruses. The HIV responsible for causing AIDS in much of West Africa is referred to as HIV-2; the HIV that causes AIDS throughout the rest of the world is referred to as HIV-1. HIV-2 and HIV-1 are so divergent in their genetic sequences that their envelope glycoproteins are often not immunologically cross-reactive.

5.6.2. **Traditional designs for an HIV-1 vaccine**

1. Live, attenuated virus
2. Inactivated viruses with adjuvants
3. Recombinant envelope protein

5.6.3. **Novel designs for an HIV-1 vaccine**

1. Plasmid DNA
2. Live, recombinant vectors:
   i. Pox viruses
   ii. Vaccinia
   iii. MVA, NYVAC
   iv. Canary pox
3. Gene-deleted adenovirus
4. Envelope subunit immunogens

5.6.4. **Recent vaccine strategies**

Four vaccine concepts have been evaluated in efficacy trials to date.

1. The VAX004 and VAX003 trials evaluated the first concept, a protein subunit vaccine.
2. The second concept, a recombinant adenovirus vector, was evaluated in the Step and HIV Vaccine Trials Network (HVTN) 503/Phambili trials.
3. The third concept, a canary pox vector prime followed by a protein subunit boost, was evaluated in the RV144 trial.
4. The fourth concept, a DNA prime followed by a recombinant adenovirus vector boost, was recently evaluated in the HVTN 505 trial.

5.6.5.**Vaccine Development**

Plasmids encoding proteins under the control of potent promoters can be immunogenic if inoculated intramuscularly in small laboratory animals. Subsequently this immunogenicity can be substantially enhanced through the delivery of these plasmids formulated with particular adjuvants or cytokines. Such immunogens have proven particularly useful in eliciting cell-mediated immune responses.DNA vaccines consist of a plasmid encoding a protein of interest. A DNA vaccine can deliver the same genes as a live-vectored vaccine without immunity developing against the vector, which may inhibit expression of the insert.

In live recombinant vectors,Genes of HIV can be inserted by molecular approaches into live, replication-competent microorganisms. The resulting recombinant microorganisms then can serve to carry these genes. Upon infection with these recombinant microorganisms, immunity is elicited to the vector and to the product of the HIV gene carried by that vector. Such immunogens have proven particularly useful for eliciting CTLs, since the HIV proteins are produced intracellularly by the replicating vector and therefore enter the MHC class I processing pathway.The microorganisms best studied as potential vaccine vectors are the pox family of viruses. The prototype member of this family is vaccinia, the replication competent virus that served as the primary vaccine virus in the worldwide smallpox eradication campaign. Other pox viruses have therefore received attention as potential HIV vaccine vectors. Perhaps the most interesting of these pox viruses is modified vaccinia Ankara (MVA). Generated from a parental vaccinia virus isolate by multiple in vitro passages, MVA carries a large number of deletions, leaving it infectious and immunogenic but highly attenuated in its pathogenicity.

Perhaps the most promising of the live recombinant vectors assessed to date as a potential HIV vaccine is the gene-deleted adenovirus that was developed as a vector for gene therapy. The serotype 5 adenovirus, made replication-incompetent by deletion or inactivation of the *E1* and *E3* genes, has demonstrated impressive immunogenicity in both murine and nonhuman primate studies. These vectors have elicited both high-titer antibody and high-frequency CTL responses in these animal models.

An approach to elicit a cellular immune response by vaccination is the use of recombinant viral vectors, in which a virus is engineered to express a gene of interest.Of these vectors, recombinant adenovirus serotype 5 (rAd5) was found to be particularly immunogenic, and was selected as the vector for the Step and HVTN 503/Phambili trials, which were the first efficacy trials to evaluate an HIV vaccine designed to stimulate T-cell responses.

Protein subunit vaccines for HIV are based on the HIV envelope. The HIV envelope is composed of glycoproteins, gp120 and gp41, which are cleaved from a gp160 precursor. The mature envelope spike forms as a trimer, composed of three gp120/gp41 complexes. Both recombinant gp160 (rgp160) and recombinant gp120 (rgp120) monomers werestudied as immunogens in early HIV vaccine clinical

trials.Another feature of the native viral envelope that may play an important role in the induction of neutralizing antibodies is glycosylation.For proper glycosylation of protein subunits, immunogens need to be produced in specific human cell lines. These cell lines were not utilized to produce the AIDSVAX vaccines. Recently, a stable envelope trimer has been developed that more closely represents antigenic properties of the native envelope trimer, including glycosylation.

Heterologous prime-boost regimens seek to augment and broaden immune responses by combining different vaccine strategies. This approach was employed in RV144, in which a canary pox viral vector prime expressing Env, Gag, and Pol (ALVAC) was followed by an AIDSVAX B/E boost (the same protein subunit vaccine used in VAX003).

The most recently conducted HIV vaccine efficacy trial, HVTN 505, is a phase IIb trial that evaluated a DNA prime followed by a rAd5-vectored boost in men.The DNA plasmid expressed Gag, Pol, Nef, and Env, and the rAd5 boost expressed Gag, Pol, and Env. This was in contrast to the rAd5 vaccine used in Step, which did not express Env. In an attempt to avoid the possibility of increased risk of HIV infection that occurred using the rAd5 vector in Step, only men who were circumcised and Ad5 sero negative were eligible for inclusion.

5.7. **Preparation of Rabies Vaccine**

5.7.1. **Introduction**

Rabies vaccine for human use prepared in cell cultures is a freeze-dried preparation of a suitable strain of fixed rabies virus grown in cell cultures and inactivated by a validated method. The vaccine is reconstituted immediately before use as stated on the label to give a clear liquid that may be colored owing to the presence of a pH indicator.

