COURSE GUIDE

BIO 415
VIROLOGY AND TISSUE CULTURE

Course Team (Course Developer) - NOUN
Prof. Babajide O. Odu (Course Reviewer) – OAU Ile-Ife
Professor Sami Michael Ayodele (Course Coordinator) - NOUN
Dr. Maureen N. Chukwu - NOUN

NATIONAL OPEN UNIVERSITY OF NIGERIA

National Open University of Nigeria
Headquarters
University Village
Plot 91, Cadastral Zone
Nnamdi Azikiwe Expressway
Jabi, Abuja

Lagos Office
14/16 Ahmadu Bello Way
Victoria Island, Lagos

e-mail: centralinfo@nou.edu.ng
URL: www.nou.edu.ng

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CONTENTS **PAGE**

BIO 415 VIROLOGY AND TISSUE CULTURE (2 UNITS)

Viruses pathogenic to man and animals with emphasis on virulence types of diseases caused methods of control. Experiments with bacteriophages and representative animal viruses to demonstrate characteristics of viruses and viral virulence. Methods of viral cultivation and identification, with special reference to tissue culture techniques

INTRODUCTION

Virology and Tissue Culture (Course Code 415) aims to take the learner through the fundamentals of viruses infecting human, animals and plants. The course intends to expose the learner to the theoretical and practical aspects of Virology.

The course describes a wide range of topics starting from the origin of viruses, to classification of viruses and giving classical examples of viruses causing economically important diseases in human. It also introduces the learner to a range of knowledge and skills available for diagnosis, effective control measure and treatment of these diseases.

The course is therefore designed to equip those planning to work in medical microbiology laboratories, and related areas such as Medical, Dental, Veterinary Medicine, Nursing, and Pharmacy. The course participants will be will be required to study online resource materials for a minimum of 2 hrs weekly and take assignments. Virology and Tissue Culture is a 2 credits units course.

The course guide tells you briefly what the course is all about, what course materials you will be using and how you can work your way through these materials. It gives you some guidance on your Tutor-Marked Assignments.

COURSE COMPETENCIES

Virology and Tissue Culture, as a course, provides the candidate with an overall view of viruses infecting humans, animals and plants; and their characteristics. This course also provides information on the symptoms of some diseases caused by specific viruses infecting humans and animals; practical diagnostic methods; and means of controlling them.

COURSE OBJECTIVES

The course aims to empower practitioners in the areas of medical microbiology, Medicine, Dentistry, Veterinary Medicine, Nursing, and

Pharmacy with required practical skills and knowledge required both in the field and in laboratories to collect samples, detect causal agents and treat diseases caused by viruses. In addition to the course objectives, each units has its own specific objectives. The candidate is advised to carefully read through and understand the specific objectives for each unit.

As you go through each unit, you should from time to time go back to these objectives to ascertain the level at which you have progressed. By the time you have finished going through this course, you should be able to:

- describe the characteristics of viruses infecting humans, animals and plants;
- know the different classes of viruses infecting humans, animals and plants;
- know the diseases caused by the viruses;
- understand the symptoms of the diseases caused by the viruses;
- know the basic skills for identification of virus diseases, and
- know basic practical techniques for managing diseases caused by viral agents.

WORKING THROUGH THIS COURSE

In this course, you will be advised to devote your time in reading through the material. You would be required to do all that has been stipulated in the course: study the course units, read the recommended reference textbooks and do all the unit(s) self- assessment exercise (s) and at some points, you are required to submit your assignment (TMAs) for assessment purpose. You should therefore avail yourself of the opportunity of being present during the tutorial sessions so that you would be able to compare knowledge with your colleagues.

STUDY UNITS

This course is divided into 3 modules with a total of fifteen units which are divided as follows:

Module 1:

Unit 1: Introduction to viruses
Unit 2: Viral Taxonomy and symmetry
Unit 3: Classification of DNA viruses
Unit 4: Classification of RNA viruses
Unit 5: Classification of RNA viruses continued

Module 2:

Unit 1: Other classes of viruses
Unit 2: Viral replication
Unit 3: Viral genetics
Unit 4: Mode of transmission and diagnosis of viral infections
Unit 5: Control and treatment of viral diseases

Module 3:

Unit 1: Case study of viral diseases
Unit 2: Cultivation of viruses
Unit 3: Purification of viral particles
Unit 4: Assessing the purity of virions and identification of a viral particle
Unit 5: Preservation of viruses and ethics in a virology laboratory

REFERENCES AND FURTHER READINGS

You would be required to do all that has been stipulated in the course: study the course units and read the recommended reference textbooks in each unit of the course material.

PRESENTATION SCHEDULE

Presentation schedule for this course will be uploaded on the online course page.

ASSESSMENT

You are required to submit your assignment (TMAs) for assessment purpose.

HOW TO GET THE MOST FROM THE COURSE

The course comes with a list of recommended textbooks. These textbooks are supplement to the course materials so that you can avail yourself of reading further. Therefore, it is advisable you acquire some of these textbooks and read them to broaden your scope of understanding.

ONLINE FACILITATION

Online facilitation for this course will hold once in a week for the period of eight weeks. The time and day for the online facilitation will be one hour as indicated in the time table

COURSE INFORMATION

Course Code:	BIO 415
Course Title:	Virology and Tissue Culture
Credit Unit:	Three (2)
Course Status:	Elective
Course Blub:	Virology and Tissue Culture, as a course designed to provide the students with an overall view of viruses infecting humans, animals and plants; and their characteristics. It also provides information on the symptoms of some diseases caused by specific viruses infecting humans and animals..
Semester:	First Semester
Course Duration:	13 weeks

Required Hours for Study: 91 hours

ICE BREAKER

I am Prof. Babajide O. Odu, Lecturer in the Obafemi Awolowo University, Ile Ife. and external facilitator in National Open University. I am currently the Director, University Research Office (URO) OAU. The links below are my research ID URL:
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https://scholar.google.com/citations?user=96zKWmgAAAAJ&hl=en
https://scholar.google.com/scholar?scilib=1&scioq=Odu+Babajide&hl=en&as_sdt=0,5
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MAIN COURSE

CONTENTS **PAGE**

MODULE 1

UNIT 1 INTRODUCTION TO VIRUSES

CONTENTS

1.1 Introduction

Virology is the study of viruses. Viruses are small, acellular entities, inert in the extracellular environment and depend on the machinery of a living host to reproduce. In this unit, we shall be looking at the origin of viruses, some terms in virology, the general characteristics of viruses and the chemical composition of viruses.

1.2 Learning Outcomes

At the end of the class student must:

- be familiar with origin of viruses, virology terms,
- understand general characteristics of viruses, and
- understand chemical composition of viruses

1.3 Origin of viruses and terms in virology

1.3.1 Origin of viruses

Virology is defined as the study of viruses. Viruses may be defined as acellular sub-microscopic organisms whose genomes consists of nucleic acid (DNA or RNA - never both); and which obligatorily replicate inside host cells using host's metabolic machinery and ribosomes, to form complete viral particles referred to as virions, which serve to protect the genome and transfer it to other cells.

Viruses are inert in the extracellular environment and only come alive in contact with a living host cell. They replicate only in the living cells. The viral nucleic acid contains all information necessary for programming infected cells to synthesize a number of virus specific macromolecules required for the production of viral progeny. The viral genome takes control of the metabolism of the host cell. Viruses infect a wide range of host cells ranging from bacteria, fungi, mycoplasma, algae, invertebrates, all higher plants and animals. Many viruses are known to infect humans.

In-Text Question

What is a virus?

Ans: A virus is an acellular sub-microscopic organism which obligatorily replicate inside a living host cell by using its metabolic machinery and ribosome.

1.3.2 Terms in virology

Capsid:

This is the protein shell or coat that encloses or surrounds the nucleic acid of the virus. Empty capsids may be by-products of replicative cycle of viruses with icosahedral symmetry.

Nucleocapsid:
This refers to the capsid together with the enclosed nucleic acid.

Virion:
This is the entire infectious unit or the complete viral particle. The virion serves to transfer the nucleic acid from cell to cell.

Viriod:
This refers to some naked genetic materials that are air-borne but lackscapsid. They also cause infection on contact with a living host cell. They mostly cause plant infections.

Envelope: This is a lipid containing membrane that surrounds some viruses. It is acquired during virus maturation by budding process through the cellular membrane. Virus enclosed glycoproteins are exposed on the surface of the envelope.

Capsomers:
This refers to morphologic unit seen in the electron microscope on the surface of the icosahedral virus particle. They represent clusters of polypeptides.

Capsomeres:
They are the protein building blocks that constitute the capsomers.
Defective virus: This refers to a virus particle that contains insufficient nucleic acid to provide for production of all essential viral components, thus infection is not produced except under certain conditions.

Replication:

Replication is the formation of biological viruses during the infection process in the target host cells. Viruses must first get into the cell before viral replication can occur.
Viroplasm: A virus factory or a modified region in an infected host cell where virus replication occurs.

Strain:

Different lines or isolates of the same virus (e.g., from different geographical locations or patients).

Type:

Different serotypes of the same virus (e.g., various antibody neutralization phenotypes).

Variant:

A virus whose phenotype differs from the original wild-type strain but the genetic basis for the difference is not known, for example, a new clinical isolate from a patient.

In-Text Question

Where is a Virion?

Ans.: Virion is the entire infectious unit or the complete viral particle. The virion serves to transfer the nucleic acid from cell to cell.

Self Assessment Exercise 1

Provide answers to the following questions in 5 minutes

1. A virus genome consists of both DNA and RNA
   True/False
2. There are viruses that infect other microorganisms
   True/False
3. Viruses have the capacity to replicate outside of their host
   True/False
4. Virus particles can be viewed under the compound microscope.

1.4 General characteristics of viruses

i. They are non-cellular and very simple in structure, consisting mainly of a nucleic acid surrounded by a protein envelope called capsid. Therefore, a unit of virus is referred to as 'a virus particle' rather than 'a virus cell'.
ii. They are devoid of the sophisticated enzymatic and biosynthetic machinery essential for independent activities of cellular life. Therefore, they can grow only inside suitable living cells. That is why; they are cultivated in the laboratory only inside living cells, unlike bacteria and fungi, which can be cultivated in the laboratory on non-living matter like nutrient agar.
iii. They are ultra-microscopic and can only be visualized under electron microscope.
iv. They do not increase in size.
v. They can pass through filters, through which bacteria cannot pass.
vi. A virus is called either 'DNA virus' or 'RNA virus' depending on whether it contains the nucleic acid DNA or RNA. A virus cannot have both DNA and RNA
vii. Viruses are not sensitive to antibiotics.
viii. Viruses can mutate.

In-Text Question

What equipment can be used to visualize viral particles? Give reason for you answer.

Answer

An electron microscope can be used to visualize viral particles. They are ultra-microscopic.

Self Assessment Exercise 2.

Provide answers to the following questions in 5 minutes

Discuss briefly the characteristics of viruses

1.5 Chemical composition of viruses

The essential components of infectious viral particles are nucleic acid (the genome) and protein. In addition, all enveloped viruses contain lipid in the envelope and carbohydrate in their glycoprotein peplomers (as well as that in the nucleic acid). The largest and most complex viruses (poxviruses, ranaviruses, and African swine fever virus) also have lipids associated with other parts of the virion.

1.5.1 Viral protein

Some virus-coded proteins are *structural,* i.e., they are part of the virion; some are *non-structural* and are concerned with various aspects of the replication cycle. A major role of structural proteins is to provide the viral nucleic acid with a protective coat. The virions of all viruses of vertebrates contain several different proteins, the number ranging from 3 in the case of the simplest viruses to over 100 in the case of the complex poxviruses. In *isometric* viruses, the structural proteins form an icosahedral capsid which sometimes encloses a polypeptide core that is intimately associated with the nucleic acid. Some virions, e.g., those of reoviruses, appear to have two concentric capsids.

The capsid proteins are assembled in the virion in groups, to form the capsomers visible in electron micrographs. Each capsomer is composed of one to six molecules of polypeptide, usually of the same kind (homopolymers) but sometimes different (heteropolymers). Capsomers from the vertices and the faces are usually composed of different polypeptides. A few viruses have a double capsid, each being composed of a different set of polypeptides. Other proteins, invariably glycoproteins, make up the peplomers projecting from the envelope; a second type of envelope protein is the nonglycosylated matrix protein that occurs as a layer at the inner surface of the lipid envelope of orthomyxoviruses, paramyxoviruses, and rhabdoviruses. One or more of the proteins on the surface of the virion has a specific affinity for

complementary receptors present on the surface of susceptible cells; the same viral protein contains the antigenic determinants against which neutralizing antibodies are made. Virions of several families carry a limited number of enzymes, transcriptases being the most important.

1.5.2 Nucleic acid

Any particular virus contains only a single kind of nucleic acid. However, this may be DNA or RNA; indeed, the RNA viruses provide the only instance in nature in which RNA is the exclusive repository of genetic information. All viral genomes are *haploid*, i.e., they contain only one copy of each gene, except for retrovirus genomes, which are *diploid.* Viral DNA or RNA can be *double-stranded* (*ds*) or *single-stranded* (*ss*). Since 1978 the genomes of many of the smaller animal viruses have been sequenced, and there are now no insuperable technical impediments to the sequencing of any viral genome. By 1985, the largest genome to be completely sequenced was that of a herpesvirus (EB virus), which consist of 172,000 base pairs (172 kilobase pairs, kbp).

When carefully extracted from the virion, the nucleic acid of viruses of certain families of both DNA and RNA viruses is infectious; i.e., when introduced into a cell it can initiate a complete cycle of viral replication, with the production of a normal yield of progeny virions. In these cases, messenger RNA (mRNA) is transcribed from the viral DNA in the nucleus, by a cellular transcriptase, or in the case of RNA viruses the genomic RNA itself functions as mRNA. In other cases, the isolated nucleic acid is not infectious even though it contains all the necessary genetic information. Among DNA viruses, failure to infect occurs if transcription requires a viral rather than a cellular transcriptase; among RNA viruses failure occurs when the viral RNA is of minus (–) sense or is double-stranded; its transcription to produce plus (+) sense mRNA then requires a virion-associated transcriptase. The (+) sense RNA of retroviruses is not infectious, because replication of the RNA occurs only after the production of a DNA provirus by a virion-associated reverse transcriptase.

i. DNA viruses

The genome of all DNA viruses consists of a single molecule, which is double-stranded except in the case of the parvoviruses, and may be linear or circular.

The DNA of papovaviruses and hepadnaviruses is circular. Within the virion, the circular DNA of the papovaviruses, like that of mitochondria and bacterial plasmids, is a supercoiled

circle, known as a superhelix. When an enzyme relieves the tension by introducing a nick into one strand, the molecule becomes a relaxed circle. One strand of the circular DNA of hepadnaviruses is shorter than the other; the genome is thus only partially double-stranded.

Most of the linear DNAs from viruses of other families have characteristics which enable them to adopt a circular configuration temporarily, presumably during replication. The two strands of poxvirus DNA are covalently cross-linked at each end, so that on denaturation, the molecule becomes a large single-stranded circle. The linear dsDNA of some herpesviruses (and the linear ssRNA of retroviruses) contains *repeat sequences* at the ends of the molecule. Following partial digestion of both DNA strands from their 5' ends by an exonuclease, the exposed single-stranded ends are complementary in their nucleotide sequences, thus providing "cohesive" or "sticky" ends, so that, if the molecule is melted, it will reanneal as a circular dsDNA. In the case of the adenoviruses, these terminal repeats are inverted; hence, even without enzymatic digestion, denatured molecules self-anneal to form single-stranded circles. Inverted terminal repeat sequences, which give rise to "hairpin" structures, are also a feature of the ssDNA parvoviruses.

Another type of terminal structure occurs in adenoviruses, hepadnaviruses, parvoviruses, and the ssRNA picornaviruses and caliciviruses, in all of which a protein is covalently linked to the 5' terminus. This has an essential function in replication of the genome.
The DNA of certain iridoviruses (genus *Ranavirus*) contains a high proportion of 5-methylcytosine instead of cytosine.

The size of viral DNA genomes ranges from 4.5 kilobases (kb) (molecular mass, $M_r = 1.5 \times 10^6$) for the small ssDNA parvoviruses to over 200 kbp ($M_r = 185 \times 10^6$) for the large dsDNA poxviruses. As 1 kb or 1 kbp contains enough genetic information to code for about one average-sized protein, we recognize as an approximation that viral DNAs contain from about 4 to 200 genes and code for 4 to 200 proteins. However, the relationship between any particular nucleotide sequence and its protein product is not as straightforward as this.

First, the DNA of most of the larger viruses - like that of cells - contains what appears to be redundant information, in the form of (1) *repeat* (*reiterated*) *sequences* and (2) *introns*, i.e., regions which are *spliced* out and discarded from the RNA transcript. On

the other hand, a single such RNA transcript may be spliced and/or cleaved in several different ways to yield several distinct mRNAs, which may be translated into different proteins. Furthermore, a given mRNA sequence may be read in two different reading frames (theoretically, up to three, because each *codon* is a triplet), giving rise to two (or three) proteins with different amino acid sequences. These fascinating examples of genetic economy are well-illustrated by the papovaviruses. Suffice it to say at this point that nowadays we cannot always talk in terms of a direct one-to-one relationship between a "gene" and its "gene-product," although such a relationship does sometimes occur.

Viral DNAs contain several kinds of noncoding sequences, in addition to introns and various types of terminal repeat sequences, described above. Consensus sequences, which tend to be conserved through evolution because they serve vital functions, include those of RNA splice sites, polyadenylation sites, RNA polymerase recognition sites and promoters, initiation codons for translation, and termination codons.

ii. RNA viruses

The genome of RNA viruses may also be single-stranded or double-stranded. Furthermore, while some occur as a single molecule, others are segmented. Arenavirus and birnavirus RNAs consist of 2 segments, bunyavirus RNA of 3, orthomyxovirus RNA of 7 or 8 (in different genera), and reovirus 10, 11, or 12 (in different genera). Each of these molecules is unique (often a single "gene"). All viral RNAs are linear; none is a covalently closed circle. However, the ssRNAs of arenaviruses and bunyaviruses have sticky ends, hence these molecules occur as circles. The genomes of ssRNA viruses have considerable secondary structure, regions of base pairing probably serving as signals controlling transcription, translation, and/or packaging into the capsid.

Single-stranded viral nucleic acid, which is generally RNA, can also be defined according to its *sense* (also known as *polarity*). If it is of the same sense as mRNA, it is said to have *positive* (+) *sense*). This is the case with picornaviruses, caliciviruses, togaviruses, flaviviruses, coronaviruses, and retroviruses. If, on the other hand, its nucleotide sequence is complementary to that of mRNA, it is said to have *negative* (–) *sense*). Such is the case with the paramyxoviruses, orthomyxoviruses, rhabdoviruses, arenaviruses, and bunyaviruses, all of which have an RNA-dependent RNA polymerase (*transcriptase*) in the virion, in order that mRNA can be transcribed. With the arenaviruses

and at least one genus of bunyaviruses one of the RNA segments is *ambisense,* i.e., part (+) sense, part (–) sense.

Where the viral RNA is of (+) sense, it is usually polyadenylated at its 3' end (in picornaviruses, caliciviruses, togaviruses, and coronaviruses, but not in flaviviruses) and capped at its 5' end (togaviruses, flaviviruses, coronaviruses). The picornaviruses and caliciviruses have a protein attached to the 5' end of the viral RNA.

The size of ssRNA viral genomes varies from 7.5 to 18 kb (M_r = 2.5 to 7 × 10^6) and that of the dsRNA viruses from 7 to 22 kbp (M_r = 4.8 to 15 × 10^6) - a much smaller range than seen with the DNA viruses. Accordingly they code, in general, for fewer than a dozen proteins. In the case of the segmented RNA genomes of orthomyxoviruses and reoviruses, one can consider most of the segments to be individual genes, each coding for one unique protein. No such simple relationship applies to the other RNA viruses. For example, the picornavirus genome [(+) sense ssRNA] is directly translated into a single "polyprotein," which is subsequently cleaved to give the several viral polypeptides.

Viral preparations often contain some particles with an atypical content of nucleic acid. Host cell DNA is found in some papovavirus particles, and cellular ribosomes are incorporated in arenaviruses. Several copies of the complete viral genome may be enclosed within a single particle, or viral particles may be formed that contain no nucleic acid (empty particles) or that have an incomplete genome (defective interfering particles).

1.5.3 Viral lipids

Lipid constitutes about 30–35% of the dry weight of enveloped viruses, the viral envelope being derived from cellular lipids. As a consequence, the composition of lipids of particular viruses differs according to the composition of the membrane lipids of the cells in which they have replicated. About 50–60% of the envelope lipid is phospholipid, and most of the remainder is cholesterol. The lipids occurring in viral envelopes have been termed "peripheral structural lipids". Extraction of these lipids with organic solvents or detergents or digestion of them with lipases results in considerable degradation of the viral particles. Clearly, such lipid components are essential for maintaining the structure of virus envelopes.

The poxviruses, ranaviruses, and African swine fever virus contain cellular lipid in their envelopes, and other lipids in the inner part of the virion. Lipid occurs in the outer membrane of poxviruses, and has a different composition from that of host cell lipids. In ranaviruses and

African swine fever virus the additional viral lipid occurs within the icosahedral capsid.

1.5.4 Viral carbohydrate and other constituents

Apart from that associated with viral nucleic acid, carbohydrate occurs as a component of viral glycoproteins, which usually occur as peplomers, with their hydrophobic ends buried in the lipid bilayer of the envelope, while their glycosylated hydrophilic ends project into the medium. Poxviruses also contain internal glycoproteins, in the membrane of the core, and one of the outer capsid proteins of rotaviruses is glycosylated.

In addition to protein, nucleic acid, lipid, and carbohydrate, some other substances are found in small amounts in highly purified preparations of certain viruses. Most of these minor components are probably adventitious elements. For example, many cells contain significant amounts of polyamines, and these cations are strongly attracted to the phosphoryl anions of viral nucleic acids, where they remain to become a part of the mature virus particle in those cases in which low particle permeability and other relationships are favorable.

In-Text Question

What are the basic components of a virus?
Ans.: A virus consists of nucleic acid (DNA or RNA), coat protein, and may contain lipids, carbohydrate.

Self Assessment Exercise 3

Provide answers to the following questions in 5 minutes

What are the functions of the main components of a virus?

1.6 Summary

- Viruses are small acellular unit that require a lining host to become living.
- They contain a nucleic acid and a protein coat, they could be enveloped or not.
- They attack a wide variety of organisms.
- Viruses are infective agents.
- Viruses can infect animals, plants, and even other microorganisms.

- Since viruses lack metabolic machinery of their own and are totally dependent on their host cell for replication, they cannot be grown in synthetic culture media.

1.7 References/Further Readings/ Web Sources

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1.8 Possible Answers to SAEs

SAE 1

1. False
2. True
3. False
4. False
5. a. A strain refers to different line or isolate of the same virus while a type is a different serotype of the same virus
   b. A virus consists of either a DNA or RNA, and has capsid (coat protein); a viroid consist of only RNA
   c. Capsomeres are protein building blocks that constitute the capsomers

SAE 2

Viruses are non-cellular and very simple in structure, consisting mainly of a nucleic acid surrounded by a protein envelope called capsid. They are devoid of the sophisticated enzymatic and biosynthetic machinery essential for independent activities of cellular life. Therefore, they can grow only inside suitable living cells. They are cultivated in the laboratory only inside living cells, unlike bacteria and fungi, which can be cultivated in the laboratory on non-living matter like nutrient agar. They are ultra-microscopic and can only be visualized under electron microscope. A virus is either 'DNA virus' or 'RNA virus' depending on whether it contains the nucleic acid DNA or RNA. Viruses can mutate.

SAE 3

A viral particle consist of the basic components: nucleic acid (single- or double-stranded RNA or DNA), a protein coat, the capsid and lipids.

The capsid (coat protein) functions as a shell to protect the viral genome from nucleases and which during infection attaches the virion to specific receptors exposed on the prospective host cell.
Lipids are essential for maintaining the structure of virus envelopes.

UNIT 2 VIRAL TAXONOMY AND SYMMETRY

CONTENTS

2.1 Introduction

In this unit we shall be looking at viral taxonomy, factors considered in classifying viruses and viral symmetry.

2.2 Learning Outcomes

At the end of the class, student must have fully understood:

- brief history of viral taxonomy
- factors considered in viral classification
- viral symmetry

2.3 Viral Taxonomy

Taxonomy is the science of classification, very dynamic and substitution and subject to change on the basis of available data. Significant development in the classification of viruses is documented in the reports of the International Committee on Nomenclature of Viruses (ICNV). The organization has however been renamed as International Committee on Taxonomy of Viruses (ICTV). The viruses infecting humans and

vertebrate animals of importance to man are most of the virus group recognized. Many of these have now been placed in families, genera and species.

A taxonomic hierarchy (Figure 1) was sought that could accommodate a virosphere-wide tree (or trees) from the roots to the tips of the branches. A formal virus classification hierarchy outlining 15 ranks, including eight principal (or primary) ranks and seven derivative (or secondary) ranks was proposed by ICTV in 2016. The eight principal ranks include four that were already in use (order, family, genus and species) and four that are new: realm, kingdom, phylum and class, which are all above the order rank. The class rank in this series is not to be confused with the 'classes' described by Baltimore, or the typological attributes of a taxonomic rank. These new principal ranks cover the entire scale of virus divergence to include the deepest virus relationships at the basal rank of realm.

The seven secondary ranks include the previously used subfamily rank and six new ranks that are derivatives of most of the remaining principal ranks. The exception is the species rank, which is currently not associated with a secondary rank, as no consensus on the definition of 'subspecies' could be reached. This new rank hierarchy and the associated nomenclature, including defined suffixes for taxa, follow those used in the Linnaean system with a single exception. The basal rank is called 'realm' in virus taxonomy, rather than 'domain' (as in other taxonomies), reflecting a complex interrelation between virus taxonomy and its counterparts for cellular organisms.

According to the 2021 release and ratification there are 6 realms, 10 kingdoms, 17 phyla, 2 subphyla, 39 classes, 59 orders, 8 suborders, 189 families, 136 subfamilies, 2224 genera, 70 subgenera, 9110 species.

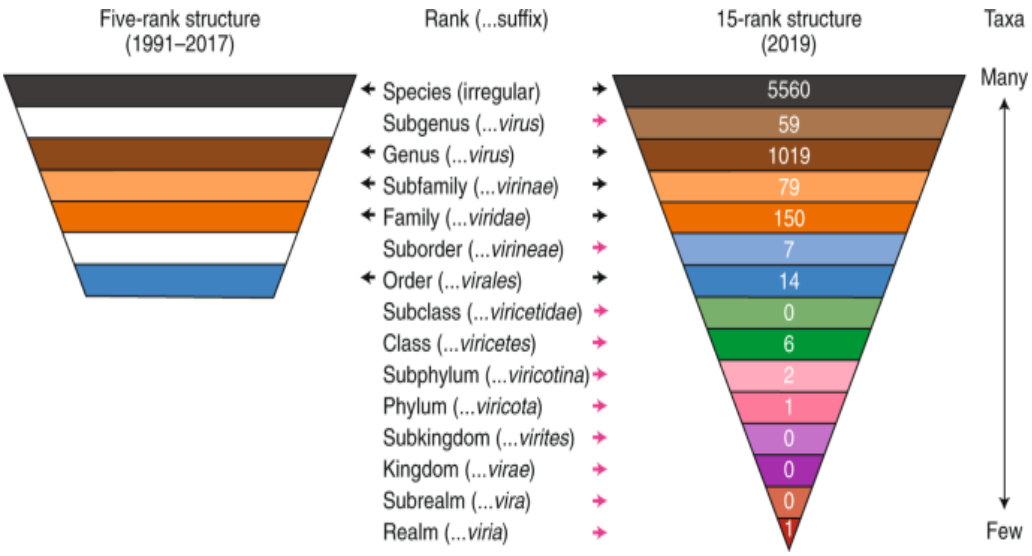


Figure 1. A comparison of the ICTV taxonomic rank hierarchy in 1991–2017 and 2019

In-Text Question (ITQ)

Make a list of the most recent ICTV proposed classification of viruses.
Ans. realms, subrealm, kingdoms, subkingdom, phyla, subphyla, classes, subclass, orders, suborders, families, subfamilies, genera, subgenera, species

Self-Assessment Exercise 1

Provide concise answer to the following question giving illustrations where necessary in 20 minutes

Using the recently proposed ICTV 15 rank classification hierarchy of viruses, present a classification of the newly emerged SARS-CoV-2 virus in this taxonomic hierarchy

2.4 Factors considered in classification of viruses

Traditionally, the nomenclature used for viruses consisted of giving the name of the disease produced in the major host followed by the word virus e.g. Pox virus

- Measles virus
- Tobacco mosaic virus

Bacteriophages were named by code symbols: using letters and numbers (such as T1, T2, c16, s13 phages etc.) which were derived from laboratory practice.

Viruses are classified into families on the basis of various factors stated below:

- The type of nucleic acid: a virus contains either RNA or DNA. The nature of the nucleic acid, single or double stranded is important as a strategy during replication.
- The size and morphology of viruses, type of virion symmetry, number of capsomers, presence or absence of an envelope.
- Presence of specific enzymes, particularly DNA and RNA polymerases concerned with genome replication.
- Susceptibility to physical and chemical agents such as ether.
- Immunologic properties.
- Natural methods of transmission.
- Host, tissue and cell tropism.

- Pathology and inclusion body formation.
- Symptomatology: This is the oldest form of viral replication and offers certain conveniences to clinicians but not satisfactory enough for virologist because the same virus may appear in several groups if it causes more than one disease.
- Sigla formation: Abbreviation from two or more names are joined together to form one name, part of the name could be derived from the type of genome and organ affected.

Examples are shown below

a. PAPOVA PA meaning Papilloma, PO meaning Polyoma, VA meaning Vacuolating agent
b. PICORNA PICO meaning Small virus, RNA meaning RNA-virus

2.4.1 Virus classification based on the method of genome replication and messenger RNA replication

The viruses can be divided into six general classes based on the nucleic acid type and the method for genome transcription.

The mRNA is defined as the RNA that binds to ribosomes and codes of protein synthesis.

CLASS 1 VIRUSES

- These viruses contain double-stranded DNA as the genome.
- In this group of viruses the DNA may be linear –e.g. adenoviruses and pox viruses; or circular –e.g. papoviruses.
- In this class of viruses, the viral DNA is replicated in the same way as cellular DNA and utilizes the host cell enzymes.
- The mRNA is transcribed in the normal way from viral DNA using the host transcriptase enzymes, into 2 types of mRNA's – early mRNA (is transcribed prior to the synthesis of viral DNA) and late mRNA (is transcribed from progeny DNA)
- The late mRNA functions in coding for structural proteins of the virus. Whereas early mRNA codes for early proteins, enzymes etc.

CLASS II VIRUSES

- The class II viruses have single-stranded DNA as a genome. An e.g. is the parvoviruses.
- Transcription and protein synthesis in this group are similar to those in class I viruses.

CLASS III VIRUSES

- This group contains double-stranded (ds) RNA as a genome. E.g. Reoviruses.
- They do not possess an envelope.
- In this group, RNA transcriptase enzymes contained in the core of the virus transcribe the (ds) RNA segments into mRNA which are then translated into proteins.
- Once the viral proteins have been formed/synthesized, the (+)RNA associates with them to form a core viral particle.
- The (+)RNA is then converted to (ds) RNA by a viral polymerase enzyme. The cycle goes on and replication continues.

CLASS IV VIRUSES

- This group of viruses contain mRNA, (+)RNA as a genome. E.g. picornaviruses and the togaviruses.
- After adsorption, penetration and uncoating, the (+)RNA associates with ribosomes to direct synthesis of viral proteins.

The viral proteins produced include the following:

1) RNA-dependent RNA polymerase (-since the cell does not contain the enzymatic machinery to replicate RNA from an RNA template – the synthesis of this polymerase is obligatory to RNA replication.

2) A protein that inhibits host cell protein synthesis. After the initial protein synthesis, the genomic RNA directs the replication of (+)RNA. Initially the genetic information in the (+)RNA must be transferred to a complementary (-)RNA strand, after which multiple copies of (+)RNA are synthesized using the polymerase enzyme. The progeny (+)RNA may become genomic RNA for progeny virus or it may serve as mRNA for the synthesis of viral proteins, which are synthesized on ribosomes.

CLASS V VIRUSES

- They possess (-)RNA as a genome i.e. they are negative strand viruses –they include orthomyxoviruses, paramyxoviruses etc.
- After adsorption, penetration and uncoating, the parental (-)RNA remains associated with a virion protein, and RNA transcriptase enzyme.

- The RNA transcriptase enzyme is a structural component of the virion and is necessary for infectivity.
- The enzyme transcribes the (-)RNA to produce mRNA's which code for the enzymes needed for replication. Some of the mRNA are translated into proteins.
- The transcriptase enzyme must also synthesize a complete (+)RNA molecule that acts as a template to replicate the (-)RNA.

CLASS VI VIRUSES

- These viruses contain (+)RNA as a genome and replicate via a DNA intermediate.
- This group is composed of the tumor viruses or retroviruses. These viruses are termed retroviruses because they contain an enzyme that transcribes DNA from an RNA template which is known as reverse transcriptase.
- Their replicative cycle is as follows: - After adsorption, penetration, and uncoating the parental (+)RNA is converted into a base-paired RNA-DNA double strand by the enzyme reverse transcriptase.
- A double-stranded DNA molecule is then synthesized from the RNA-DNA double strand/duplex by the same enzyme.
- Thus the genetic information contained in the parental (+)RNA is transferred to a double-stranded DNA.
- The double-stranded DNA then becomes circular and gets integrated into the host genome. This virus DNA is called a provirus and it is replicated along with the cell DNA and transferred to each daughter cell.
- The viral DNA is transcribed into mRNA, which is translated into proteins.
- At the same time, the viral (+)RNA directly behaves like mRNA and is directly translated into viral proteins.

In-Text Question (ITQ)

What are the phenotypic characteristics for classification of viruses.
Ans. Viruses are classified by phenotypic characteristics, such as morphology, nucleic acid type, mode of replication, host organisms, and the type of disease they cause.

Self_Assessment Exercise 2

Provide answer to the following questions giving diagrammatic illustrations where necessary in 20 minutes

1. Differentiate between DNA and RNA viral genomes
2. Draw a labelled structure of DNA molecule

2.5 Symmetry in Virus

Symmetry refers to the way in which capsomere units are arranged in viral capsid. The use of electron microscope and x-ray diffraction techniques have made it possible to resolve the differences in the basic morphology of viruses. Heavy metal stains e.g. potassium phosphotungstate is used in emphasizing the viral surface structure. The heavy metal permeates the virus particle like a cloud and brings out the surface structure of the viruses by virtue of negative staining. There are three basic symmetry in viral morphology.

In the simpler viruses the virion consists of a single molecule of nucleic acid surrounded by a protein coat, the *capsid;* the capsid and its enclosed nucleic acid together constitute the *nucleocapsid.* In some of the more complex viruses the capsid surrounds a protein core, and in other viruses the capsid is surrounded by a lipoprotein *envelope*. The capsid is composed of morphological units called *capsomers*, which are held together by noncovalent bonds. Individual capsomers, which consist of one or more polypeptide molecules, are usually visible by electron microscopy. In helical nucleocapsids, the viral nucleic acid is folded throughout its length in a specific relationship with the capsomers, but there is no such specific relationship between RNA and protein in the small icosahedral picornaviruses.

Within an infected cell, the capsomers of the simpler viruses self-assemble to form the capsid. The manner of this assembly is strictly defined by the nature of the bonds formed between individual capsomers, which imparts symmetry to the capsid.

2.5.1 Cubic or Icosahedral

The icosahedron is one of the five classical “Platonic solids” of geometry; it has 12 vertices (corners) and 20 faces, each an equilateral triangle (Figure 2). It has axes of two-, three-, and fivefold rotational symmetry, passing through its edges, faces, and vertices, respectively. The icosahedron is the optimum solution to the problem of constructing, from repeating subunits, a strong structure to enclose a maximum

volume. The same principles were applied by the architect Buckminster Fuller to the construction of icosahedral buildings ("geodesic domes").

Only certain arrangements of the repeating morphological units, the capsomers, can fit into the faces, edges, and vertices. In adenovirus particles, for example, capsomers on the faces and edges bond to six neighboring capsomers and are called *hexamers;* those at the vertices bond to five neighbors and are called *pentamers*. In some viruses both hexamers and pentamers consist of the same polypeptide; in others they are different. The varied arrangements of hexamers between pentamers have been systematically codified by Caspar using terms such as "P-number" and "T-number.

In a practical sense, the examination of negatively stained icosahedral virions in the electron microscope, and analysis of their capsomer arrangement, can often provide immediate and unambiguous information for the identification of a virus as a member of a known family-or, in very rare instances, as a candidate prototype for a new family. For example, the visualization of a non-enveloped virion with a row of four hexamers in line between vertex pentamers would identify a virus as an adenovirus.

The recent demonstration by X-ray crystallography of the atomic resolution structure of two picornaviruses (poliovirus and rhinovirus) has provided a remarkable insight into the organization and assembly of their virions, the location of the antigenic sites involved in neutralization, and aspects of their penetration into cells. Similar detail can be expected as these new technical capabilities are applied to other viruses and to problems of replication, assembly, and pathogenesis.

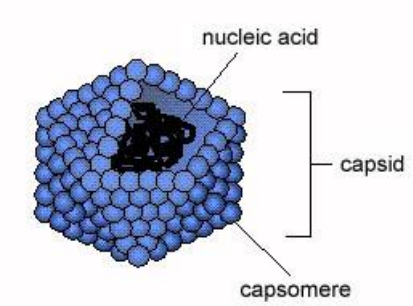
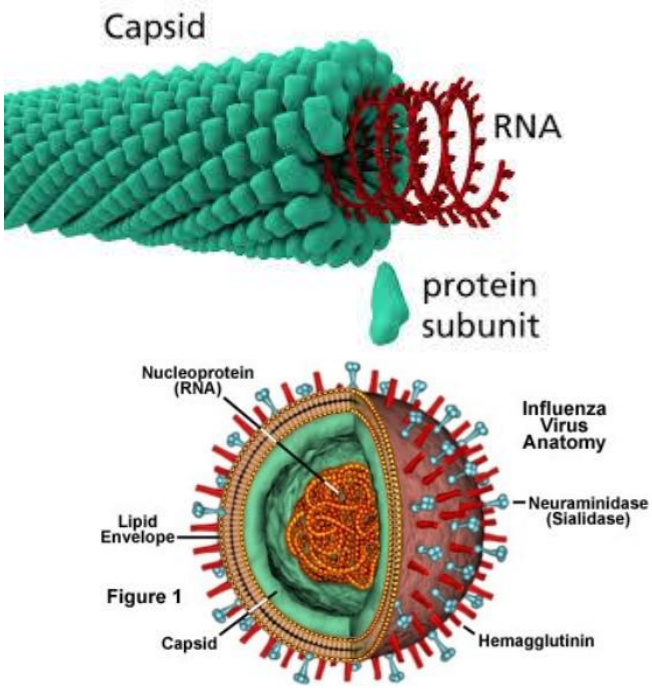


Figure 2. Structure of an icosahedral virus

2.5.2 Helical

The nucleocapsids of several RNA viruses have a different type of symmetry: the capsomers and nucleic acid molecule(s) self-assemble as a helix (Figure 3). In all such viruses each capsomer consists of a single polypeptide molecule. The plant viruses with helical nucleocapsids are

rod shaped and naked (non-enveloped, Figure 3(a)). However, in all animal viruses helical nucleocapsids are wound into a coil and enclosed within a lipoprotein envelope, possibly to give the very long nucleocapsids stability (Figure 3(b)). The capsomere and nucleic acid are wined together to form helical or spiral tube like structure. Most of the helical viruses are enveloped and all are RNA viruses. The typical virus with helical symmetry is tobacco mosaic virus (TMV), which is a RNA virus with 2130 identical capsomeres arranged in a helix. Helical viruses are of two types: naked helical structure and enveloped helical structure.



a. A naked helical virus b. An enveloped helical virus

Figure 3. Structures of a helical virus.