5.7.2. **Production**

The production of the vaccine is based on a virus seed-lot system and, if a cell line is used for virus propagation, a cell-bank system. The production method shall have been shown to yield consistently vaccines that comply with the requirements for immunogenicity, safety and stability. Unless otherwise justified and authorized, the virus in the final vaccine shall not have undergone more passages from the

master seed lot than was used to prepare the vaccine shown in clinical studies to be satisfactory with respect to safety and efficacy; even with authorised exceptions, the number of passages beyond the level used for clinical studies shall not exceed five. The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for immunosera and vaccines for human use.

Substrate for virus propagation

The virus is propagated in a human diploid cell line, in a continuous cell line approved by the competent authority or in cultures of chick-embryo cells derived from a flock free from specified pathogens.

Seed lots

The strain of rabies virus used shall be identified by historical records that include information on the origin of the strain and its subsequent manipulation. Working seed lots are prepared by not more than five passages from the master seed lot. Only a working seed lot that complies with the following tests may be used for virus propagation.

a. **Identification**. Each working seed lot is identified as rabies virus using specific antibodies.

b. **Virus concentration**. The virus concentration of each working seed lot is determined by a cell culture method using immunofluorescence, to ensure consistency of production.

c. **Extraneous agents.** The working seed lot complies with the requirements for virus seed lots. If the virus has been passaged in mouse brain, specific tests for murine viruses are carried out.

Virus propagation and harvest

All processing of the cell bank and subsequent cell cultures are done under aseptic conditions in an area where no other cells are handled. Approved animal (but not human) serum may be used in the media, but the final medium for maintaining cell growth during virus multiplication does not contain animal serum; the media may contain human albumin complying

with the monograph on *Human albuminsolution.* Serum and trypsin used in the preparation of cell suspensions and media are shown to be free from infectious extraneous agents; trypsin complies with the monograph on *Trypsin*. The cell culture media may contain a pH indicator such as phenol red and approved antibiotics at the lowest effective concentration. Not less than 500 ml of the cell cultures employed for vaccine production are set aside as uninfected cell cultures (control cells). The virus suspension is harvested on one or more occasions during incubation. Multiple harvests from the same production cell culture may be pooled and considered as a single harvest. Only a single harvest that complies with the following requirements may be used in the preparation of the inactivated viral harvest.

a. **Identification**. The single harvest contains virus that is identified as rabies virus using specific antibodies.

b. **Virus concentration**. Titrate for infective virus in cell cultures; the titre is used to monitor consistency of production.

c. **Control cells**. The control cells of the production cell culture from which the single harvest is derived comply with a test for identification and with the requirements for extraneous agents.

Purification and inactivation

The virus harvest may be concentrated and/or purified by suitable methods; the virus harvest is inactivated by a validated method at a fixed, well defined stage of the process which may be before, during or after any concentration or purification. The method shall have been shown to be capable of inactivating rabies virus without destruction of the immunogenic activity. If betapropiolactone is used, the concentration shall at no time exceed 1:3500.Only an inactivated viral suspension that complies with the following requirements may be used in the preparation of the final bulk vaccine.

a. **Inactivation**. Carry out an amplification test for residual infectious rabies virus immediately after inactivation or using a sample frozen immediately after inactivation and stored at −70 °C. Inoculate a quantity of inactivated viral suspension equivalent to not less than 25 doses of vaccine into cell cultures of the same type as those used for production of the vaccine. Make a passage after 7 days. Maintain the cultures for a further 14 days and then examine the cell cultures for rabies virus using an immunofluorescence test. No rabies virus is detected.

b. **Residual host-cell DNA**. If a continuous cell line is used for virus propagation, the content of residual host-cell DNA, determined using a suitable method, is not greater than 100 pg per single human dose.

Final bulk vaccine

The final bulk vaccine is prepared from one or more inactivated viral suspensions. An approved stabilizer may be added to maintain the activity of the product during and after freeze-drying. Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

a. **Glycoprotein content**. Determine the glycoprotein content by a suitable immunochemical method, for example, single-radial immunodiffusion, enzyme-linked immunosorbent assay or an antibody-binding test. The content is within the limits approved for the particular product.

b. **Sterility.** The final bulk vaccine complies with the test for sterility, carried out using 10 ml for each medium.

Final lot

The final bulk vaccine is distributed aseptically into sterile containers and freeze-dried to a moisture content shown to be favorable to the stability of the vaccine. The containers are then closed so as to avoid contamination and the introduction of moisture. Only a final lot that complies with each of the requirements given below under Identification, Tests and Assay may be released for use. Provided that the test for inactivation has been carried out with satisfactory results on the inactivated viral suspension and the test for bovine serum albumin has been carried out with satisfactory results on the final bulk vaccine, these tests may be omitted on the final lot.

Identification

The vaccine is shown to contain rabies virus antigen by a suitable immunochemical method using specific antibodies, preferably monoclonal; alternatively, the assay serves also to identify the vaccine.

Tests

a. **Inactivation**. Inoculate a quantity equivalent to not less than 25 human doses of vaccine into cell cultures of the same type as those used for production of the vaccine. Make a passage after 7 days. Maintain the cultures for a further 14 days and then examine the cell cultures for rabies virus using an immunofluorescence test. No rabies virus is detected.

b. **Bovine serum albumin**. Not more than 50 ng per single human dose, determined by a suitable immunochemical method.

c. **Sterility** The vaccine complies with the test for sterility.

d. **Bacterial endotoxins**: less than 25 IU per single human dose.

e. **Pyrogens.** The vaccine complies with the test for pyrogens. Unless otherwise justified and authorised, inject into each rabbit a single human dose of the vaccine diluted to ten times its volume.

f. **Water.** Not more than 3.0 per cent, determined by the semi-micro determination of water.