2.5.3 Complex symmetry

Some virus are more complex, being composed of several separate capsomere with separate shape and symmetry. They do not have either icosahedral or helical symmetry due to complexity of their capsid structure. Examples of this are the Pox virus and Bacteriophage.

2.5.3.1Binal symmetry

This is a type of complex symmetry. Some viruses such as T-phage (T2, T4 etc) have complex symmetry including head and tail (Figure 4). The most complicated virus in terms of structure are some bacteriophage which possess icosahedral head and helical tail. Such structure is called binal symmetry.

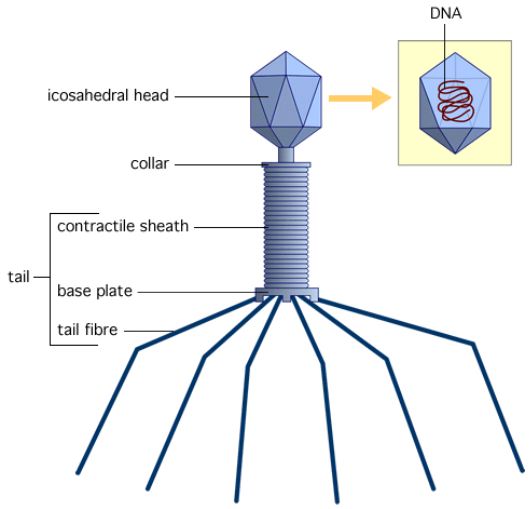


Figure 4. Structure of T-even bacteriophage

2.5.4 Viral Envelopes

Viral envelopes are acquired at host cell membranes - some at the plasma membrane, others at internal cell membranes such as the nuclear membrane, endoplasmic reticulum, and Golgi complex - during the maturation of the virus by the process known as “budding”. The lipids of the viral envelope are derived directly from the cell, but the proteins in the envelope are virus coded. One kind is the glycoprotein *peplomer* (*peplos* = envelope) or spike. These peplomers can often be seen clearly in electron micrographs as projections from the outer surface of the envelope. The other kind of envelope protein, *matrix protein*, is nonglycosylated and is found on the inside of the envelope of virions of several families; it provides added rigidity. The envelope of rhabdoviruses is closely applied to a bullet-shaped matrix protein that encloses a helical nucleocapsid. Arenaviruses, bunyaviruses, and coronaviruses have no matrix protein and consequently are rather more pleomorphic than other enveloped viruses.

Envelopes are not restricted to viruses of helical symmetry; some icosahedral viruses (ranaviruses, African swine fever virus, herpesviruses, togaviruses, flaviviruses, and retroviruses) have envelopes. The infectivity of most enveloped viruses depends on the integrity of the envelope, but some poxviruses have an envelope which is not necessary for infectivity.

In-Text Question (ITQ)

What is a bacteriophage?

Answer

A virus which parasitizes a bacterium by infecting it and reproducing inside it. Bacteriophages
are much used in genetic research, they have the following features: Polyhedral head, Helical tail, Fibers for attachment. They are considered either lytic or temperate, and are often associated with virulence genes in bacteria.

Self-Assessment Exercise 3

Provide concise answers to the following questions giving illustration where necessary in 20 minutes

1. What are the reasons for studying the structure, dynamics and physical and (bio)chemical properties of virus particles?
2. Differentiate between non-envelope and enveloped virus particles

2.6 Summary

- Classification of viruses is documented in the reports of the International Committee on Nomenclature of Viruses (ICNV) now International Committee on Taxonomy of Viruses (ICTV)
- Several factors are considered in classifying viruses such as shape of viral genome, sensitivity of the virus to organic solvents, type of nucleic acid, symptomatology amongst a host of others.
- Viral symmetry can either be icosahedral, helical, or complex.

2.7 References/Further reading/Web Sources

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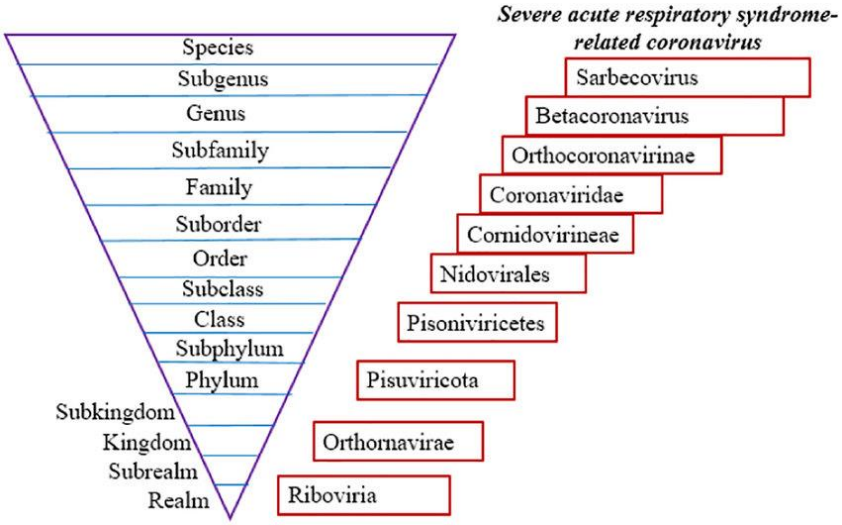
2.8 Possible Answers to Self-Assessment Exercises

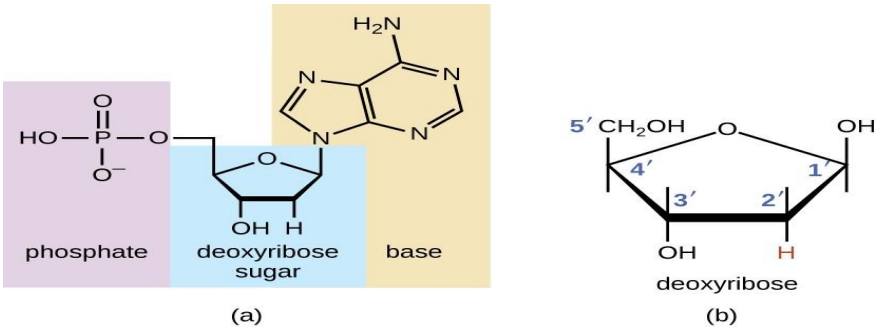
SAE 1



SAE 2

1. All major DNA viruses have genomes that are single molecules of DNA and have a linear or circular configuration. The RNA may be single linear molecule like Picornaviruses while others like Orthomyxovirus have their genome consisting of several segments of RNA that may be loosely associated within the virion.

2.

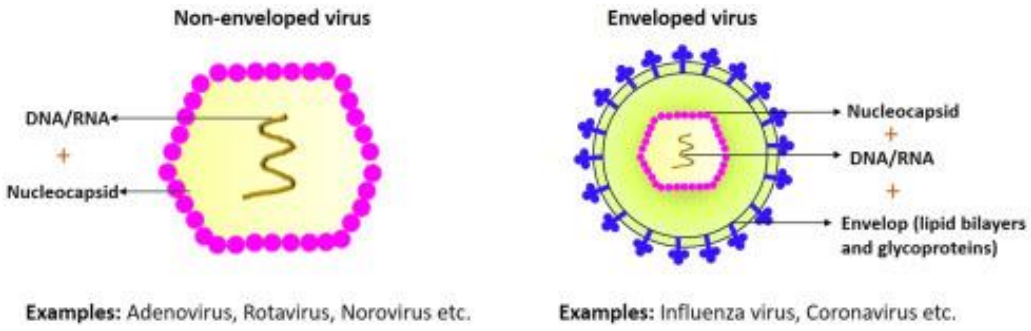


SAE 3

1.

i. Virus particles constitute excellent models to understand and learn to manipulate molecular self-assembly.
ii. Virus particles are paradigms to understand structure-function relationships in biomacromolecular assemblies and biological machines.
iii. A profound knowledge of virus structure, dynamics and properties is essential to understand the life cycles of viruses.
iv. Virus particles, their components and the processes in which they participate provide novel targets for the design of antiviral agents.
v. Understanding the structural determinants of virus stability, dynamics and function may facilitate the rational manipulation of virus particles to develop new or improved vaccines, gene therapy vectors, and nanoparticles for drug delivery or other biomedical or bio/nanotechnological uses.

2. These differences reflect different mechanisms of cell entry and different pathways of assembly and maturation. Enveloped viruses enter by membrane fusion, either from an internal compartment following an endocytic step, or at the cell surface. Non-enveloped viruses require some form of membrane perforation.



UNIT 3 CLASSIFICATION OF DNA VIRUSES

CONTENTS

3.1 Introduction

We shall be considering the classification of viruses based on the type of nucleic acids in their genome. In this unit, we shall look into the various classes of DNA viruses.

3.2 Learning Outcomes

At the end of the class, student must be familiar and have understood of the different classes and characteristics of DNA viruses.

3.3 Parvoviridae

They are very small DNA viruses with particle size of about 18-22nm in diameter with icosahedral symmetry of 32 capsomers. They are non-enveloped with single stranded DNA (ssDNA) of molecular weight (MW) of 1.5-2.0 × 10^6, G+C content of 41-53%. The physicochemical properties of the virion includes MW of 5.5 = 6.2 10^6; $S_{20}W$ = 110-122; buoyant density of 1.39-1.42g/dm^3 in cesium chloride (CsCl). The mature virion/particle is stable in the presence of lipid solvent, pH of 3.9 and in most species at 56^{o}C for at least 60 min. The virion contains 3 polypeptides, MW of 60-90 × 10^3 and could be demonstrated in the genera parvovirus and dependovirus. Densoviruses have 4 structural polypeptides. Viral replication takes place in only actively dividing cells while capsid assembly occurs in the nucleus of the infected cells. Members of this group are parvovirus group (Parvovirus), Dependovirus

(adeno-associated virus group) and insect parvovirus group (Densovirus). Members of the Dependovirus group require a "helper virus" (adenoviruses and herpesviruses) co-infection for efficient replication. Parvovirus replicates autonomously and preferentially encapsulates negatively sensed ssDNA. In the genera Dependovirus and Densovirus, the complementary +ve and –ve strands are encapsulated with about the same frequency.

In-Text Question (ITQ)

Briefly describe the structure of a Parvovirus.

Ans. Parvovirus virions are 23–28 nanometers (nm) in diameter and consist of the genome enclosed inside a capsid that is icosahedral in shape with a rugged surface. The capsid is composed of 60 structurally equivalent polypeptide chains derived from the C-terminal end of a VP protein's sequence, interlocking extensively to form an icosahedron with 60 asymmetric, superficial triangular units.

Self-Assessment Exercise 1

Provide answers to the following questions in 10 minutes

Briefly describe the disease cause by a named member of the Parvoviridae and how it is transmitted.

3.4 Papovaviridae

The name is derived from the sigla 'PA'= papilloma; PO = polyoma and VA = vacuolating agent (SV40). Members of this family are the genera polyomavirus and papillomavirus. Virions are small in size of about 40-55nm nn diameter, heat stable, ether and acid resistant. They have an icosahedral symmetry with 72 capsomers in skew arrangement, though filamentous forms occur. The virus particle is non-enveloped containing a molecule of circular double stranded DNA (dsDNA) with molecular weight of 3-5 × 106, G+C content= 40-50%. About 6-9 polypeptides are found in the, MW of 3.82 × 102. Infections by these viruses are airborne but some forms of human papilloma virus are sexually transmitted. These agents have a slow cycle, stimulate cell DNA synthesis and replicate in differentiating cells. They produce late and chronic infections in their natural host and all can induce tumors in some animal species.

In-Text Question (ITQ)

Give a brief description of the Papovaviridae.

Ans. The Papovaviridae family is comprised of two genera: papillomaviruses and polyomaviruses. The family name is derived from the names of three prototypical members: rabbit papilloma virus, mouse polyoma virus, and simian virus 40 (SV40), originally called vacuolating virus.

Self-Assessment Exercise 2

Provide answer to the following question in 10 minutes

What are the diseases caused by Papovaviridae?

3.5 Adenoviridae

Adenoviruses were first isolated from human adenoid tissue. Members of this family are the genera

- Mammalian Adenoviruses (Mastadenovirus), the prefix 'Mast' is derived from the Greek word 'Mastos', meaning 'breast'.
- Avian Adenovirus

Adenoviruses are non-enveloped isometric particles with icosahedral symmetry of about 70-90nm in diameter with 252 capsomers, 8-9 nm in diameter. Twelve vertex capsomers (penton bases) carry one or two filamentous projections, 240 non-vertex (hexons) capsomers are different from penton bass and fibres. These fibres are glycoprotein nature. The physicochemical properties of adventure are: MW = 170 x 10^6 buoyant density in CsCl = 1.32 - 1.35g/cm^3. The virion is stable on storage in frozen state, and not inactivated by lipid solvent. The Nucleic acid is a single linear molecule of DNA ct MW 20 - 25 x 10^6 for viruses isolated from mammalian (M) - Species OR 30 x 10^6 from avian (A) Species. A viral coded terminal protein is covalently linked to each 5i end. The sequence of the human adenovirus 2 genome is 35,937 hp and contains an inverted terminal Repetition (ITR) of 103bp. ITR's of 50-200bp's are found in all virus sequenced. G + C content varies from 48 - 61 % (mastadenoviruses) and 54-55 % for aviadenoviruses. At least 10 polypeptides with MW's of 5 - 121) x 10^3 are found in the M-Species. At least 41 types infect humans, especially in mucous membrane, and some types can persist in lymphoid tissue. Some adenoviruses cause acute respiratory diseases, phyaryngitis and conjunctivitis. Some human adenoviruses can induce tumors in new horn hamsters. There are many serotypes that infect animals. Characteristic Cytopathic effect (CPE) without lysis occurs during multiplication in cell culture. After attachment of infectious particle to cell receptocytes by the glycoproteins, the virus gain entry into cell by endocytosis. Transcription, DNA replication, DNA replication, and virus assembly takes place in the cell nucleus. It has been observed that there is

intranuclear inclusions, containing DNA, viral antigens and virions paracystalline array or otherwise. Transmission could be by direct, or in direct from throat, faeces, eye or urine, depending on the serotype.

In-Text Question (ITQ)

What are the means of transmission of the diseases causes by members of the Adenoviridae?
Ans. Transmission could be by direct, or in direct from throat, faeces, eye or urine, depending on the serotype

Self-Assessment Exercise 3

Provide answer to the following question in 10 minutes

Make a list of the genera of the family Adenoviridae giving the host(s) of each.

3.6 Herpesviridae

The name was derived from the Greek word "Herpes", "herpetos" meaning creeping, or crawling creature, from the nature of herpes febrilis lesions in infected patients. There are three sub families in this family.

i. Herpes Simplex Virus group called Alpha Herpesvirinae.
ii. Cytomegalovirus group called Beta Herpesvirinae.
iii. Lymphoproliferative Virus group called Gamma Herpesvirinae

Their host range in variable, from very wide to very narrow e.g. warm and cold-blooded vertebrates and invertebrates. The virion is 120 – 200nm in diameter and consists of 4 structural components. The capsid is 100 – 110nm in diameter, has 162 capsomeres arranged as an icosahedron (150 hexameric and 12 pentameric Capsomeres). The capsomeres are hexagonal in cross section with a hole running half way down the long axis. The tegument surrounding the capsid consists of globular material which is frequently asymmetrically distributed and may be variable in amount.

They are enveloped viruses with them envelope being a bilayer membrane surrounding the tegument, and has surface projections. The core consists of a fibrillar spool on which the nucleic acid (DNA) is wrapped. The ends of the fibers are anchored to the underside of the capsid shell. Tie intact envelope is impermeable to negative stain. The physicochemical properties of the virion are: MW is > 1000 x 10^6, buoyant density in CsCl = 1.20 – 1.29g/cm^3. Herpesviruses contain one

molecule of ds DNA, 120 – 220kbp with G + C content of 35-75%. They contain more than 20 structural proteins, MW = 12000 to > 222,000.

Herpesviridae are transmitted by contact between moist mucosal surfaces, trans-placentally, intrapartum, breast milk, transfusions, airborne or waterborne. Herpesviruses may remain latent in their primary hosts for the lifetime of those of those hosts, usually in ganglial or lymphoblastoid cells. Human Herpesviruses include herpes simpler types 1 & 2 (oral and genital lesions), Varicella - Zooster Virus (shingles and Chicken pox), cytomegalovirus (CMV), and Epstein-Barr virus (infectious mononucleosis and association with human neoplasm).

In-Text Question (ITQ)

What is the host range of the family Herpesviridae?

Ans. Warm and cold blooded vertebrate and invertebrate including mammals, birds, fish, reptiles, amphibians, and mollusk

Self-Assessment Exercise 4

Provide answer to the following question in 5 minutes

List the viruses classified as herpesviridae that are causing diseases in human

3.7 Poxviridae

The name was derived from the old English Poc, Pocc-, (Plural of pock) meaning Pustule or Ulcer as one of the symptoms shown on infected individuals. They are large, somewhat pleomorphic, brick-shaped or ovoid virion, 230-450nm x 140-260nm, with external coat containing lipid and tubular or globular protein structures, enclosing one or two lateral bodies and a core, which contains the genome. They are enveloped viruses with some being ether resistant and some other member ether sensitive. The nucleic acid is a single molecule of ds DNA, 130 - 375 kbp with variable G+C contents. In vertebrate poxviruses, the G+C = 35-64% while it is 20% in the entmo poxviruses. More than 100 polypeptides are detected in the virion of poxviruses. The virion core contains several enzymes concerned with transcription, and modifications of nucleic acids and proteins. The lipid content of the virion is about it 4%by, weigh it (e.g. in Vaccinia virus) while the carbohydrate is about 3% by weight. They replicate in the cytoplasm producing type B reactive occurs both with and between genera of vertebrate poxviruses. They are transmitted by airborne, contact,

fomites, and by arthropods as vectors. The family poxviridae is made up of 2 sub-families: Poxviruses of vertebrates (CHORDOPOX VIRINAE)

The word ‘chordo’ is from the word Chordate. The genera in this subfamily are

(a) Orthopox virus - Ortho - front the Greek word ‘Orthos’ meaning straight, correct.
(b) Para Poxvirus - Para means ‘by side of’
(c) Avipox Virus - Avi means Ave) front Latin word “AVIS”
(d) Capripox Virus - Capri from Latin word “Ceper’ means Goat
(e) Leporipox Virus - Lepori from Latin word “Lepus, leporis” means Hare
(f) Suipox Virus - Sui from Latin word ‘sus’ means Swine
(g) Molluscscipox Virus - Mollusci from Latin word Molluscum means Cam or snail
(h) Yatapox Virus - ‘Yata’ derived from the sigla Yaba poxvirus and Tanapox Virus

Entomopoxvirinae: Pox viruses infecting insects. “Entomo” was derived from Greek word ‘Entomon meaning insect. This subfamily contains probably 3 genera namely: Entomopoxviruis A, B and C.

In-Text Question (ITQ)

How are Poxviridae transmitted?

Answer

They are transmitted by airborne, contact, fomites, and by arthropods as vectors. They are transmitted between individuals by several routes: by aerosol and droplets (variola virus),by introduction of virus into small skin abrasions after direct or indirect contact with an infected animal (orf virus, milker's nodule virus), and in the case of some animal poxviruses mechanically by biting.

Self-Assessment Exercise 5

Provide answer to the following question in 10 minutes

List the genera in sub-family Poxviridae

3.8 Hepadnaviridae

The name was derived from the sigla 'HEPA' because of its affinity for liver cells .i.e. heap tropism, DNA meaning the type of genome i.e. deoxyribonucleic acid (DNA). They are spherical in shape, 40-4nm in diameter, with no surface projections. They of which is 7nm in diameter and detergent sensitive. The Nucleocapsid (icosahedral) is made up of 180 capsomeres arranged with T = 3 symmetry made up of one major, polypeptide species. The virion envelope is antigenically similar to the nucleic acid - free 22nm lipoprotein particles (HBs Ag) that occur naturally n the sera of infected patients.

The physicochemical properties are S = 280; buoyant density in CsCl = 1.24 - 1.26 g/crn^3, (surface antigen particles without core = 1.1.8g/cm. The virus is unstable in acid pH; infectivity is retained for 6 months at 30-32°C or 1.0 hours at 60°C. The nucleic acid is a single circular molecule of partially ds and partially ssDNA with molecular weight of 1.6- 106, S2 = 155; G+C content is 48%. One strand (negative sense, complementary to mRNA) is full length (3.02 - 3.32kb) and the other varies in length from 1.7 - 2.8kb. Length of clone DNA (fully double stranded) = 3.2 kbp. The virion coat is composed of following virus - coded proteins: S-proteins (P24, 0P27), M-proteins (GP33, GP36) L-proteins (P39, GP42). The virions are probably derived from the ER. Carbohydrates have also been demonstrated in the 22nm HBs Ag particle and virions as N-linked glycans. HBsAg, HBcAg, HBeAg are the antigens. However, the S-proteins are sufficient to simulate protective immunity. The two genera in this family are: Orthohepadnavirus (i.e. Hepatitis - B virus group) and Avihepadnavirus (i.e. Duck hepatitis B Virus group).

In-Text Question (ITQ)

What are the natural hosts of the family Hepadnaviridae?
Ans. Man, apes and birds

Self-Assessment Exercise 6

Provide answer to the following question in 10 minutes

Write short note on DNA Viruses

3.9 Summary

- DNA viruses contain double stranded helix in their genomes
- They infect mostly animal cells
- They are pathogenic to man and other vertebrates

3.10 References/Further reading/Web Sources

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3.11. Possible Answers to Self-Assessment Exercises

SAE 1

Name of the disease: Parvovirus infection face rash
It is a bright red rash on the cheeks is a distinctive sign of parvovirus infection. Parvovirus infection is a common and highly contagious childhood illness. It is sometimes called slapped-cheek disease because

of the distinctive face rash that develops. It spreads through respiratory secretions, such as saliva, sputum, or nasal mucus, when an infected person coughs or sneezes. Parvovirus B19 can also spread through blood or blood products.

SAE 2

Papovaviruses are responsible for a variety of abnormal growths in animals: warts (papillomas) in humans, dogs, and other animals; cervical cancer in women; tumours (polyomas) in mice; and vacuoles (open areas) in cells of monkeys.

SAE 3

The family Adenoviridae currently comprises five genera:

(1) Mastadenovirus, comprising viruses that infect only mammalian species, including bats, dogs, ruminants, horses, humans, swine, and mice
(2) Aviadenovirus, comprising viruses that infect only birds
(3) Atadenovirus, which includes viruses that infect a broad host range, including reptiles, birds, opossums, and ruminants
(4) Siadenovirus, which includes adenoviruses of birds, reptiles, and amphibians;
(5) Ichtadenovirus, which includes adenoviruses of fish.

SAE 4

There are eight herpesviruses for which humans are the primary host. They are the herpes simplex virus 1, herpes simplex virus 2, varicella-zoster virus, Epstein-Barr virus, cytomegalovirus, Human herpesvirus-6, Human herpesvirus-7, and Kaposi's sarcoma herpes virus.

SAE 5

(a) Orthopox virus
(b) Para Poxvirus
(c) Avipox virus
(d) Capripox virus
(e) Leporipox virus
(f) Suipox virus
(g) Molluscscipox virus
(h) Yatapox virus

SAE 6

A DNA virus is a virus that has a genome made of deoxyribonucleic acid (DNA) that is replicated by a DNA polymerase. They can be divided between those that have two strands of DNA in their genome, called double-stranded DNA (dsDNA) viruses, and those that have one strand of DNA in their genome, called single-stranded DNA (ssDNA) viruses. dsDNA viruses primarily belong to two realms: Duplodnaviria and Varidnaviria, and ssDNA viruses are almost exclusively assigned to the realm Monodnaviria, which also includes dsDNA viruses. Additionally, many DNA viruses are unassigned to higher taxa. Viruses that have a DNA genome that are replicated through an RNA intermediate by a reverse transcriptase are separately considered reverse transcribing viruses and are assigned to the kingdom Pararnavirae in the realm Riboviria.

DNA viruses are ubiquitous worldwide, especially in marine environments where they form an important part of marine ecosystems, and infect both prokaryotes and eukaryotes. They appear to have multiple origins, as viruses in Monodnaviria appear to have emerged from archaeal and bacterial plasmids on multiple occasions, though the origins of Duplodnaviria and Varidnaviria are less clear. Prominent disease-causing DNA viruses include herpesviruses, papillomaviruses, and poxviruses.

UNIT 4 CLASSIFICATION OF RNA VIRUSES

CONTENTS

4.1 Introduction

In this unit, we shall continue our study on viral classification by looking into eight RNA viruses.

4.2 Learning Outcomes

At the end of the class, student must be familiar and have understood the different classes and characteristics of RNA viruses.

4.3 Picornaviridae

The name is derived as Time word ‘PICO” is prefix meaning (micro or small). RNA is the sigla from Ribonucleic Acid). The family consists of five genera:

(a) Enterovirus — Enterovirus group (i.e. found in the intestine) e.g. Poliovirus
(b) Hepatovirus — Hepatitis A virus group (affinity for liver)
(c) Cardiovirus — EMC Virus group (affecting the heart).
(d) Rhinovirus — Common cold virus group (affecting time Nose)
(e) Aphthovirus — Foot & Mouth disease virus group (causing vesicles in the mouth)

The virions in this family are Icosahedral (T = 1) with no envelope. The core consist of RNA and a small protein 313VPg covalently linked to its 5-end. The electron micrograph (EM) reveals almost a featureless surface with no projections. Hydrated native particles are 30nm it (from 22-30nm. The virion MW = 8-9 x 10^6: S20w = 140–165: buoyant density in CsCl = 1.33-1.45g/cm^3depending mainly on genus. Some species are unstable below pH6, many are less stable at low tonic strength than at high.

They are insensitive to ether, chloroform, or non-ionic detergents. They are inactivated by light when grown with or in the presence of dyes such as natural red and proflavin. The Nucleic acid is a molecule of infectious positively sensed ssRNA of MW: 2.4 – 2.7 x 10^6. A poly A tract, heterogeneous in length is transcribed onto the 3'-terminus. A protein VPq (MW=2,400) is linked covalently to the 5-terminus, Capsid of 60 protein submits (Protomers) each consisting of 4 polypeptides (three of MW=24-41x10 and one of MW=5.5 - 13.5x10) derived by cleavage of a single polyprotein. Protomers vary from 80kDa for aphthovirus to 97 for poliovirus and some may he incompletely cleared. The inner capsid polypeptide 1A (VP4) has a molecule of myristic acid covalently attached to the amino terminal end. Some strains of poliovirus may carry 60 it to molecules each of a sphingosine-like molecules. Native virions are antigenically specific (designated 'N or 'D') but after gently heating are converted to group specificity (designated 'H').

Replication of viral RNA occurs in complexes associated with cytoplasmic membranes apparently via two distinct replicative intermediates (RIs). One complex uses positive strand RNA and the other uses negative strand RNA as template. Functional proteins are mainly produced from a single large (MW=240-250x10^3) poly protein by post-translational cleavage. Coat protein is encoded by the 5'half; VPg, proteases and polymerases or polymerase factors are encoded downstream. Genetic recombination, complementation and phenotypic mixing occur.

Most species in this family are host specific. The group infecting humans are rhinoviruses (more than, 100 serotypes causing common colds) and enteroviruses (polio, coxsackie, and echoviruses). Those infecting animals include foot and mouth disease of cattle and encephalomy-o-carditis of rodents.

In-Text Question (ITQ)

Where does the replication of Picornavirus take place?

Answer

Cytoplasm of the host cell

Self-Assessment Exercise 1

Provide answer to the following question in 10 minutes

List the genera that are members of the Picornaviridae and give examples of the diseases they cause

4.4 Caliciviridae

The Caliciviridae are a family of "small round structured" viruses, members of Class IV of the Baltimore scheme. Caliciviridae bear resemblance to enlarged picornavirus and was formerly a separate genus within the Picornaviridae. They are positive-sense, single-stranded RNA which is not segmented. Thirteen species are placed in this family, divided among eleven genera. Diseases associated with this family include feline calicivirus (respiratory disease), rabbit haemorrhagic disease virus (often-fatal haemorrhages), and Norwalk group of viruses (gastroenteritis). Caliciviruses naturally infect vertebrates, and have been found in a number of organisms such as humans, cattle, pigs, cats, chickens, reptiles, dolphins and amphibians. The calciviruses have a simple construction and are not enveloped. The capsid appears hexagonal/spherical and has icosahedral symmetry (T=1 or T=3) with a diameter of 35–39 nm.

Caliciviruses are not very well studied because until recently, they could not be grown in culture, and they have a very narrow host range and no suitable animal model. However, the recent application of modern genomic technologies has led to an increased understanding of the virus family. A recent isolate from rhesus monkeys - Tulane virus - can be grown in culture, and this system promises to increase understanding of these viruses.

In-Text Question (ITQ)

What are the diseases caused by the members of the Caliciviridae family?

Answer

Respiratory diseases, haemorrhagic diseases, and gastroenteritis

Self-Assessment Exercise 2

Provide answer to the following question in 5 minutes

1. What are the symptoms of calicivirus?
2. List the genera of the family Caliciviridae

4.5 Reoviridae

The genera of this family are the reovirus group (i.e. the Orthoreovirus, Orbivirus, Coltivirus, Rotavirus, Aquareovirus); the cytoplasmic polyhedrosis virus group (cypovirus); plant reovirus subgroup 1 (phytoreovirus); plant reovirus subgroup 2 (Fijivirus) and plant reovirus subgroup 3.

The virus is an Icosahedral particles with diameter range of 60-80 nm. One or two outer protein coats and an inner protein coat are present. Transcriptase activity is associated with the core. The Virion Molecular weight is 120x10 buoyant density in CsCl = 1 .36-1.39 g/cm^3. They are ether resistant because they are non-enveloped. The Nucleic acid is I0-12 segmented linear dsRNA with MW of 0.2-3.0 x 10^6. The total MW = 12-20x10^6 which is about 14-22% by weight of the virus particle. Each RNA segment has one open reading frame (ORE) encoding a protein requiring no further processing. About 6-10 proteins are found in the virus particle, M1vVs = 15-155x10^3 including transcriptase and messenger RNA-capping enzymes. Some of the proteins are glycosylated. Viral replication takes place in the cytoplasm with the presence of viroplasmas in the cytoplasm of infected cells. Genetic recombination occurs very efficiently by genome reassortment.

Reoviruses of human include rotaviruses, which cause infantile gastroenteritis and have distinctive wheel-shaped appearance. Antigenically, similar reoviruses infect many animals. Orbiviruses constitute a distinct subgroup that includes Colorado tick fever virus of humans and other agents that infect plants, insects, and animals (blue-tongue of cattle and sheep).

In-Text Question (ITQ)

Is Reoviridae positive sense?

Answer

Viruses in the family Reoviridae have genomes consisting of segmented, double-stranded RNA (dsRNA). Because of this, replication occurs exclusively in the cytoplasm, and the virus encodes several proteins

which are needed for replication and conversion of the dsRNA genome into positive-sense RNAs.

Self-Assessment Exercise 3

Provide answer to the following question in 5 minutes

1. List the genera of the family Reoviridae
2. What diseases does Reoviridae cause?

4.6 Arboviridae

Arbovirus is an informal name for any virus that is transmitted by arthropod vectors. The term *arbovirus* is a portmanteau word (*ar*thropod-*bo*rne *virus*). *Tibovirus* (*ti*ck-*bo*rne *virus*) is sometimes used to more specifically describe viruses transmitted by ticks, a superorder within the arthropods. Arboviruses can affect both animals (including humans) and plants. In humans, symptoms of arbovirus infection generally occur 3-15 days after exposure to the virus and last three or four days. The most common clinical features of infection are fever, headache, and malaise, but encephalitis and viral hemorrhagic fever may also occur.

Arboviruses maintain themselves in nature by going through a cycle between a host, an organism that carries the virus, and a vector, an organism that carries and transmits the virus to other organisms. For arboviruses, vectors are commonly mosquitoes, ticks, sandflies and other arthropods that consume the blood of vertebrates for nutrition or developmental purposes. Vertebrates which have their blood consumed act as the hosts, with each vector generally having an affinity for the blood of specific species, making those species the hosts.

Transmission between the vector and the host occurs when the vector feeds on the blood of the vertebrate, wherein the virus that has established an infection in the salivary glands of the vector comes into contact with the host's blood. While the virus is inside the host, it undergoes a process called amplification, where the virus replicates at sufficient levels to induce viremia, a condition in which there are large numbers of viruses present in the blood. The abundance of viruses in the host's blood allows the host to transmit the virus to other organisms if its blood is consumed by them. When uninfected vectors become infected from feeding, they are then capable of transmitting the virus to uninfected hosts, resuming amplification of virus populations. If viremia is not achieved in a vertebrate, the species can be called a "dead-end host", as the virus cannot be transmitted back to the vector.

An example of this vector-host relationship can be observed in the transmission of the West Nile virus. Female mosquitoes of the genus *Culex* prefer to consume the blood of passerine birds, making them the hosts of the virus. When these birds are infected, the virus amplifies, potentially infecting multiple mosquitoes that feed on its blood. These infected mosquitoes may go on to further transmit the virus to more birds. If the mosquito is unable to find its preferred food source, it will choose another. Human blood is sometimes consumed, but since the West Nile virus does not replicate that well in mammals, humans are considered a dead-end host.

Person-to-person transmission of arboviruses is not common, but can occur. Blood transfusions, organ transplantation, and the use of blood products can transmit arboviruses if the virus is present in the donor's blood or organs. Because of this, blood and organs are often screened for viruses before being administered. Rarely, vertical transmission, or mother-to-child transmission, has been observed in infected pregnant and breastfeeding women. Exposure to used needles may also transmit arboviruses if they have been used by an infected person or animal. This puts intravenous drug users and healthcare workers at risk for infection in regions where the arbovirus may be spreading in human populations.

In-Text Question (ITQ)

What are the vectors of the Family Arborviridae?

Ans. vectors are commonly mosquitoes, ticks, sandflies and other arthropods that consume the blood of vertebrates for nutrition or developmental purposes.

Self-Assessment Exercise 4

Provide answer to the following question in 5 minutes
What are the clinical features of an Arbovirus infection?

4.7 Togaviridae

The names derived from the Latin word ‘toga’ meaning “gown”, “Cloak”
Members of the genera in this family are:

(a) Alphavirus — Arbovirus group A
(b) Rubivirus — Rubella Virus. (Rubi means Reddish)
(c) Arterivirus — Equine arteritris virus.

The virions are spherical, 60-70nrn in diameter, with an envelope tightly applied to a proven or presumed Icosahedral nucleocapsid 35-40nm in diameter. Surface projections are demonstrable in most togaviruses. The virion buoyant density in sucrose is $1.2g/cm^3$, S20w=280. The Nucleic acid is a single molecule of positively sensed ssRNA, MW= 4×10^6 which is 8-9% by weight of the virus. The genes for non-structural proteins are located at the 5' end. The 5' terminus is capped, and the 3' end is polyadehylated, there are two or 3 envelope proteins one or more of which are glycosylated, and a small core protein. Members are antigenically related. The virus specific glycoproteins are inserted in the lipoprotein envelope whose lipids are cell-derived. Replication is in the cytoplasm amid mature by budding. They infect arthropods as well as a wide range of vertebrates.

Alphaviruses are mainly arthropod borne viruses and responsible for such diseases in humans as encephalitis, and arthritis. The sole member of the Rubivirus genus is the Rubella virus, which is the causative agent for the common childhood disease, rubella. Togaviviridae has a worldwide distribution. The rubella virus, which is the causative agent for the common childhood disease rubella, has a global distribution. Certain alphaviruses may have limited dispersal due to the geographic distribution of the principal arthropod vectors. For example, the Western equine encephalitis, Eastern equine encephalitis, and Venezuelan equine encephalitis viruses have principal vectors belonging to Culex species of mosquitoes found in the Americas and therefore have a distribution in this region of the world. Togavirus genomes contain a single, positive-sense RNA molecule. The complete genome is 9700-11800 nucleotides long, with the rubella virus genome (approximately 9.8 kbp) being shorter than that of the alphaviruses. Genomes also possess a 5' cap and a 3' poly (A) tail. Togavirus particles are comprised of an envelope and a nucleocapsid.

These virions are 70 nm in diameter and are spherical to pleomorphic in shape with glycoprotein projections dispersed along the surface. Replication begins with the attachment of the viral glycoproteins to host cell receptors. The virus is then absorbed into the cell. Acidification of endocytic vesicles induces fusion and uncoating of the virion resulting in the release of the RNA genome into the cytosol. Proteins are then translated and processed enabling replication to occur. Progeny virions are assembled in the cytoplasm at the plasma membrane of the host cell and are released by budding. Arenavirus infections begin with onset of fever and myalgia, followed by the development of rashes and/ or additional complications. Alphaviruses are predominantly transmitted through the bite of an infected mosquito. Rubella is transmitted through airborne aerosol droplets.

In-Text Question (ITQ)

How many alphaviruses are there?

Ans. There are 32 alphaviruses, which infect various vertebrates such as humans, rodents, fish, birds, and larger mammals such as horses, as well as invertebrates.

Self-Assessment Exercise 5

Provide answer to the following question in 10 minutes
Write a short not on the Family Togaviridae

4.8 Flaviviridae

The name is derived from 'FLAVI' meaning yellow. The members of the genera of are

a) Flavivirus – includes Yellow fever virus, West Nile virus, Dengue virus, and Zika virus
b) Hepacivirus - includes Hepacivirus C (hepatitis C virus) and Hepacivirus B (GB virus B)
c) Pegivirus - includes Pegivirus A (GB virus A), Pegivirus C (GB virus C), and Pegivirus B (GB virus D)
d) Pestivirus - includes Pestivirus A (bovine viral diarrhea virus 1) and Pestivirus C (classical swine fever virus, previously hog cholera virus). Viruses in this genus infect nonhuman mammals.

The virion is spherical in shape, 40-6-nm in diameter. They are enveloped viruses, S_{20w} = 140 - 200. The nucleic acid is single molecule of infectious single stranded, positively sensed RNA. The structural and non-structural proteins are derived from the 51 - and 31-terminal sequences respectively. There are two or three membrane-associated problems and a core protein found in the virion. The membrane-associated proteins are inserted in the lipoprotein envelope whose lipids are cell derived. Replication takes place in the cytoplasm and in association with membranes, and matures into cytoplasmic vesicles. Most members are transmitted by blood-sucking orthropods. Mature virions accumulate within cisternae of the endoplasmic reticulum.

In-Text Question (ITQ)

List the genera of the family Flaviviridae giving examples of diseases caused by of each

Answer

a) Flavivirus –Yellow fever , West Nile disease, Dengue fever, and Zika virus disease
b) Hepacivirus - includes Hepacivirus C (hepatitis C virus) and Hepacivirus B (GB virus B)
c) Pegivirus - includes Pegivirus A (GB virus A), Pegivirus C (GB virus C), and Pegivirus B (GB virus D)
d) Pestivirus - includes Pestivirus A (bovine viral diarrhea virus 1) and Pestivirus C (swine fever).

Self-Assessment Exercise 6

Provide answer to the following question in 10 minutes

Write a short note on the life cycle of family Flaviviridae?

4.9 Arenaviridae

The name 'Arena' is derived from Latin word 'arenosus' meaning 'Sandy' because of the appearance of the viral particles in EM sections. Members of this family are:

(a) Lymphocytic Choriomeningitis (LCM) i.e. Lassa, Mobala, Mopeia and Ippy virus.
(b) Tacarbe Complex: i.e. Tacaribe, Junin, Macupo, Amapari, Parana, Tamiami, Pichinde, Latino and Flexal virus.

They are enveloped viruses; spherical to pleomorphic particles, 50-300 nm in diameter (usually 110-130nm). The dense lipid bi-layer envelope has surface projections 10 nm long and club-shaped. Varying numbers of ribosome like particles (20-25nm diameter) appear tree within the envelope. Isolated nucleocapsids vary in lengths from 450-1300nm. The buoyant density in sucrose is 1.17 - 1.18g/cm^3 in CsCI = 1.19-1.20g/cm^3, in amidotrizoate compounds = 1.14g/cm^3 compounds = 114g, S_{20w} = 325-500. The virus is rapidly inactivated below pH 5.5 and above pH 5.5, also rapidly inactivated at 56°C and by solvents. They are highly sensitive to UV and gamma radiation. The genome is two virus negative sensed specific ssRNA molecules, L and S (MWs = 2.2-2.8x10^5 and 1.1x10^6 respectively, and three RNAs of cell origin, = 28, l8 and 4-6.' The nucleocapsid contain one glycosylated polypeptide (MW=63-72x10) associated with the RNA as part of RNP complex. One glycosylated polypeptide with MW=34-44x10^{3}is found in all members of the family and a second glycosylated polypeptide of MW=44-72x10^3 noted II in some but not other members. At least 3 distinct antigenic

molecules are known. Antigens on the surface glycoprotein (MW=34-44x10) are involved in virus neutralization. Most, if not all, arenaviruses probably have limited cell killing potential. Host range varies from animals, mammals and humans.

In-Text Question (ITQ)

What disease does Arenaviridae cause?

Ans. They include Chapare virus, a severe or fatal hemorrhagic fever, found in Bolivia, as well as Guanarito virus found in Venezuela. Old World viruses occur in the Eastern Hemisphere — Africa, Europe, and Asia. Lassa fever, which can cause mild to severe disease in people

Self-Assessment Exercise 7

Provide answer to the following question in 10 minutes

Write a brief note on the family Arenaviridae?

4.10 Coronaviridae

They have petal-shaped projections arranged in fringe like a solar corona. The name (Prefix "Corona") is derived from the Latin word "Crown" from appearance of surface projections in negatively stained electron micrographs. The members of tile family are Human coronavirus, Mirune hepatitis virus, Porcine I Haemagglutinating encephalomyelitis virus, Porcine transmissible gastroenteritis virus, bovine coronavirus, canine coronavirus, Feline infectious peritonitis virus, Turkey, rat and Rabbit coronavirus. The viral particle is spherical or pleomorphic enveloped particles, 60-220nm in diameter. There are club-shaped surface projections, 12-24nm in length protruding from the envelope. There is presence of Ribonucleoprotein (RNA) structure seen by negative staining as helix of a 9-13nm or strands of 9nm in diameter. The Buoyant density is 1.15-1.180g/cm^3 in sucrose. They are sensitive to ether, chloroform, and detergents. The envelope spikes (but not haemagglutinin esterase protein of BCV) are removed by bromelain. The nucleic acid is a molecule of infectious unsegemented positively sensed ssRNA, MW of 9.0-11.0x 10^6. IBV genome is 27kb, MHV=33kb. The genome is adehylated at 3'-terminus but MHV genomic RNA is known to be capped at 5'-end. About 3 or 4 proteins are found in all coronaviruses.

(1) The spike(S) protein, MW = 170-220x10^3. This could be cleaved into two subunits: N-terminal (S1) and C-terminal (S2).
(2) The membrane (M) protein, MW of main species is 23-29x10^3.

(3) The Nucleocapsid (N) protein. MW 47-60x $10^3$and it is phosphorylated and associated with INA.
(4) The haemagglutinin – Esterase (HE) protein. Membrane fusion and esterase activity is associated with S and HE protein respectively. The enveloped of the viral particle is lipid in nature and s-protein is acylated.

The S- and N-proteins are glycosylated. There are 3 or 4 major antigens corresponding to each virion protein. The S and HE proteins are predominant antigens involved in neutralization. The genomic RNA behaves as the mRNA for RNA polymerase responsible for amplification of genome and production of sub-genomic mRNAs. The Virion matures in the cytoplasm by budding through the ER and Golgi membranes. There is no budding at plasmalemma.

The infection coronaviruses is restricted to natural vertebrate host and are often associated with respiratory or gastrointestinal organ. Transmission is by respiratory, facial-oral routes. Biological vectors and mechanical transmission is also possible.

In-Text Question (ITQ)
What are the constituents o the viral envelope of the family Coronaviridae?

Ans. The viral envelope contains two virus-specified glycoprotein species, known as the spike (S) and membrane (M) proteins. The spike protein makes up the large surface projections (sometimes known as peplomers), while the membrane protein is a triple-spanning transmembrane protein.

Self-Assessment Exercise 8

Provide answer to the following question in 10 minutes

1. How do RNA viruses work?
2. Why is Covid-19 a RNA virus?

4.11 Summary

- RNA viruses are mostly pathogenic to plants
- Some contain enzymes called reverse transcriptase, which enable them to synthesis a second strand while infecting their host.

4.12 References/Further reading/Web Sources

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4.13 Possible Answers to Self-Assessment Exercises

SAE 1

(a) Enterovirus —Poliovirus
(b) Hepatovirus — Hepatitis A
(c) Cardiovirus —heart diseases
(d) Rhinovirus — Common cold
(e) Aphthovirus — Foot and Mouth disease

SAE 2

1. Typical signs include sneezing, nasal discharge, ocular discharge, conjunctivitis, ulceration of the tongue, lethargy and fever. Signs

may last from a few days to a few weeks and vary in severity. In young kittens the virus may also cause pneumonia.

2. The Caliciviridae family contains five genera: Norovirus, Sapovirus, Lagovirus, Vesivirus, and Nebovirus

SAE 3

1. Orthoreovirus, Orbivirus, and Rotavirus. Most orthoreoviruses are nonpathogenic, but the genera Orbivirus and Rotavirus contain several important pathogens.
2. Reoviruses have been associated with upper respiratory infections, enteritis, fever, and febrile exanthema in childhood.

SAE 4

The clinical feature or symptoms of an Arbovirus infection are fever, headache, and malaise, but encephalitis and viral hemorrhagic fever may also occur

SAE 5

The Togaviridae is a family of small, enveloped viruses with single-stranded, positive-sense RNA genomes of 10-12 kb. Within the family, the genus Alphavirus includes a large number of diverse species, while the genus Rubivirus includes the single species Rubella virus. Members are antigenically related. The virus specific glycoproteins are inserted in the lipoprotein envelope whose lipids are cell-derived. Replication is in the cytoplasm amid mature by budding. They infect arthropods as well as a wide range of vertebrates. Togaviruses are important veterinary pathogens, and are transmitted via mosquitoes. Some of them are zoonotic viruses, including Venezuelan equine encephalitis virus (VEEV). Rubella virus is the only member of togavirus family that causes significant disease in human—German measles.

SAE 6

Viral replication is cytoplasmic. Entry into the host cell is achieved by attachment of the viral envelope protein E to host receptors, which mediates clathrin-mediated endocytosis. Replication follows the positive-stranded RNA virus replication model. Positive-stranded RNA virus transcription is the method of transcription. Translation takes place by viral initiation. The virus exits the host cell by budding. Humans and mammals serve as the natural hosts. The virus is transmitted via vectors (ticks and mosquitoes).

SAE 7

The virions of this family are spherical to pleomorphic, are of 50–300 nm in diameter, with a dense lipid envelope, and have a surface layer covered by club-shaped projections, of 8–10 nm in length. The genome consists of two single stranded, ambisense RNA molecules, L and S, of lengths of about 7.5 kb and 3.5 kb, respectively and variable amounts of full-length viral-complementary RNAs (predominantly S) and viral subgenomic mRNA species have been reported in virus preparations. The most abundant structural protein is the nucleoprotein (N or NP), a nonglycosylated polypeptide (ca. 63 kDa) found tightly associated with the virus genomic RNA in the form of a ribonucleoprotein complex or nucleocapsid structure. The lipids represent about 20% of virion dry weight and are similar in composition to those of the host plasma membrane. Carbohydrates in the form of complex glycans on GP1 and GP2 represent about 8% of virion dry weight. The process of infection involves attachment to cell receptors, entry via the endosomal route, uncoating, and mRNA transcription in the cytoplasm of infected cells. The viral envelope glycoproteins are synthesized in cells as a single mannose-rich precursor molecule that is proteolytically cleaved and processed to contain complex glycans during transport to the plasma membrane.

SAE 8

1. Retroviruses use reverse transcriptase to transform their single-stranded RNA into double-stranded DNA. It is DNA that stores the genome of human cells and cells from other higher life forms. Once transformed from RNA to DNA, the viral DNA can be integrated into the genome of the infected cells.
2. Like many other viruses, SARS-CoV-2 is an RNA virus. This means that, unlike in humans and other mammals, the genetic material for SARS-CoV-2 is encoded in ribonucleic acid (RNA). The viral RNA is sneaky: its features cause the protein synthesis machinery in humans to mistake it for RNA produced by our own DNA.

UNIT 5 CLASSIFICATION OF RNA VIRUSES CONTINUED

CONTENTS

5.1 Introduction

We shall continue in the study of RNA viruses. The last 8 groups of RNA viruses we shall be considering today include the Retroviridae, a special group of RNA viruses that can make DNA from their RNA genome using the enzyme Reverse transcriptase.

5.2 Learning Outcomes

At the end of the class, student must be familiar and have understood the different classes and characteristics of RNA viruses.

5.3 Retroviridae

They are enveloped viruses of about 90 - 120nm in diameter whose genome contains duplicate copies of high molecular weight, SSRNA of the same polarity as viral mRNA. A retrovirus is a type of virus that inserts a copy of its RNA genome into the DNA of a host cell that it invades, thus changing the genome of that cell. Once inside the host cell's cytoplasm, the virus uses its own reverse transcriptase enzyme to produce DNA from its RNA genome, the reverse of the usual pattern, thus *retro* (backwards). The new DNA is then incorporated into the host cell genome by an integrase enzyme, at which point the retroviral DNA

is referred to as a provirus. The host cell then treats the viral DNA as part of its own genome, transcribing and translating the viral genes along with the cell's own genes, producing the proteins required to assemble new copies of the virus.

Although retroviruses have different subfamilies, they have three basic groups: the oncoretroviruses (oncogenic retroviruses), the lentiviruses (slow retroviruses) and the spumaviruses (foamy viruses). The oncoretroviruses are able to cause cancer in some species, the lentiviruses are able to cause severe immunodeficiency and death in humans and other animals, and the spumaviruses are benign and not linked to any disease in humans or animals.
Many retroviruses cause serious diseases in humans, other mammals, and birds. Human retroviruses include HIV-1 and HIV-2, the cause of the disease AIDS. Also, human T-lymphotropic virus (HTLV) causes disease in humans. The murine leukaemia viruses (MLVs) cause cancer in mouse hosts. Retroviruses are valuable research tools in molecular biology, and they have been used successfully in gene delivery systems.

In-Text Question (ITQ)

What are the means of transmission of Retroviridae?

Ans. Cell-to-cell, Fluids, Airborne

What group of diseases does Retroviridae cause?

Ans. Oncogenic diseases (cancer), immunodeficiency syndromes (HIV), multiple sclerosis

Self-Assessment Exercise 1

Provide answer to the following question in 10 minutes

1. Why is virus called retrovirus?
2. Which three viruses are the most prominent human retroviruses?

5.4 Bunyaviridae

Name derived from “Bunyamwera” where it was first isolated. The viral genera in this family are:

- Bunyamwera Super Group - Btiniavirus
- Sandfly Fever and Uukuniemi group - Phlehovirus
- Nairobi Sheep disease group - Nairovirits
- Hantaan group - Haittavirus

- Tomato Spotted Wilt group – Tospovirus (a plant virus).

The viral particles are spherical or pleomorphic. They are enveloped particles of about 80 - 100 nm in diameter, with glycoprotein surface projections, ribonucleo capsids composed of 3 circular, helical strands, 2 - 2.5nm in diameter and sometimes super-coiled, 0.2 - 3m in length depending on the arrangement. The viral MW = 300 - 400 X 10, S_{20w} =350-500, buoyant density in CsCl = 1.2g/cm^3. They are sensitive to lipid solvents and detergents.

The nucleic is made of 3 molecules; Large (L), Medium (M) and small (S), negatively or ambisensed ssRNA. The MW of L = 2.2 – 4.9 x 10^6 (6.5 - 14.4kb): M= 1.0 - 2.3 x 10^6 (3.2-6.3 kb): while 50.28 x 0.8 x 10^6 (0.8 - 2.0kb). The constitute 1-2% by weight of a whole particle. Differences exist between terminal nucleotide sequences of gene segments of viruses of different genera. Ends are hydrogen bonded, RNA and nucleocapsids is circular. Usually, 4- proteins consisting of 2 external glycoproteins (01 and 02), a nucleocapisd protein (N) and a Large (L) protein which is presumably a transcriptase are known. Transcriptase activity is present it the virion. The Lipid is 20-30% by weight with the lipoprotein envelope derived from host cell. The carbohydrate is 2-7% by weights of which are mainly glycoproteins and glycolipids. Hemagglutinin as the neutralizing antigenic determinants are present on the viral glycoproteins. Complement fixing antigenic determinants is principally associated with N-protein. Cell fusion has been shown to be induced by these viruses at low pH. Most species cause CPE but Hantaviruses do no cause CPE. Some members have ion-dependent haemaglutinating activity. The virus replicate in the cytoplasm. The Host RNA sequences have been shown to prime viral mRNA synthesis. Genetic re-assortment is known for certain members. The virus mature by budding into smooth surface vesicles in or near the Golgi region but maturation at the plasma membrane has also been observed.

These viruses infect various arthropods and or warm or cold blooded vertebrates. They are transmitted by Mosquitoes, ticks, phlebotomine flies and other arthropod vectors. They have been demonstrated to be transmitted venereally. Aerosol infections also occur and could also be disseminated by avian host and, or vector movements. Hantaviruses are transmitted by Rodents. They cause haemorrhagic fevers.

Bunyaviruses that cause disease in humans include:

- California encephalitis virus, La Crosse encephalitis virus, Jamestown Canyon virus and Snowshoe hare virus vector: mosquitoes Family: Peribunyaviridae

- Hantavirus reservoir: small mammals or rodents vector: aerosolized excreta from these mammals Family: Hantaviridae
- Crimean – Congo hemorrhagic fever reservoir and vector: ticks, amplifying hosts and vector: small mammals, domestic mammals Family Nairoviridae
- Rift Valley fever reservoir: bats vector: mosquitoes amplifying hosts: small mammals, domestic mammals Family: Phenuiviridae
- Bwamba Fever reservoir: monkeys vector: mosquitoes, amplifying hosts: donkeys Family: Peribunyaviridae
- Severe fever with thrombocytopenia syndrome
- Lassa fever and Argentine hemorrhagic fever reservoir: rodents vector: aerosolized excreta from these mammals Family: Arenaviridae

In-Text Question (ITQ)

Which organisms are natural host of members of the Bunyaviridae?

Ans. arthropods, plants, protozoans, and vertebrates.

Self-Assessment Exercise 2

Provide answer to the following question in 10 minutes

1. How is Bunyaviridae transmitted?
2. Briefly describe Bunyaviruses

5.5 Orthomyxoviridae

Orthomyxoviridae is a family of negative-sense RNA viruses. The name is derived from the sigla; ORTHO from Greek 'Orthos meaning straight; 'MYXO-" from Greek 'Myxa' meaning mucus, relating to the activity of hemagglutinin and neuraminidase.. It includes seven genera:

Alphainfluenzavirus, Betainfluenzavirus, Deltainfluenzavirus, Gammainfluenzavirus, Isavirus, Thogotovirus, and Quaranjavirus. The first four genera contain viruses that cause influenza in birds (avian influenza) and mammals, including humans. Isaviruses infect salmon; the thogotoviruses are arboviruses, infecting vertebrates and invertebrates (such as ticks and mosquitoes). The Quaranjaviruses are also arboviruses, infecting vertebrates (birds) and invertebrates (arthropods).

The Nucleocapids of helical symmetry and diameter of 9 – 15 nm are enclosed within a lipoproteins of different sizes classes (50–130 nm in length) with loop at each end, are extractable from virions or infected cells. The virion is pleomorphic, 20-120nm in diameter. Arrangement

within virion uncertain, although coils of about 4-20 turns of a 7 nm thick material are sometimes seen in partially disrupted virus. M-1 protein is believed to form a layer inside the Lipid bilayer, with HA and NA glycoproteins projecting about 10-14 nm from the surface. About 500 spikes project from the surface of a spherical virion. Most are HA, with NA clusters interposed irregularly, but usually in the ratio 4 or 5 to 1 of HA and NA respectively. The HA spikes are rods, 13.5 nm in length and 4nm in diameter. The NA glycoprotein has a box-shaped head, 10 x 10 x 6 nm, attached to a slender stalk about l00 nm long projecting from the membrane. Each NA subunit is composed of 6 topologically identical beta sheets arranged in the formation of propeller. Cores containing MI, RNP and P-proteins may be generated by controlled chemical disruption of virions. Orthomyxoviruses are enveloped viruses.

In-Text Question (ITQ)

List the genera comprising the Orthomyxoviridae

Answer

Alphainfluenzavirus, Betainfluenzavirus, Deltainfluenzavirus, Gammainfluenzavirus, Isavirus, Thogotovirus, and Quaranjavirus.

Self-Assessment Exercise 3

Provide answer to the following question in 10 minutes

1. What is importance of family Orthomyxoviridae?
2. Briefly describe the structure of Influenza B virus

5.6 Paramyxoviridae

The name “PARAMYXO” is derived from the sigla PARA from Greek ‘para’ meaning ‘by the side of’ and ‘myxo’ from Greek ‘myxa’ meaning mucus, relating to the activity of haemagglutinin and neuraminidase. Paramyxoviridae is a family of negative-strand RNA viruses in the order *Mononegavirales* Vertebrates serve as natural hosts. Diseases associated with this family include measles, mumps, and respiratory tract infections. The family has four subfamilies, 17 genera, and 78 species, three genera of which are unassigned to a subfamily. The four subfamilies are:

a. *Avulavirinae*, which contains three genera and 22 species
b. *Metaparamyxovirinae*, which contains one genus and one species

c. *Orthoparamyxovirinae*, which contains eight genera and 34 species
d. *Rubulavirinae*, which contains two genera and 18 species

A number of important human diseases are caused by paramyxoviruses. These include mumps, as well as measles, which caused around 733,000 deaths in 2000. The human parainfluenza viruses (HPIV) are the second most common causes of respiratory tract disease in infants and children. There are four types of HPIVs, known as HPIV-1, HPIV-2, HPIV-3 and HPIV-4. HPIV-1 and HPIV-2 may cause cold-like symptoms, along with croup in children. HPIV-3 is associated with bronchiolitis, bronchitis, and pneumonia. HPIV-4 is less common than the other types, and is known to cause mild to severe respiratory tract illnesses.

Paramyxoviruses are also responsible for a range of diseases in other animal species, for example canine distemper virus (dogs), phocine distemper virus (seals), cetacean morbillivirus (dolphins and porpoises), Newcastle disease virus (birds), and rinderpest virus (cattle). Some paramyxoviruses, such as the henipaviruses, are zoonotic pathogens, occurring naturally in an animal host, but also able to infect humans.

The virus particles are pleomorphic, usually roughly spherical, 150nm or more in diameter. Filamentous forms are common. They are enveloped, incorporating 2 or 3 virus glycoproteins and 1 or 2 unglycosilated proteins. There are surface projections of about 8 – 12 nm in length, spaced 7-10 nm apart according to genus, contain virus glycoproteins. Nucleocapsid is helical in symmetry, 13-l8 nm in diameter and 5.5–7nm pitch to according to genus; length up to 1µm in some genera. The virus particle MW = > 500 x 10^6, much more for pleomorphic multiploid virions, S_{20w} at least 100; buoyant density in sucrose = 1.1-l.20 g/cm^3. They are sensitive to Lipid solvents, non-ionic detergents, formaldehyde and oxidizing agents. The nucleic acid is a single molecule of non-segmented negatively sensed SSRNA of WM= 5-7 x 10^6 which is about 0.5% by weight of virus particle.

In-Text Question (ITQ)

What diseases do members of the Paramyxoviridae cause?

Ans. measles, mumps, and respiratory tract infections.

Self-Assessment Exercise 4

Provide answer to the following question in 10 minutes

1. Why is the group called paramyxovirus?
2. Is paramyxovirus positive or negative-sense?

5.7 Rhabdoviridae

Rhabdoviridae is a family of negative-strand RNA viruses in the order *Mononegavirales*. Vertebrates (including mammals and humans), invertebrates, plants, fungi and protozoans serve as natural hosts. Diseases associated with member viruses include rabies encephalitis caused by the rabies virus, and flu-like symptoms in humans caused by vesiculoviruses. The name is derived from Ancient Greek *rhabdos*, meaning rod, referring to the shape of the viral particles. The family has 40 genera, most assigned to three subfamilies, namely Alpharhabdovirinae, Betarhabdovirinae, and Gammarhabdovirinae.

The viral particles are 100-430nm long and 45–100nm in diameter with surface projections (G-proteins), 5-10nm and long 3nm in diameter. A central axial channel is seen in thin section of the virion. There is characteristic cross–striations (spacing 4.5—5.0 nm see in negatively stained and thin-sectioned particles. Truncated particles of 0.1-0.5 of the length of the virus may be common except perhaps in members infecting plants. Abnormally long an double–length particles and tandem formations are sometimes observed. The inner nucleocapsid is 50nm in diameter, with helical symmetry consist of an RNA + N protein complex together with L-and NS-proteins, surrounded by an envelope containing M-protein. The nucleocapsid contains transcriptase activity and is infections. It coils to a helical structure which is 20 x 700 nm. The MW of virus particle is 300-1,000 x 10^6, S_{20w}= 550-1,000; buoyant density in CsCl =1.19-1.20 g/cm^3' and in sucrose, 1.17–1.19g/cm^3. The viral infectivity is stable at pH range 5–10 but rapidly x–irradiation. Since they are enveloped viruses, they are sensitive to Lipid solvents. The nucleic acid is a molecule of non-segmented, linear, negatively sensed ssRNA (non-infections). The MW of the genome is 3.5-4.6 x 10^6 which is about 1-2% by weight of virus at id S_{20w}= 38-45. The proteins are 65-75% by weight of the virus. Transcriptase and other enzyme activities and present in virus. The Lipid contents is about 15—25% by weight of virus and the lipid composition is dependent on the host cell. The carbohydrate content is about 3% by weight of virus and is associated with surface projections, glycolipids, minor variation with the host cell type.

In-Text Question (ITQ)

What type of virus is the rabies virus?

Answer

Rabies virus belongs to the order Mononegavirales, viruses with a nonsegmented, negative-stranded RNA genomes. Within this group, viruses with a distinct bullet shape are classified in the Rhabdoviridae family, which includes at least three genera of animal viruses, Lyssavirus, Ephemerovirus, and Vesiculovirus.

Self-Assessment Exercise 5

Provide answer to the following question in 10 minutes

1. How are Rhabdoviruses transmitted?
2. What diseases does Rhabdoviridae cause?

5.8 Toroviridae

Torovirus is a genus of enveloped, positive-strand RNA viruses in the order Nidovirales and family Tobaniviridae. They primarily infect vertebrates, especially cattle, pig, and horse. Diseases associated with this genus include gastroenteritis, which commonly presents in mammals. Torovirus is the only genus in the monotypic subfamily Torovirinae. Torovirus is also a monotypic taxon, containing only one subgenus, Renitovirus. They are pleomorphic, biconcave disk, kidney, and rod-shaped viral particles of about 20–140nm diameter containing an elongated tubular capsid with helical symmetry. They are developed which hears some peplomers of spikes. The virus is table at pH 2.5 and 9.7. The buoyant density in sucrose is 1.16-1.17g/cm^3 and S_{20w} = 380-400. The genome is a polyadenylated linear, non-segmented, positively segmented, positively sensed ssRNA that acts as a mRNA (infectious) of about >20kb.

Three major proteins are known in the virus particle. The nucleocapsid MW= 180 x 103while the envelope is 26 x 30^3 and the peplomer dimer derived from 200x10^3 precursor) is 80-100 x 10^3. Lipids are present in form of envelope with the glycosylated protein peplomer embedded in it as the only carbohydrate. Replication take place in the cytoplasm with the 31-coterminal rested set of 5-subqenoinic mRNA detected. The polymerase gene contains 2 overlapping ORFs; the downstream one expected by ribosomal frame–shifting during translation of genomic RNA. Budding of preformed tubular capsids is through Golgi

membranes and E.R. but host cell nuclear function is required. The transmission is probably via the faecal oral route.

In-Text Question (ITQ)

What group of animals do Toroviruses infect?

Ans. They infect vertebrates, especially cattle, pig, and horse.

Self-Assessment Exercise 6

Provide answer to the following question in 10 minutes

List the species of viruses in the family Toroviridae
What are the symptoms of Torovirus infection in the different hosts?

5.9 Filoviridae

Filoviridae is a family of single-stranded negative-sense RNA viruses in the order Mononegavirales. Two members of the family that are commonly known are Ebola virus and Marburg virus. Both viruses, and some of their lesser known relatives, cause severe disease in humans and nonhuman primates in the form of viral hemorrhagic fevers. The natural reservoirs of these viruses or their source are unknown. However, Monkey, Mouse, Guinea Pig and Hamster have been experimentally infected in the laboratory. The virus particle is pleomorphic, appearing as a long filamentous forms (Sometimes with extensive branching) or as U-Shaped, 6-Shaped or circular forms. They vary greatly in length (up to 14,000nm), but of uniform diameter (80nm).

Surface projections (about 7nm in length and spaced at 10nm intervals) are presented on the virion, Virions purified by Rate-zonal-gradient centrifugation are infectious, uniform and bacilliform in shape; Ebola (970nm) and Marburg (790nm) long. They are enveloped viruses. Inside the envelope is a nucleocapsid with a dark central axis (20nm in diameter) surrounded by a helical tubular capsid (50nm in diameter) bearing cross-striations with a periodicity of 5nm. The 20nm-central axis, also seen in infected cells appears to be the virion RNA. A structure with buoyant density 1.32g/cm3 in CsCl is released from virions by detergent treatment and probably represented the viral RNP Within the nucleocapsid is an axial channel of 10-l5nm with nucleocapsid proteins (N and VP 30) proteins L and VP35. The whole virion MW = 300 600 x 60^6, S_{20w} of long particles very high but infectious bacilliform particles = 1,400S buoyant density is 1. 14g/cm^3 in Potassium tartrate. Infectivity is stable of room temperature but destroyed in 30nm at 600^OC. They are also sensitive to Lipid solvents.

The genome is a molecule of linear, negatively sensed SSRNA (non-infectious), MW=1.1% by weight of the virus, seven proteins designated as L.G.N VP40, VP35, VP30 and VP24 are known. The G-protein is very large and two are associated with RNA (N and VP30. Lipids are present in form of envelope while glycolipids and surface projections (glycosylated) as the carbohydrates present. The virus cannot be neutralized in vitro.

In-Text Question (ITQ)

What disease does Filoviridae cause?

Answer

Viruses in the family Filoviridae can cause severe haemorrhagic fever in people and nonhuman primates (such as monkeys and gorillas) and may spread in other animals, such as bats.

Self-Assessment Exercise 7

Provide answers to the following questions in 10 minutes

1. What are the symptoms of Filoviruses?
2. What disease does Filoviridae cause?

5.10 Birnaviridae

Birnaviridae is a family of double-stranded RNA viruses. Salmonid fish, birds and insects serve as natural hosts. There are currently 11 species in this family, divided among seven genera. Diseases associated with this family include infectious pancreatic necrosis in salmonid fish, which causes significant losses to the aquaculture industry, with chronic infection in adult salmonid fish and acute viral disease in young salmonid fish.

Viruses in family Birnaviridae are non-enveloped, with icosahedral single-shelled geometries, and T=13 symmetry. The diameter is around 70 nm. The genome is composed of linear, bi-segmented, double-stranded RNA. It is around 5.9–6.9 kbp in length and codes for five to six proteins. Birnaviruses encode the following proteins: RNA-directed RNA polymerase (VP1), which lacks the highly conserved Gly-Asp-Asp (GDD) sequence, a component of the proposed catalytic site of this enzyme family that exists in the conserved motif VI of the palm domain of other RNA-directed RNA polymerases.

The large RNA segment, segment A, of birnaviruses codes for a polyprotein (N-VP2-VP4-VP3-C) that is processed into the major structural proteins of the virion: VP2, VP3 (a minor structural component of the virus), and into the putative protease VP4. VP4 protein is involved in generating VP2 and VP3. recombinant VP3 is more immunogenic than recombinant VP2.

Infectious pancreatic necrosis virus (IPNV), a birnavirus, is an important pathogen in fish farms. Analyses of viral proteins showed that VP2 is the major structural and immunogenic polypeptide of the virus. All neutralizing monoclonal antibodies are specific to VP2 and bind to continuous or discontinuous epitopes. The variable domain of VP2 and the 20 adjacent amino acids of the conserved C-terminal are probably the most important in inducing an immune response for the protection of animals. Non structural protein VP5 is found in RNA segment A. The function of this small viral protein is unknown. It is believed to be involved in influencing apoptosis, but studies are not completely concurring. The protein cannot be found in the virion.

Viral replication is cytoplasmic. Entry into the host cell is achieved by cell receptor endocytosis. Replication follows the double-stranded RNA virus replication model in the cytoplasm. Double-stranded RNA virus transcription is the method of transcription in cytoplasm. The virus is released by budding. Salmonid fish (Aquabirnavirus), young sexually immature chickens (Avibirnavirus), insects (Entomobirnavirus), and blotched snakehead fish (Blosnavirus) as the natural host. Transmission routes are contact.

In-Text Question (ITQ)

What disease does Birnaviridae cause?
Infectious bursal disease virus (IBDV) belongs to the genus Avibirnavirus, family Birnaviridae, with a double-stranded, segmented RNA genome. IBDV affects chickens at young ages and causes an acute, immunosuppressive disease, infectious bursal disease.

Self-Assessment Exercise 8

Provide answer to the following question in 10 minutes
Briefly discuss the Family Birnaviridae

5.11 Summary

- There are 16 RNA virus families.
- The HIV virus is a member of the family Retroviridae.

5.12 References/Further Reading/Web Resources

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5.13 Possible Answers to Self-Assessment Exercises

SAE 1

1. While transcription was classically thought to occur only from DNA to RNA, reverse transcriptase transcribes RNA into DNA. The term "retro" in retrovirus refers to this reversal (making DNA from RNA) of the usual direction of transcription.
2. HIV-1, HIV-2, and HTLV-1 Genomes: All 3 major human retroviruses also encode a set of auxiliary genes, known as

accessory genes, which are dispensable for replication in certain in vitro cell culture systems

SAE 2

1. Bunyaviridae are transmitted by hematophagous arthropods including mosquitoes, midges, flies, and ticks. The viral incubation period is about 48 hours. Symptomatic infection typically causes non-specific flu-like symptoms with fever lasting for about three days.
2. Bunyaviruses are enveloped, segmented, negative-strand RNA viruses. Virion structure is relatively simple; structural proteins include a nucleocapsid (N) protein and two transmembrane glycoproteins (Gn and Gc). The L polymerase (RNA-dependent RNA polymerase) is also present in the virion.

SAE 3

The Orthomyxoviridae is a family of viruses that possess segmented, single-stranded, and negative-sense RNA genome. It contains influenza A virus, which is one of the most important pathogens to our public health.

1. The Influenza B virus capsid is enveloped while its virion consists of an envelope, a matrix protein, a nucleoprotein complex, a nucleocapsid, and a polymerase complex. It is sometimes spherical and sometimes filamentous. Its 500 or so surface projections are made of hemagglutinin and neuraminidase.

SAE 4

1. Paramyxoviridae is from the Greek word *para* which means “by the side of” and *myxa* “mucus” is a family of negative-strand RNA viruses in the order Mononegavirales. Vertebrates serve as natural hosts. Diseases associated with this family include measles, mumps, and respiratory tract infections.
2. The paramyxovirus genome is made up of a single strand of negative-sense nonsegmented RNA (ribonucleic acid). An endogenous RNA polymerase is present as well and is necessary for the transcription of the negative-sense strand into a positive-sense strand, thereby enabling proteins to be encoded from the RNA.

SAE 5

1. The majority of rhabdoviruses are transmitted by arthropods to vertebrate or plant hosts, but lyssaviruses (e.g. rabies virus) and novirhabdoviruses (e.g. infectious hematopoietic necrosis virus) have evolved to circulate among vertebrates without a biological vector, and sigmaviruses (e.g. Drosophila melanogaster).
2. The viruses in the group are responsible for rabies and vesicular stomatitis of cattle and horses.

SAE 6

1. The species of the family Toroviridae are
   Equine Torovirus
   Porcine Torovirus
   Bovine Torovirus

2. The disease causes diarrhoea, pyrexia, dehydration, lethargy and depression in all ages of cattle. In calves it causes anorexia, mucoid faeces and the following neurological signs; generalised weakness, paralysis, inability to stand, trembling and sudden death. It can also cause respiratory problems such as laryngitis, tracheitis and pneumonia. Young, colostrum-deprived calves are particularly at risk.
   In cats, diarrhoea and protruding nictitating membranes have been associated with feline torovirus infections.
   Pigs can shed the torovirus without showing any symptoms of disease

SAE 7

1. Filoviruses cause a severe hemorrhagic fever in human and non-human primates. Disease onset is sudden, with fever, chills, headache, myalgia, and anorexia. These symptoms may be followed by abdominal pain, sore throat, nausea, vomiting, cough, arthralgia, diarrhea, and pharyngeal and conjunctival vasodilitation.
2. Viruses in the family Filoviridae can cause severe hemorrhagic fever in people and nonhuman primates (such as monkeys and gorillas) and may spread in other animals, such as bats.

SAE 8

Birnaviridae is a family of double-stranded RNA viruses, Salmonid fish, birds and insects serve as natural hosts. There are currently 11 species in this family, divided among seven genera. Diseases associated with this

family include infectious pancreatic necrosis in salmonid fish, which causes significant losses to the aquaculture industry, with chronic infection in adult salmonid fish and acute viral disease in young salmonid fish.

Glossary

AIDS – Acquired immuno deficiency syndrome
CPE – Cytopathic Effect
$CsCl_2$ – Caesium chloride
DNA – Deoxyribonucleic acid
dsDNA – Double stranded Deoxyribonucleic acid
dsRNA - Double stranded Ribonucleic acid
g - gram
g/cm^3 – gram per cubic meter
HA - Heamagglutinin glycoproteins
HIV – Human immunodeficiency virus
HPIV - human parainfluenza virus
HTLV -human T-lymphotropic virus
ICNV - International Committee on Nomenclature of Viruses
ICTV - International Committee on Taxonomy of Viruses
IPNV - Infectious pancreatic necrosis virus
MLV - Murine leukaemia virus
mRNA – messenger Ribonucleic acid
MW – Molecular weight
NA - neuraminidase glycoprotein
nm - Nanometer
ORFs – Open reading frame
pH – Potential of Hydrogen
RNA – Ribonucleic acid
RNP complex – Ribonucleo Protein complex
S_{20w} – Normalized sedimentation coefficient
ssDNA - Single stranded Deoxyribonucleic acid
ssRNA - Single stranded Ribonucleic acid
TMV – Tobacco mosaic virus
UV – Ultra violet
VP – Viral protein

End of Module Questions

Multiple Choice Questions

1. The information necessary for programming infected cells for the production of viral progenies are located in the _____. a) capsid, b) polypeptide, c) nucleic acid, d) capsomers

2. The morphologic unit seen in the electron microscope on the surface of the icosahedral virus particle is _____. a) nucleocapsid, b) capsomers, c) capsomeres, d) capsid
3. Viruses are also sensitive to antibiotic like bacteria a) True, b) False
4. A virus particle contains both the DNA and RNA a) True, b) False
5. The major role of the structural protein of a virus is _____ a) for replication of the virus, b) for infection purposes, c) as protective coat, d) for transcription
6. Herpes virus consists of how many kilo base pairs of sequences? a) 120 kbp, b) 156 kbp, c) 198 kbp, d) 172 kbp
7. The genome of most DNA viruses consist of a single molecule which is double stranded a) True, b) False
8. The genome of RNA viruses may also be single-stranded and occur as a single molecule a) True, b) False
9. The following are RNA viruses except _____ a) caliciviruses, b) papovaviruses, c) orthomyxoviruses, d) bunyaviruses
10. Lipid constitutes about _____ percent of the dry weight of enveloped viruses? a) 50 – 60, b) 20 – 25, c) 30 – 35, d) 38 – 42
11. The lipids occurring in viral envelopes can also be termed _____ a) cholesterol, b) peripheral structural lipids, c) soluble lipids, d) cellular lipids
12. The infective materials in viruses are the _____ a) phospholipids, b) capsid, c) nucleic acid, d) glycoprotein
13. Viruses possess metabolic machinery of their own to replicate a) True, b) False
14. Which of the following is not part of the ranking according to the 2016 taxonomic hierarchy? a) species, b) subfamily, c) subgenus, d) subphylum
15. The basal rank in the 2021 taxonomic hierarchy for viruses is _____ a) species, b) realm, c) kingdom, d) class
16. The first virus to be identified and named is _____ a) Tomato mosaic virus, b) Cucumber mosaic virus, c) Measles virus, d) Tobacco mosaic virus
17. Joining of two or more abbreviations to form the name of a virus is referred to as _____ a) sigla formation, b) immunologic formation, c) Baltimore classification
18. Phenotypic characterization of viruses includes the following except _____ a) nucleic acid type, b) mode of replication, c) immunologic properties, d) host organism
19. Virus symmetry refers to the way _____ are arranged in the capsid. a) capsomeres, b) coat protein, c) genome, d) capsomers

20. The icosahedral structure of the virus geometry has _____ vertices or corners a) 12, b) 16, c) 8, d) 10
21. Helical viruses are strictly enveloped structure a) True, b) False
22. An example of binal symmetry is _____ a) Poxvirus, b) measles virus, c) bacteriophage, d) Tobacco mosaic virus
23. Virus envelopes are acquired at the following except _____ a) nuclear membranes, b) endoplasmic reticulum, c) phospholipids, d) golgi complex
24. The mature viral particles of the family Parvoviridae are stable in lipid solvent. a) True, b) False
25. Which of the following is a member of the family Parvoviridae? a) Cypovirus, b) coltivirus, c) densovirus, d) Badnavirus
26. Members of the Dependovirus group require a helper virus co-infection for efficient transmission. a) True, b) False
27. The virions of Parvoviridae contain how many polypeptides? A) 5, b) 4, c) 3, d) 2
28. Parvoviridae are non-enveloped and single stranded RNA. a) True, b) False
29. Adenoviruses are transmitted by the following except _____ a) airborne, b) faeces, c) eye droplets, d) urine
30. Herpes Simplex virus group is a _____ Herpesvirinae sub-family. a) beta, b) alpha, c) gamma, d) none of the above
31. Herpesviruses are transmitted by the following except _____. a) transfusion, b) water borne, c) insect vector, d) trans-placentally
32. Hepadnaviruses have affinity for which of the following? a) kidney, b) heart, c) liver, d) brain
33. Enteroviruses infect which part of the body? a) intestines, b) liver, c) mouth, d) legs
34. Aphthoviruses infect which of the following part of the body a) intestines, b) nose, kidney, d) mouth
35. Caliciviruses are non-enveloped RNA viruses a) True, b) False
36. The family Reoviridae include the following genera except _____ a) potyviruses, b) reoviruses, c) cryptoviruses, d) Fijiviruses
37. Phytoreoviruses infect which of the following? A) monkey, b) dogs c) plants, d) cats
38. Arboviruses are transmitted by which of the following? A) waterborne, b) airborne, c) arthropods, d) physical contact
39. The family Togaviridae includes the following except _____. a) Potexvirus, b) Alphavirus, c) Rubivirus, d) Arterivirus
40. Retroviruses are known to integrate their RNA genome into the DNA of the host cell a) True, b) False

41. The Family Retrovirivae includes the following sub-families except _____ a) Phytoretroviruses, b) oncoretroviruses, c) lentiviruses, d) spumaviruses
42. Tomato spotted wilt virus is a member of which of the following families? A) Potyviridae, b) Phytoviridae, c) Bunyaviridae, d) Fijiviridae
43. Viruses, outside their host cells, survive as _____. a) bacteria, b) virions, c) algae, d) protozoa
44. Which of the following is not the category of virus genome? a) dsDNA, b) ssRNA, c) tsDNA, d) dsRNA
45. Most of the plant viruses have _____. a) ssRNA, b) ssDNA, c) dsDNA, d) dsRNA
46. . Which of the following is the most common capsid shape of the virus? a) Cube, b) Rod, c) Cone, d) Icosahedron
47. Who is the father of Virology? a) Martinus Beijerinck, b) Dmitri Ivanovsky, c) John Ellerman, d) Frederick Twort
48. Who discovered viruses? a) John Ellerman, b) Frederick Twort, c) Dmitri Ivanovsky d) Martinus Beijerinck
49. Which of the following viruses help dependovirus for replication? a) Influenza virus, b) Reovirus, c) Rhinovirus, d) Adenovirus
50. . Which of the following viruses are icosahedrons? a) Isometric virus, b) Simple virus, c) Filamentous virus, d) Complex virus.