5.7.3. **Assay**

The potency of rabies vaccine is determined by comparing the dose necessary to protect mice against the effects of a lethal dose of rabies virus, administered intracerebrally, with the quantity of a reference preparation of rabies vaccine necessary to provide the same protection. For this comparison a reference preparation of rabies vaccine, calibrated in International Units, and a suitable preparation of rabies virus for use as the challenge preparation are necessary. The International Unit is the activity contained in a stated quantity of the International Standard. The equivalence in International Units of the International Standard is stated by the World Health Organization.

a. ***Selection and distribution of the test animals***.

Use in the test healthy female mice about 4 weeks old, each weighing 11 g to 15 g, and from the same stock. Distribute the mice into six groups of a size suitable to meet the requirements for validity of the test and, for titration of the challenge suspension, four groups of five.

b. ***Preparation of the challenge suspension.***

Inoculate mice intracerebrally with the CVS strain of rabies virus and when the mice show signs of rabies, but before they die, sacrifice them, remove the brains and prepare a homogenate of the brain tissue in a suitable diluent. Separate gross particulate matter by centrifugation and use the supernatant liquid as the challenge suspension. Distribute the suspension in small volumes in ampoules, seal and store at a temperature below −60 °C. Thaw one ampoule of the suspension and make serial dilutions in a suitable diluent. Allocate each dilution to a group of five mice and inject intracerebrally into each mouse 0.03 ml of the dilution allocated to its group. Observe the mice for 14 days. Calculate the LD50 of the undiluted suspension using the number in each group that, between the fifth and fourteenth days, die or develop signs of rabies.

c. ***Determination of potency of the vaccine to be examined.***

Prepare three fivefold serial dilutions of the vaccine tobe examined and three fivefold serial dilutions of thereference preparation. Prepare the dilutions such that themost concentrated suspensions may be expected to protect more than 50 per cent of the animals to which they are administered and the least concentrated suspensions may be expected to protect less than 50 per cent of the animals to which they are administered. Allocate the six dilutions one to each of the six groups of mice and inject intraperitoneally into each mouse 0.5 ml of the dilution allocated to its group. After 7 days, prepare three identical dilutions of the vaccine to be examined and of the reference preparation and repeat the injections. Seven days after the second injection, prepare a suspension of the challenge virus such that, on the basis of the preliminary titration, 0.03 ml contains about 50 LD50.

Inject intracerebrally into each vaccinated mouse 0.03 ml of this suspension. Prepare three suitable serial dilutions of the challenge suspension. Allocate the challenge suspension and the three dilutions one to each of the four groups of five control mice and inject intracerebrally into each mouse 0.03 ml of the suspension or one of the dilutions allocated to its group. Observe the animals in each group for 14 days and record the number in each group that die or show signs of rabies in the period 5 days to 14 days after challenge. The test is not valid unless : for both the vaccine to be examined and the reference preparation the 50 per cent protective dose lies between the largest and smallest doses given to the mice; the titration of the challenge suspension shows that 0.03 ml of the suspension contained not less than 10 LD50 ; the statistical analysis shows a significant slope and no significant deviations from linearity or parallelism of the dose-response lines ; the confidence limits (P = 0.95) are not less than 25 per cent and not

more than 400 per cent of the estimated potency. The vaccine complies with the test if the estimated potency is not less than 2.5 IU per human dose.

VACCINE ▼ AGE GROUP ▶	19-21 years	22-26 years	27-49 years	50-59 years	60-64 years	≥ 65 years

5.7.4. **Labelling**

The label states the biological origin of the cells used for the preparation of the vaccine.

5.8. **Immunization dosage for adults**

Recommended Adult Immunization Schedule—United States - 2013

Note: These recommendations must be read with the footnotes that followcontaining number of doses, intervals between doses, and other important information.

Influenza	1 dose annually					
Varicella	2 doses					
Human papillomavirus (HPV) Female	3 doses					
Human papillomavirus (HPV) Male	3 doses					
Zoster					1 doses	
Measles, mumps, rubella (MMR)	1 or 2 doses					
Hepatitis A	2 doses					
Hepatitis B	3 doses					

For all persons in this category who meet the age requirements and who lack documentation of vaccination or have no evidence of previous infection;zoster vaccine recommended regardless of prior episode of zoster

Recommended if some other risk factor is present (e.g., on the basis of medical, occupational, lifestyle, or other indication)

No recommendation

5.9. **Immunization dosage for Children**

Recommended immunization schedule for persons aged 0 through 18 years – 2013.