Theory Questions

1. An RNA virus contains the following sequence: A U C C C G A A U, What will be the sequence on the complementary strand in its cDNA.
2. List five families of RNA viruses important to man.
3. In a tabular form, list 10 medically important viruses and infection caused by each
4. In a tabular form, list 10 medically important viruses and their characteristics
5. In a tabular form, make a list of all the classes of strandedness of the nucleic acid, giving examples of the family, size and diseases caused.

Answers to MCQ Questions (Module 1)

S/N	Answer	S/N	Answer	S/N	Answer	S/N	Answer
1	c	16	d	31	c	46	d
2	b	17	a	32	c	47	a
3	b	18	c	33	a	48	c
4	b	19	a	34	d	49	d

5	c	20	a	35	a	50	a
6	d	21	b	36	a		
7	a	22	c	37	c		
8	b	23	c	38	c		
9	b	24	a	39	a		
10	c	25	c	40	a		
11	b	26	b	41	a		
12	c	27	c	42	c		
13	b	28	b	43	b		
14	c	29	a	44	c		
15	b	30	b	45	a		

Answers to Theory Questions (Module 1)

1. cDNA contains T A G G G C T T A

2. Five families of RNA viruses important to man

a. Reoviridae
b. Picornaviridae
c. Flaviviridae
d. Retroviridae
e. Rhabdoviridae

3. List of 10 medically important viruses and infection caused by each

S/N	Virus	Infection caused
1	Influenza virus	Respiratory diseases
2	Human Papilloma virus	Cancer of the cervix and warts
3	Human immune deficiency virus	AIDS
4	Dengue virus	Febrile diseases
5	Lassa virus	Hemorrhagic fever
6	Mumps	Meningitis and parotitis
7	Hepatitis A and B virus	Hepatitis
8	Cytomegalovirus	Infectious mononucleosis and congenital anomalies
9	Rubella virus	German measles and congenital anomalies
10	Adenovirus	Respiratory diseases, Exanthema and conjunctivitis

4. list of 10 medically important viruses and their characteristics

S/N	Virus group	Size of virion (nm)	Symmetry	Shape	Envelope
1	Reovirus	40-70	RNA, Cubic	Spherical	Yes

			or Icosahedral		
2	Rhabdovirus	130-300 x 70-80	RNA, helical	Elongated, bullet shaped	Yes
3	Retrovitus	100	RNA, helical	Spherical	Yes
4	Herpesvirus	120-150	DNA, cubic	Spherical	Yes
5	Poxvirus	300-450 x 170-260	DNA, complex	Brick shaped	No
6	Adenovirus	70-90	DNA, cubic	Spherical	No
7	Ebola virus	130-2600 x 80-100	RNA, pleomorphic	Filametous, U-shaped, ring shaped	No
8	Hepatitis A	27	RNA, cubic	Spherical	No
9	Hepatitis B	42	DNA, double capsid shell	Spherical	No
10	Papovavirus	45-55	DNA, cubic	Spherical	No

5. List of all the classes of strandedness of the nucleic acid, giving examples of the family, size and diseases caused.

Properties	Viral Family	Size	Examples of diseases caused
single-stranded DNA; naked; polyhedral capsid	Parvoviridae	18-25 nm	parvoviruses (roseola, fetal death, gastroenteritis; some depend on coinfection with adenoviruses)
double-stranded, DNA; naked; polyhedral capsid	Papovaviridae; circular dsDNA	40-57 nm	human papilloma viruses (HPV; benign warts and genital warts; genital and rectal cancers)
	Adenoviridae; dsDNA	70-90 nm	adenoviruses (respiratory infections, gastroenteritis, infectious pinkeye, rashes, meningoencephalitis)
double-stranded, circular DNA; enveloped; complex	Poxviridae	200-350 nm	smallpox virus (smallpox), vaccinia virus (cowpox), molluscipox virus (molluscum contagiosum-wartlike skin lesions)
double-stranded	Herpesviridae	150-200	herpes simplex 1 virus (HSV-1; most oral herpes;

DNA; enveloped; polyhedral capsid		nm	herpes simplex 2 virus (HSV-2; most genital herpes), herpes simplex 6 virus (HSV-6; roseola), varicella-zoster virus (VZV; chickenpox and shingles), Epstein-Barr virus (EBV; infectious mononucleosis and lymphomas), cytomegalovirus (CMV; birth defects and infections of a variety of body systems in immunosuppressed individuals)
	Hepadnaviridae	42 nm	hepatitis B virus (HBV; hepatitis B and liver cancer)
(+)single-stranded RNA; naked; polyhedral capsid	picornaviridae	28-30 nm	enteroviruses (poliomyelitis), rhinoviruses (most frequent cause of the common cold), Noroviruses (gastroenteritis), echoviruses (meningitis), hepatitis A virus (HAV; hepatitis A)
(+)single-stranded RNA; enveloped; usually a polyhedral capsid	Togaviridae	60-70 nm	arboviruses (eastern equine encephalitis, western equine encephalitis), rubella virus (German measles)
	Flaviviridae	40-50 nm	flaviviruses (yellow fever, dengue fever, St. Louis encephalitis), hepatitis C virus (HCV; hepatitis C)
	Coronaviridae	80-160 nm	coronaviruses (upper respiratory infections and the common cold; SARS)
(-)single-stranded RNA; enveloped; pleomorphic	Rhabdoviridae; bullet-shaped	70-189 nm	rabies virus (rabies)
	Filoviridae; long and filamentous	80-14,000 nm	Ebola virus, Marburg virus (hemorrhagic fevers)

	Paramyxoviridae; pleomorphic	150-300 nm	paramyxoviruses (parainfluenza, mumps); measles virus (measles)
(-) strand; multiple strands of RNA; enveloped	Orthomyxoviridae	80-200 nm	influenza viruses A, B, and C (influenza)
	Bunyaviridae	90-120 nm	California encephalitis virus (encephalitis); hantaviruses (Hantavirus pulmonary syndrome, Korean hemorrhagic fever)
	Arenaviridae	50-300 nm	arenaviruses (lymphocytic choriomeningitis, hemorrhagic fevers)
produce DNA from (+) single stranded RNA using reverse transcriptase; enveloped; bullet-shaped or polyhedral capsid	Retroviridae	100-120 nm	HIV-1 and HIV-2 (HIV infection/AIDS); HTLV-1 and HTLV-2 (T-cell leukemia)
dsRNA; naked; polyhedral capsid	Reoviridae	60-80 nm	reoviruses (mild respiratory infections, infant gastroenteritis); Colorado tick fever virus (Colorado tick fever)

MODULE 2

UNIT 1 OTHER CLASSES OF VIRUSES

CONTENTS

 1.1 Introduction

We shall begin this module by looking into a special order of viruses known as mononegaviriales, they contain a single negative sensed RNA in their genome. We shall also be looking at satellite structures in the genome of viruses and a quick look into the world of viroids.

 **1.2 Learning Outcomes**

At the end of the class, student must be familiar and have understood the order mononegavirales, viroids and satellites associated with plant viruses

 **1.3 The Order Mononegavirales**

Derivation of the name is as follows:‘Mono’ from Greek word ‘Monos’ meaning single. ‘Nega’ from negative strand or negatively sensed RNA (the genome). ‘Virales’ from the viral orders which ends with the suffix - ‘ales’. This order encompasses the three viral families of eukaryotic

viruses possessing linear, monosegemented negatively sensed RNA genome. The three viral families are:

The Filoviridae, Paramyxoviridae and Rhabdoviridae, all of which had been discussed earlier, generally their MW ranges from 300 - 100 x 10^6; S_{20w} = 550 - > 1000. Buoyant density in sucrose = 1.18–120g/cm^3 their genome MW = 3.5–7 × 10^6 and constitute about 0.5-20% of particle weight. Their Lipid content is about 15-25% by weight and the composition is dependent on the host cell.

The carbohydrate is 3-6% by weight where known. The pathogenic potential in human tends to be characteristic of family: Haemorrhagic fever (Filoviridal); Respiratory and neurological disease (Paramyxoviridae); mild febrile to fatal neurological disease (Rhabdoviridae). Replication occurs by synthesis of a complete positive sense RNA anti-genome. Maturation of the independently assembled helical nucleocapsids occurs by budding through the host membranes and investment by a host derived Lipid envelope containing trans-membrane virus proteins.

A virus is a member of the order Mononegavirales if

- its genome is a linear, typically (but not always) non-segmented, single-stranded, non-infectious RNA of negative polarity; possesses inverse-complementary 3' and 5' termini; and is not covalently linked to a protein;
- its genome has the characteristic gene order 3'-UTR–core protein genes–envelope protein genes–RNA-dependent RNA polymerase gene–5'-UTR (3'-N-P-M-G-L-5') (there are, however, some exceptions);
- it produces 5–10 distinct mRNAs from its genome via polar sequential transcription from a single promoter located at the 3' end of the genome; mRNAs are 5' capped and polyadenylated;
- it replicates by synthesizing complete antigenomes;
- it forms infectious helical ribonucleocapsids as the templates for the synthesis of mRNAs, antigenomes, and genomes;
- it encodes an RNA-dependent RNA polymerase (RdRp, L) that is highly homologous to those of other mononegaviruses; and/or
- it typically (but not always) produces enveloped virions with a molecular mass of 300–1,000×10^6; an S_{20W} of 550–>1,045; and a buoyant density in CsCl of 1.18–1.22 g/cm^3.

In-Text Question (ITQ)

What type of viruses are the Mononegavirales?

Ans. Mononegavirales, known as nonsegmented negative-sense (NNS) RNA viruses, are a class of pathogenic and sometimes deadly viruses that include rabies virus (RABV), human respiratory syncytial virus (HRSV), and Ebola virus (EBOV).

Self-Assessment Exercises 1

Provide answer to the following question in 10 minutes

1. List the families of the Order Mononegavirales
2. What are the symptoms of paramyxovirus?

1.4 Viriods

These are small infectious agents that cause various plants diseases. Viroids are encapsidated low molecular weight, covalently closed circular, single stranded infectious positively sensed RNAs. The non-denatural viroid molecules adopt extensive internal base pairing to give rod-like structures that is 50nm long. They are denatured by cooperative melting to single-stranded circules of 100nm contour length. The molecular weight = 80-122 x 10 S_{20w} 8 – 10; Tm in 10mM Na^+ is 50 C; density in cesium sulphate is 1 .6g/cm^3. They comprises about 246 to cover 370 nucleotides. They are rich it G+C content except few members, with central conserved regions. They comprise highly base-paired rod like structure with unique properties. Each is arranged into 26 double stranded regions separated by 25 regions of unpaired bases embodied in single - stranded internal loops there is a loop at each end of the rod-like molecule.

1.4.1 Biological properties of viroids

Host range: Some viroids have wide host ranges in the angiosperms but others, particularly members of the family *Avsunviroidae*, have narrow host ranges. CCCVd and coconut tinangaja viroid (CTiVd) infect monocotyledons. Old cultivars of grapevine and citrus can harbor at least five different viroids. A single nucleotide substitution converts PSTVd from a non-infectious RNA to one that is infectious for *Nicotiana tabacum*.

Symptoms: Some viroids have devastating effects, for example, CCCVd has killed millions of coconut palms in the Philippines, while others cause epinasty, rugosity, chlorosis and necrosis on leaves, internode shortening of stems leading to stunted plants, bark cracking, deformation and color alterations of fruits and storage organs, and delays in foliation, flowering and ripening. A few viroids induce only

mild or no symptoms. Symptom expression is generally more pronounced at high temperature and light intensities.

Molecular determinants of specific symptoms have been mapped in the genome of members of both families. Small sequence changes can convert a severe strain into a symptomless strain (or *vice versa*). Direct interaction of the genomic viroid RNA with host factors, and involvement of or interference with the plant RNA silencing machinery, have been suggested as primary event(s) of symptom induction.

Transmission: Viroids of horticultural species are transmitted mainly by vegetative propagation. In plants propagated via seeds, they may be transmitted mechanically or through seed or pollen. Only tomato planta macho viroid (TPMVd) is known to be efficiently transmitted by aphids.

Movement: To invade plants systematically, viroids must be able to move from the initially infected cells to the surrounding ones and then to the vascular system. Three different types of movement can be considered: intracellular, cell-to-cell and long-distance. PSTVd movement into the nucleus appears to be a cytoskeleton-independent process that is mediated by a specific and saturable receptor and involves recognition of a conserved sequence and/or structural motif in the upper central conserved region. How members of the family *Avsunviroidae* are transported into chloroplasts is unknown. Mutational analysis of PSTVd has shown that many of the loops in its rod-like secondary structure play a role in movement from cell-to-cell and across tissue boundaries, possibly by creating pockets that bind host proteins. *In vitro,* hop stunt viroid (HpSVd) can form a ribonucleoprotein complex with the phloem protein 2, a dimeric lectin able to move from cell-to-cell through plasmodesmata and toward sink tissues in the assimilate stream. These properties, together with its ability to bind RNA, suggest that this lectin facilitates the systemic movement of viroids. Access of PSTVd to floral and vegetative meristems is impaired, most likely by an RNA silencing mechanism. In contrast, a chloroplast replicating viroid, PLMVd, is able to enter the apical meristem of its natural host.

Cross protection: Interactions at the level of symptom expression and viroid accumulation have been detected in plants co-infected by two strains of a viroid or even by two different viroids sharing extensive sequence similarities. Interactions of this class have been observed in the case of members belonging to both viroid families, suggesting the possible existence of more than one mechanism of cross-protection between viroids. Alternatively, because RNA-mediated cross-protection in plant viruses is mechanistically similar to post-transcriptional gene

silencing, cross-protection between viroids might occur through a similar mechanism in both families.

In-Text Question (ITQ)

What are viroids?

Answer

Viroids are infectious agents that consist only of naked RNA without any protective layer such as a protein coat. Viroids infect plants (but no other forms of life) and are replicated at the expense of the host cell. Viroid genomes are small single-stranded circles of RNA that are only 250 - 400 bases long.

Self-Assessment Exercises 2

Provide answer to the following question in 10 minutes

Write short note on viroids

1.5 Satellites

Several viruses, as obligate parasites to the host plants, are associated with even smaller molecular parasites, sometimes being commensal or even beneficial, and are among the simplest life forms, namely, satellite RNAs (satRNAs) and satellite viruses. These satRNAs are short RNA molecules, usually <1,500 nt, that depend on cognate helper viruses for replication, encapsidation, movement, and transmission, but most share little or no sequence homology to the helper viruses. In contrast, satellite viruses are satRNAs that encode and are encapsidated in their own capsid proteins (CPs). Certain satRNAs code for nonstructural proteins, but most satRNAs do not encode any functional protein products and are therefore thought to exert their biological functions through direct RNA interactions. Recent advances in research into satRNAs and satellite viruses have resulted in deeper insights into the molecular biology of these small replicating entities and to certain practical applications in modern biotechnology.

Satellite viruses and satRNAs have attracted much interest over the past decades, mainly for the following reasons: (1) they can modulate - attenuate or exacerbate - the symptoms caused by their cognate helper viruses; (2) they do not encode their own RNA-dependent RNA polymerases (RdRps) for their own replication and apparently use

replication machineries similar to those of the helper viruses and thus have great potential as surrogate systems for the study of the replication mechanisms of their cognate helper viruses; (3) they can alter − usually reduce − the accumulation of their cognate helper viral RNAs and are thus considered the molecular parasites of the helper viruses; and (4) they can accumulate to high levels in host plants and thus in some cases can be developed into high-level expression vectors for foreign genes. Because of these features, satRNAs and satellite viruses are good biological systems for the study of the molecular biology of viruses. Indeed, current studies of satRNAs and satellite viruses have provided further insights into several key subjects in molecular virology. In addition, recent studies have revealed the roles of an antiviral defense system of host plants, RNA silencing or posttranscriptional gene silencing (PTGS) in the pathogenicity and molecular biology of satRNAs. This information has altered our views of satRNAs and satellite viruses as being purely parasitic to the helper viruses.

Satellites are basically nucleic acid molecules that depend on co-infection of a host cell with a helper virus for their multiplication. Satellite nucleic acids have no appreciable sequence homology with their helper virus genome and are not a part of its genome. The satellites are different and district nucleic acids from other types of dependent nucleic acid such as sub-genomic nucleic acids e.g. defective interfering and messenger it molecules; genome parts and transmission-defective but independently replicating viruses. Some satellites may contribute advantageous character to their helper virus; the distinction between these and genome parts is sometimes no clear cut. Most reported satellites are associated with plant viruses and these have been arbitrarily classified into 4-types according to physical and messenger properties of the satellite RNA. These are;

1. TYPE A: The RNA is large (>0.7kb) and encodes a capsid protein that fond in satellite specific particles.
2. TYPE B: The RNA is large (>0.7kb) and encodes a non structural protein.
3. TYPE C: The RNA is small (< 0.7kb), lacks significant mRNA properties
4. TYPE D: The RNA is small (<0.7kb), lacks mRNA activity and forms circular molecules during replication.

Most records of satellites are of those associated with plant viruses. Satellite have also been found associated with a viruses of other taxonomic groups, e.g. Bacteriophage p4, which is a dsDNA satellite virus dependent on Bacteriophage p2 adeno associated viruses (Dependeovirus:

Parvoviridae) ssDNA satellite viruses dependent on adenoviruses or herpes viruses, hepatitis delta virus which is large, but circular, satellite RNA dependent on hepatitis B virus and a ssRNA satellite virus which is associated with chronic bee-paralysis virus. By definition, all satellites share the following features: they depend on helper viruses, at least for replication; they are not part of the helper viral genome and are not required for the infection cycle of their helper viruses (with at least one exception: the satRNA associated with *Groundnut rosette virus* (GRV); and they share little or no nucleotide sequence similarity with their cognate helper viruses, which distinguishes them from subgenomic or defective RNAs (D-RNAs).

In-Test Question (ITQ)

What type of virus is a satellite?

Ans. Satellite viruses (SVs) are subviral pathogens that are entirely dependent upon the replication machinery of their helper viruses.

Self-Assessment Exercises 3

Provide answer to the following question in 10 minutes

What is the difference between viroids and satellites?

1.6 Summary

- Viriods are small infectious agents
- They infect plants causing disease
- Satellites require a helper virus for infection of a host cell.

1.7 References/Further Readings/Web Sources

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1.8 Possible Answers to Self-Assessment Exercises

SAE 1

1. The order ***Mononegavirales*** comprises four families of viruses namely:

   Bornaviridae,
   Rhabdoviridae,
   Filoviridae,
   and Paramyxoviridae.

2. The symptoms of the Family paramyxoviridae include the following:

- nervous signs, including trembling wings and heads, and twisting of the neck.
- partial paralysis of wings and legs (birds may fall over on landing and be unable to feed)
- unusually wet and liquidy faeces (diarrhoea) that are often greenish in colour.

SAE 2

Viroids are infectious agents that consist only of naked RNA without any protective layer such as a protein coat. Viroids infect plants (but no other forms of life) and are replicated at the expense of the host cell. Viroid genomes are small single-stranded circles of RNA that are only 250–400 bases long. Viroids are non-coding circular RNA molecules with rod-like or branched structures. They are often ribozymes, characterized by catalytic RNA. They can perform many basic functions of life and may have played a role in evolution since the beginning of life on Earth. They can cleave, join, replicate, and undergo Darwinian

evolution. Furthermore, ribozymes are the essential elements for protein synthesis of cellular organisms as parts of ribosomes. Thus, they must have preceded DNA and proteins during evolution. Here, we discuss the current evidence for viroids or viroid-like RNAs as a likely origin of life on Earth. As such, they may also be considered as models for life on other planets or moons in the solar system as well as on exoplanets.

SAE 3

Satellite RNAs and viroids are sub-viral pathogens of plants. Satellite RNAs are dependent on their HVs for replication and encapsidation. Viroids do not encode any proteins and are replicated by cellular enzymes. Some commonly shared replication features exist between satellite RNAs and viroids.

UNIT 2 VIRAL REPLICATION

CONTENTS

2.1 Introduction

Viral replication is the formation of biological viruses during the infection process in the target host cells. Viruses must first get into the cell before viral replication can occur. Through the generation of abundant copies of its genome and packaging these copies, the virus continues infecting new hosts. Replication between viruses is greatly varied and depends on the type of genes involved in them. Most DNA viruses assemble in the nucleus while most RNA viruses develop solely in cytoplasm.

The unique feature of virus multiplications is that soon after interaction with host cell, the infecting virion is disrupted and its measurable infectivity lost. The phase of this growth is called the ECLIPSE PERIOD. This duration depends on both the particular virus and the host cell, and ends with the formation of the first infectious progeny of virus particles. The eclipse period is actually one of intense synthetic activity as the cell is re-directed toward fulfilling the needs of the viral ‘pirate’. Viruses have evolved a variety of different strategies for accomplishing multiplication in their host cells. Although the details vary from one viral family to, the other, the general outline of the replication cycles is similar. The replication process could be broken down into seven stages.

2.2 Learning Outcomes

At the end of the class, student must have fully understood the concept of viral replication and the seven stages involved in the process.

2.3 Adsorption/Attachment

This is the first step on virus infection, during which there is interaction of a virion with a specific receptor site on the surface of the cell to be infected. It is to be noted that receptor molecules differ for different viruses, e. g. poliovirus is able to attach only to cells in CNS/ and intestinal tract of primates, HIV binds to the CD4 receptor on cells of human immune system. Each susceptible cell probably contains at least 1,000,000 receptor sites for a given virus. Adsorption is best achieved at 37°C but could also take place at 4°C but very slow. Adsorption is also enhanced by presence of Magnesium (Mg^{2+}) or Calcium (Ca^{2+}) ions.

In-Test Question (ITQ)

What is adsorption in viral replication?

Attachment, or adsorption, occurs between the viral particle and the host cell membrane. A hole forms in the cell membrane, then the virus particle or its genetic contents are released into the host cell, where viral reproduction may commence.

Self-Assessment Exercises 1

Provide answers to the following questions in 10 minutes

1. Attachment is the first stage in replication of viruses
   Yes/No
2. Attachment of virus during replication can also be described as adsorption. Yes/No
3. The receptor molecules needed for attachment during replication are same for all viruses. Yes/No

2.4 Penetration/Entry

The mode of penetration of entry of viruses into the host cell is complex in nature. It is accomplished by receptor-mediated endocytosis with uptake of the ingested virus particles within endosomes. With syncytia

producing viruses, it is by fusion of virus envelope with the cell membrane. Penetration in some other node is well known.

In-Test Question (ITQ)

How do viruses penetrate a host cell?

The virus attacks the host cell by first attaching to a specific receptor site on the membrane of the host cell. Next, the viral nucleic acid, either DNA or RNA, enters the host cell, either naked, leaving the protein capsid behind, or with the capsid.

Self-Assessment Exercises 2

Provide answers to the following questions in 10 minutes

How does a non enveloped virus enters a cell?

2.5 Uncoating

Uncoating occurs concomitantly with or shortly after penetration. At this stage, there is physical separation of the viral nucleic acid (or in some cases, internal nucleocapsid) from the outer structural components of the virion. The infectivity in the parental virus is lost at this point. Viruses are only infectious agents for which dissolution of the infecting agent is an obligatory step in the replicative pathway. Uncoating is done enzymatically (from lysosome).

In-Test Question (ITQ)

What is Uncoating and how is it carried out?

Ans.: It is a process by which the viral DNA/RNA separates from the viral capsid. The virus then exploits the host's RNA/DNA/protein synthesis machinery. Viral subunits are then assembled, allowing the progeny viruses to be released, as the host cell disintegrates.

Self-Assessment Exercises 3

Provide answers to the following questions in 10 minutes

Where does viral uncoating occur?

2.6 Transcription

At his stage, a specific mRNA must be transcribed from time viral nucleic acid for successful expression and duplication of genetic information contained in the viral genome. The mRNA produced serves as replicative intermediate from the viral genome. The process of transcription could be carried out by either the host cell mechanism or a virus-specified-enzyme. The patterns of transcription may differ before (early) at and after (late) virus nucleic acid replication. The primary transcripts are often spliced to remove intron sequences between expressed exons and transcription is sometimes overlapping with different starting and/or termination points within one gene to produce proteins from the same nucleic acid sequence. Virus mRNA generally

(i) Contains leader sequence
(ii) Capped at the 5-’ end
(iii) Polyadenylated at time 3’ terminus.

Some viruses carry RNA polymerases to synthesize mRNA. RNA viruses of this type are called Negative-sense since their single stranded RNA genome is complementary to mRNA which is designated positive strand (positiveness).
In-Test Question (ITQ)

What is the basic process of transcription?

Ans.: Transcription is the process in which a gene's DNA sequence is copied (transcribed) to make an RNA molecule. RNA polymerase is the main transcription enzyme. Transcription begins when RNA polymerase binds to a promoter sequence near the beginning of a gene (directly or through helper proteins).

Self-Assessment Exercises 4

Provide answers to the following questions in 10 minutes

Briefly explain transcription of viruses?

2.7 Synthesis of Viral components

This is the stage where use the host cell component to translate the viral mRNA. All the virus specified macromolecules are synthesized in a highly organized sequences. The virus mRNA is translated on cell ribosomes to produce two types of virus-protein:

(a) The structural proteins which make up the virus particle.
(b) Non-structural protein, mainly enzymes for virus genome replications. This type of protein is not found in the viral particle.

In some virus infections, notably those involving double stranded, DNA- containing viruses, early viral proteins are synthesized soon after infection and late proteins are made only late in infecting viral DNA synthesis. Early genes may or may not be shut off when late products are made. In contrast, most of the genetic information of RNA viruses is expressed at the same time. In addition to these temporal controls, quantitative controls also exist, since not all virus proteins are made in the same amounts. Virus specific protein may regulate the extent of transcription of the genome or translation of viral mRNA.

Small animal viruses and bacteriophage are good models for studies of gene expression. The widest variation in strategies of gene expression is found among RNA viruses. Some virions carry polymerases, some system utilize sub-genomic messages, sometimes generated by splicing (orthomyxoviruses, retroviruses) and some viruses synthesize large polyprotein precursors that are processed and cleaved to generate the final gene involved in these processes varies from group to group. The intercellular sites where the different events in virus replication take place vary from group to group. However, some general statements could be made:

(a) Viral Proteins are synthesize in the cytoplasm poly ribosomes composed of virus-specific mRNA and host cell ribosomes
(b) Viral DNA is usually replicated in the nucleus
(c) Viral genomic RNA is generally duplicated in the cell cytoplasm, although there are exceptions.

In-Test Question (ITQ)

Can viruses synthesize their own components?

Answer

Without a host cell, viruses cannot carry out their life-sustaining functions or reproduce. They cannot synthesize proteins, because they lack ribosomes and must use the ribosomes of their host cells to translate viral messenger RNA into viral proteins.

Self-Assessment Exercises 5

Provide answers to the following questions in 10 minutes

Which cell molecules will be used to make viral proteins?

2.8 Assemblage/Morphogenesis

The newly synthesized viral genomes and capsid polypeptides assemble together to form progeny viruses. Icosahedral capsid can condense in the absence of nucleic acid, whereas nucleocapsids of viruses with helical symmetry cannot form without viral RNA. There are no special mechanisms for the release of non-enveloped viruses; the infected cells eventually lyses and release the virus particles. Assembly of viral proteins may take place in the cytoplasm or (as in most enveloped viruses) at the plasma membrane, After assemblage of viruses viral structural and essential proteins sub units, the viral becomes mature and ready for liberation or release.

In-Test Question (ITQ)

What is virus morphogenesis?

Answer

Virus morphogenesisoccurs at the inner nuclear envelope, and enveloped virus particles accumulate in perinuclear spaces. In protoplasts treated with tunicamycin, morphogenesis is interrupted and nucleocapsids accumulate in the nucleoplasm.

Self-Assessment Exercises 6

Provide answers to the following questions in 10 minutes

What is assembly in viral replication?

2.9 Maturity and release

On maturation, viral particles escape from the cell in several ways but most importantly done in two ways:

(a) Bursting out in the cell thereby killing the host cells.
(b) Budding out of the host cell which does not necessarily kill the cell.

Enveloped viruses mature by budding process, Virus-specific envelope glycoproteins are inserted into cellular membranes, viral nucleocapsids then bud through the membrane at these modified sites and, in so doing acquire an envelope. Budding frequently occur at the plasma membrane but may involve other membranes cell. It is important to note that enveloped viruses are not infectious until they acquired their envelopes. Therefore, infectious progeny virions typically do not accumulate within the infected cell.

Virus maturation is sometime a faculty process. Excess amounts of viral components may accumulate and be involved in the formation of inclusion bodies in the cell. As a result of the profound deleterious effects of virus replication, cellular cytopathic effects eventually develop and the cell dies. However, there are instances in which the cell is not damaged by the virus and long-term persistent infections evolve. The basic fact is that each virus effectively utilizes whichever cellular process is necessary to achieve its multiplication and morphogenesis.

In-Test Question (ITQ)

What is maturation in virus replication?

Answer

Virion maturation is a process that takes place after separation of the viral infectious particle from host cell by budding or sealing of spherical capsid. It consist in irreversible rearrangement and/or cleavage of viral proteins that are activating the virion to be competent for reinfection.

Self-Assessment Exercises 7

Provide answers to the following questions in 10 minutes

Make a diagrammatic representation of virus replication

2.10 Summary

- Viral replication is essential for the survival and multiplication of viral particle
- The viral particle must be able to effectively bypass the host defence for effective replication.

2.11 References/Further Reading/Web Resources

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2.12 Possible Answers to Self-Assessment Exercises

SAE 1

1. Yes
2. Yes
3. No
4. No

SAE 2

Non-enveloped viruses can enter the cytosol by directly penetrating the plasma membrane, as well as through a variety of endocytic mechanisms leading to penetration of internal membrane(s). Internal membranes crossed by non-enveloped viruses include the endosomal membrane (e.g. adenovirus)

SAE 3

In the cytoplasm, viruses can be exposed to cellular cues and processes which culminate in the completion of uncoating and release of the viral genome from the capsid. The viral genome is transported to the site of replication, which may be in the cytosol, on cytoplasmic membranes or in the nucleus.

SAE 4

Genome transcription is a critical stage in the life cycle of a virus, as this is the process by which the viral genetic information is presented to the host cell protein synthesis machinery for the production of the viral proteins needed for genome replication and progeny virion assembly.

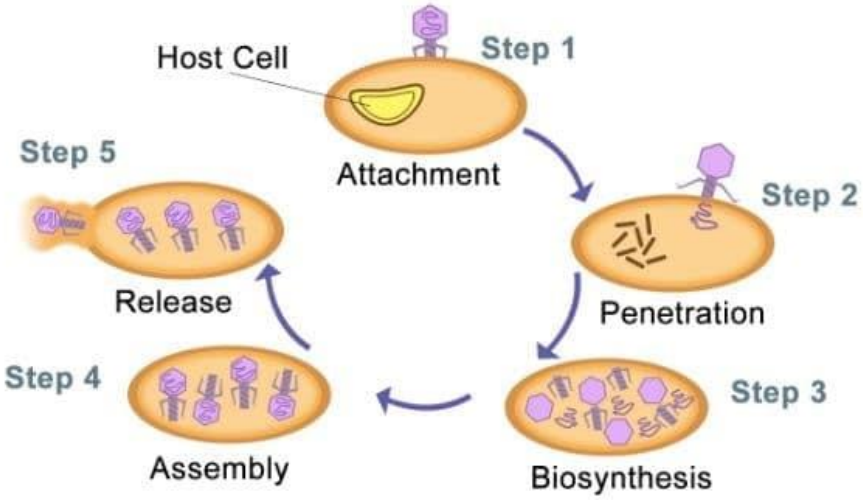
SAE 5

DNA, which is the makeup of the genome of most organisms, can be replicated, but also transcribed into RNA molecules, which are then translated into proteins. Proteins go on to perform functions, such as the capsid of a virus is made of proteins that provide a structure to encapsulate the viral genome.

SAE 6

After the synthesis of viral genome and proteins, which can be post-transcriptionally modified, viral proteins are packaged with newly replicated viral genome into new virions that are ready for release from the host cell. This process can also be referred to as maturation.

SAE 7


Host Cell
Step 1
Attachment
Step 2
Penetration
Step 3
Biosynthesis
Step 4
Assembly
Step 5
Release

UNIT 3 VIRAL GENETICS

CONTENTS

3.1 Introduction

Viruses are simple entities, lacking an energy-generating system and having very limited biosynthetic capabilities. The smallest viruses have only a few genes; the largest viruses have as many as 200. Genetically, however, viruses have many features in common with cells. Viruses are subject to mutations, the genomes of different viruses can recombine to form novel progeny, the expression of the viral genome can be regulated, and viral gene products can interact. By studying viruses, we can learn more about the two mechanisms by which viruses and their host cells function: mutation and recombination.

3.2. Learning Outcomes

At the end of the class, student must have fully understood the concept of viral genetics and the mechanisms by which genetic changes occur in viruses.

3.3 Mutations

Mutations arise by one of three mechanisms: (1) by the effects of physical mutagens (UV light, x-rays) on nucleic acids; (2) by the natural behaviour of the bases that make up nucleic acids (resonance from keto

to enol and from amino to imino forms), and (3) through the fallibility of the enzymes that replicate the nucleic acids. The first two mechanisms act similarly in all viruses; hence, the effects of physical mutagens and the natural behaviour of nucleotides are relatively constant. However, viruses differ markedly in their mutation rates, which is due primarily to differences in the fidelity with which their enzymes replicate their nucleic acids. Viruses with high-fidelity transcriptases have relatively low mutation rates and vice versa.

3.3.1 Mutation Rates and Outcomes

DNA viruses have mutation rates similar to those of eukaryotic cells because, like eukaryotic DNA polymerases, their replicatory enzymes have proofreading functions. The error rate for DNA viruses has been calculated to be 10^{-8} to 10^{-11} errors per incorporated nucleotide. With this low mutation rate, replication of even the most complex DNA viruses, which have 2×10^5 to 3×10^5 nucleotide pairs per genome, will generate mutants rather rarely, perhaps once in several hundred to many thousand genome copies. The RNA viruses, however, lack a proofreading function in their replicatory enzymes, and some have mutation rates that are many orders of magnitude higher - 10^{-3} to 10^{-4} errors per incorporated nucleotide. Even the simplest RNA viruses, which have about 7,400 nucleotides per genome, will generate mutants frequently, perhaps as often as once per genome copy.

Not all mutations that occur persist in the virus population. Mutations that interfere with the essential functions of attachment, penetration, uncoating, replication, assembly, and release do not permit misreplication and are rapidly lost from the population. However, because of the redundancy of the genetic code, many mutations are neutral, resulting either in no change in the viral protein or in replacement of an amino acid by a functionally similar amino acid. Only mutations that do not cripple essential viral functions can persist or become fixed in a virus population.

In-Test Question (ITQ)

1. Do viruses have a high rate of mutation?
2. What is the meaning of mutation rate?

Answer:

1. Many viruses have high rates of evolution. These high evolutionary rates have been attributed to the large population sizes, short generation times, and high mutation rates of viruses.

Mutation rate, specifically, is an important determinant of evolutionary rate across taxa (1–4).

2. The rate of mutation is the probability that a given base pair or a larger region of DNA changes with time. For practical reasons mutations are usually detected by changes in phenotype per unit of time indicated as cell generations (1) or days (2).

3.3.2 Phenotypic Variation by Mutations

Mutations that alter the viral phenotype but are not deleterious may be important. For example, mutation can create novel antigenic determinants. A mutation in the hemagglutinin gene of influenza A virus can give rise to a hemagglutinin molecule with an altered antigenic site (epitope). Provided the attachment function of the new hemagglutinin is intact, the mutant virus may be able to initiate an infection in an individual immune to viruses expressing the previous hemagglutinin. For example, from 1968 to 1979, mutations altered 10 percent of the amino acids in the influenza virus hemagglutinin serotype H3 molecule. This relatively modest mechanism of antigenic change through mutation, called antigenic drift, may allow a virus to outflank host defenses and cause disease in previously immune individuals.