Vaccines	Birth	1 month	2 months	4 months	6 months	9 months	12 months	15 months	18 months	19 - 23 months	2-3 yrs	4-6 yrs	7-10 yrs	11-12 yrs	13-15 yrs	16-18 yrs
Hepatitis B	1st dose	2nd dose			3rd dose											
Rotavirus			1st dose	2nd dose												
Haemophilus influenzae type b			1st dose	2nd dose			3rd or 4th dose									
Inactivated Poliovirus			1st dose	2nd dose	3rd dose							4th dose				
Influenza (IIV; LAIV)					Annual vaccination (IIV only)							Annual vaccination (IIV or LAIV)				
Measles, mumps, rubella (MMR)						1st dose						2nd dose				
Varicella (VAR)						1st dose						2nd dose				

Hepatitis A (HepA)							2 dose series								
Human papillomavirus (HPV2: females only; HPV4: males and females)														(3-dose series)	

Unit Vquestion
The hepadnaviruses such as hepatitis B virus are quite different from other DNA viruses with respect to g
Which of the following virus is always detectable after infections?
Viroids are composed of
_____ suggested the use of agar as a solidifying material for microbiological media
_____ established principles for germ theory of disease
Complement fixation test for Syphilis was introduced by _____
Vaccination against Rabies was first introduced by _____
_____ is considered as Father of Modern Microbiology
Anaerobic microorganisms were first discovered by
The causative agent of tuberculosis was first discovered by
Which of the following is not the contribution of Pasteur?
The theory of spontaneous generation was disproved by
The first virus to be crystallized was
Present day classification in biology was established by the work of _____
_____ is the arranging of organisms into related groups.
Berkefeld filters are prepared by mixing diatomaceous earth with
Asbestos is chemically composed of
Phenol was first used as an antiseptic by _____
The widely used fumigant is _____
An example of a nonionizing type of radiation which is microbicidal is ____
Ultra violet light is strongly absorbed by _____ with in a cell.
Membrane filters are manufactured from _____
Capillary pore membranes have pores produced by ____
The microbial media are solidified by using
- - - - - - is the universal bacterial medium
Give an example for a mechanical device for removing microorganism from a solution
Ultra violet light has a wavelength of between.
Which process force out electrons out of their shells in organic molecules
In ultra sonic vibration forms cavities is known as____
The process of destroying pathogens is called ____
Which phenolic compound hexachlorophene which used in toothpaste
__ is the time in minutes needed to reduce the number of viable microorganisms
Name the isotope which release the x-rays, v-raysand cosmic rays
The oncogene theory refers to
In cell culture, measles virus may lead to
A change from lysogeny to lysis is generally not induced by
The viral DNA is removed from the host's chromosomes and the lytic cycle occurs. The process is called
The lysogenic state is governed by the activity of the regulatory region of the lambda phage genomes; this
The capsomeres consist of a number of proteins subunits or molecules called
In order for a virus to replicate
Which of the following viruses belong to family Flaviviridae?
Which of the following viruses show/s transformation of infected cells?
Which of the following may affect proteins and nucleic acids, but not viruses?
The viral DNA of the temperate phage, instead of taking over the functions of the cell's genes, is incorpor

Which of the following statements is not true of viruses?
Which of the following viruses belong/s to family caliciviridae?
In the simplest capsid, there is a capsomere at each of the 12 vertices; this capsomere, which is surrounde
The size of viruses is usually measured in
The prophage that have no site specificity for insertion and may even be able to insert multiple copies of t
Enzyme neuraminidase is carried by which of the following viruses?
Lysozyme (an endolysin) which will lyse the bacterial cell, releasing the mature virions is present in
Which of the following is continuous cell line?
The repressor protein, since the cell is resistant to lysis from externally infecting phage, is also called
Which of the following virus is susceptible to chloroform?
Group E phages have
The phage possesses a gene that codes for a repressor protein which makes the cell resistant to lysis initiat
The bacterial viruses having head made up of large capsomeres, but no tail is morphologically classified a
The process by which phage reproduction is initiated in lysogenized culture is called
Area of lysis on a bacterial lawn culture produced by a phage is known as
The procapsid is assembled with the aid of _____ proteins.
Which of the following is/are synthesized from late mRNA?
Which capsid symmetry is exhibited by most of the phages?
Contractile sheath of the tail is present in which of the following phages?

Opt 1	Opt 2
DNA-dependent DNA polymerase	reverse transcriptase
Hepatitis B virus	Herpes simplex virus
single-stranded DNA	double-stranded DNA
Fanny Hesse	Rous
Jaco Henk	Lymc
Wasserman	Fleming
Ricketts	Louis Pasteur
Fanny Hesse	Rous
Robert Koch	John Needham
Robert Koch	Paul Ehrlich
Vaccine for the prevention of anthrax	Vaccination against rabies
Spallanzani	Louis Pasteur
Rabies	TMV
Carolus Linnaeus	Robert Koch
Classification	Distribution
asbestos	aluminium
magnesium silicate	mercuric sulphate
Louis Pasteur	John Tyndall
ethylene	chlorine
gamma rays	UV rays
DNA	ribosomes
cellulose nitrate	poly carbonate and polyester
Irradation	filtration
Agar	Peplone
Nutrient Agar	Tryptone agar
Hot air oven	Autoclave
300-400nm	200-300nm
Ultra sonic vibration	Ionization radiation
Cavitation	Ionization radiation
Tyndallization	Pasteurization
Ipan	Mum
Steam	hot air
Uranium-60	Cobalt-60
how chemicals inactivate viruses when applied	how viruses replicate in host cells
nuclear pyknosis	transformation of cells
ultraviolet light	chemicals
spontaneous induction	inductive infection
immunity repressor	immunity operon
protomers	caproprotein
the capsid must enter the host cell cytoplasm	the host cell must be undergoing mitosis
Rubella virus	Yellow fever virus
Hepatitis B virus	T cell lymphotronic virus type I
Denaturation	Enzyme treatment
lysogeny	spontaneous induction

Viruses have been successfully grown in pure cu	All viruses are obligatory intracellular parasi
Hepatitis B virus	Hepatitis D virus
hexon	polyhedra
centimeters	micrometers
λ phage enzyme	λ DNA
Human immunodeficiency virus	Epstein-Barr virus
immediate early phage gene	late genes
HeLa	HEp-2
immunity repressor	immunity operon
Herpes	Influenza
single stranded DNA	double stranded DNA
the prophage	lytic infection by other viruses
A	B
infection	integration
pock	plaque
ladder	framing
Phage structural proteins	Proteins that help with phage assembly witho
Helical	Icosahedral
T3	T2

Opt 3	Opt 4
Rnase H	DNA ligase
Varicella-zoster virus	Cytomegalovirus
single-stranded RNA	double-stranded RNA
Pasteur	Reed
Khorana	Kreb
Ricketts	Bordet
Bordet	Ehrlich
Louis Pasteur	Twort
Louis Pasteur	Ferdinand Cohn
Louis Pasteur	Metchnihoff
Discovery of anthrax bacilli	Isolation of bacteria responsible for chicken cho
John Tyndall	Jenner
T4	Pox
Alexander Fleming	Pasteur
Corrulation	Differentiation
sand and clay	Copper
manganous chloride	mercapto ethanol
Joseph lister	Robert koch
formaldehyde	carbon-di-oxide
X- rays	sun rays
cell wall	cytoplasm
cellulose diacetate	cellulose
evaporation	respiration
Yeast extract	Tryptone
Macconkey agar	Mnnitol salt agar
waterbath	Filter
100-400nm	50-100nm
Pasteurization	Tyndallization
Pasteurization	Tyndallization
Disinfection	Sterilization
Dial	Phisohex
L-value	D- value
Tween-80	Cl
how viruses transform normal cells into tumor cell	Retransform of tumor cells
syncytium formation	rounding and aggregation of cells
irradiation	alcohol
resultant induction	spontaneous infection
operon repressor	Lac operon
bprocapsid	depressor
the genome must be released in the cytoplasm	the host cell must lack a cell membrane
Hepatitis C virus	Herpes virus
Epstein-Barr virus	Adeno virus
Pressure	Retro virus
lytic phase	induction

All viruses have either DNA or RNA as their gene	Viruses probably arose from small fragments of
Hepatitis E virus	Hepatitis D virus
icosahedral	helical
nanometers	millimeters
Phage Mu	Phage Mn
Influenza virus	Adenovirus
delayed early genes	Early genes
KB	ML2
operon repressor	Operon depresssor
Measles	HSV
single stranded RNA	double stranded RNA
Both (a) and (b)	Lysogenic cycle
C	D
repression	induction
pox	colony
scaffolding	form
Proteins involved in cell lysis and phage release	lipids
Complex	Cylinder
P22	P322

Opt 5	Answer
	DNA-dependent DNA polymerase
	Hepatitis B virus
	single-stranded DNA
	Fanny Hesse
	Jaco Henk
	Wasserman
	Louis Pasteur
	Louis Pasteur
	John Needham
	Louis Pasteur
lera	Isolation of bacteria responsible for chicken cholera
	Spallanzani
	TMV
	Carolus Linnaeus
	Classification
	asbestos
	manganous chloride
	Joseph lister
	formaldehyde
	UV rays
	DNA
	Cellulose nitrate
	filtration
	Agar
	Tryptone agar
	Filter
	100-400nm
	Ionization radiation
	Cavitation
	Disinfection
	Ipan
	D- value
	Cobalt-60
	how viruses transform normal cells into tumor cells
	syncytium formation
	alcohol
	spontaneous induction
	immunity repressor
	caproprotein
	the genome must be released in the cytoplasm
	Yellow fever virus
	T cell lymphotronic virus type I
	Denaturation
	lysogeny

cellular chromosomes	Viruses have been successfully grown in pure cultures
	Hepatitis E virus
	hexon
	nanometers
	Phage Mu
	Influenza virus
	late genes
	HeLa
	immunity repressor
	Measles
	double stranded RNA
	the prophage
	D
	infection
	plaque
	scaffolding
	Proteins that help with phage assembly without becomi
	Icosahedral
	T2

in test tubes

ing part of the virion structure

Reg. No._____

[17MBU201]

KARPAGAM ACADEMY OF HIGHER EDUCATION

(Under Section 3 of UGC Act 1956)

COIMBATORE – 641 021

B.Sc. DEGREE EXAMINATION

FIRST INTERNAL EXAMINATION, JANUARY 2018

SECOND SEMESTER

MICROBIOLOGY

VIROLOGY

Time: 2 hours **Maximum: 50 marks**

PART A – (20 x 1 = 20 marks)

Answer all the questions

1. Virus means____
   A. Pellet B. Poison
   C.Protein D.Incomplete
2. The complete protein-nucleic acid complex is called as_____
   A.Capsid B. Protein coat
   C. Nucleocapsid D. Nucleic acid
3. Virus which requires second virus for its replication is called as_____
   A. Defective virus B. Direct virus
   C. Temperate virus D. Provirus
4. Infectious virus particle is called as _____
   A.Virion B. Viriod
   C.Prion D.Capsid
5. Virus is classified based on _____
   A.DNA B. RNA
   C. DNA & RNA D. Host
6. _____ classified virus into seven classes
   A.David Baltimore B. Edward jenner
   C. Montangier D. Felix
7. Virus replicates by _____ mechanism
   A. Host B. Own
   C.Cell D. Direct
8. Yolk sac inoculation is used for which virus cultivation?
   A.HIV B. Pox
   C. HSV D. Influenza
9. For propagation, viruses depend on_____cells
   A. Host B. Other
   C. Own D. Neighbour.
10. Proteins associated with nucleic acid is called as
   A. Proteins B. Nucleous
   C. Nucleoproteins D. Capsid
11. Envelope comes from____
   A. Virus B. Host
   C. Protein D. Nucleic acid
12. Single type of capsomeres stacked around a central axis form a _____
   A.Capsid B. Protein coat
   C. Nucleocapsid D. Nucleic acid
13. _____ symmetry is seen in animal virus