In-Test Question (ITQ)

When a virus mutates what are the changes that occur?

Answer

As a virus replicates, its genes undergo random “copying errors” (i.e. genetic mutations). Over time, these genetic copying errors can, among other changes to the virus, lead to alterations in the virus' surface proteins or antigens.

3.3.3 Vaccine Strains from Mutations

Mutation has been a principal tool of virologists in developing attenuated live virus vaccines. For example, the Sabin vaccine strains of poliovirus were developed by growing polioviruses in monkey kidney cells. Mutation and selection produced variant polioviruses that were adapted for efficient replication in these cells. Some of the mutations in these variants affected the genes coding for the poliovirus coat proteins in such a way as to produce mutants unable to attach to human neural cells but still able to infect human intestinal cells. Infection of human intestinal cells does not produce paralytic disease but does induce immunity. Poliovirus vaccine strains 1 and 2 have multiple mutations in

the coat proteins and are very stable. The type 3 vaccine strain is less stable and is subject to back-mutations (reversions) that restore neural virulence. This vaccine strain therefore causes paralytic disease in one out of every several million vaccinated individuals. Despite the possibility of back-mutations, the generation and selection of attenuated viral mutants remains an important mechanism for producing viral vaccines.

In-test Question (ITQ)

What is the importance of mutation in Virology?

Ans.: Mutation of viruses has been exploited to produce attenuated live virus vaccines for the treatment of many infections in humans and animals. It has also been used to introduce less virulent strains of the same virus that has over time reduce the capability of more virulent ones in the population.

Self-Assessment Exercises 1

Provide short answers to the following questions in 10 minutes

1. Are virus mutations normal?
2. What is a variant of a virus?

3.4 Recombination

Viral recombination occurs when viruses of two different parent strains co-infect the same host cell and interact during replication to generate virus progeny that have some genes from both parents. Recombination generally occurs between members of the same virus type (e.g., between two influenza viruses or between two herpes simplex viruses). Two mechanisms of recombination have been observed for viruses: independent assortment and incomplete linkage. Either mechanism can produce new viral serotypes or viruses with altered virulence.

3.4.1 Recombination by Independent Assortment

Independent assortment occurs when viruses that have multipartite (segmented) genomes trade segments during replication. These genes are unlinked and assort at random. Recombination by independent assortment has been reported, for example, for the influenza viruses and other orthomyxoviruses (8 segments of single-stranded RNA) and for the reoviruses (10 segments of double-stranded RNA). The frequency of recombination by independent assortment is 6 to 20 percent for orthomyxoviruses. Independent assortment between an animal and a

human strain of influenza virus during a mixed infection can yield an antigenically novel influenza virus strain capable of infecting humans but carrying animal-strain hemagglutinin and/or neuraminidase surface molecules. This recombinant can infect individuals that are immune to the parent human virus. This mechanism results in an immediate, major antigenic change and is called antigenic shift. Antigenic shifts in influenza virus antigens can give rise to pandemics (worldwide epidemics) of influenza. Such antigenic shifts have occurred relatively frequently during recent history. Because the number of different serotypes of hemagglutinin and neuraminidase are limited, a given strain reappears from time to time. For example, the H1N1 influenza virus strain was responsible for the 1918 to 1919 influenza pandemic that caused 20 million deaths. The same virus also caused pandemics in 1934 and in 1947, then disappeared after 1958 and reappeared in 1977. The reappearance of virus strains after an absence is believed to be the result of recombination events involving the independent assortment of genes from two variant viruses.

In-test Questions (ITQ)

Explain the term Independent Assortment in Viruses

Answer

Independent assortment is the random exchange of segments within the genomes of viruses having multipartite genomes. Independent assortment occur during replication of viruses in the cell of the common host of the viruses.

How does reassortment occur during viral replication?
Reassortment only occurs when multiple viruses co-infect the same cell, and replicate their progeny segments in the same cytoplasm. The progeny virions are assembled from a mix of segments from the parental virions, creating a novel assortment of genome segments – a hybrid – into a capsid

3.4.2 Recombination of Incompletely Linked Genes

Recombination also occurs between genes residing on the same piece of nucleic acid. Genes that generally segregate together are called linked genes. If recombination occurs between them, the linkage is said to be incomplete. Recombination of incompletely linked genes occurs in all DNA viruses that have been studied and in several RNA viruses.

In DNA viruses, as in prokaryotic and eukaryotic cells, recombination between incompletely linked genes occurs by means of a break-rejoin

mechanism. This mechanism involves the actual severing of the covalent bonds linking the bases of each of the two DNA strands in a DNA molecule. The severed DNA strands are then rejoined to the DNA strands of a different DNA molecule that has been broken in a similar site. Recombination rates for herpesviruses, which are DNA viruses that replicate in the nucleus of infected cells, approximate those expected for a eukaryotic genome of the size of the herpesvirus genome. Herpesviruses have an average recombination frequency of 10 to 20 percent for any two loci. However, the rate of recombination between a specific pair of genetic loci depends on the distance between them and varies from less than 1 percent to approximately 50 percent. Measurement of the recombination frequencies for different loci can be used to map the virus genome. In this type of genetic map, loci with high recombination frequencies are far apart and loci with low recombination frequencies are close together.

Recombination has been shown to occur in several positive-sense single-stranded RNA virus groups: retroviruses, picornaviruses, and coronaviruses. That is initially surprising, as recombination between RNA molecules has not been observed in prokaryotic or eukaryotic cells. In retroviruses, recombination actually occurs at the point in replication when the retrovirus genome is in a DNA form and takes place by the same break-rejoin mechanism as in cells and DNA viruses. Recombination can occur both between two related retroviruses and between the retrovirus DNA and the host cell DNA. Recombination between two retroviruses gives rise to novel viral progeny with reassorted genes. Recombination between retroviruses and the host cell can give rise to novel viral progeny that carry nonviral genes. If these host genes code for growth factors, growth factor receptors, or a number of other specific cellular proteins, the recombinant retroviruses may be oncogenic.

In picornaviruses and coronaviruses, recombination takes place at the level of the interaction of the viral RNA genomes and is not believed to occur by a break-rejoin mechanism. The mechanism is currently believed to be a copy-choice mechanism. Copy-choice may occur in these RNA viruses because the viral RNA polymerase binds to only a few bases of the template RNA at any one time. Such a weak interaction of the polymerase with the template RNA would permit the polymerase, carrying its RNA strand, to disassociate from the original template nucleic acid strand and then associate with a new template RNA strand. Recombination frequencies in the range of 0.2 to 0.4 percent have been reported. Therefore, the efficiency of this mechanism of recombination is low.

In test Questions (ITQ)

What is viral recombination?

Answer

Viral recombination occurs when viruses of two different parent strains co-infect the same host cell and interact during replication to generate virus progeny that have some genes from both parents.

3.4.3 Phenotypic Variation from Recombination

Viral recombination is important because it can generate novel progeny viruses that express new antigenic and/or virulence characteristics. For example, the novel progeny viruses may have new surface proteins that permit them to infect previously resistant individuals; they may have altered virulence characteristics; they may have novel combinations of proteins that make them infective to new cells in the original host or to new hosts; or they may carry material of cellular origin that gives them oncogenic potential.

In-test Questions (ITQ)

How does recombination affect phenotype?

Recombination can rearrange entire genes and even larger units of organization. It thus has potentially much greater effects on the phenotype than mutations, in particular point mutations of single nucleotides.

3.4.4 Vaccines and Gene Therapy through Recombination

Recombination is being used experimentally by virologists to create new vaccines. Vaccinia virus, a DNA virus of the poxvirus group, was used as a live vaccine in the eradication of smallpox. Recombinant vaccinia viruses are being developed that carry vaccinia virus DNA recombined with DNA from other sources (exogenous DNA). For example, vaccinia virus strains carrying DNA coding for bacterial and viral antigens have been produced. It is expected that after vaccination with the recombinant vaccinia virus, the bacterial or viral antigen (immunogen) will be produced. The presence of this immunogen will then stimulate specific antibody production by the host, resulting in protection of the host from the immunogen. Studies with these live, recombinant vaccinia viruses are currently under way to determine whether inoculation of the skin with the recombinant virus can induce a protective host antibody response to the bacterial or viral antigens. Other studies are investigating

the use of live, recombinant adenoviruses containing bacterial or viral genes to infect the gastrointestinal tract and induce both mucosal and systemic immunity.

In a similar manner, recombinant viruses are also being developed that carry normal human genes. It is envisioned that such recombinant viruses could be useful for gene therapy. Target diseases for gene therapy span a wide range, including diabetes, cystic fibrosis, severe combined immunodeficiency syndrome, etc. Indeed, treatment of cystic fibrosis patients with replication deficient, recombinant adenoviruses bearing a normal copy of the cystic fibrosis transmembrane regulator gene has already been approved.

If these studies give positive results, such directed generation of recombinant viruses may become an important tool in the development of vaccines and for gene therapy.

In-Test Question

Answer

How does viral recombination aid in viral evolution?

Ans. Recombination events can be an evolutionary advantage for the virus when they help it evade host immune defenses, for example by changing surface protein antigenicity. Recombination with host or other organism occurs when a viral genome acquires sequences from a cellular organism.

Self-Assessment Exercise 2

Provide Answers to the following question in 10 minutes

1. Why do viruses recombine?
2. What is viral reassortment and how does it contribute to virus

3.5 Summary

- Mutations and recombinations are essential for the survival and emergence of new virus strains.
- Genetic changes can be exploited for the control virus disease.

3.6 References/Further Readings/Web Sources

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3.7 Possible Answers to Self-Assessment Exercises

SAE 1

1. Mutation is part of being a virus. Viruses mutate to adapt to their surroundings and more effectively move from host to host. Mutations can cause viruses to better evade our immune systems, treatments and vaccines. A mutation can help the virus gain traits that better help it reproduce quickly or adhere better to the surface of human cells.
2. This means that, over time, the virus may start to differ slightly in terms of its genetic sequence. Any changes to the viral genetic sequence during this process is known as a mutation and viruses with new mutations are sometimes called variants. Variants can differ by one or multiple mutations.

SAE 2

1. Viruses are in a perpetual arm race with their hosts. Camouflage is a common strategy viruses use to escape to the immune system (either innate or adaptive) of their hosts. This generally translates in a propensity to develop replication strategies that are, at different extents, prone to the insertion of mutations in their genome. Accumulation of mutations is nevertheless limited by the need to maintain viability and its own genetic identity. Keeping the subtle equilibrium between these two contrasting forces is vital for viruses, it often influences their pathogenic potential, and can be at the origin of outbreaks of infection of relevance for public health.
   Recombination is an important source of genetic variability in viruses, particularly for viruses possessing an RNA genome. The remarkable power of recombination resides in its ability, in a single infectious cycle, to generate new combinations of mutations. This is important at two regards: one is that recombination does not generate new mutations but reshuffles pre-existing ones, whose compatibility with viral survival has already been established. This is expected to increase the probability of having a viable recombinant progeny. On the other hand, the fact that, in general, several mutations are simultaneously introduced through the recombination process, is expected to favour the opposite outcome: that a high proportion of recombinant products will not be viable. Finally, recombination in concert with natural selection, can be responsible of combining advantageous mutations, as well as removing deleterious ones, by far the most abundant type of mutations found in nature.

2. Virus reassortment, or simply reassortment, is a process of genetic recombination that is exclusive to segmented RNA viruses in which co-infection of a host cell with multiple viruses may result in the shuffling of gene segments to generate progeny viruses with novel genome Combinations

UNIT 4 MODE OF TRANSMISSION AND DIAGNOSIS OF VIRAL INFECTIONS

CONTENTS

4.1 Introduction

Transmission occurs when the virus leaves its **reservoir** or host through a **portal of exit**, conveyed by some **mode of transmission**, and enters through an appropriate **portal of entry** to infect a **susceptible host**. This sequence is sometimes called the chain of infection. These modes of transmission of viruses, which include direct transmission, animal-animal transmission and arthropod transmission, shall be considered. We shall also consider the methods of diagnosing viral infections.

4.2 Learning Outcomes

At the end of the class, student must be familiar and have understood modes of viral transmission and various methods used in diagnosis of viral infections.

4.3 Mode of transmission

Different viruses have evolved ingenious and complicate mechanisms of survival in nature and transmission from one host to the next. The mode of transmission depends the nature of the interaction between virus and host. A virus may be transmitted from its natural reservoir to a

susceptible host in different ways. There are different classifications for modes of transmission. Here is one classification:

- Direct
  - Direct contact
  - Droplet spread
- Indirect
  - Airborne
  - Vehicle borne
  - Vector borne (mechanical or biologic)

4.3.1 Direct transmission

In direct transmission, viral particles are transferred from a reservoir to a susceptible host by direct contact or droplet spread. This occurs through skin-to-skin contact, kissing, and sexual intercourse. Direct contact also refers to contact with soil or vegetation harbouring infectious organisms. **Droplet spread** refers to spray with relatively large, short-range aerosols produced by sneezing, coughing, or even talking. Droplet spread is classified as direct because transmission is by direct spray over a few feet, before the droplets fall to the ground. Examples of microorganisms that are spread by droplet transmission are: influenza, colds and respiratory syncytial virus (RSV).

In-test Question (ITQ)

Write short not on direct transmission of viruses giving example
Ans.: In direct transmission, an infectious agent, the virus particle, is transferred from a reservoir to a susceptible host by direct contact or droplet spread. Direct contact occurs through skin-to-skin contact, kissing, and sexual intercourse. Direct contact also refers to contact with soil or vegetation harbouring infectious organisms. Examples of direct contact are touching, kissing, sexual contact, contact with oral secretions, or contact with body lesions. Indirect contact infections spread when an infected person sneezes or coughs, sending infectious droplets into the air.

4.3.2 Indirect transmission

This refers to the transfer of an infectious agent from a reservoir to a host by suspended air particles, inanimate objects (vehicles), or animate intermediaries (vectors). This is further classified into:

a) **Airborne transmission which** occurs when infectious agents are carried by dust or droplet nuclei suspended in air. Airborne dust includes material that has settled on surfaces and become

resuspended by air currents as well as infectious particles blown from the soil by the wind. Droplet nuclei are dried residue of less than 5 microns in size. In contrast to droplets that fall to the ground within a few feet, droplet nuclei may remain suspended in the air for long periods of time and may be blown over great distances. Measles, for example, has occurred in children who came into a physician's office after a child with measles had left, because the measles virus remained suspended in the air.

b) **Vehicles** that may indirectly transmit an infectious agent include food, water, biologic products (blood), and fomites (inanimate objects such as handkerchiefs, bedding, or surgical scalpels). A vehicle may passively carry a pathogen - as food or water may carry hepatitis A virus.

c) **Vectors** such as arthropods may carry an infectious agent through purely mechanical means or may support growth or changes in the agent.

In-Test Question (ITQ)

What is indirect mode of transmission of viruses?
Ans.: Indirect transmission refers to the transfer of an infectious agent from a reservoir to a host by suspended air particles, inanimate objects (vehicles), or animate intermediaries (vectors).

Self-Assessment Exercise 1

Provide answers to the following questions in 20 minutes

1. What are various modes of transmission of communicable disease?
2. Is Covid an airborne virus?

4.4 Diagnosis of viral infection

An accurate virus diagnosis invariably requires laboratory testing of clinical specimens for the presence of virus, viral antigens, or specific antibodies. The past few decades have seen a major revolution in the operation of virus diagnostic laboratories and in their role in clinical patient management. Virus isolation has been largely replaced by sensitive nucleic acid detection assays and the measurement of specific antibodies at a very high level of sensitivity and specificity.
The modern virus diagnostic laboratory is characterized by high-test throughputs, rapid turnaround times, and a close liaison with clinical staff. Many of the older and slower diagnostic approaches such as animal inoculation, virus isolation in cell culture, and serological

demonstration of a four-fold rise in antibody titre, are now of minor importance, if practiced at all.

4.4.1 Virus Isolation

A wide variety of samples can be used for virological testing. The type of sample sent to the laboratory often depends on the type of viral infection being diagnosed and the test required. Proper sampling technique is essential to avoid potential pre-analytical errors. For example, different types of samples must be collected in appropriate tubes to maintain the integrity of the sample and stored at appropriate temperatures (usually 4°C) to preserve the virus and prevent bacterial or fungal growth. Sometimes multiple sites may also be sampled.

Types of samples include the following:

- Nasopharyngeal swab
- Blood
- Skin
- Sputum, gargles and bronchial washings
- Urine
- Semen
- Faeces
- Cerebrospinal fluid
- Tissues (biopsies or post-mortem)
- Dried blood spots

For example, a nasal mucus test may be done to diagnose rhinovirus.

In recent years, virus isolation has changed from its role as the “gold standard” of the diagnostic laboratory, to occupying more of a research or historical role. Newer techniques, particularly sensitive antigen EIAs and PCRs, do not suffer from the drawbacks of virus isolation that included high cost, slowness, false negatives due to virus being inactivated during transport or complexed with antibody, cell lines losing sensitivity, and increased concerns about live virus transmission to staff. Many diagnostic laboratories no longer try to isolate viruses in cell culture; virus isolation in cell culture is mostly limited to research laboratories or where there is a need to grow up large amounts of virus. Even bulk antigen production is now often done preferentially by expression of recombinant antigens, and unknown agents can be sought using molecular techniques such as random-priming PCR. Similarly, techniques for animal inoculation, for example, suckling mice, and egg inoculation procedures, are only performed where a laboratory has a specialist need, for example, influenza virus to be used in vaccine production is grown in embryonated hens’ eggs.

In-test Question (ITQ)

What is the importance of isolating virus particles?

Answer

Virus isolation is necessary for preparation of the sample in adequate quantity and quality for identification. Samples are collected in many forms and quality which are not appropriate for diagnosis, and isolating the virus particles will improve the quality and provide adequate quantity for identification and detection.

4.4.2 Specimen

All specimens must be safely contained for transport to the lab. Each specimen must be clearly labeled and should be accompanied by relevant information. Isolation of active virus requires proper collection of appropriate specimens, their preservation, both en-route to and in the laboratory, and inoculation in suitable cell cultures, susceptible animals or embryonated eggs. The various specimens to be collected in a particular viral disease, the agent involved and the various or appropriate diagnostic test could be summarized in the control. A positive reaction is taken to indicate resistance to infection with some viruses.

In-test Question (ITQ)

What is a specimen.

Answer

A specimen is sample collected from the target organ most closely associated with clinical symptoms to identify the etiologic agent responsible for the patient's disease. They are labelled and transport appropriately without compromising the quality. It is important to select the appropriate specimens, collect the specimen carefully to optimize recovery of the infectious agent, and transport the specimens as directed so as to maintain viability and minimize overgrowth with contaminating organisms.

4.4.3 Neutralization test (Nt-test)

Virus neutralizing antibodies is determined or measured by adding serum containing these antibodies to a suspension of virus and then inoculating the mixture into specific cell cultures. The presence of neutralizing antibodies is demonstrated if the cell culture fails to develop

CPE while control cell cultures which have received virus plus a serum free of Neutralizing antibody develop CPE. To establish a diagnosis, one looks for a significant rise in antibody titer - (4 fold or greater is desirable) during the course of the infection. Neutralizing antibodies can persist for year and their presence may indicate a infection in a given individual. The Nt-tests are useful in serologic, epidemiology, in which it is important to know which viral agents have infected a given population in the past. Although Nt - tests principle is simple but are expensive in time and materials and must be standardized for each viral agent. Among the variables to be considered are:

1. Selection of cell culture, experimental animal or embryonated egg.
2. Rate of inoculation of virus-serum mixture.
3. Age of test animals.
4. Stability of the test virus.
5. Reproducibility of the end-point.
6. Relative heat-stability of the specific antibody and possible, interfering substance in serum.
7. Addition of an accessory factor found in freshe4ôrmal serum of the homologous species.
8. Use of one concentration of virus and varying dilutions (and the relationship between varying concentrations of each).
9. Temperature of the neutralizing mixtures.
10. Incubation time for the mixture.

In-test Question (ITQ)

How can a virus be detected in cell culture?

Answer

In neutralization testing, the virus-infected cells are incubated with antibodies of known viral specificity; an aliquot of the mixture is then inoculated into susceptible cell cultures, and the cell cultures are observed for evidence of viral proliferation.

4.4.4 Identification of Virus, Viral Antigen, or Viral Genome

Direct detection of virus material in a patient sample has become the method of choice for a very large number of different infections. These methods have the advantage of speed, as they do not rely on virus culture or a rise in antibody titre. The sensitivity and specificity of antigen detection methods have increased greatly through use of high-quality reagents and solid phase reagent supports allowing ease of washing and signal detection; the sensitivity of nucleic acid detection

has advanced from early dot blot and Southern blot assays to the widespread and reliable use of PCR.

Direct Detection of Virions by Electron Microscopy: The morphology of most viruses is sufficiently characteristic to allow assigning many viruses to the correct family by appearance in the electron microscope. Moreover, viruses that have never been cultured may nevertheless be recognized. During the 1970s electron microscopy was the means to the discovery in faeces of several new groups of previously non-cultivated viruses. The human rotaviruses, caliciviruses, astroviruses, hepatitis A virus, and previously unknown types of adenoviruses and coronaviruses were all initially identified in this way. The method can be quick for one or several samples only, but is impractical for large batch testing.

Enzyme Immunoassay (EIA): The introduction of EIA, also known as enzyme-linked immunosorbent assay (ELISA), revolutionized diagnostic virology in the days before the development of polymerase chain reaction (PCR), and still has widespread specific uses. EIAs can be designed in different formats to detect antigen or antibody. The high sensitivity of the method means that less than 1ng of viral antigen per milliliter can be detected in specimens taken directly from a patient.

Radioimmunoassay and Time-Resolved Fluoroimmunoassay: Radioimmunoassay (RIA) predates EIA. The only significant difference is that the label is not an enzyme but a radioactive isotope such as ^{125}I, and the bound antibody is measured in a gamma counter. RIA is a highly sensitive and reliable assay that lends itself well to automation, but the cost of the equipment and the health hazard of working with radioisotopes argue against its use in small laboratories.

Latex Particle Agglutination: Perhaps the simplest of all immunoassays is the agglutination by antigen of small latex beads previously coated with antiviral antibody. The test can be read by eye within minutes.

Immunochromatography: Immunochromatography or lateral flow tests involve the migration of an antigen, or antigen–antibody complexes, through a support, for example, nitrocellulose film, filter paper, or agarose.

Detection of Antigen in Tissues: Viral (or non-viral) antigens can be detected in fixed or frozen tissue sections, or in exfoliated cells, using the same principles as above, where the cells or tissues on a glass slide fill the role of the solid support. Commonly used indicators include fluorescein, horseradish peroxidase, and alkaline phosphatase.

Detection of Viral Nucleic Acids

Nucleic acid detection is now widely applied by use of polymerase chain reaction (PCR) assays, combined with advances in oligonucleotide synthesis, standardized automated procedures for nucleic acid extraction, and real-time detection of PCR products. Rapid advances in nucleic acid sequencing technology and the availability of sequence databases have greatly enhanced analysis of the results obtained.

Nucleic Acid Hybridization: From the time of the development of the first nucleic acid hybridization techniques, a variety of test formats were applied to viral nucleic acid detection.

Polymerase Chain Reaction: PCR constitutes one of the greatest advances in molecular biology. It enables a single copy of any gene sequence to be enzymatically amplified *in vitro* at least a million-fold within a few hours. Thus viral DNA extracted from a very small number of virions or infected cells can be amplified to the point where it can be readily identified. PCR can also be used to detect viral RNA by including a preliminary step in which reverse transcriptase is used to convert RNA to DNA.

Microarray Technologies: Another technological advance that is impacting the field of diagnostics is the use of microarrays or microchips for nucleic acid detection. The microchip is a solid support matrix onto which have been "printed" spots, each containing one of several hundred to several thousand unique oligonucleotides.

Next-Generation Deep Sequencing: "Deep" sequencing, or "next-generation" sequencing refers to novel techniques of DNA sequencing directly from DNA fragments without the need for cloning in vectors, allowing the generation of enormous amounts of sequence data at high speed and low cost from a single run. A good example is the detection of different strains and variants of SARS-Cov 2 (COVID-19). This revolutionised molecular virology worldwide.

Applications of Serology

Serological tests are used for many important purposes apart from diagnosing or excluding acute or chronic infection. These assays are valuable in many contexts because these provide indications of both clinical and subclinical infections, thereby giving a truer record of *total* number of infections. The finding of antibodies to a virus in a single sample carries very different clinical implications depending on the virus. The screening for immunity is very useful to document successful immunization in an individual, and to check the coverage and efficacy

of vaccination in a population. In addition, testing the susceptibility of the close contacts of an individual with a potentially dangerous infection allows for the protection of at-risk (non-immune) individuals by segregation or immunization, and provides a baseline for subsequent monitoring as to the course of infection.

In-Test Question

When is Serology-based detection method the most appropriate for viral infection?

Answer

Serology-based detection methods will be desirable when any of the following situation is in place:

- a previous symptomatic / asymptomatic infection has been observed;
- In response to cross reacting antigen; and
- after vaccination.

In all these cases the level of the antibody will remain near the same for the entire duration of current illness.

Self-Assessment Exercise 2

Provide answer to the following question in 20 minutes

What are the quality assurance and control measures to be considered in laboratory diagnosis of viral infections?

4.5 Summary

- There are modes of transmitting viruses namely, direct transmission, animal-animal transmission and arthropod transmission.
- Good diagnostic virology depends on rapid communication between the physician and the laboratory and on the quality of specimens and information supplied to the laboratory.
- Antibody testing or isolation of virus are used in diagnosing viral infections

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4.7 Possible Answers to Self-Assessment Exercises

SAE 1

1. A communicable disease is one that is spread from one person to another through a variety of ways that include: contact with blood and bodily fluids; breathing in an airborne virus; or by being bitten by an insect.
2. Spread of COVID-19 occurs via airborne particles and droplets. People who are infected with COVID can release particles and droplets of respiratory fluids that contain the SARS CoV-2 virus into the air when they exhale (e.g., quiet breathing, speaking, singing, exercise, coughing, sneezing).
3. Direct contact transmission involves immediate contact between two people (or with an animal). Indirect contact transmission involves an object that becomes contaminated by touch then spreads the infection by touch.

SAE 2

The ability of the laboratory to provide accurate diagnostic results is essential for effective clinical management of patients, solving of outbreaks and for responsible decision making. Therefore monitoring ongoing quality assurance (QA) and improvement in all aspects of the laboratory is crucial. This involves the managing and monitoring of all services and processes related to releasing a diagnostic results. Processes that should be monitored relate to the pre-analytical phase *i.e.* specimen transport, collection and storage; the analytical phase *i.e.* testing and monitoring of the laboratory procedures and environment as well as the post analytical phase *i.e.* of result reporting and result interpretation. QA ensures annual assessment of staff competency, calibration and servicing of equipment as well as the quality of diagnostic tests. Clinical laboratories are strictly regulated by appointed agencies and are audited according to specific standards set forth by the International Organization for Standardization (ISO) and the International Electrotechnical Commission (IEC).

The primary quality control (QC) concern in a molecular laboratory is the specimen and nucleic acid quality or integrity, assay sensitivity and specificity, as well as the false positive tests because of PCR contamination. The RNA and DNA integrity can be insured through use of RNase and DNase free reagents and consumables, in addition to

handling specimens on ice. While, PCR contamination can be avoided through physical organization of the laboratory and workflow, separating work areas and equipment; relevant PCR controls should be included in each run to ensure correct interpretation of the results. The use of uracil N-glycosylase (UNG) in PCR reactions provides for chemical control for carry over contamination.

It is vital that when PCR diagnostics are undertaken, every effort is made to minimize contamination and that these assays are tested in a laboratory environment in which staff are well trained and competent for this type of work. Using an accredited laboratory ensures the diagnostic findings are reliable.

UNIT 5 CONTROL AND TREATMENT OF VIRAL DISEASES

CONTENTS

5.1 Introduction

In this unit, we shall be looking into the roles and uses of interferons in managing viral diseases as well as anti-viral drugs.

5.2 Learning Outcome

At the end of the class, student must be familiar and have understood the mode of actions of inteferons and antiviral drugs and their various types.

5.3 Interferons (IFN)

Interferons are host-coded proteins (non-toxic antiviral agent) that inhibit viral replication, they are produced by intact animals or cultured cells in response to viral infection or other inducers. They are produced by all vertebrates' species but generally, normal cells do not produce or synthesize interferons unless they are induced to do so. They are believed to be the body's first line of defence against potent insult by viruses to the cells. IFN modulates humoral and cellular immunity and have broad cell growth regulatory activities. IFNs also have various other functions: they activate immune cells, such as natural killer cells and macrophages; they increase host defenses by up-regulating antigen presentation by virtue of increasing the expression of major histocompatibility complex (MHC) antigens. Interferons could also be induced by dsRNA, bacteria endotoxin and small molecules such as

Tilorone. RNA-viruses are stronger inducers of IFN than DNA-viruses. All viruses are inhibited by IFN but RNA-viruses are more susceptible to it than DNA-viruses. IFN are active only in cells of the same animal species in which it was formed. There are 3-main types of IFN, Alpha, Beta and Gamma interferons. They are similar in size hut differ distinctly in antigenic nature. Alpha IFN and Beta-IFN are resistant to low pH. Beta-IFN and Gamma-IFN are glycosylated but the sugars are not necessary for biologic activity, so cloned IFN produced in bacteria are biologically active. The different classes of IFN are produced by different cell types. Alpha-IFN is synthesized predominantly by leukocytes, Beta-IFN are produce mainly by fibroblasts while gamma IFN are produced only by lymphocytes. Due the fact that the amounts of IFN synthesized by induced cells are quite small it has been difficult to purify and characterize the proteins. With recombinant-DNA techniques, cloned IFN genes are being expressed in large amount in bacteria and yeast, and the availability of genetically engineered IFN makes clinical studies feasible.

5.3.1 Mode of Action of IFN

IFN are always host species-specific in function. By contrast, IFN-activity is not specific for a given virus, the replication of a wide variety of viruses can be inhibited. When IFN is added to cell prior to infection, there is marked inhibition of viral replication but nearly normal cell function. IFN are extremely potent, so that very small amount is required for function. It has been estimated that fewer than 50 molecules of IFN per cell are sufficient to induce the antiviral states.

The mechanism of action of IFN is still poorly understood. It is however established that IFN is not the antiviral agent; rather IFN induces an antiviral state by promoting the synthesis of other proteins that actually inhibit viral replication. IFN act by binding to cell surface receptors, with alpha-lFN and beta-IFN sharing a common receptor and gamma recognizing a distinct receptor. This binding triggers the synthesis of several enzymes believed to be instrumental in the development of the antiviral state. These cellular enzymes subsequently block viral reproduction by inhibiting the translation of viral mRNA into viral protein. The mode of IFN action is from two points.

1. Degradation of Viral mRNA: This is basically done by an enzyme, endonuclease. The enzyme is activated by the presence of oligonuleotide synthetase, 2-5A synthetase both of which is needed for oligoadenulic acid, 2,5-oligoadenulic acid, a formation which in turn, degrades the viral mRNA.
2. Inhibition of protein synthesis: A protein kinase phosphorylates and inactivates a cellular initiation factor, elF-2) and thus

prevents the formation of the initiation complex needed for translation of viral proteins. Interferons may also affect viral assembly, perhaps as a result of change at the plasma membrane.

It is noteworthy that IFN has been shown to have toxic side effects even when purified material is tested. Gastro intestinal and nervous system side effects proportionate of the dose given are common. Bone marrow suppression also may occur. Theoretically, IFN inducers could be administered therapeutically but every inducer that have been carefully studied have also been found to be toxic.

In-test Question (ITQ)

How do interferons work in the body?

Ans.:Interferons are proteins that are part of your natural defenses. They tell your immune system that germs or cancer cells are in your body. And they trigger killer immune cells to fight those invaders. Interferons got their name because they "interfere" with viruses and keep them from multiplying.

5.3.2. Uses of IFN

1. They have been shown to be effective against (prevents) Rhinovirus when administered intranasally.
2. They inhibit vaccinia infection when administered intradermally.
3. Chronic active hepatitis due to hepatitis B-virus could be also be prevented but not a dramatic effect and also herpes-keratitis Zoster and cytomegalovirus infections.
4. IFN has been shown to have significant effect on several human tumors e.g. Sarcoma, breast cancer, lymphoma, myeloma.
5. IFN is at present used for trials in cancer patients.

The properties and types of human interferons are presented in the table below (Table 1).

Table 1. Properties of human interferons

S/N	PROPERTY	TYPE		
		ALPHA	BETA	GAMMA
1	Current nomenclature	IFN α	IFN β	IFN γ
2	Former designation	Leukocytes	Fibroblast	Immune
3	Number of genes that codes for that family	14	>2	1

4	Principal cell source	Leukocytes	Fibroblast	Lymphocytes
5	Size of protein (MW)	17,000	17,000	17,000
6	Inducing agent	Viruses	Mitogens	
7	Stability at pH 2.0	Stable	Stable	Labile
8	Glycosylated	No	Yes	yes
9	Introns in genes	No	No	Yes

In-Test Question

What is the role of interferon in viral infection?

Ans.: Summary. Interferons can alter the course of virus infections by inhibiting virus replication at the intracellular level and by modifying the aspecific and specific immune response to viral antigens in body fluids and on cellular surfaces.

Self-Assessment Exercise 1

Provide short answers to the following questions in 20 minutes

1. How does interferon act against viruses?
2. How do interferons protect against infection in healthy cells?
3. What are the biological effects of interferons?
4. Why is interferon so important
5. What are the functions of interferons?

5.4 Anti-viral drugs

Since viruses are obligate intracellular parasites, a good antiviral agent must be capable of selectively inhibiting viral functions without damaging the host cells. Molecular virology studies have now succeeded in identifying virus specific functions that can serve as realistic targets for inhibition. Theoretically, any stage in viral replicative cycle could be a target for antiviral therapy. Recently, compounds have been found that are of value on treatment of viral diseases, while other compounds appear promising. Seven antiviral drugs are currently licensed for use, i.e. Acyclovir, Amantadine, doxuridine, Trifluriridine, Vidarabine, Ribavirin, and Azidothymidine. All these have one or more side effects on the host, hence an ideal antiviral agents remain to be developed.

The majority of available antiviral agents are nucleoside analogues. Analogues inhibit nucleic acid replication by inhibition of enzymes of the metabolic pathways for purines and pyrimidines or by inhibition of

polymerases for nucleic acid replication. In addition, some analogues can be incorporated into the nucleic acid and block further synthesis or alter its function. Analogues can inhibit cellular enzymes as well as virus-encoded enzymes. The new types of analogues are those able to inhibit specifically virus-encoded enzymes, with minimal inhibition of analogues of host cell enzymes.

In-Test Question (ITQ)

What are examples of antiviral drugs?

Answer

Zanamivir, peramivir, and oseltamivir are active against both influenza A and influenza B. Zanamivir is given by inhalation only, peramivir is given intravenously, and oseltamivir can be given orally. These drugs are inhibitors of neuraminidase, a glycoprotein on the surface of the influenza virus.

Self-Assessment Exercise 2

Provide short answers to the following questions in 10 minutes

1. What is best medicine for viral infection?
2. What antibiotics treat viral infections?

5.5 Vaccination

Vaccination is the administration of a vaccine to help the immune system develop immunity from a disease. Vaccines contain a microorganism or virus in a weakened, live or killed state, or proteins or toxins from the organism. In stimulating the body's adaptive immunity, they help prevent sickness from an infectious disease. When a sufficiently large percentage of a population has been vaccinated, herd immunity results. Herd immunity protects those who may be immunocompromised and cannot get a vaccine because even a weakened version would harm them. Immunity to viral infection is based on the development of an immune response to specific antigens located on the surface of virus-particles or virus infected cells. For enveloped viruses, the important Antigens are the surface glycoproteins. Vaccines are available for the prevention of several significant human diseases hut certain general principles apply to most virus vaccine as for use in the prevention of human disease. Viruses that have a viremic mode of spread e.g. polio, hepatitis and measles are controlled by serum antibodies. Cell mediate immunity also is involved in protection against systemic infection e.g. Measles, herpes.

Neither vaccination nor recovery from natural infection always results in total protection against a later infection with the same virus. Control can be achieved by limiting the multiplication of virulent virus upon subsequent exposure and preventing its spread to target organs where the pathologic damage is done. e.g polio and measles viruses must be kept from the brain and spiral cord; Rubella virus must be kept from the embryo. There are various types of vaccine available for use against viral infections and diseases (Table 2):

1. Killed-Virus Vaccine: These are made of purifying viral preparations to a (certain extent and then inactivating viral infectivity in a way that does minimal damage to the viral structural proteins. This done by treating the virus with mild formalin. Killed virus vaccines prepared from a virion stimulate the development of circulating antibody against the viral protein coats; thus conferring some degree of resistor ace. For some diseases, only this type of vaccine is available for its prevention.

2. Live-Attenuated Virus Vaccine: Here the viruses used here are virus mutants that antigenically overlap with the wild-type but are restricted in some steps in the pathogenesis of disease. The development of virus strains suitable for live virus vaccines previously was done by selecting naturally attenuated strains or by cultivating the virus serially in various hosts and cultures in the hope of deriving an attenuated strain fortuitously. The search for such strains is now being approached by laboratory manipulations aimed at specific, planned genetic alterations in the virus (e.g. Rabies Influenza, respiratory syncytial viruses).

Attenuated virus vaccines have the advantages of acting like the natural infection with regard to their effect on immunity. They multiply in the host and stimulate longer-lasting antibody production, to induce a good cell-mediated response, and to induce antibody production and resistance at the portal of entry.

The proper usage, strong and maintenance of vaccine in an important factor that determines its efficacy.

Table 2. Principal vaccines used in the prevention of viral diseases of human

S/N	Disease	Source of vaccine	Condition of virus	Route of administration
1	Yellow fever	Tissue culture and eggs (17D strain)	Live attenuated	Subcutaneous
2	Hepatitis B	HBsAg from recombinant DNA	Sub-unit	Subcutaneous

		yeast		
3	Adenovirus 6	human diploid cell cultures	Live attenuated	Oral by enteric coated capsule
4	Influenza	Highly purified or sub-units from chick embryo allantoic fluids	Killed	Subcutaneous
5	Japanese B encephalitis	Formalized mouse brain, tissue culture	Killed	Subcutaneous

In-Test Question (ITQ)

What are the possible routes of administration of vaccines?

A vaccine administration may be oral, by injection (intramuscular, intradermal, subcutaneous), by puncture, transdermal or intranasal. Several recent clinical trials have aimed to deliver the vaccines via mucosal surfaces to be up-taken by the common mucosal immunity system, thus avoiding the need for injections.