A.Helical, B. Icosahedral
C.Radical D. Spiral

14. Capsomers with the triangular faces are surrounded by six others are called as _____
A. Tetrads B. Trions
C. Hexons D. Pentons

15. The size of viruses is usually measured in
A. Centimetres B. Micrometers
C. Nanometers D. Millimeters

16. Which of the following is the agent associated with the development of neurodegenerative disease in livestock and humans?
A. Prions B. Viroids
C. Virions D. Virinos

17. Enzyme neuraminidase is carried by which of the following viruses?
A. HIV B. EBV
C. Influenza virus D. Adenovirus

18. Repressor protein, since the cell is resistant to lysis from externally infecting phage, is also called
A.Immunity repressor B. Immunity operon
C. Operon repressor D. Lac operon

19. Which of the following virus is susceptible to chloroform?
A. Herpes B. Influenza
C. Measles D. Human viruses

20. Area of lysis on a bacterial lawn culture produced by a phage is known as
A. Pock B. Plaque
C. Pox D. Colony

PART –B (3 x 2 = 6 marks)

21. What is capsid and list the types of symmetry?
22. What is reverse transcriptase enzyme?
23. What is Baltimore classification? List out.

PART –C (3 x 8 = 24 marks)

24. A. Explain in short about the viral cultivation methods.

(or)

B. Write in detail about classification of DNA viruses

25. A. Explain in short about the viral purification.

(or)

B. Give a detailed note on viral structure and symmetry with necessary examples and images.

26. A. Give a detailed note on lytic and lysogenic phages.

(or)

B. What is the one step growth curve experiment? Explain in detail.

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COIMBATORE – 641 021
B.Sc. DEGREE EXAMINATION
FIRST INTERNAL EXAMINATION ANSWER KEY
VIROLOGY

PART A – (20 x 1 = 20 marks)

1. Poison

2. nucleocapsid

3. Defective virus

4. virion

5. DNA &RNA

6. David Baltimore

7. Host

8. Pox

9. Host

10. Nucleoproteins

11. Host

12. Capsid

13. Icosahedral

14. Hexons

15. Nanometres

16. Viriods

17. Influenza virus

18. Immunity repressor

19. Measles

20. Plaque

Part-B

21. What is capsid and list the types of symmetry?

Single type of capsomeres stacked around a central axis form a capsid. The capsid is of three types-icosahedral-helical-complex.

22. What is reverse transcriptase enzyme?

Conversion of RNA template to form DNA MOLECULE-RETROVIRUSES.

23. What is Baltimore classification? List out.

i. dsDNA viruses
ii. ssDNA viruses
iii. dsRNA viruses
iv. (+) sense ssRNA viruses (codes directly for protein)
v. (-) sense ssRNA viruses
vi. RNA reverse transcribing viruses
vii. DNA reverse transcribing viruses

PART –C (3 x 8 = 24 marks)

24. A. Explain in short about the viral cultivation methods.

viruses do not reproduce- independent of living host cells However, the cultivation of viruses can be done in different ways

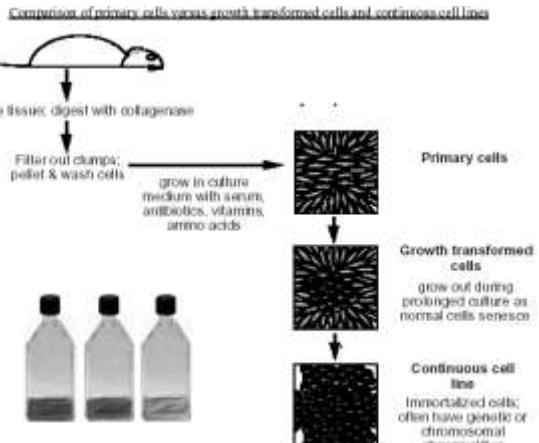
- Cultivation of animal viruses
- Cultivation of plant viruses
- Cultivation of bacteriophages.

a. Cultivation of Animal Viruses- In Animal Cells Human volunteers, Reed and colleagues- yellow fever.
b. Monkeys- Landsteiner & Popper (viruses.(1909) - polio viruses.
c. White mice- Theiler (1903).
d. Animals still in use
    1. Suckling mice
    2. Rabbits