Self-Assessment Exercise 3

Provide answer to the following question in 10 minutes

Discuss the types of Vaccine Platforms available

5.6 Current development in vaccine production

At least nine different technology platforms are under research and development to create an effective vaccine against COVID-19. Most of the platforms of vaccine candidates in clinical trials are focused on the coronavirus spike protein (S protein) and its variants as the primary antigen of COVID-19 infection, since the S protein triggers strong B-cell and T-cell immune responses. Platforms developed in 2020 involved nucleic acid technologies (nucleoside-modified messenger RNA and DNA), non-replicating viral vectors, peptides, recombinant proteins, live attenuated viruses, and inactivated viruses.

mRNA vaccines: Several COVID-19 vaccines, including the Pfizer-BioNTech and Moderna vaccines, have been developed to use RNA to stimulate an immune response. When introduced into human tissue, the vaccine contains either self-replicating RNA or messenger RNA (mRNA), which both cause cells to express the SARS-CoV-2 spike protein. This teaches the body how to identify and destroy the corresponding pathogen. RNA vaccines often, but not always, use

nucleoside-modified messenger RNA. The delivery of mRNA is achieved by a coformulation of the molecule into lipid nanoparticles which protect the RNA strands and help their absorption into the cells.

Adenovirus vector vaccines: These vaccines are examples of non-replicating viral vector vaccines, using an adenovirus shell containing DNA that encodes a SARS-CoV-2 protein. The viral vector-based vaccines against COVID-19 are non-replicating, meaning that they do not make new virus particles, but rather produce only the antigen which elicits a systemic immune response. Authorized vaccines of this type are the Oxford-AstraZeneca COVID-19 vaccine, the Sputnik V COVID-19 vaccine, Convidecia, and the Janssen COVID-19 vaccine.

Inactivated virus vaccines: Inactivated vaccines consist of virus particles that are grown in culture and then killed using a method such as heat or formaldehyde to lose disease producing capacity, while still stimulating an immune response. Authorized vaccines of this type are the Chinese CoronaVac and the Sinopharm BIBP and WIBP vaccines; the Indian Covaxin.

Subunit vaccines: Subunit vaccines present one or more antigens without introducing whole pathogen particles. The antigens involved are often protein subunits, but can be any molecule that is a fragment of the pathogen. The authorized vaccines of this type are the peptide vaccine EpiVacCorona, MVC-COV1901, and Corbevax.

Intranasal: Intranasal vaccines target mucosal immunity in the nasal mucosa which is a portal for viral entrance to the body. These vaccines are designed to stimulate nasal immune factors, such as IgA. In addition to inhibiting the virus, nasal vaccines provide ease of administration because no needles (and the accompanying needle phobia) are involved. Nasal vaccines have been approved for other infections, such as influenza. As of 2021, only one nasal vaccine, *Flumist* (USA); *Fluenz Tetra* (European Union), had been authorized in the United States and Europe for use as an influenza vaccine.

Other types: Additional types of vaccines that are in clinical trials include virus-like particle vaccines, multiple DNA plasmid vaccines, at least two lentivirus vector vaccines, a conjugate vaccine, and a vesicular stomatitis virus displaying the SARS-CoV-2 spike protein.

In-Test Question

Using SARS-Cov-2 as an example, briefly discuss how vaccines work

Answer

SARS-CoV-2 is the virus that causes COVID-19. The **spike protein** on the surface of SARS-CoV-2 is an example of an **antigen**. Vaccines are the best way to train our immune system to recognize viruses, or pieces of viruses, called **antigens**. Our immune system creates **antibodies** and other defenses to protect us. When a vaccinated person is exposed to SARS-CoV-2, their immune system will recognize the viral antigens and spring into action to keep them healthy. There are many different types of vaccines, as seen in the diagram above.

Self-Assessment Exercise 4

Provide answer to the following question in 10 minutes
Distinguish between vaccination and inoculation

5.7 Summary

- Inteferons are host-coded proteins (non-toxic antiviral agent) that inhibit viral replication, they are produced by intact animals or cultured cells in response to viral infection or other inducers
- They act by degrading viral mRNA and inhibiting protein synthesis.
- Seven antiviral drugs are currently licensed for use, i.e. Acyclovir, Amantadine, Doxuridine, Trifluriridine, Vidarabine, Ribavirin, and Azidothymidine.
- All these have one or more side effects on the host, hence an ideal antiviral agents remain to be developed

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5.9 Possible Answers to Self-Assessment Exercises

SAE 1

1. Interferons (IFNs) - the body's first line of antiviral defence - are cytokines that are secreted by host cells in response to virus infection. By inducing the expression of hundreds of IFN-stimulated genes, several of which have antiviral functions, IFNs block virus replication at many levels.
2. Interferons block viral reproduction in healthy cells through the production of antiviral proteins.
3. Interferons (IFNs) are potent pleiotropic cytokines that broadly alter cellular functions in response to viral and other infections. These alterations include changes in protein synthesis,

proliferation, membrane composition, and the nutritional microenvironment.

4. Interferons (IFNs) constitute the first line of defense against microbial infections particularly against viruses. They provide antiviral properties to cells by inducing the expression of hundreds of genes known as interferon-stimulated genes (ISGs).
5. All interferons share several common effects: they are antiviral agents and they modulate functions of the immune system. Administration of Type I IFN has been shown experimentally to inhibit tumour growth in animals, but the beneficial action in human tumours has not been widely documented. In addition, interferons induce production of hundreds of other proteins - known collectively as interferon-stimulated genes (ISGs) - that have roles in combating viruses and other actions produced by interferon. They also limit viral spread by increasing p53 activity, which kills virus-infected cells by promoting apoptosis. The effect of IFN on p53 is also linked to its protective role against certain cancers.

Another function of interferons is to up-regulate major histocompatibility complex molecules, MHC I and MHC II, and increase immunoproteasome activity. All interferons significantly enhance the presentation of MHC I dependent antigens. Interferon gamma (IFN-gamma) also significantly stimulates the MHC II-dependent presentation of antigens. Higher MHC I expression increases presentation of viral and abnormal peptides from cancer cells to cytotoxic T cells, while the immunoproteasome processes these peptides for loading onto the MHC I molecule, thereby increasing the recognition and killing of infected or malignant cells. Higher MHC II expression increases presentation of these peptides to helper T cells; these cells release cytokines (such as more interferons and interleukins, among others) that signal to and co-ordinate the activity of other immune cells.

Interferons can also suppress angiogenesis by down regulation of angiogenic stimuli deriving from tumor cells. They also suppress the proliferation of endothelial cells. Such suppression causes a decrease in tumor angiogenesis, a decrease in its vascularization and subsequent growth inhibition. Interferons, such as interferon gamma, directly activate other immune cells, such as macrophages and natural killer cells.

SAE 2

1. Antiviral medications help the body fight off harmful viruses. The drugs can ease symptoms and shorten the length of a viral

infection. Antivirals also lower the risk of getting or spreading viruses that cause herpes and HIV.

2. Antibiotics do not work for viral infections. There are antiviral medicines to treat some viral infections. Vaccines can help prevent you from getting many viral diseases.

SAE 3

All vaccine platforms are designed to train our immune system. There are two categories of COVID-19 vaccines:

(a) Component Viral Vaccines and
(b) Whole Virus Vaccines.

a) Component Viral Vaccines

- **Protein Subunit**: Contains isolated and purified viral proteins
- **Virus-like particles (VLP)**: Contains viral proteins that mimic the structure of the virus, but no genetic material
- **DNA-based** and **RNA-based**: Contains viral genetic material (such as mRNA) which provides the instructions for making viral proteins
- **Non-replicated viral vector**: Contains viral genetic material packaged inside another harmless virus that **cannot** copy itself
- **Replicating viral vector**: Contains viral genetic material packaged inside another harmless virus that **can** copy itself

b) Whole Virus Vaccines

- **Inactivated**: Contains copies of the virus that have been killed (inactivated)
- **Live-Attenuated**: Contains copies of the virus that have been weakened (attenuated)

SAE 4

Distinguish between vaccination and inoculation

The term inoculation is often used interchangeably with vaccination. However, while related, the terms are not synonymous. Vaccination is treatment of an individual with an attenuated (i.e. less virulent) pathogen or other immunogens, whereas inoculation, also called variolation in the context of smallpox prophylaxis, is treatment with unattenuated variola virus taken from a pustule or scab of a smallpox sufferer into the superficial layers of the skin, commonly the upper arm. Variolation was often done 'arm-to-arm' or, less effectively, 'scab-to-arm', and often

caused the patient to become infected with smallpox, which in some cases resulted in severe disease.

Glossary

CCCVd - Coconut cadang-cadang *viroid*
CNS – Central nervous system
COVID-19 – Coronavirus disease 19
CPE – Cytopathic effect
$CsCl_2$ – Caesium chloride
CTiVd - coconut tinangaja viroid
D-RNAs - defective RNAs
dsRNA – Double stranded Ribonucleic acid
EBOV - Ebola virus
EIA - Enzyme Immunoassay
ELISA - enzyme-linked immunosorbent assay ()
GRV - *Groundnut rosette virus*
H1N1 – Hemagglutinin 1 neuraminidase 1
HRSV -human respiratory syncytial virus
IFN - Interferons
MHC - major histocompatibility complex ()
mRNA – messenger Ribonucleic acid
NNS - nonsegmented negative-sense
Nt-test - Neutralization test
PCR Polymerase chain reaction
pH - Potential of Hydrogen
PLMVd – Peach latent mosaic viroid
PSTVd – Potato spindle tuber viroid
PTGS- Posttranscriptional gene silencing
RABV - rabies virus
RdRp - RNA-dependent RNA polymerase
RdRps - RNA-dependent RNA polymerases
RNA – Ribonucleic acid
RSV - respiratory syncytial virus
SARS-Cov 2 - Severe acute respiratory syndrome coronavirus 2
satRNA – Satellite RNA
ssDNA – Single stranded Deoxyribonucleic acid
SVs - Satellite viruses
TPMVd - Tomato planta macho viroid
UTR – Untranslated regions

End of Module Questions

Multiple Choice Questions

1. Viroids are made up of both single stranded positively-sensed RNAs and DNAs molecules. a) True, b) False
2. Viroids infect only plants. a) True, b) False
3. Symptom expression in viroid-infected plants are made more pronounced by _____. a) drought and light intensity, b) high temperature and drought, c) high temperature and light intensity, d) low temperature and light intensity
4. Viroids infecting plants are transmitted primarily by the following except _____ a) planthoppers, b) aphids, c) seed, d) mechanically
5. Satellite viruses can only replicate in the host in the presence of a helper-virus. a) True, b) False
6. The sequence of transmission of a virus from one host to another is called _____. a) means of transportation, b) chain of infection, c) transmission partway, d) epidemiology
7. The appropriate temperature for the storage of virus samples is ____ degree Celsius. a) 10, b) 20, c) 4, d) 27
8. Which of the following phase determines the specificity of the virus? a) Uncoating, b) Release, c) Attachment, d) Penetration
9. Which of the following is not a category of the method of virus detection? a) Nucleic acid detection, b) Serology, c) Hematology, d) Multiplication
10. Which of the following organelle prevents the entry of viruses in plant cells? a) Cell wall, b) Golgi bodies, c) Plasma membrane, d) Mitochondria
11. Satellite viruses do not encode enzymes for replication. a) True, b) False
12. Which of the following is not a property of an ideal vaccine? a) It should be genetically stable, b) It should have private support, c) It should be affordable, d) It should not have any side effects
13. The process of producing a virus which causes a reduced amount of disease for use as a live vaccine is called _____. a) attenuation, b) avirulent, c) virulent, d) adjuvant
14. Which of the following vaccine contains a mutant strain of a virus that has been derived from a wild-type virulent strain? a) Inactivated virus vaccines, b) Live recombinant virus vaccines, c) Virion subunit vaccines, d) Live attenuated virus vaccines
15. Which of the following viruses are made by the mass production of virulent viruses? a) Inactivated virus vaccines, b) Live recombinant virus vaccines, c) Virion subunit vaccines, d) Live attenuated virus vaccines

16. Which of the following antiviral drug is used to treat the infection from herpes simplex virus? a) Aciclovir, b) Azidothymidine, c) Dideoxycytidine, d) Dideoxyinosine
17. Which of the following is used to treat the infection from cytomegalovirus? a) Dideoxycytidine, b) Azidothymidine, c) Ganciclovir, d) Dideoxyinosine
18. Which of the following is used in the treatment of infection from RNA viruses? a) Zidovudine, b) Ribavirin, c) Ritonavir, d) Enfuvirtide
19. The fusion of _____ and _____ occurs during the entry of viral genome into the cell. a) lipid bilayer and plasma membrane, b) lipid bilayer and cell wall, c) carbohydrate layer and cytoplasm, d) virus and host cell
20. The entry of picornaviruses is inhibited by zanamivir. a) True, b) False
21. . Replicases are used in _____. a) translation, b) replication, c) transcription, d) post-translation
22. The error rate during RNA replication is approximately _____. a) 10^{-5} to 10^{-7}, b) 10^{-7} to 10^{-9}, c) 10^{-3} to 10^{-5}, d) 10^{-1} to 10^{-3}
23. 6. Which of the following is programmed cell death? a) Necrosis, b) Toxicity, c) Apoptosis, d) Infection
24. The active form of the reverse transcriptase enzyme is a _____. a) tetramer, b) trimer, c) dimer, d) monomer
25. Interferon-α is synthesized by lymphocytes. a) True, b) False
26. Which of the following family does Measles virus belong to?, a) Flaviviridae, b) Bunyaviridae, c) Filoviridae, d) Paramoxyviridae
27. Which of the following organelle prevents the entry of viruses in plant cells? a) Cell wall, b) Golgi bodies, c) Plasma membrane, d) Mitochondria
28. Which of the following virus promotes cell death by apoptosis? a) Rubella virus, b) HSV, c) Vaccinia virus, d) Myxoma virus
29. Which of the following organ does influenza virus infects? a) heart, b) liver, c) respiratory system, d) kidney
30. Which of the following virus is spread by arthropods? a) HIV, b) Influenza virus, c) Arbovirus, d) Rhinovirus
31. Which of the following virus infects the gastrointestinal tract? a) Rubella virus, b) Norwalk virus, c) Mumps virus, d) Parvovirus
32. . Which of the following is used in the treatment of infection from RNA viruses? a) Enfuvirtide, b) Ritonavir, c) Ribavirin, d) Zidovudine
33. Which of the following is the genome of poliovirus? a) dsRNA, b) dsDNA, c) ssDNA, d) ssRNA
34. From which of the following rhinovirus cannot be isolated? a) sputum, b) throat, c) fecal, d) nose

35. The herpesvirus virion's inner core is surrounded by _____. a) amorphous legument, b) icosahedral capsid, c) envelope, d) spikes
36. The herpes virus contains _____ genome. a) linear dsDNA, b) circular dsDNA, c) linear ssDNA, d) circular ssDNA
37. Which of the following subfamily contains the Epstein-Barr virus? a) Alphaherpesviridae, b) Betaherpesviridae, c) Gammaherpesviridae, d) Thetaherpesviridae
38. The Epstein-Barr virus replicates in the epithelial cells of the _____. a) pancreatic glands, b) salivary glands, c) skin, d) kidney
39. The virions of retrovirus are _____. a) conical, b) rod-shaped, c) spherical, d) rhombical
40. Which of the following causes AIDS in rhesus monkeys? a) HIV, b) Spumavirus, c) SIV, d) DIV

Theory Questions

1. Write short note on viral replication
2. List three major information needed in estimating the size of a viral genome.
3. Write short note on reverse transcription
4. Write short note kinds of phenotypic changes seen in mutants.
5. In a tabular form, five (5) viral infections, specimen and diagnostic test.
6. List methods of detecting viral nucleic acids
7. Discuss host-cell response to viral infections

Answers to MCQ Questions (Module 2)

S/N	Answer	S/N	Answer	S/N	Answer	S/N	Answer
1	b	11	a	21	b	31	b
2	a	12	b	22	c	32	c
3	c	13	a	23	c	33	d
4	a	14	d	24	c	34	c
5	a	15	a	25	a	35	b
6	b	16	a	26	d	36	a
7	c	17	a	27	a	37	c
8	c	18	b	28	a	38	b
9	c	19	a	29	c	39	c
10	a	20	b	30	c	40	c

Answers to Theory Question (Module 2)

1. Short note on viral replication

Viral replication is the formation of biological viruses during the infection process in the target host cells. Viruses must first get into the cell before viral replication can occur. Through the generation of abundant copies of its genome and packaging these copies, the virus continues infecting new hosts.

Replication between viruses is varied and depends on the type of genes involved. Most DNA viruses assemble in the nucleus; most RNA viruses develop solely in cytoplasm. Viral populations do not grow through cell division, because they are acellular. Instead, they hijack the machinery and metabolism of a host cell to produce multiple copies of themselves, and they assemble inside the cell.
The life cycle of viruses differs greatly between species but there are six common basic stages:

Attachment is a specific binding between viral capsid proteins and specific receptors on the host cellular surface. This specificity determines the host range of a virus. For example, HIV can infect only a limited range of human leukocytes. Its surface protein, gp120, specifically interacts only with the CD4 molecule – a chemokine receptor – which is most commonly found on the surface of CD4+ T-Cells. This mechanism has evolved to favor those viruses that infect only cells within which they are capable of replication. Attachment to the receptor can force the viral envelope protein to undergo either changes that result in the fusion of viral and cellular membranes, or changes of non-enveloped virus surface proteins that allow the virus to enter.

Penetration follows attachment. Virions enter the host cell through receptor-mediated endocytosis or membrane fusion. This is often called *viral entry*. The infection of plant and fungal cells is different from that of animal cells. Plants have a rigid cell wall made of cellulose, and fungi one of chitin, so most viruses can get inside these cells only after trauma to the cell wall. However, nearly all plant viruses (such as tobacco mosaic virus) can also move directly from cell to cell, in the form of single-stranded nucleoprotein complexes, through pores called plasmodesmata. Bacteria, like plants, have strong cell walls that a virus must breach to infect the cell. However, since bacterial cell walls are much less thick than plant cell walls due to their much smaller size, some viruses have evolved mechanisms that inject their

genome into the bacterial cell across the cell wall, while the viral capsid remains outside.

Uncoating is a process in which the viral capsid is removed: This may be by degradation by viral or host enzymes or by simple dissociation. In either case the end-result is the release of the viral genomic nucleic acid.

Replication of viruses depends on the multiplication of the genome. This is accomplished through synthesis of viral messenger RNA (mRNA) from "early" genes (with exceptions for positive sense RNA viruses), viral protein synthesis, possible assembly of viral proteins, then viral genome replication mediated by early or regulatory protein expression. This may be followed, for complex viruses with larger genomes, by one or more further rounds of mRNA synthesis: "late" gene expression is, in general, of structural or virion proteins.

2. The number of genes in a viral genome can be estimated if the following are known:
The triplet code,
Molecular weight and
Average size of protein.

3. Short note on reverse transcription

This is the process by which RNA viruses such as Retroviruses make a second DNA strand using the enzyme reverse transcriptase before integrating their genome into their host genome. It is reveres transcription because we DNA is made from RNA as opposed to the order in the central dogma of molecular biology where RNA is made from DNA.

4. Short note kinds of phenotypic changes seen in mutants.
Conditional lethal mutants: These mutants multiply under some conditions but not others (whereas the wild-type virus grows under both sets of conditions) (i) temperature sensitive (ts) mutants - These will grow at low temperature e.g. 31 ^{o}C but not at e.g. 39 ^{o}C, wild type grows at 31 and 39 ^{o}C. It appears that the reason for this is often that the altered protein cannot maintain a functional conformation at the elevated temperature. (ii) host range - These mutants will only grow in a subset of the cell types in which the wild type virus will grow - such mutants provide a means to investigate the role of the host cell in viral infection
Plaque size: Plaques may be larger or smaller than in the wild type virus, sometimes such mutants show altered pathogenicity.

Drug resistance: This is important in the development of antiviral agents - the possibility of drug resistant mutants arising must always be considered

Enzyme-deficient mutants: Some viral enzymes are not always essential and so we can isolate viable enzyme-deficient mutants; e.g. herpes simplex virus thymidine kinase is usually not required in tissue culture but it is important in infection of neuronal cells
"Hot" mutants: These grow better at elevated temperatures than the wild type virus. They may be more virulent since host fever may have little effect on the mutants but may slow down the replication of wild type virions

Attenuated mutants: Many viral mutants cause much milder symptoms (or no symptoms) compared to the parental virus - these are said to be attenuated. These have a potential role in vaccine development and they are also useful tools in determining why the parental virus is harmful.

5. Five (5) viral infections, specimen and diagnostic test.

S/N	Syndrome/Virus	Specimen	Diagnostic test
1	Rhinovirus	Nasopharyngeal washing/swab	Cell culture (HEL)
2	Mumps	Nasopharyngeal washing/swab and urine	Cell culture (PMK)
3	Rubella virus	Nasopharyngeal washing/swab and blood	Cell culture (AGMR and Vero)
4	Monkey pox, cow pox, vaccinia and	Vesicle blood	Embryonated eggs and electron microscopy
5	Rabies virus	Saliva and brain biopsy	Sucking mice Unit 4

6. List of methods of detecting viral nucleic acids

Different techniques used to study viruses

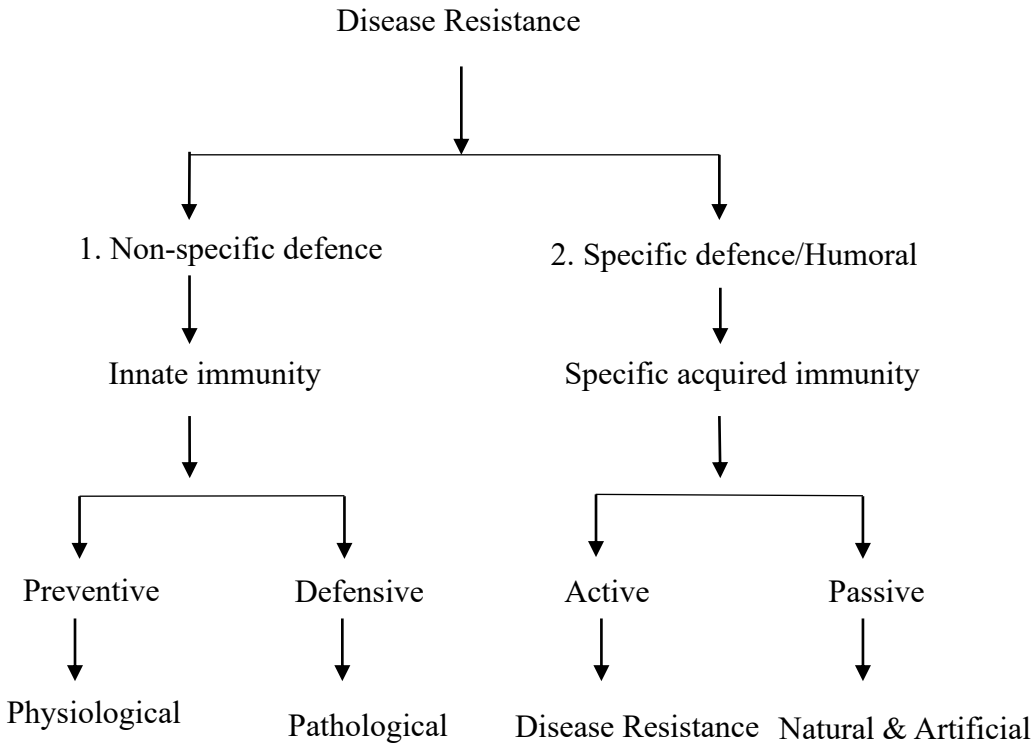
- Agarose gel electrophoresis
- Polyacrylamide gel electrophoresis (PAGE)
- Sodium dodecyl sulfate added to denature proteins (SDS-PAGE)
- ELISA
- Western Blot
- Northern Blot
- Southern Blot

- Column Chromatography
- Centrifugation
- Ultracentrifugation

7. Discuss host-cell response to viral infections

1. Non-specific defence or innate immunity is also divided into two:

(a) Preventive mechanisms: This embraces the normal physiological processes designed to protect the host from it environmental microorganisms
(b) Defensive mechanisms: This is a response stimulated by infecting microbe causing injury to tissues.
2. Specific defence or Humoral immunity: This cell response against a particular organism in order for the infecting microbe to be eliminated and to memorize it against future occurrence. This is an antibody mediated response.



MODULE 3

UNIT 1 CASE STUDY OF VIRAL DISEASES

CONTENTS

1.1 Introduction

In this unit, we shall be looking at the case study of two human viral diseases namely AIDS and Rabies. We shall look into their epidemiology, history, management, diagnosis amongst others.

1.2 Learning Outcomes

At the end of the class, student must be familiar and have understood the virology, clinical presentation, diagnosis and treatment of representative viral diseases.

1.3 Acquired Immunodeficiency Syndrome (AIDS)

1.3.1 Brief History and Origin

The first news story on the disease appeared May 18, 1981, in the gay newspaper New York Native. AIDS was first clinically reported on June 5, 1981, with five cases in the United States. The initial cases were a cluster of injecting drug users and gay men with no known cause of impaired immunity who showed symptoms of Pneumocystis carinii pneumonia (PCP), a rare opportunistic infection that was known to occur in people with very compromised immune systems. Soon thereafter, an unexpected number of homosexual men developed a previously rare skin cancer called Kaposi's sarcoma (KS). Many more cases of PCP and KS emerged, alerting U.S. Centers for Disease Control and Prevention (CDC) and a CDC task force was formed to monitor the outbreak.

In the early days, the CDC did not have an official name for the disease, often referring to it by way of diseases associated with it, such as lymphadenopathy, the disease after which the discoverers of HIV originally named the virus. They also used Kaposi's sarcoma and opportunistic infections, the name by which a task force had been set up in 1981. At one point the CDC referred to it as the "4H disease", as the syndrome seemed to affect heroin users, homosexuals, haemophiliacs, and Haitians. The term GRID, which stood for gay-related immune deficiency, had also been coined. However, after determining that AIDS was not isolated to the gay community, it was realized that the term GRID was misleading, and the term AIDS was introduced at a meeting in July 1982. By September 1982 the CDC started referring to the disease as AIDS.

In 1983, two separate research groups led by Robert Gallo and Luc Montagnier declared that a novel retrovirus may have been infecting people with AIDS, and published their findings in the same issue of the journal Science. Gallo claimed a virus which his group had isolated from a person with AIDS was strikingly similar in shape to other human T-lymphotropic viruses (HTLVs) that his group had been the first to

isolate. Gallo's group called their newly isolated virus HTLV-III. At the same time, Montagnier's group isolated a virus from a person presenting with swelling of the lymph nodes of the neck and physical weakness, two characteristic symptoms of AIDS. Contradicting the report from Gallo's group, Montagnier and his colleagues showed that core proteins of this virus were immunologically different from those of HTLV-I. Montagnier's group named their isolated virus lymphadenopathy-associated virus (LAV). As these two viruses turned out to be the same, in 1986, LAV and HTLV-III were renamed HIV.

The origin of HIV/AIDS and the circumstances that led to its emergence remain unsolved. Both HIV-1 and HIV-2 are believed to have originated in non-human primates in West-central Africa and were transferred to humans in the early 20th century. HIV-1 appears to have originated in southern Cameroon through the evolution of SIV(cpz), a simian immunodeficiency virus (SIV) that infects wild chimpanzees (HIV-1 descends from the SIVcpz endemic in the chimpanzee subspecies *Pan troglodytes troglodytes*). The closest relative of HIV-2 is SIV (smm), a virus of the sooty mangabey (*Cercocebus atys atys*), an Old World monkey living in coastal West Africa (from southern Senegal to western Ivory Coast). New World monkeys such as the owl monkey are resistant to HIV-1 infection, possibly because of a genomic fusion of two viral resistance genes. HIV-1 is thought to have jumped the species barrier on at least three separate occasions, giving rise to the three groups of the virus, M, N, and O.

There is evidence that humans who participate in bushmeat activities, either as hunters or as bushmeat vendors, commonly acquire SIV. However, SIV is a weak virus which is typically suppressed by the human immune system within weeks of infection. It is thought that several transmissions of the virus from individual to individual in quick succession are necessary to allow it enough time to mutate into HIV. Furthermore, due to its relatively low person-to-person transmission rate, SIV can only spread throughout the population in the presence of one or more high-risk transmission channels, which are thought to have been absent in Africa before the 20th century.

In-Test Question

What is the origin of HIV?

Answer

Scientists believe that HIV originally came from a virus particular to chimpanzees in West Africa during the 1930s, and originally transmitted

to humans through the transfer of blood through hunting. Over the decades, the virus spread through Africa, and to other parts of the world.

1.3.2 Virology

HIV is the cause of the spectrum of disease known as HIV/AIDS. HIV is a retrovirus that primarily infects components of the human immune system such as $CD4^+$ T cells, macrophages and dendritic cells. It directly and indirectly destroys $CD4^+$ T cells. HIV is a member of the genus Lentivirus, part of the family Retroviridae. Lentiviruses share many morphological and biological characteristics. Many species of mammals are infected by lentiviruses, which are characteristically responsible for long-duration illnesses with a long incubation period. Lentiviruses are transmitted as single-stranded, positive-sense, enveloped RNA viruses. Upon entry into the target cell, the viral RNA genome is converted (reverse transcribed) into double-stranded DNA by a virally encoded reverse transcriptase that is transported along with the viral genome in the virus particle. The resulting viral DNA is then imported into the cell nucleus and integrated into the cellular DNA by a virally encoded integrase and host co-factors. Once integrated, the virus may become latent, allowing the virus and its host cell to avoid detection by the immune system. Alternatively, the virus may be transcribed, producing new RNA genomes and viral proteins that are packaged and released from the cell as new virus particles that begin the replication cycle anew.

Two types of HIV have been characterized: HIV-1 and HIV-2. HIV-1 is the virus that was originally discovered (and initially referred to also as LAV or HTLV-III). It is more virulent, more infective, and is the cause of the majority of HIV infections globally. The lower infectivity of HIV-2 as compared with HIV-1 implies that fewer people exposed to HIV-2 will be infected per exposure. Because of its relatively poor capacity for transmission, HIV-2 is largely confined to West Africa.

In-Test Question

What is the difference between HIV and AIDS?

Answer

The difference between HIV and AIDS is that HIV is a virus that weakens your immune system. AIDS is a condition that can happen as a result of an HIV infection when your immune system is severely weakened. You can’t get AIDS if you aren’t infected with HIV. Thanks to treatment that slows down the effects of the virus, not everyone with

HIV progresses to AIDS. But without treatment, almost all people living with HIV will advance to AIDS.

1.3.3 Signs and symptoms

Acute infection: The initial period following the contraction of HIV is called acute HIV, primary HIV or acute retroviral syndrome. Many individuals develop an influenza-like illness or a mononucleosis-like illness 2–4 weeks after exposure while others have no significant symptoms. Symptoms occur in 40–90% of cases and most commonly include fever, large tender lymph nodes, throat inflammation, a rash, headache, tiredness, and/or sores of the mouth and genitals. The rash, which occurs in 20–50% of cases, presents itself on the trunk and is maculopapular, classically. Some people also develop opportunistic infections at this stage. Gastrointestinal symptoms, such as vomiting or diarrhoea may occur. Neurological symptoms of peripheral neuropathy or Guillain–Barré syndrome also occur. The duration of the symptoms varies, but is usually one or two weeks.

Owing to their nonspecific character, these symptoms are not often recognized as signs of HIV infection. Even cases that do get seen by a family doctor or a hospital are often misdiagnosed as one of the many common infectious diseases with overlapping symptoms. Thus, it is recommended that HIV be considered in people presenting with an unexplained fever who may have risk factors for the infection.

Clinical latency: The initial symptoms are followed by a stage called clinical latency, asymptomatic HIV, or chronic HIV. Without treatment, this second stage of the natural history of HIV infection can last from about three years to over 20 years (on average, about eight years). While typically there are few or no symptoms at first, near the end of this stage many people experience fever, weight loss, gastrointestinal problems and muscle pains. Between 50% and 70% of people also develop persistent generalized lymphadenopathy, characterized by unexplained, non-painful enlargement of more than one group of lymph nodes (other than in the groin) for over three to six months.

Although most HIV-1 infected individuals have a detectable viral load and in the absence of treatment will eventually progress to AIDS, a small proportion (about 5%) retain high levels of $CD4^+$ T cells (T helper cells) without antiretroviral therapy for more than five years. These individuals are classified as "HIV controllers" or long-term non-progressors (LTNP). Another group consists of those who maintain a low or undetectable viral load without anti-retroviral treatment, known as "elite controllers" or "elite suppressors". They represent approximately 1 in 300 infected persons.

Acquired immunodeficiency syndrome: Acquired immunodeficiency syndrome (AIDS) is defined as an HIV infection with either a $CD4^+$ T cell count below 200 cells per µL or the occurrence of specific diseases associated with HIV infection. In the absence of specific treatment, around half of people infected with HIV develop AIDS within ten years. The most common initial conditions that alert to the presence of AIDS are pneumocystis pneumonia (40%), cachexia in the form of HIV wasting syndrome (20%), and oesophageal candidiasis. Other common signs include recurrent respiratory tract infections.

Opportunistic infections may be caused by bacteria, viruses, fungi, and parasites that are normally controlled by the immune system. Which infections occur depends partly on what organisms are common in the person's environment. These infections may affect nearly every organ system.

People with AIDS have an increased risk of developing various viral-induced cancers, including Kaposi's sarcoma, Burkitt's lymphoma, primary central nervous system lymphoma, and cervical cancer. Kaposi's sarcoma is the most common cancer, occurring in 10% to 20% of people with HIV. The second-most common cancer is lymphoma, which is the cause of death of nearly 16% of people with AIDS and is the initial sign of AIDS in 3% to 4%. Both these cancers are associated with human herpesvirus 8 (HHV-8). Cervical cancer occurs more frequently in those with AIDS because of its association with human papillomavirus (HPV). Conjunctival cancer (of the layer that lines the inner part of eyelids and the white part of the eye) is also more common in those with HIV.

Additionally, people with AIDS frequently have systemic symptoms such as prolonged fevers, sweats (particularly at night), swollen lymph nodes, chills, weakness, and unintended weight loss. Diarrhoea is another common symptom, present in about 90% of people with AIDS. They can also be affected by diverse psychiatric and neurological symptoms independent of opportunistic infections and cancers.

In-Test Question

What does HIV do to an infected person?

Answer

HIV infects white blood cells of your immune system called CD4 cells, or helper T cells. It destroys CD4 cells, causing your white blood cell

count to drop. This leaves you with an immune system that can't fight off infections, even those that wouldn't normally make you sick.

HIV initially makes you feel sick with flu-like symptoms. Then it can hide in your body for a long time without causing noticeable symptoms. During that time, it slowly destroys your T-cells. When your T-cells get very low or you begin to get certain illnesses that people with healthy immune systems don't get, HIV has progressed to AIDS.

AIDS can cause rapid weight loss, extreme tiredness, mouth or genital ulcers, fevers, night sweats and skin discolorations. Other illnesses and cancers often happen in people living with AIDS and can cause additional symptoms.

1.3.4 Transmission

HIV is spread by three main routes: sexual contact, significant exposure to infected body fluids or tissues, and from mother to child during pregnancy, delivery, or breastfeeding (known as vertical transmission). There is no risk of acquiring HIV if exposed to faeces, nasal secretions, saliva, sputum, sweat, tears, urine, or vomit unless these are contaminated with blood. It is also possible to be co-infected by more than one strain of HIV - a condition known as HIV superinfection.

Sexual: The most frequent mode of transmission of HIV is through sexual contact with an infected person. However, an HIV-positive person who has an undetectable viral load as a result of long-term treatment has effectively no risk of transmitting HIV sexually. The existence of functionally noncontagious HIV-positive people on antiretroviral therapy was controversially publicised in the 2008 Swiss Statement, and has since become accepted as medically sound.

Globally, the most common mode of HIV transmission is via sexual contacts between people of the opposite sex; however, the pattern of transmission varies among countries. As of 2017, most HIV transmission in the United States occurred among men who had sex with men (82% of new HIV diagnoses among males aged 13 and older and 70% of total new diagnoses). In the US, gay and bisexual men aged 13 to 24 accounted for an estimated 92% of new HIV diagnoses among all men in their age group and 27% of new diagnoses among all gay and bisexual men.

Body fluids: The second-most frequent mode of HIV transmission is via blood and blood products. Blood-borne transmission can be through needle-sharing during intravenous drug use, needle-stick injury, transfusion of contaminated blood or blood product, or medical

injections with unsterilized equipment. The risk from sharing a needle during drug injection is between 0.63% and 2.4% per act, with an average of 0.8%. The risk of acquiring HIV from a needle stick from an HIV-infected person is estimated as 0.3% (about 1 in 333) per act and the risk following mucous membrane exposure to infected blood as 0.09% (about 1 in 1000) per act. This risk may, however, be up to 5% if the introduced blood was from a person with a high viral load and the cut was deep. In the United States intravenous drug users made up 12% of all new cases of HIV in 2009, and in some areas more than 80% of people who inject drugs are HIV-positive.

HIV is transmitted in about 90% of blood transfusions using infected blood. In developed countries the risk of acquiring HIV from a blood transfusion is extremely low (less than one in half a million) where improved donor selection and HIV screening is performed; for example, in the UK the risk is reported at one in five million and in the United States it was one in 1.5 million in 2008. In low-income countries, only half of transfusions may be appropriately screened (as of 2008), and it is estimated that up to 15% of HIV infections in these areas come from transfusion of infected blood and blood products, representing between 5% and 10% of global infections.[13][77] It is possible to acquire HIV from organ and tissue transplantation, although this is rare because of screening.

Unsafe medical injections play a role in HIV spread in sub-Saharan Africa. In 2007, between 12% and 17% of infections in this region were attributed to medical syringe use. The World Health Organization estimates the risk of transmission as a result of a medical injection in Africa at 1.2%. Risks are also associated with invasive procedures, assisted delivery, and dental care in this area of the world.

People giving or receiving tattoos, piercings, and scarification are theoretically at risk of infection but no confirmed cases have been documented. It is not possible for mosquitoes or other insects to transmit HIV.

Mother-to-child: HIV can be transmitted from mother to child during pregnancy, during delivery, or through breast milk, resulting in the baby also contracting HIV. As of 2008, vertical transmission accounted for about 90% of cases of HIV in children. In the absence of treatment, the risk of transmission before or during birth is around 20%, and in those who also breastfeed 35%. Treatment decreases this risk to less than 5%.

Antiretrovirals when taken by either the mother or the baby decrease the risk of transmission in those who do breastfeed. If blood contaminates food during pre-chewing it may pose a risk of transmission. If a woman

is untreated, two years of breastfeeding results in an HIV/AIDS risk in her baby of about 17%. Due to the increased risk of death without breastfeeding in many areas in the developing world, the World Health Organization recommends either exclusive breastfeeding or the provision of safe formula. All women known to be HIV-positive should be taking lifelong antiretroviral therapy.

In-Test Question

How does HIV spread?

Ans.: You can get HIV through the blood, semen, vaginal fluids, breast milk and rectal fluids of an infected person. People of all sexes and sexual orientations can get infected with and spread HIV. The virus can enter your body through your mouth, anus, penis, vagina or broken skin. It can't get through your skin unless you have a cut or wound. Pregnant people with HIV can also give it to their babies. Having sex without a condom and sharing needles to take drugs are the most common ways that HIV spreads. Even if you feel fine, you can still give HIV to others.