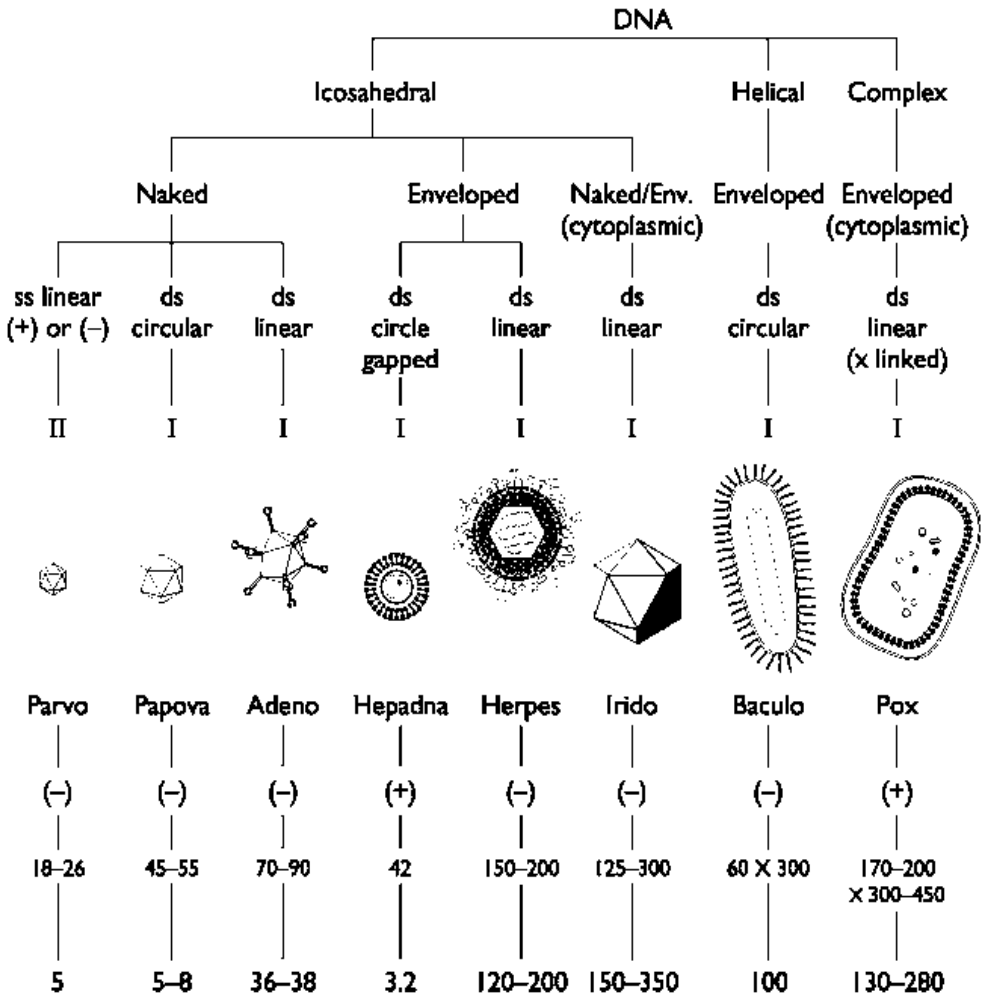
In Chick-Embryo

In Cell Cultures

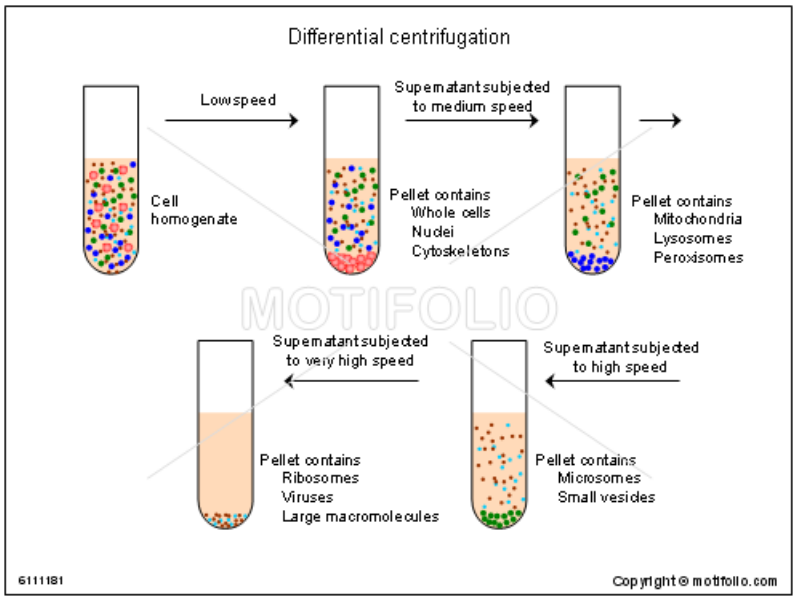
Tissue cultures - 3 types. Primary cell culture -Diploid culture -Continuous cell lines



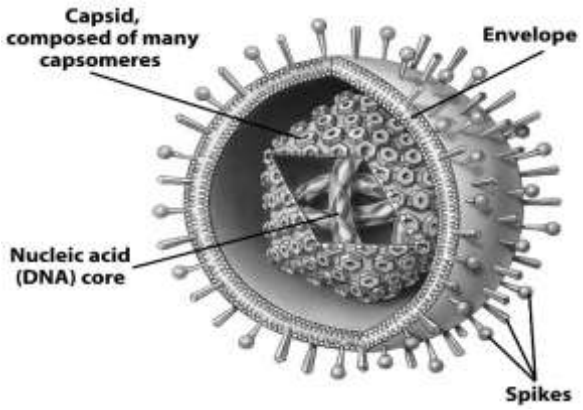
B. Write in detail about classification of DNA viruses



25. A. Explain in short about the viral purification.



B. Give a detailed note on viral structure and symmetry with necessary examples and images.



When a single virus is in its complete form and has reached full infectivity outside of the cell, it is known as a virion.

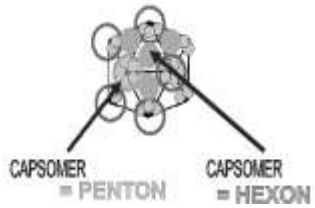
Viral components

- Viral Nucleic acids
- Viral Capsid
- Viral Envelope
- Viral Spikes

Nucleocapsid- core of a virus particle - genome plus a complex of proteins

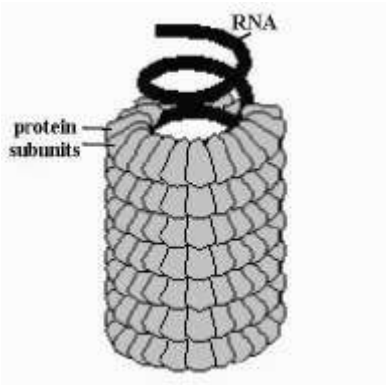
1. Icosahedral symmetry
2. Helical symmetry-
3. Complex structureS

ICOSAHEDRAL SYMMETRY



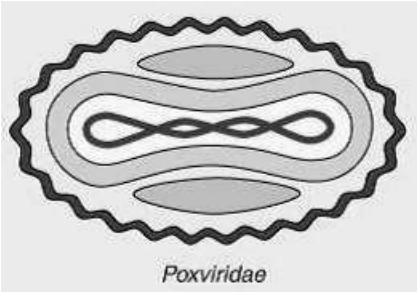
Icosahedral symmetry- 12 vertices, 20 faces (each an equilateral triangle) with the approximate outline of a sphere and two types of capsomeRS.

Helical symmetry



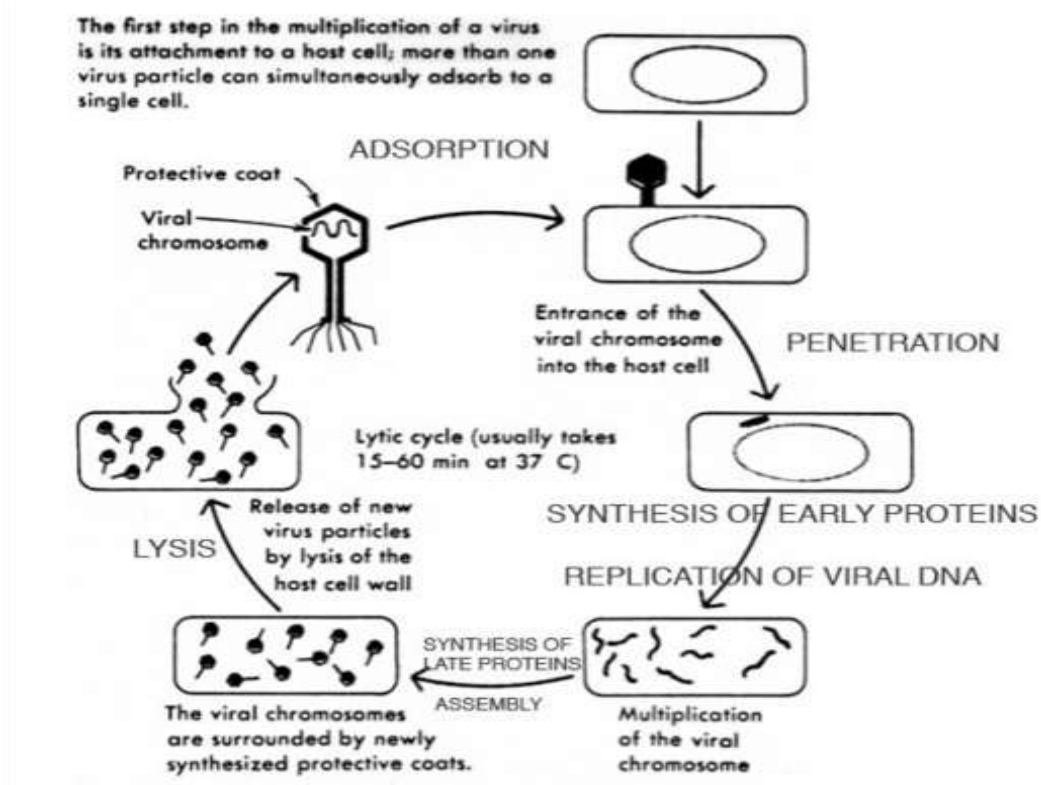
E.g. Rabies virus, Influenza virus, Para influenza virus, Mumps, Measles

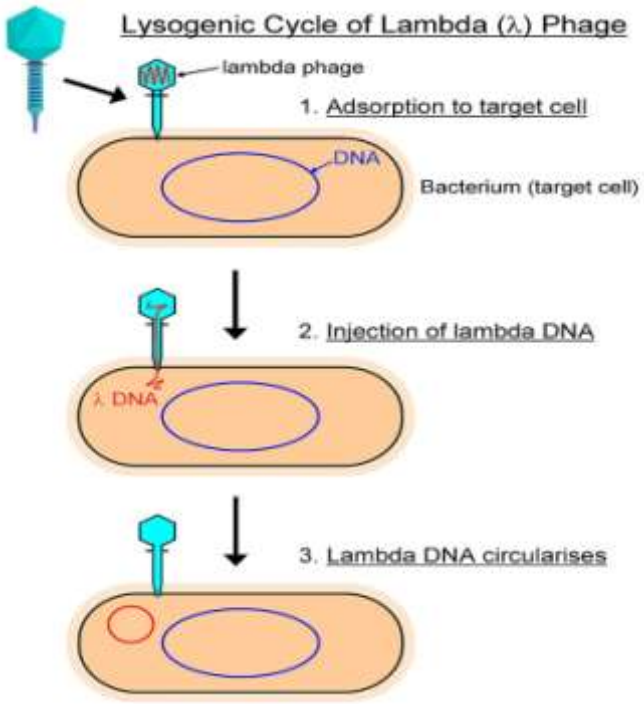
Complex structures



E.g. Poxviruses

26. A. Give a detailed note on lytic and lysogenic phages.





B. What is the one step growth curve experiment? Explain in detail.