1.3.5 Diagnosis

HIV/AIDS is diagnosed via laboratory testing and then staged based on the presence of certain signs or symptoms. HIV screening is recommended by the United States Preventive Services Task Force for all people 15 years to 65 years of age, including all pregnant women. Additionally, testing is recommended for those at high risk, which includes anyone diagnosed with a sexually transmitted illness. In many areas of the world, a third of HIV carriers only discover they are infected at an advanced stage of the disease when AIDS or severe immunodeficiency has become apparent.

HIV testing: Most people infected with HIV develop specific antibodies (i.e. seroconvert) within three to twelve weeks after the initial infection. Diagnosis of primary HIV before sero-conversion is done by measuring HIV-RNA or p24 antigen. Positive results obtained by antibody or PCR testing are confirmed either by a different antibody or by PCR.

Antibody tests in children younger than 18 months are typically inaccurate, due to the continued presence of maternal antibodies. Thus HIV infection can only be diagnosed by PCR testing for HIV RNA or DNA, or via testing for the p24 antigen. Much of the world lacks access to reliable PCR testing, and people in many places simply wait until either symptoms develop or the child is old enough for accurate antibody testing. In sub-Saharan Africa between 2007 and 2009, between 30% and 70% of the population were aware of their HIV status.

In 2009, between 3.6% and 42% of men and women in sub-Saharan countries were tested; this represented a significant increase compared to previous years.

AIDS could be diagnosed in the laboratory as follows

1. Cellular test to evaluate the ratio of helper (T4) and suppressor (T8) lymphocyte subtypes.
2. Antibody tests to detect HIV-antibodies.
3. ELISA, of which most laboratory implored. The type of ELISA for HIV-antibody testing is called COMPETITIVE ELISA.

In-Test Question

How is HIV diagnosed?

Answer

HIV is diagnosed with either a test of your blood or your spit (saliva). You can take a test at home, in a healthcare provider's office or at a location that provides testing in your community.

If your test comes back negative, no further testing is required if:

- You haven't had a possible exposure in the previous three months before testing with any kind of test.
- You haven't had a possible exposure within the window period for a test done with a blood draw. (Ask your healthcare provider if you are unsure what the window period is for a test you took.)

If you have had a possible exposure within three months of testing, you should consider retesting to confirm the negative result. If your test comes back positive, the lab may do follow-up tests to confirm the result.

1.3.6 Prevention

Sexual contact: Consistent condom use reduces the risk of HIV transmission by approximately 80% over the long term. When condoms are used consistently by a couple in which one person is infected, the rate of HIV infection is less than 1% per year. There is some evidence to suggest that female condoms may provide an equivalent level of protection. Application of a vaginal gel containing tenofovir (a reverse transcriptase inhibitor) immediately before sex seems to reduce infection rates by approximately 40% among African women. By contrast, use of the spermicide nonoxynol-9 may increase the risk of transmission due to its tendency to cause vaginal and rectal irritation. Circumcision in Sub-

Saharan Africa reduces the acquisition of HIV by heterosexual men by between 38% and 66% over 24 months". Programs encouraging sexual abstinence do not appear to affect subsequent HIV risk. Evidence of any benefit from peer education is equally poor. Comprehensive sexual education provided at school may decrease high-risk behaviour.

Pre-exposure: Antiretroviral treatment among people with HIV whose CD4 count ≤ 550 cells/μL is a very effective way to prevent HIV infection of their partner (a strategy known as treatment as prevention, or TASP). TASP is associated with a 10- to 20-fold reduction in transmission risk. Pre-exposure prophylaxis (PrEP) with a daily dose of the medications tenofovir, with or without emtricitabine, is effective in people at high risk including men who have sex with men, couples where one is HIV-positive, and young heterosexuals in Africa.

Post-exposure: A course of antiretrovirals administered within 48 to 72 hours after exposure to HIV-positive blood or genital secretions is referred to as post-exposure prophylaxis (PEP). The use of the single agent zidovudine reduces the risk of a HIV infection five-fold following a needle-stick injury. PEP treatment is recommended after a sexual assault when the perpetrator is known to be HIV-positive, but is controversial when their HIV status is unknown. The duration of treatment is usually four weeks and is frequently associated with adverse effects-where zidovudine is used, about 70% of cases result in adverse effects such as nausea (24%), fatigue (22%), emotional distress (13%) and headaches (9%).

Programs to prevent the vertical transmission of HIV (from mothers to children) can reduce rates of transmission by 92–99%. This primarily involves the use of a combination of antiviral medications during pregnancy and after birth in the infant, and potentially includes bottle feeding rather than breastfeeding. If replacement feeding is acceptable, feasible, affordable, sustainable and safe, mothers should avoid breastfeeding their infants; however, exclusive breastfeeding is recommended during the first months of life if this is not the case. If exclusive breastfeeding is carried out, the provision of extended antiretroviral prophylaxis to the infant decreases the risk of transmission. In 2015, Cuba became the first country in the world to eradicate mother-to-child transmission of HIV.

In-Test Question

How can infection with HIV be prevented?

Answer

A condom is the most effective form of protection against HIV and other STIs. It can be used for vaginal and anal sex, and for oral sex performed on men. HIV can be passed on before ejaculation through pre-cum and vaginal secretions, and from the anus. Most importantly, Abstinence for unmarried individuals will greatly reduce the incidence of the virus

1.3.7 Treatment

There is currently no cure, nor an effective HIV vaccine. Treatment consists of highly active antiretroviral therapy (HAART), which slows progression of the disease. As of 2010, more than 6.6 million people were receiving HAART in low- and middle-income countries. Treatment also includes preventive and active treatment of opportunistic infections. As of March 2020, two people have been successfully cleared of HIV. Rapid initiation of antiretroviral therapy within one week of diagnosis appear to improve treatment outcomes in low and medium-income settings.

Antiviral therapy: Current HAART options are combinations (or "cocktails") consisting of at least three medications belonging to at least two types, or "classes", of antiretroviral agents. Initially, treatment is typically a non-nucleoside reverse transcriptase inhibitor (NNRTI) plus two nucleoside analog reverse transcriptase inhibitors (NRTIs). Typical NRTIs include: zidovudine (AZT) or tenofovir (TDF) and lamivudine (3TC) or emtricitabine (FTC). As of 2019, dolutegravir/lamivudine/tenofovir is listed by the WHO as the first-line treatment for adults, with tenofovir/lamivudine/efavirenz as an alternative. Combinations of agents that include protease inhibitors (PI) are used if the above regimen loses effectiveness.

Benefits of treatment include a decreased risk of progression to AIDS and a decreased risk of death. In the developing world, treatment also improves physical and mental health. With treatment, there is a 70% reduced risk of acquiring tuberculosis. Additional benefits include a decreased risk of transmission of the disease to sexual partners and a decrease in mother-to-child transmission.

Cabotegravir combined with rilpivirine (Cabenuva) is a complete regimen for the treatment of human immunodeficiency virus type 1 (HIV-1) infection in adults to replace a current antiretroviral regimen in those who are virologically suppressed on a stable antiretroviral regimen with no history of treatment failure and with no known or suspected resistance to either cabotegravir or rilpivirine.

No anti-HIV vaccine is available till date. Various approaches toward development a vaccine are being investigated. Vaccine development against HIV is difficult because it mutates rapidly, undergoes latency, at id resists the immune responses that usually control viral infections. HIV also showed a marked variation, especially in the envelope antigens, yet parts of the envelope proteins and most core protein are served.

In-Test Questions

Is there a cure for HIV?

Answer

There is currently no cure for HIV, but there are many treatment options that can slow the progression of HIV significantly.

How is HIV treated?

Answer

HIV is treated with a combination of medicines (pills) taken by mouth every day. This combination of pills is called antiretroviral therapy (ART). Taking a combination of types of pills, rather than just one, is the most effective way to keep HIV from multiplying and destroying your cells. There are also combination pills that have several medications in a single pill. Your healthcare provider will carefully select a combination specifically for you. The goal of ART is to reduce HIV in the blood (viral load) to an amount that's not detectable by an HIV test and to slow HIV's weakening of your immune system.

Self-Assessment Exercise 1

Provide answers to the following questions in 20 minutes

1. What are the causes of acquired immunodeficiency syndrome?
2. Where did HIV come from?

1.4 Rabies

The disease is as old as man existence. The etiological agent was first discovered by Lew Pasteur et al. (1884) when he attenuated the virus. The virus rabies belongs to the family Rhandoviridae and genus

Lyssavirus. The genome of the virus is a single stranded RNA, Linear, non-segmented, and negative sense with molecular weight of 4 million. The virion is enveloped. The infection is zoonostic, and is transmitted to man via the bite of infected animal e.g. Dogs. It causes a lethal form of encephalitis.

1.4.1 Epidemiology

Dogs, cats, bats and wild animals such as foxes, wolfs, skunks etc are natural hosts of rabies. It could also be found in rodents and cattle. In Europe, exposure to rabies is most common from cats than dogs except in Turkey where dog rabies is a particular problem. Infection by bite of vampire bats in central and South America is also a problem rabies spread. Animal that remains healthy after l0 days of bite can be regarded as being free of the virus. Viruses are present in the saliva of infected animal usually 4 days before the onset of symptoms of the disease. Usually 15% of bitten individuals by a rabid animal developed the disease. Rabies is more common after bites on the head or neck rather than bites/wounds on limb.

Rabies as far back as 1921 almost been eradicated in Britain due to quarantine law on imported animals. Rabies is present in wild animals in all continents of the world except in Australia (Antarctica). Case to case spread of human patients is not a source of infection. Rabies infection due to corneal transplantation had been reported. The spread of rabies virus is by the bite of infected vampire has been reported in the West Indies and Central and South America.

In-Test Question

Which animals are the natural hosts of rabies?

Answer

Dogs, cats, bats and wild animals such as foxes, wolfs and skunks are natural hosts of rabies.
Which population of humans is at greatest risk for rabies?

Answer

Children are often at greatest risk from rabies. They are more likely to be bitten by dogs, and are also more likely to be severely exposed through multiple bites in high-risk sites on the body. Severe exposures make it more difficult to prevent rabies unless access to good medical care is immediately available.

1.4.2 Pathogenesis and Pathology

Following an animal bite, the virus multiplies in the peripheral tissue o the wound and spread to the CNS via the nerves. The viruses attack the neuromuscular and neurotendonal Spindle of the nerves and bud oft to the CNS which is the seat of the virus. The disease is virtually always fatal, often leading to death some cases of recovery had been reported but rare.

Death follows convulsion. There is little or no lesions of the virus in the CNS with little evidence of destructive effects on cells but the main changes are the typical intra-cytoplasmic inclusions within the neurones called the NEGRI BODIES. They are specifically found in the perivascular mononuclear infiltration and are stained by sellers stain.

In-Test Question

What part of the body does rabies affect?

Answer

Rabies is a rare but serious disease caused by a virus. It affects the nerves and brain. The virus is usually transmitted by a bite from an infected animal. Rabies can be prevented if the bitten person gets treatment quickly.

1.4.3 Clinical Features/Symptoms

Rabies infection begin with non-specific constitutional pro-dronatal including fever, fatigue, musculo-skeletal pains, occular pains, nervousness, hypersensitivity to stimulus by mainly excitement with tremor muscular contractions and convulsions: typically spasm of muscles of swallowing hence the old name of the disease-HYDROPHOBIA. There is increase in sensitivity of the sensory nervous system. The infected animal especially dogs bark at anything and easily infuriated or excited hence the classical syndrome of FURIOUS RABIES. There is presence of the virus in the saliva, skin, eyes as well as the brain. At this stage, there is hyper-salivation, hyperpyrexia,' excessive sweating, hypotension and tarchychadia which are all the autonomic disturbance of the infected animal/individuals. This stage is followed by the dumb condition of the animal. Here, all the conditions in furious stage are aggravated and the animal now becomes dumb-like and paralyzed. There is no hydrophobia in the dumb stage.

In-Test Question

What are the clinical signs of rabies in animals?
Answer

Signs progress within days to cerebral dysfunction, cranial nerve dysfunction, ataxia, weakness, paralysis, seizures, difficulty breathing, difficulty swallowing, excessive salivation, abnormal behavior, aggression, and/or self-mutilation.

1.4.4 Incubation Period

The incubation period is usually long, taking about 4-12 weeks when the wound is on the limb but sometime take much more time. The incubation is however shorter if the wound is on the head or neck (about 10 days).

In-Test Question

How long does it take for appearance of symptom from the time of rabies infection?

Answer

The incubation period for rabies is typically 4-12 weeks but may vary from 1 week to 1 year, depending on factors such as the location of virus entry and viral load.

1.4.5 Isolation/Diagnosis

Rabies virus could be isolated from specimens of brain, tissue, CSE saliva and urine. The specimens are inoculated into healthy susceptible lab-mice intra-cerebrally. Observe for paralysis and convulsion. Post-mortem demonstration of Negri bodies in the brain cells and immunoflouresence demonstration of Rabies antibody with rabies antiserum is diagnostic.
Proper diagnosis of Rabies is as follows:

1. Direct demonstration of virus in smear or brain tissues by election microscopy or immunoflourescence demonstration of Rabies virus antigen.
2. Examination of brain smear by Selle's Stain to demonstrate inclusion bodies (NEGRI BODIES) which are stained red.
   Molecular analysis of viral genome by polymerase chain reaction (PCR) is possible. Complement fixation or Neutralizing

antibodies, test on infected animal serum to detect rabies antibodies is diagnostic.

In-Test Question

What tests are used to diagnose rabies?

Answer

Tests are performed on samples of saliva, serum, spinal fluid, and skin biopsies of hair follicles at the nape of the neck. Saliva can be tested by virus isolation or reverse transcription followed by polymerase chain reaction (RT-PCR). Serum and spinal fluid are tested for antibodies to rabies virus.

1.4.6 Treatment and Controls

Passive immunization is done by injection of human anti-rabies immunoglobulin while active immunization should be started after passive immunization. Long incubation of rabies is a suitable disease for prophylactic immunization after exposure.

Live attenuated vaccine made from human diploid cells is now the vaccine of choice and could be administered as a means of treatment. It is given subcutaneously in 6 doses of 0, 3,7,14, 30 and 90 days. This produces effective protection with high level of neutralizing antibodies. Simple vaccine and Fermin vaccine were the early vaccine used but produces side effects which include severe neuropamlytic effects due to allergic encephalomyelitis. This is clue to repeated injection of nervous tissues. However, Duck-embryo-vaccine though less used now produces reduced risks of neurological side effects.

The Control of rabies could he by:

(1) Government introduction of quarantine on the influx of dog and other domestic animals into the country.
(2) Campaign on vaccination of domestic animals as done in some countries like Latin America.
(3) -exposure vaccination is desirable for all persons who are at high risk of contact with rabid animals within the country and about travel 1mg to other countries.
(4) A booster vaccine dose every 2 years to have their serum tested for rabies neutralizing antibodies every 2 years before booster does is administered (if serum titre is found inadequate) is needed.

In-Test Question

How is rabies transmitted to human?

Answer

Rabies is a deadly virus spread to people from the saliva of infected animals, usually transmitted through a bite.

Self-Assessment Exercise 2

Provide answer to the following question in 10 minutes

1. What is the name of the rabies virus?
2. What are the symptom presentations in a man infected with rabies?
3. What causes rabies virus?

1.5 Summary

- There is no known cure for AIDS, vaccine are under development.
- Vaccination is possible against rabies.
- Both caused by RNA viruses.

1.6 References/Further Readings/Web Sources

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1.7 Possible Answers to Self-Assessment Exercises

SAE 1

1. The causes of AIDS include: having unprotected sex with an HIV infected partner, sharing drug needles with someone who is infected by HIV, and the virus passing on from an expectant mother to her baby, during or before birth or while breastfeeding the baby, through breast milk.

2. The following have been established from investigations by scientists

- HIV infection in humans came from a type of chimpanzee in Central Africa.
- The chimpanzee version of the virus (called simian immunodeficiency virus, or SIV) was probably passed to humans when humans hunted these chimpanzees for meat and came in contact with their infected blood.
- Studies show that HIV may have jumped from chimpanzees to humans as far back as the late 1800s.
- Over decades, HIV slowly spread across Africa and later into other parts of the world. We know that the virus has existed in the United States since at least the mid to late 1970s.

SAE 2

1. Rabies virus (RABV), the prototype lyssavirus, is responsible for the vast majority of all human rabies cases. However, all lyssaviruses can cause indistinguishable fatal encephalitis both in humans and other mammals.

2. An infected person may experience on or more of the following symptom: fever, headache, excess salivation, muscle spasms, paralysis and mental confusion
   Pain areas: in the muscles
   Whole body: dizziness, fatigue, fever, loss of appetite, or malaise
   Psychological: delirium, fear, or hallucination
   Muscular: muscle spasms or paralysis with weak muscles
   Sensory: pins and needles or sensitivity to light
   Behavioural: aggression or irritability
   Gastrointestinal: nausea or vomiting
   Also common: anxiety, brain death, coma, difficulty swallowing, dilated pupils, drooling, headache, hypersalivation, mental confusion, seizure, or stiff neck

3. The rabies virus causes a rabies infection. The virus spreads through the saliva of infected animals. Infected animals can spread the virus by biting another animal or a person. In rare cases, rabies can be spread when infected saliva gets into an open wound or the mucous membranes, such as the mouth or eyes.

UNIT 2 CULTIVATION OF VIRUSES

CONTENTS

2.1 Introduction

We shall be looking into various means of detecting infected viral cells and cultivation of virus.

2.2 Learning Outcomes

At the end of the class, student must be familiar and have understood methods cultivating viruses and how to identify virus-infected cells.

2.3 Virus cultivation: Purposes and methods

- Viruses are obligate intracellular parasites so they depend on host for their survival.
- They cannot be grown in non-living culture media or on agar plates alone, they must require living cells to support their replication.
- The primary purposes of virus cultivation is:
- To isolate and identify viruses in clinical samples. Demonstration of virus in appropriate clinical specimens by culture establishes diagnosis of viral diseases.
- To do research on viral structure, replication, genetics and effects on host cell.
- To prepare viruses for vaccine production.
- Isolation of virus is always considered as a gold standard for establishing viral etiology of a disease.

- Most of the viruses can be cultivated in
  - Experimental animals
  - Embryonated eggs or
  - Tissue culture.

2.3.1 Specimens for culture of virus

Collection of appropriate clinical specimens depends on type of the viral disease. For example, cerebrospinal fluid (CSF) is the specimen of choice for diagnosis of viral infections of the central nervous system (CNS) caused by arboviruses, picornavirus, or rabies virus. In general, specimens for virus isolation should be collected within 4 days after onset of illness as virus shedding decreases rapidly after that time. With only a rare exception, virus cultures are not worthwhile for specimens collected more than 7 days after the onset of illness.

In-test Question

What are the different specimens used for laboratory tests?

Answer

The types of biological samples accepted in most clinical laboratories are: serum samples, virology swab samples, biopsy and necropsy tissue, cerebrospinal fluid, whole blood for PCR, and urine samples. These are collected in specific containers for successful processing in the laboratory.

2.3.2 Animal inoculation

- Mouse is most frequently used for isolation of viruses by animal inoculation.
- In addition, rabbits, hamsters, newborn or suckling rodents are also used.
- Experimental animals are rarely used for cultivation of viruses but play an essential role in study of pathogenesis of viral infections and that of viral oncogenesis.
- Intracerebral, subcutaneous, intraperitoneal, or intranasal routes are various routes of inoculation.
- After inoculation, the animals are observed for signs of disease or death.
- The infected animals are then sacrificed and infected tissues are examined for the presence of viruses by various tests, and also for inclusion bodies in infected tissues.
- Furthermore, infant (suckling) mice are used for isolation of coxsackie virus and rabies virus.

In-test Question

Can inoculation be done on living organisms?

Answer

Live inoculation was first used on human volunteers for the study of yellow fever virus. Animals of choice for cultivating viruses include monkeys, rabbits, guinea pigs, rats, hamsters, and mice. This plays an essential role in the study of pathogenesis of viral infections and that of viral oncogenesis.

2.3.3 Embryonated Eggs

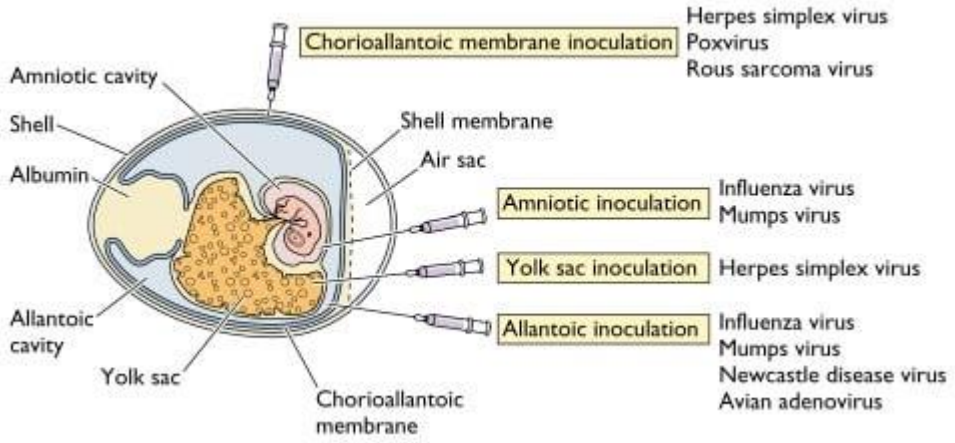

Figure 5. Pictorial representation of Embryonated Eggs showing sites of inoculating different viruses

- Embryonated chick egg was used first for cultivation of viruses by Goodpasture in 1931.
- The method further developed by Burnet was used for cultivation of viruses in different sites of the embryonated egg.
- Usually, 8–11 days' old chick eggs are used for culture of viruses.
- The viruses are isolated in different sites of the egg, such as yolk sac, amniotic cavity, and allantoic cavity, and chorioallantoic membrane (CAM).
- Many of these viruses cause well-defined and characteristic foci, providing a method for identification, quantification, or assessing virus pathogenicity.
- The embryonated egg is also used for growing higher titre stocks of some viruses in research laboratories and for vaccine production.
- Yolk sac: Yolk sac inoculation is used for cultivation of Japanese encephalitis, Saint Louis encephalitis, and West Nile virus. It is also used for growth of chlamydia and rickettsia.

- Amniotic cavity: Inoculation in the amniotic cavity is used mainly for primary isolation of influenza virus.
- Allantoic cavity: Inoculation in the allantoic cavity is used for serial passages and for obtaining large quantities of virus, such as influenza virus, yellow fever (17D strain), and rabies (Flury strain) viruses for preparation of vaccines. For production of rabies virus, duck eggs were used due to their bigger size than that of hen's egg. This helped in production of large quantities of rabies virus, which are used for preparation of the inactivated non-neural rabies vaccine.
- Chorioallantoic membrane: Inoculation of some viruses on CAM produced visible lesions known as pocks. Each infectious virus particle produces one pock. The pox viruses, such as variola or vaccinia are identified by demonstration of typical pocks on the CAM inoculated with the pox virus. Nowadays, in a virology laboratory, chick embryo inoculation has been replaced by cell cultures for routine isolation of viruses.

In-test Questions

When was the embryonated chicken egg first used for virus cultivation?

Answer

The embryonated chicken egg was first used for the cultivation of viruses by Good Pasteur and Burnet (1931). Before the development of cell lines for the culture of viruses, egg inoculation was one of the preferred methods of virus cultivation.

What is the importance of embryonated eggs?

Answer

Embryonated eggs are among the most useful and available forms of living animal tissue for the isolation and identification of animal viruses, for titrating viruses, and for quantity cultivation in the production of viral vaccines.

2.3.4 Tissue Culture

- Cell culture is most widely used in diagnostic virology for cultivation and assays of viruses.
- The tissue culture was first applied in diagnostic virology by Steinhardt and colleagues in 1913.

- They maintained the vaccinia virus by culture in tissues of rabbit cornea. Subsequently, Maitland (1928) used cut tissues in nutrient media for cultivation of vaccine viruses.
- Enders, Weller, and Robins (1949) were the first to culture poliovirus in tissue cultures of non-neural origin. Since then, most of the virus had been grown in tissue culture for diagnosis of viral diseases.
- Different types of tissue cultures are used to grow viruses. Tissue culture can be of three different types as follows:

i. Organ Culture

This was used earlier for the isolation of some viruses, which appear to show affinity for certain tissue organs. For example, coronavirus, a respiratory pathogen, was isolated in the tracheal ring organ culture. In this method, small bits of the organs are maintained in vitro for days and weeks preserving their original morphology and function. Nowadays, organ culture is not used.

ii. Explant Culture

In this method, components of minced tissue are grown as explants embedded in plasma clots. Earlier, adenoid tissue explant cultures were used for isolation of adenoviruses. This method is now seldom used in virology.

iii. Cell Culture

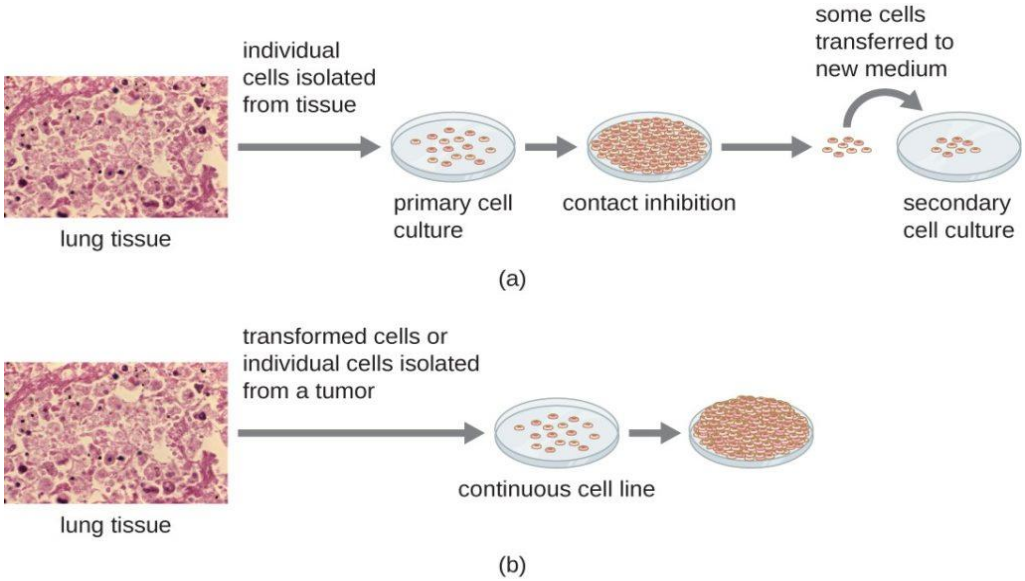

Figure 6. Pictorial representation of method employed in cell culture.

- Cell culture is now routinely used for growing viruses.
- In this method, tissues are dissociated into component cells by treatment with proteolytic enzymes (trypsin or collagenase) followed by mechanical shaking.
- The cells are then washed, counted, and suspended in a growth medium containing essential amino acids and vitamins, salts, glucose, and a buffering system. This medium is supplemented by up to 5% of foetal calf serum and antibiotics.
- The cell suspension is dispensed in glass or plastic bottles, tables, or Petri dishes.
- On incubation, the cells adhere to the glass surfaces and divide to form a confluent monolayer sheet of cells covering the surface within a week.
- The cell culture may be incubated either as a stationery culture or as a roller drum culture. The latter is useful for growth of some fastidious viruses due to better aeration by rolling of the culture bottle in special roller drums.
- The cell cultures are classified into three different types based on their origin, chromosomal characters, and number of generations for which they can be maintained.

Primary cell culture:

- These are a culture of normal cells obtained freshly from the original tissues that have been cultivated in vitro for the first time and that have not been sub-cultured.
- These cell cultures can be established from whole animal embryo or from selected tissues from adult, newborn, or embryos.
- These cells have the normal diploid chromosomal number and are capable of only limited growth (5–10 divisions) in culture.
- They cannot be maintained in serial culture, but can be sub-cultured to obtain large number of cells.
- Monkey kidney cell culture, human embryonic kidney cell culture, and chick embryo cell culture are the common examples of primary cell culture.
- Primary monkey kidney cell cultures are highly useful for the primary isolation of myxovirus, paramyxovirus, many enteroviruses, and some adenoviruses.

Diploid cell strains:

- Diploid cell strains are of a single cell type that retains their original diploid chromosome number and karyotype. However, they have specific characteristics and compositions and are usually composed of one basic cell type.
- They are usually fibroblasts and can be cultured for maximum 50 serial passages before they undergo senescence (die off) or undergo a significant change in their characteristics.

- Diploid cells derived from human fibroblasts are useful for isolation of some fastidious viruses.
- They are also used for production of vaccines; for example, WI-38 human embryonic, lung cell stem is used for the cultivation of fixed rabies virus, and human foetal diploid cells for isolation of adenovirus, picornaviruses, HSV, CMV, and VZV.

Continuous cell lines:

- Continuous or immortal cell lines are cells of a single type, which are derived from cancerous tissue and are capable of continuous serial cultivation indefinitely without senescing.
- The cells are usually derived from diploid cell lines or from malignant tissues and have altered and irregular number of chromosomes.
- Immortalization may occur spontaneously or can be induced by chemical mutagens, tumorigenic viruses, or oncogens. Hep-2, HeLa, and KB derived from human carcinoma cervix, human epithelioma of larynx, and human carcinoma of nasopharynx and other cell lines are excellent for recovery of a large number of viruses.
- These cell lines have been used extensively for the growth of a number of viruses. These cell lines are usually stored at -70°C for use when necessary or are maintained by serial subculture.
- The type of cell line used for virus culture depends on the sensitivity of the cells to a particular virus; for example, Hep-2 cell line is excellent for the recovery of respiratory syncytial viruses, adenoviruses, and HSV.
- Most of the viruses can be isolated by using one of these cell lines.

In-Test Question (ITQ)

What supports viral cultivation?

Answer

Viral cultivation requires the presence of some form of host cell (whole organism, embryo, or cell culture). Viruses can be isolated from samples by filtration. Viral filtrate is a rich source of released virions. Bacteriophages are detected by presence of clear plaques on bacterial lawn.

Self-Assessment Exercise 1

Provide answer to the following question in 5 minutes

Can virus be artificially cultured?

2.4 Detection of virus infected cells

Growth of viruses in cell cultures can be detected by the following methods:

i. Cytopathic effect:

- Many viruses can be detected and initially identified by observation of the morphological changes in the cultured cells in which they replicate.
- The CPE produced by different types of viruses are characteristic and help in the initial identification of virus isolates.
- Nuclear shrinking, vacuoles in the cytoplasm, syncytia formation, rounding up, and detachment are the examples of alteration of morphology of the cells.
- Most CPEs can be demonstrated in unfixed and unstained monolayer of cells under low power of microscope.
- For example, adenoviruses produce large granular changes resembling bunches of grapes, SV-14 produces well-defined cytoplasmic vacuolation, measles virus produces syncytium formation, herpes virus produces discrete focal degeneration, and enteroviruses cause crenation of cells and degeneration of the entire cell sheet.

ii. Hemadsorption:

- Hemadsorption is the process of adsorption of erythrocytes to the surfaces of infected cells which serves as an indirect measurement of viral protein synthesis.
- This property is made use of to detect infection with non-cytocidal viruses as well as the early stage of cytocidal viruses.
- Viruses, such as influenza virus, parainfluenza virus, mumps virus, and togavirus, when infect cell lines code for the expression of red cell agglutinins, which are expressed on the infected cell membrane during infections.
- These hemagglutinins bind some erythrocytes to the infected cell surface.
- Sometimes, viruses can be detected by agglutination of erythrocytes in the culture medium.

iii. Heterologous interference:

- This property is used to detect viruses that do not produce classic CPEs in the cell lines.
- In this method, the growth of non-CPE-producing virus in cell culture can be tested by subsequent challenge with a virus known to produce CPEs.

- The growth of the first virus will inhibit infection by the cytopathic challenge virus by interference.
- For example, rubella virus usually does not produce any CPE, but prevents the replication of picornaviruses, which is inoculated as a cytopathic challenge virus.

iv. Transformation:

- Oncogenic viruses that are associated with formation of tumors induce cell transformation and loss of contact inhibition in the infected cell lines.
- This leads to surface growth that appears in a piled-up fashion producing microtumors.
- Examples of such oncogenic viruses that produce transformation in cell lines are some herpes viruses, adenoviruses, hepadnaviruses, papovavirus, and retroviruses.

v. Light microscopy:

- Viral antigens in infected cell cultures are demonstrated by staining virus-infected cells of tissue sections with specific viral antibody conjugated with horseradish peroxidase.
- This is followed by addition of hydrogen peroxide along with a benzidine derivative substance.
- In a positive reaction, a red insoluble precipitate is deposited on the cell line, which is demonstrated by examination under ordinary light microscope.

vi. Immunofluorescence:

- Direct immunofluorescence using specific antibodies is frequently used to detect viral antigens in inoculated cell lines for identification of viruses.

vii. Electron microscopy:

- The viruses can also be demonstrated in infected cell lines by EM.

viii. Presence of inclusion bodies:

- In the course of virus multiplication within cells, virus-structures called inclusion bodies may be produced. In many viral infections, the inclusion bodies are the site of development of the virions (referred to as the virus factory).
- Variations in the appearance of inclusion materials depend largely upon the tissue fixative used. The presence of inclusion bodies may be of considerable diagnostic importance.
- The intracytoplasmic inclusion in nerve cells, the Negri bodies is pathognomonic for rabies.

ix. Chromosome alterations:

- One of the consequences of infection of cells by certain viruses is derangement of the karyotype (chromosome).
- Breakage, fragmentation, re-arrangement of the chromosomes, abnormal chromosomes and changes in chromosome number may occur.
- Cells transformed by viruses also exhibit random chromosomal abnormalities.
- Particular chromosomal alterations, including translocations, inversions and deletions are frequently observed in human cancer cells especially specific types of Leukaemia.
- More than 20 cellular oncogenes have been localized to specific human chromosomes, many are located at bands that are involved in translocations or deletions.

In-Test Question

What is Cytopathic effect?

Ans. Cytopathic effect (CPE) is the structural changes in a host cell resulting from viral infection. CPE occurs when the infecting virus causes lysis (dissolution) of the host cell or when the cell dies without lysis because of its inability to reproduce.

Self-Assessment Exercise 2

Provide answers to the following questions in 20 minutes

1. What is Nucleic Acid Amplification Test?
2. What is Hemadsorption inhibition test?
3. What is viral interference and its application?
4. Advantages of electron microscopy

2.5. Summary

The best method for culturing viruses is cell cultures, as even the most difficult virus can grow in cell lines suitable as host.

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2.7 Possible Answers to Self-Assessment Exercises

SAE 1

Unlike bacteria and other microorganisms which can be grown in artificial media, viruses cannot be grown on artificial media but must be grown in living cells. Viruses cannot be grown on an ordinary culture medium because they are not actually living organisms.

SAE 2

1. Polymerase chain reaction (PCR) is an NAAT used to detect the presence of viral DNA in a patient's tissue or body fluid sample. PCR is a technique that amplifies (i.e., synthesizes many copies) of a viral DNA segment of interest.

2. A quantitative hemadsorption-inhibition test was developed to estimate myxovirus serum antibodies within 24 h by determining the serum dilution inhibiting hemadsorption in 50% of the infected cells. Hemadsorption occurs when erythrocytes added to a myxovirus-infected tissue culture are adsorbed to the host-cell surface. The erythrocytes combine with virus-specific material at the cell surface, since the reaction can be prevented by prior treatment of the tissue culture with immune serum.

3. Virus interference is defined as the host resistance to a superinfection caused by a pathogenic virus causing obvious signs of disease and/or mortality due to the action of an interfering virus abrogating the replication of the former virus. Different degrees of inhibition of the superinfecting virus can occur.

4. Magnification and higher resolution – as electrons rather than light waves are used, it can be used to analyze structures which cannot otherwise be seen. The resolution of electron microscopy images is in the range of up to 0.2 nm, which is 1000x more detailed than light microscopy.

UNIT 3 PURIFICATION OF VIRAL PARTICLES

CONTENTS

3.1 Introduction

In this unit we shall consider methods of purifying viral particles. Special attention will be given to centrifugation as method of purification of virus particles.

3.2 Learning Outcomes

Student must have understood the methods of purifying viral nucleic acids and factors considered in identifying a viral particle.

3.3 Purification

Virus purification techniques involve filtration, dialysis, precipitation, ultracentrifugation, chromatographic and flow field-flow fractionation methods that are optimized and used together and sequentially in different combinations.

For purification, the starting material is usually large volumes of tissue culture medium, body fluids or infected cells. Pure virus is important so as to have meaningful studies on the properties and molecular biology of the virion. The first frequently involved concentration of the virus particles by precipitation with ammonium sulphate, ethanol or polyethylene glycol or by ultra-filtration. Haemagglutination and elution can be used to concentrate orthomyxoviruses. Once concentrated, virus particles can then be separated from materials by differential centrifugation, density gradient centrifugation, column chromatography and electrophoresis.

More than one-step is usually necessary to achieve adequate concentration. A preliminary purification will remove non-virus material: The first step may include centrifugation while the final purification step always involves density gradient centrifugation. The band of purified virus may be detected by optical methods, by following radioactivity if the virus is radio-labelled, or by assaying infectivity.

Viruses can also be purified by high-speed centrifugation in density gradients of Cesium Chloride ($CsCl_2$), potassium tartrate, potassium citrate of Sucrose. The gradient material of choice is the one that is least toxic to the virus. The virus particles migrate to the equilibrium position where the density of the solution is equal to their buoyant density and form a visible band, Virus bands are harvested by puncture through the bottom of the plastic centrifuge tube and assayed for infectivity.
In column chromatography, virus is bound to substance such as DEAE or phosphor-cellulose and then eluted by changes in pH or Salt concentration. Zone electrophoresis permits the separation of virus particles from contaminant on the basis of charge. Specific antisera also can he used to remove virus particles from host materials.

Icosahedral viruses are easier to purify than enveloped viruses because enveloped viruses contains variable amounts of envelope per particle, the virus population is heterogeneous in both size amid density. It is very difficult to achieve complete purity of viruses. Small amounts of cellular material tend to adsorb to particles and this co purity with the virion. The minimal criteria for purity are a homogenous appearance in electron micrographs and the failure of additional purification procedure to remove contaminants without reducing infectivity.

3.3.1 Centrifugation

Centrifugation as a purification and characterization procedure:
Ultracentrifuge: A centrifuge is capable of generating large centrifugal fields by rotating samples at 20,000-100,000 rpm. Centrifugal forces of greater than 100,000 X gravity can be generated.

Sedimentation coefficient

- Rate at which a macromolecule sediments under a defined gravitational force.
- This parameter is influenced by both the molecular weight and shape of a macromolecule (larger and more spherical sed. faster).
- The basic unit is the Svedberg (S) which is 10^{-13} sec.
- This value can be used to estimate molecular weights in conjunction with other values.

Buoyant density - Density at which a virus or other macromolecule neither sinks nor floats when suspended in a density gradient (e.g., $CsCl_2$ or sucrose).
The Svedberg equation is

$$S = \frac{v}{\varpi^2 r} = \frac{m(1 - v\rho_m)}{f} = \frac{\phi(\rho_p - \rho_m)}{f}$$

Where:

S= Sedimentation coefficient
v = velocity
r = radius, i.e. distance from center of rotation
m = mass (grams)
v= partial specific volume of particle (in nm)
r = density of solvent (g/cm^3)
f = frictional coefficient between particle and solvent. For a globular protein, $f \approx 1$ (f_p = frictional coefficient of the particle; f_m = frict. coeff. of solvent).

Types of sedimentation medium

1. Aqueous Buffer (Water based) - Can be used to separate molecules with widely different S values (ex. Nuclei from ribosomes)
2. Sucrose or glycerol gradients or cushions (iso-kinetic or rate-zonal) - A fixed concentration or a linear gradient of these agents in buffer is used. The compounds increase the density and viscosity of the medium therefore, decreasing the rate at which macromolecule sediment through them and preventing the sedimentation molecules with densities less than the medium. General approach is to pour a "cushion" of material at the bottom of the centrifuge tube and centrifuge the virions onto the cushion (cushion need not always be used). By controlling the time and speed of centrifugation a significant purification can be obtained. Since most macromolecules have greater densities than these media separation is based on S values. This can be used to separate molecules with relatively close S values.
3. $CsCl_2$ gradient centrifugation (isopycnic or buoyant density) - A linear gradient of these compounds in buffer is prepared in the centrifuge tube. As the concentration of the compound is increased the density of the medium increases in the tube. Density is low at the top and high at the bottom. Macromolecule centrifuged through will form a band at a position equal to their buoyant density. Useful for separating molecules of different

densities even when the densities are very close. Drawback is that $CsCl_2$ can permanently inactivate some viruses.

Other medium are Histodenz gradient, Sorbitol cushion and Glycerol-tartrate gradient which are used specifically for different viruses.

In-Test Question

Why is purification of viruses important?

Answer

Viruses need to be purified for many studies in which properties or structure of the virus must be distinguished from those of the host cells or culture medium, such as analyses of structure of viral polypeptides and function of membrane glycoproteins.

Self-Assessment Exercise 1

Provide answers to the following questions in 20 minutes

Write short note on 2 types of sedimentation medium used in purification of viruses

3.4 Summary

- Viruses can be purified from tissue culture mediums.
- Virus can then be separated from materials by differential centrifugation, density gradient centrifugation, column chromatography and electrophoresis.
- Viruses can also be purified by high speed centrifugation in density gradients of Cesium Chloride ($CsCl_2$), potassium tartrate, potassium citrate of sucrose.
- Icosahedral viruses are easier to purify than enveloped viruses because enveloped viruses contains variable amounts of envelope per particle, the virus population is heterogeneous in both size amid density

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3.6. Possible Answers to Self-Assessment Exercises

SAE 1

Two types of centrifugation medium namely sucrose cushion or gradient and $CsCl_2$ gradient centrifugation

a. Sucrose cushions or gradient - A fixed concentration or a linear gradient of sucrose is used. Increasing the density and viscosity of the medium decreases the rate at which virus sediments through them. In general a cushion of sucrose is prepared at the bottom of the centrifuge tube and the sample containing virus is overlaid over the cushion. Since most viruses have greater densities than sucrose, separation is based on S values. This method can be used to separate molecules with relatively close S values. Sometime glycerol is also used in place of sucrose. In the case of gradient, different concentration of sucrose is prepared one of which will house a band of the pure viral particles during after centrifugation.

b. $CsCl_2$ gradient centrifugation - A linear gradient of $CsCl_2$ in buffer is prepared in the ultracentrifuge tube. As the concentration of the $CsCl_2$ is increased the density of the medium increases in the tube so that density is low at the top and high at the bottom. Viral particle centrifuged through this medium will form a band at a position equal to their buoyant density. These are useful to separate viruses of different densities. Limitation of this method is that $CsCl_2$ can permanently inactivate some viruses.

UNIT 4 ASSESSING THE PURITY OF VIRIONS AND IDENTIFICATION OF A VIRAL PARTICLE

CONTENTS

4.1 Introduction

This unit covers assessing the purity of virions and how to identify a viral particle. You will learn the factors to consider when identifying a viral particle.

4.2 Learning Outcomes

Student must be familiar with the different methods used in assessing the purity of viral particles, how to identify viral particles and factors considered in identifying a viral particle.

4.3 Methods for assessing the purity of virions

4.3.1 Spectrophotometric analysis

This is measured using the Ultra-violet (UV) absorption of the purified virus sample at 260 and 280 nm. This ratio ($A_{260/280}$) is a characteristic of a pure virus and is dependent on the amount of nucleic acid and protein in the virus. The number can be used to estimate the amount in the preparation. Nucleic acid absorbs light about twice as well at 260 vs 280 and vice-versa for protein (Figure 7).

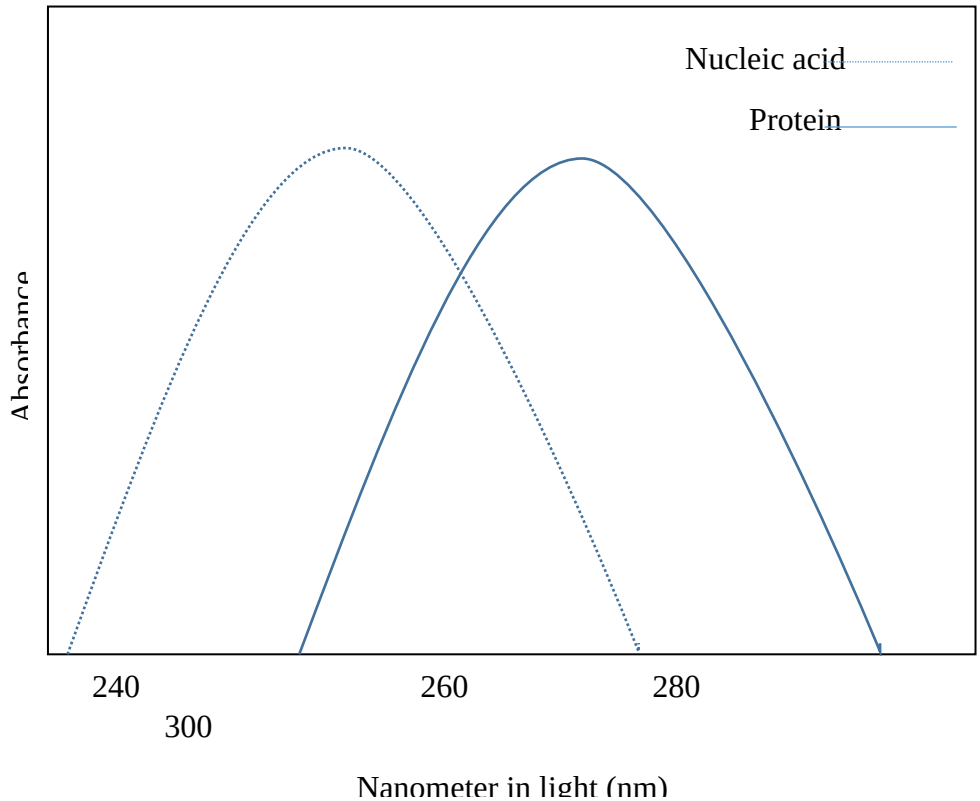

Figure 7. Graphical representation of spectrophotometric reading of Ultra-violet (UV) absorption at 260 nm and 280 nm.

In-test Questions (ITQ)

What is spectrophotometry used for?

Answer

Spectrophotometry applications are useful to measure the absorbance, reflectance, and transmission of light by gases, liquids, and solids.

How is spectrophotometry used in lab?

Answer

It is used to measure the amount of light that passes through a sample material and, by comparison to the initial intensity of light reaching the sample, they indirectly measure the amount of light absorbed by that sample. Spectrophotometers are designed to transmit light of narrow wavelength ranges.

What is the use of spectrophotometer in the laboratory?

Answer

Spectrophotometers measure light intensity as a function of wavelength and are commonly used to measure the concentration of a compound in an aqueous solution.

4.3.2 Serological methods

Antibodies to viral proteins are used to characterize, detect, or quantify virions. Antibodies can be made in several ways. Whole virus (possibly attenuated/ modified so cannot cause disease) can be injected into animals (rabbit or mouse) and monoclonal (single type of antibody generally recognizes a single epitope) or polyclonal (several different antibodies that may recognize several epitopes). A second approach is to purify or clone individual viral proteins and inject these directly. Methods available for using antibodies include ELISA (Enzyme-linked immunosorbent assay), RIA (radioimmune assay), RIPA (radioimmune precipitation assay), western blotting, direct precipitation of virus with antibody, neutralization of viral infectivity, complement fixation by the virus-antibody complex, and others.

In-test Question (ITQ)

What are antibody serology tests?

Answer

Antibody serology tests check for the presence or level of specific antibodies in the blood. Antibodies are proteins that your immune system makes to fight foreign substances. These substances are often pathogens, (disease-causing germs) such as viruses and bacteria.

What are types of serological evidence?

Answer

There are different types of serological tests, flocculation tests, neutralization tests, hemagglutinin-inhibition tests, enzyme-linked immunosorbent assays (ELISAs), and chemiluminescence immunoassays.

What are serological methods?

Answer

Serological methods are used for measuring the antibody response while the presence of virus can be demonstrated by cultivation or demonstration of specific antigens or gene sequences.

4.3.3 Electron microscopy

Method allows the visualization of single virus particles. It is based on the principle of electron scattering. A beam of electrons is focused on the sample. Electrons within the specimen will scatter the electron beam. The scattering effect is enhanced by the presence of heavy, electron rich metal ions (i.e. gold, platinum) within the sample. This is why the sample is coated with a solution containing a heavy metal. Resolution in the nm range (10^{-9} meter) is possible. Negative staining (Sodium phosphotungstate or uranyl acetate that will stain background but not the virus particles) or shadowing techniques (place specimen on support and direct a vaporized heavy metal across the sample at an angle. This creates a region where relatively little metal deposits just behind the viral particle (resulting in a shadow).

In-test Question (ITQ)

What are the major disadvantages of Electron microscopy?
Ans.: The main disadvantages are cost, size, maintenance, researcher training and image artifacts resulting from specimen preparation. This type of microscope is a large, cumbersome, expensive piece of equipment, extremely sensitive to vibration and external magnetic fields.
What is difference between light and electron microscope?

Answer

The main difference between light microscope and electron microscope is that beam of electrons is used for magnifying the image of an object while visible light is used in the light microscope to magnify images of tiny areas of materials or biological specimens.

4.3.4 X-ray crystallography

X-ray crystallography involves the analysis of crystallized virus. Virus crystals are symmetrical structures composed of many iso-metric viruses. The atoms of the crystal will diffract X-rays in a structure dependent manner. This approach has been used to analyze the structure of the viruses at the molecular level. Resolution at the Armstrong level (10^{-10} meters, in the bond length range) is possible.

In-Test Questions

What is X-ray crystallography used for?

The aim of x ray crystallography is to obtain a three dimensional molecular structure from a crystal. A purified sample at high concentration is crystallised and the crystals are exposed to an x ray beam.

Self-Assessment Exercise 1

Provide answers to the questions in 20 minutes

1. Discuss electron microscopy (EM) as means of identification of viral particles
2. List the serological-based methods used in assessing the purity of viral particles during centrifugation
3. How does electron microscope affect cells?

4.4 Identification of a viral particle

The identification of viral particles is as previously discussed in Module 2 Unit 4. A purified physical particle should fulfill the following criteria before it is identified as a virus particle:

1. The particle can be obtained only from infected cells or tissues.
2. Particles obtained from various sources are identical, regardless of the cellular species in which virus is grown.
3. The degree of infective activity of the preparation varies directly with the number of particles present.
4. The degree of destruction of the physical particle by chemical or physical means is associated with a corresponding loss of virus activity.
5. Certain properties of the particles and infectivity must be shown to be identical, such as their sedimentation behavior in the ultracentrifuge and their pH stability curves
6. The absorption spectrum of the purified physical particle in the ultraviolet range should coincide with ultraviolet inactivation spectrum of the virus.
7. Antisera prepared against the infective virus should react with the characteristic particles and vice versa. Direct observation of an unknown virus can be accomplished by electron microscopic examination of aggregate formate in a mixture of antisera and crude virus suspension.

8. The particles should be able to induce the characteristic disease in-vivo (if such experiments are feasible).
9. Passage of the particles in tissue culture should result in the production of progeny with biologic and serologic properties of the virus.

In-Test Question

What are the different methods of identification of viral particles?

Answer

Electron Microscopy
Histology/cytology
Virus isolation
Nucleic acid-based methods (PCR, RT-PCR, Real time RT-PCR)
Serological-based methods (ELISA, haemagglutination inhibition assay, Neutralization assay).

Self-Assessment Exercise

Provide answer to the question in 10 minutes

Why is virus quantification important?

4.5 Summary

- A purified physical particle should fulfill nine important criteria before it is identified as a virus particle as listed in the unit content.
- Spectrophotometery, serological assays, electron microscopy and X-ray crystallography are methods of assessing the purity of a viral particle.

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4.7 Possible Answers to Self-Assessment Exercises

SAE 1

1. Although this is one of the oldest techniques it is not routinely used in diagnostic laboratories anymore. Electron microscopy (EM) is the only method available for directly visualizing the

virus, and therefore has many applications beyond being purely diagnostic. The visualization of viruses with EM involves negative staining of the clinical specimen. Negative staining of the clinical sample is a relatively straightforward; inexpensive technique that would represent a "catch all" method of viral identification. EM could be particularly useful in identifying fastidious or non-cultivable virus in specimens, providing they have a high virus concentration with a sensitivity limit of approximately 10^6 viral particles per milliliter of specimen, making a negative result difficult to interpret. While the sensitivity could be increased by ultracentrifugation or antibody-induced clumping, a further limitation is the lack of specificity, as EM can only identify up to the family level whereafter, other methods would have to be applied for a specific diagnosis. Although the major advantage of EM is the speed with which a result could be obtained (30 min), the high cost of the instrument and specialized training and expertize needed, coupled with the lack of sensitivity and specificity, does not make this a viable option for routine diagnostics. There are two types of EM such as Transmission EM and Scanning EM.

2. Enzyme-linked immunosorbent assay (ELISA), radioimmune assay (RIA), radioimmune precipitation assay (RIPA), western blotting, direct precipitation, neutralization test
3. Electron microscopes use a beam of electrons instead of beams or rays of light. Living cells cannot be observed using an electron microscope because samples are placed in a vacuum.

SAE 2

Viral quantification involves the counting of viruses or viral molecules in a known volume to determine their concentration. It plays an essential role in studies carried out in the fields of recombinant protein production, viral vaccine production and infectious disease.

UNIT 5 PRESERVATION OF VIRUSES AND ETHICS IN A VIROLOGY LABORATORY

CONTENTS

5.1 Introduction

In rounding off this course, we shall look into methods of preserving viruses such as freezing and lyophilization and ethics in a virology laboratory. Viruses can also be store in cryo storage facility for longer year.

5.2 Learning Outcomes

Student must have understood the methods of preserving viruses and the dos and don’ts in a virology laboratory.

5.3 Preservation of viruses

The preservation of virus is an important sensitive area in virology. It is necessary to preserve viruses after being purified for research purposes and in the development of vaccines. Viruses cannot be preserved on ordinary laboratory media as in most bacteria or fungi. They are preserved as follows.

5.3.1 Freezing

Virus infectivity is retained well at temperatures below -60°C. Many freezers can reliably maintain these ultra-low temperatures. In many virology laboratories, -70°C (or more recently -80°C) is the favoured temperature, partly because viruses are known to survive for decades at -

70°C and partly because modern freezers do not have to work at their maximum capacity to maintain this temperature thereby increasing their reliability. Viruses should be frozen rapidly and this is most readily accomplished by storing only small volumes (0.1 to 0.5 ml) of virus suspension. If retention of virus infectivity is not essential, for example in cases where the sample is required as an antigen in an ELISA test, it can be stored for many years at -20°C without loss of antigenic activity even though the infectivity might be significantly reduced. Long term storage at -20°C of acetone or paraformaldehyde-fixed virus infected cells on glass coverslips is a very convenient method of retaining specific virus antigens for sero-diagnostic purposes. Proteins in the form of serum or other biological material, in buffered isotonic salt solution or tissue culture medium, can be used to preserve infectivity of most viruses held at ultra-low temperatures. It is good practice to thaw frozen virus samples rapidly by placing the cryotubes in a water bath at 37°C. Thawing should be carried out just before the virus is to be used unless it is known that the virus has good thermos-stability characteristics when held at laboratory temperatures. The cryotubes should be removed from the water bath immediately after thawing is completed and placed at 4°C until they are needed. If possible, virus stocks for long-term preservation should be backed up by storage in more than one ultra-low temperature freezer.

In-test Question (ITQ)

Virus survive freezer?

Answer

On the other end of the spectrum, at 40 degree C, or 104 degree F, and 80% humidity, the viruses survived for less than 6 hours. This suggests that coronaviruses survive better on surfaces at colder temperatures. It is also expected that the virus would survive being frozen.

5.3.2 Freeze-drying viruses for long term preservation (Lyophilization)

This is probably the most satisfactory method of preserving viruses for very long periods. There are several variations in the technical procedures depending on the specific design of the freeze-drying equipment. For small numbers of samples and small volumes of virus, the simplest and most effective method involves only one vacuum stage because the glass ampoules are placed directly onto the branched exhaust manifold of the freeze dryer. We use either 5 ml or 2 ml glass ampoules and always ensure that the volume of sample occupies less than one third of the total ampoule volume. The ampoules are designed

with a long thin neck and a snap-off point at the shoulder i.e. above the body of the ampoule. It is quite common practice to produce a small restriction in the neck of each ampoule by heating it before use, to simplify the process of sealing it under vacuum after the freeze-drying process is complete. It is worthwhile practicing this technique on different sized ampoules before starting the preservation work. A good seal is very important for long-term preservation.

This procedure consists of rapid freezing at low temperature (in a bath containing Alcohol and dry ice) and dehydration from the frozen state at high vacuum; 1.0-50% of normal plasma or serum in the fluid menstruum protects the virus to be frozen and dried. The plasma or serum must not contain neutralizing antibodies. Skimmed milk also another "protective" menstruum in which virus-containing material may he suspended. The use of Di-nitrogen oxide (N_2O) which maintains a temperature of -160^{o}C is also a better way of preserving viruses for many years.

Some general rules for preservation apply to most viruses.

1. Freeze-dried preparations of virus can be maintained for decades at 4°C in the dark and lower temperatures increase the storage time. Although this principle has not been tested exhaustively for every known virus, it has been demonstrated with very many different viruses.

2. Virus infectivity is retained for very long periods in liquid nitrogen. This is not the most convenient/cost effective method of storing viruses for most purposes. However, most viruses will survive almost indefinitely in liquid nitrogen. It is important to enclose the individual cryotubes in a heat shrinkable tubing, such as Nunc - CryoFlex tubing.
3. Proteins are effective protectants for virus cryopreservation. The suspending medium of choice for many viruses is tissue culture medium containing added serum or other proteins, at concentrations up to or greater than 10%. The proteins are believed to provide protection of virus infectivity when samples are frozen or freeze-dried.
4. It is good practice to preserve small volumes of virus suspension. In general, virus infectivity is maintained more effectively when samples are preserved in small volumes because the processes of freezing, thawing, freeze-drying and reconstituting virus samples can be carried out much more quickly. Rapid freezing and thawing or reconstitution of a virus preparation is less harmful to the virus than slow freezing, thawing and reconstitution.

5. High titre virus preparations are preferable for long-term storage. Since the half-life of a virus held either at low temperature or freeze-dried is unaltered by the quantity of infectious virus in the preparation, it follows that a high titre virus preparation will retain viability for longer than a low titre preparation.
6. Dry ice should only be used to preserve viruses in totally sealed containers. The optimal pH for virus storage is between pH 7.0 and 8.0. Viruses are relatively labile at pH 6.0 or below. It is therefore unwise to store virus preparations in unsealed containers on dry ice since the released carbon dioxide is absorbed through the joint between the cap and the cryotube and the absorbed carbon dioxide reduces the pH of the preserved virus suspension.
7. Viruses can be preserved for long periods as nucleic acid. The purified nucleic acid of positive stranded RNA viruses (i.e. those in which the viral RNA is the messenger RNA) and many DNA viruses (those that do not enclose essential enzymes in their structure) is infectious. This principle can be utilised to preserve these viruses for very long period of time. The ethanol precipitated RNA and DNA can be stored almost indefinitely at 4°C (or lower temperatures) under ethanol. The ethanol is important for long term storage of RNA, to inhibit enzymes that breakdown RNA. DNA can be stored either under ethanol or as dried DNA. This method of virus preservation is one of the most effective available but is not really very widely used. Virus frozen as nucleic acid can probably be preserved almost indefinitely and since it can be stored in extremely small volumes, many samples can be maintained without the need for large volumes of storage capacity.

In-Test Question (ITQ)

How long is virus stable at 4°C?

Answer

Virus can be stored at 4°C for a short time (less than a week) before using after reception. Since Lentiviruses are sensitive to freeze-thawing and the titre drops with repeated freeze-thawing, aliquot viral stock should be stored at -80°C freezer immediately upon arrival for long-term usage.

Self-Assessment Exercise 1

Provide Answer to the following question in 10 minutes

How do you freeze a virus?

5.4 Ethics in a virology laboratory

The virology laboratory is a place where the scientist needs to take special caution in addition to normal laboratory practices:

- You must wear a sterile laboratory coat every time.
- You must wear a shoe cover.
- You must not eat in the laboratory.
- You must not wear make-ups, jewelry or wear your hair down in the laboratory.
- The work-benches must be free of unnecessary items such as bags.
- Nose mask and sterile gloves must be available at all times.
- You must not talk when working with RNA viruses as RNAases are everywhere and may degrade your RNA genome.
- Safety signs must be in appropriate places and on chemicals.
- Proper storage of chemicals before and after use.
- Proper labeling of samples and chemicals.
- The laboratory must be clean at all times.

5.4.1 Laboratory safety

Although virology can be considered one of the less hazardous human occupations compared with building, mining, or driving a car, it is also true that many cases of serious illness and over 700 deaths from laboratory-acquired infection have been recorded over the years, particularly from togaviruses, flaviviruses, arenaviruses, and filoviruses. In many countries, including the United States, European Union, Canada, and Australia, pathogens are classified into categories 1 to 4 based on properties of pathogenicity, transmissibility, host range, the availability of treatment and vaccination, etc. These categories are endorsed by the World Health Organization. An appropriate level of containment and handling practice is specified for each risk category, ranging from Biosafety level 1 (BSL 1) - standard microbiological practice - for category 1 organisms, through to BSL 4 - maximum security facilities and containment protocols - for category 4 viruses and organisms. In research laboratories, work usually involves known agents where the hazards are predictable and safety procedures appropriate to the level of hazard can be applied. However, diagnostic laboratories

accept human samples of unknown infective status that are in a practical sense an extension of the patient. These may contain HIV, hepatitis B or C virus, or other common or exotic infective agents. The usual approach is to regard all unknown biological specimens as potentially infectious, and to apply a level of containment appropriate to the majority of agents likely to be present, for example, BSL2; individual samples from known or suspected cases of greater hazard, for example, Ebola, are handled at a higher containment level once these are identified. Similarly, material from laboratory or wild animals, particularly primates, may contain unknown pathogens with a danger to humans.

For all containment levels, good laboratory technique is an essential aspect for avoiding laboratory hazards. Use of elaborate safety equipment, negative air pressure management, etc. will not compensate for sloppy or unsafe practices. Rigorous aseptic technique must be practiced. Mouth pipetting is banned. Laboratory coats must be worn at all times, gloves must be used for handling potentially infectious materials, and special care taken with sharp objects. Rigorous attention must be given to sterilization, where possible by autoclaving, of all potentially infectious waste as well as used equipment. Special arrangements for the disposal of "sharps" are essential. Spills are cleaned up with an appropriate chemical disinfectant. Staff should be immunized against such diseases as hepatitis B and poliomyelitis as a matter of routine and, when vaccines are available, against more exotic agents in those special laboratories handling such viruses. Limitations should be placed on the type of work undertaken by pregnant or immunosuppressed employees.

It is also important to inactivate the infectivity of any particularly dangerous viruses employed as antigens in serological tests other than virus neutralization (e.g., arenaviruses, rhabdoviruses, togaviruses, or flaviviruses). This can be done, without destroying antigenicity, by γ-irradiation, or by photodynamic inactivation with ultraviolet light in the presence of psoralen or related compounds. Viral proteins produced by recombinant DNA technology provide a simple, standardized, and safe alternative to whole virus as antigen in many serological tests.

In-Test Question

What are the risks in a virology laboratory?

Answer

In addition to safety risks associated with any clinical laboratory, such as chemical, fire, electrical, and radioactive hazards, the microbiology laboratory presents the hazard of exposure to infectious agents, or

biohazards. Biohazards are biological substances that may present a health risk to humans.

Self-Assessment Exercise 2

Provide Answer to the following question in 10 minutes

1. What are the most common laboratory safety problem?
2. What are the 3 most common ways accidents and incidents occur in the laboratory?

5.5 Summary

- Lyophilisation and freezing are methods of preserving viruses
- Good laboratory practice is important in getting good result from virus studies.

5.6 References/Further Readings/Web Sources

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5.7 Possible Answers to Self-Assessment Exercises

SAE 1

Virus samples are preserve in the following order

i. Do aliquot and freeze at -80^{o}C for long term storage if the virus is not used within ~1 week.
ii. Thaw on ice just before use.
iii. Use virus within ~ 6 months of storage at -80^{o}C unless the titre is much higher than needed.

SAE 2

1. Never store or consume food or drinks in labs where hazardous materials are used. This goes for keeping your lunch, snacks and sodas in refrigerators made for chemicals. Safety Eyewashes should be cleaned and flushed weekly.
2. Generally, accidents result from the following factors:

- Fires.
- Electrical short circuits and shocks.
- Leaks and spills.
- Unplanned storage.
- Use of defective material handling equipment.
- Careless handling of containers.

Glossary

AIDS - Acquired Immunodeficiency Syndrome

BSL 1 - Biosafety level 1
BSL 4 - Biosafety level 1
CAM - chorioallantoic membrane
CDC - Centers for Disease Control and Prevention
CMV - Cytomegalovirus
CNS – Central nervous system
$CsCl_2$ - Caesium Chloride
CSE – Cytopathic effect
CSF - cerebrospinal fluid
DEAE - Diethylaminoethyl
DNA – Deoxyribonucleic acid
ELISA - Enzyme-linked immunosorbent assay
EM – Electron microscope
GRID - Gay-related immune deficiency
HAART - highly active antiretroviral therapy
HHV-8 - human herpesvirus 8
HIV – Human immunodeficiency virus
HPV - human papillomavirus
HSV – Herpes simplex virus
HTLV - human T-lymphotropic virus
KS - Kaposi's sarcoma
LAV - lymphadenopathy-associated virus
LTNP - long-term non-progressor
N_2O - Di-nitrogen oxide
nm - Nanometer
NNRTI - non-nucleoside reverse transcriptase inhibitor
NRTI - nucleoside analog reverse transcriptase inhibitor
PCP - Pneumocystis carinii pneumonia
PCR – Polymerase chain reaction
PrEP - Pre-exposure prophylaxis
RIA - radioimmune assay
RIPA - radioimmune precipitation assay
RNA – Ribonucleic acid
RT-PCR – Reverse transcriptase polymerase chain reaction
SIV - Simian immunodeficiency virus
SIVcpz - Simian immunodeficiency virus chimpanzee subspecies (*Pan troglodytes troglodytes*)
SIVsmm - Simian immunodeficiency virus sooty mangabey (*Cercocebus atys atys*)
TASP - treatment as prevention
UV - Ultra-violet
VZV – Vericellar zooster virus

End of Module Questions

Multiple Choice Questions

1. Which of the following therapy is used to halt HIV replication? a) Gene therapy, b) Chemotherapy, c) HAART therapy, d) Physiotherapy
2. HIV-1 is a member of _____ family. a) retroviridae, b) arenaviridae, c) filoviridae, d) flaviviridae
3. Which of the following member of the lentivirus family causes pneumonia? a) HIV-1, b) HIV-2, c) Visna-Maedi, d) Bovine immunodeficiency
4. . HIV is not transmitted through _____. a) saliva, b) breast milk, c) vaginal fluids, d) blood
5. Group of people with a high risk of contracting HIV infection are children below 5 years of age. a) True, b) False
6. On which of the following cells, HIV virus attacks? a) Red Blood Cells, b) Neuroglial cells, c) Platelets, d) Hair follicles
7. Which of the following techniques is not used to diagnose HIV virus? a) PCR, b) Western Blot, c) ELISA, d) Widal test
8. Which of the following drug is used for the treatment of AIDS? a) Azidothymidine, b) Nevirapine, c) Acetaminophen, d) Alprazolam
9. . HIV-I is more common in Africa and HIV-II is more common in India and USA. a) True, b) False
10. When is AIDS day celebrated? a) 1st December, b) 1st November, c) 1st May, d) 1st August
11. Full-form of HIV is _____. a) Human Infecting Virus, b) Haemorrhage Inducing Virus, c) Human Immunodeficiency Virus, d) Hay fever Inducing Virus
12. AIDS virus belongs to which of the following group of viruses? a) Reovirus, b) Retrovirus, c) Rhinovirus, d) Ribovirus
13. When was AIDS first clinically reported as a disease? A) December 1, 1980, b) May 18, 1981, c) June 5, 1981, d) May 30, 1981
14. Who were the first set of people discovered to be manifesting symptoms of AIDS infection? a) prostitutes, b) homeless children, c) gay men, d) care givers
15. HAART was discovered in _____. a) 1998, b) 1999, c) 1994, d) 1995
16. HIV-1 was first known as _____. a) HHV, b) LAV, c) HSV, d) EBV
17. HIV-1 is haploid. a) True, b) False
18. Which of the following is not an opportunistic serious disease contracted in HIV infected patients? a) Kaposi’s sarcoma, b) Toxoplasmosis, c) Leukoplakia, d) Influenza

19. People with AIDS have an increased risk of developing various viral-induced cancers. ac) True, b) False
20. Which of the following has the tendency to reduce the risk of HIV transmission? a) reduced sexual partners, b) use of condoms, c) use of spermicide, d) government monitored circumcision
21. Which country was the first to eradicate mother-to-child transmission of HIV? a) USA, b) Canada, c) Germany, d) Cuba
22. Rabies was first discovered by which of the following people? a) Montagnier, b) Robert Gallo, c) Lew Pasteur, d) Linneaus
23. Which of the following is not a natural host of rabies? a) cats, b) foxes, c) goats, d) wolfs
24. The genome of rabies virus is _____ a) ssDNA, b) ssRNA, c) dsDNA, d) dsRNA
25. Rabies virus can be transmitted to man through _____ from an infected animal. a) bite, b) insect vector, c) airborne, d) waterborne
26. Rabies virus infection is present in all continents of the world except in _____ a) Europe, b) North America, c) Australia, d) Asia
27. Rabies virus could be isolated from all of the following except a) faecal sample, b) brain, c) saliva, d) urine
28. Which of the following animal is most appropriate for virus isolation through inoculation a) sheep, b) horse, c) rabbit, d) mouse
29. Viruses can be introduced to laboratory animals through any of the following except _____ a) intracerebral, b) intranasal route, c) orally, d) subcutaneously
30. Who was the first to apply tissue culture in diagnostic virology? A) Maitland, b) Pasteur, c) Steinhardt, d) Robin
31. The essence of purifying virus particles it to have meaningful studies on the properties of virions. a) True, b) False
32. Virus materials can be separated from materials by the following except _____. a) electrophoresis, b) differential centrifugation, c) column chromatography, d) material filtration
33. What does spectrophotometric analysis measure? a) x-ray, b) ultra-violet absorbance, c) gamma ray, d) alpha rays
34. What is the wavelength at which purified viruses are measured in spectrophotometric analysis? a) 450/480 nm, b) 220/240nm, c) 260/260, d) 180/220 nm
35. Serological method of virus diagnosis is based on the detection of _____ a) viral nucleic acid, b) viral protein, c) none of the above, d) all of the above
36. Antibodies produced from viral proteins can be used to do the following except _____ virions. a) characterize, b) detect, c) attenuate, d) quantify

37. Viruses can be preserved the same way as fungi and bacteria. a) True, b) False
38. Long term storage of virus samples is appropriate at _____ degree Celsius. a) 37, b) 20, c) 4, d) -20
39. Virology laboratory ethics include the following except _____. a) nose mask and sterile gloves must be available at all times, b) safety signs must be in appropriate places and on chemicals, c) listening to music, d) proper storage of chemicals before and after use
40. Maximum security facilities and containment protocols as prescribed by the WHO is _____. A) BSL 2, b) BSL 4, c) BSL 1, d) BSL 3

Theory Questions

1. List 5 diseases, host, site of infection and agents of such diseases caused by prions
2. Discuss the prevention and control of AIDS
3. Discuss viral pathogenesis
4. Discuss the parthenogenesis of two named virus

Answers to MCQ Questions (Module 3)

S/N	Answer	S/N	Answer	S/N	Answer	S/N	Answer
1	c	11	c	21	d	31	a
2	a	12	b	22	c	32	d
3	c	13	c	23	c	33	b
4	a	14	c	24	b	34	c
5	b	15	d	25	a	35	b
6	b	16	b	26	c	36	c
7	d	17	b	27	a	37	b
8	a	18	d	28	d	38	d
9	b	19	a	29	c	39	c
10	a	20	b	30	c	40	b

Answers to Theory Questions (Module 3)

1. List of 5 diseases, host, site of infection and agents of such diseases caused by prions

Agent	**Host**	**Site of Infection**	**Disease**
Scrapie	Sheep	Central nervous system	Scrapie spongiform encephalopathy
Kuru agen	Humans	Central nervous system	Kuru spongiform encephalopathy
Creuzfeldt-	Humans	Central nervous	Creuzfeldt-Jacob

Jacob Agent		system	spongiform encephalopathy
Mad cow agent	Cows and humans	Central nervous system	Mad cow spongiform encephalopathy
Chronic wasting disease agent	Deer and elk	Central nervous system	Chronic wasting disease

2. Prevention and control of AIDS

There is currently no effective method for the prevention or cure of this devastating disease, though several, antiviral drugs are being tested. Without intervention by drugs or vaccines, the way to avoid epidemic spread of HIV is to have a life-style that minimize or eliminates high-risk factors discussed above. It is striking that the disease has not occurred among medical and health-care workers who are for AIDS patients but do not have life-styles which place them in the high-risk groups. No cases have been documented to result from such common exposures as sneezing, coughing, sharing meal, or other casual contacts.

Because HIV may be transmitted in blood, all donor blood should be tested for antibody and, when such tests become commercially available, for virus or for viral antigens. Properly conducted antibody tests appear to detect almost all HIV-1 carriers. Since the introduction of widespread screening of blood donors for virus exposure and the rejection of contaminated blood, transmission by blood transfusion has virtually disappeared. HIV-2 Infections are exceedingly rare in the USA and currently do not pose a threat to the blood supply.

Public health authorities have recommended that persons reported to have an HIV infection be provided the following information and advice.

1. The prognosis overlong term for an infected individual is unknown. However, available data indicate that most persons will remain infected for life and many will develop the disease.
2. All tough asymptomatic, these it individuals may transmit HIV to others. Regular medical evaluation and follow-up are advised, especially for those who develop signs or symptoms suggestive AIDS
3. Infected persons should refrain from donating blood, plasma, body organs, other tissue or sperm.
4. There is a risk of infecting other by sexual intercourse (Vaginal or anal, by oral-genital contact, or by sharing of needles. The

consistent and proper use of condoms reduce transmission ion of the virus, though prevention is not absolute.

5. Toothbrushes, razors, or other implements that could become contaminated with blood should not be shared.
6. Seropositive women or women with seropositive sexual partners are themselves at increased risk of acquiring AIDS. If they become pregnant their oil-spring also are at high risk of acquiring AIDS.
7. After accidents that result in bleeding, contaminated surface should be cleanse with household bleach fresh diluted 1.10 in water.
8. Devices that have punctured the skin, e.g. hypodermic and acupuncture needles, should be steam sterilized by autoclaving before reuse or should be safely discarded. Whenever possible, disposable needless and equipment should be used.
9. When seeking medical or dental care for inter-current illness, infected persons should inform those responsible for their care that they are seropositive, so that appropriate evaluation can be undertaken and precautions taken to prevent transmission to others.
10. Testing for HIV antibody should be offered to persons who may have been infected as a result of their contact with seropositive individuals (e.g. sexual partners, persons with whom needles have been shared, infants born seropositive mothers).
11. Most persons with positive test for HIV do not need to consider a change in employment unless their work involves significant potential for exposing others to blood or other fluids. There is no evidence of viral transmission by blood handling.
12. Seropositive persons in the health care professions who perform invasive procedures or have skin lesions should take precautions similar to those recommended for hepatitis B carriers to protect patients from the risk of infection.
13. Children with positive tests should be allowed to attend school, since casual person- person contact of schoolchildren poses no risk. However, a more restricted environment is advisable for preschool children or children who lack control of their body secretions, display behavior, or have oozing lesions.

The health education messages for the general public have been summarized as follows:

1. Any sexual intercourse (outside of mutually monogamous HIVantibody-negative relationships) should be protected by a condom.
2. Do no share unsterile needles or syringes

3. All women who have been potentially exposed should seek HIV antibody testing before becoming pregnant and, if the test is positive, should consider avoiding pregnancy. HIV seropositivity in pregnant women can be considered grounds for termination of pregnancy if the woman so wishes.

Without a vaccine or treatment, the prevention of AIDS relies on the success of education projects involving behavioural changes at least for the immediate future.

4. Viral pathogenesis

Viral pathogenesis is the process by which a viral infection leads to disease. Viral pathogenesis is an abnormal situation of no value to the virus. The majority of viral infections are subclinical. It is not in the interest of the virus to severely harm or kill the host. The consequences of viral infections depend on the interplay between a number of viral and host factors.

Outcome of Viral Infection

Acute Infection

- Recovery with no residue effects
- Recovery with residue effects e.g. acute viral encephalitis leading to neurological sequelae.
- Death
- Proceed to chronic infection

Chronic Infection

- Silent subclinical infection for life e.g. CMV, EBV
- A long silent period before disease e.g. HIV, SSPE, PML
- Reactivation to cause acute disease e.g. herpes and shingles.
- Chronic disease with relapses and excerbations e.g. HBV, HCV.
- Cancers e.g. EBV, HTLV-1, HPV, HBV, HCV, HHV-8.

Factors in viral pathogenesis

- Effects of viral infection on cells (Cellular Pathogenesis)
- Entry into the Host
- Course of Infection (Primary Replication, Systemic Spread, Secondary Replication)
- Cell/Tissue Tropism
- Cell/Tissue Damage
- Host Immune Response
- Virus Clearance or Persistence

5. parthenogenesis of two named virus

i. Rubella virus

It is transmitted by the respiratory route and replicates upper/lower respiratory tract and then local lymphoid tissues. A viraemia occurs following an incubation period of 2 weeks and the virus spreads throughout the body.

Clinical Features

- Maculopapular rash due to immune complex deposition
- Lymphadenopathy
- Fever
- Arthropathy (up to 60% of cases)

Rubella virus enters the fetus during the maternal viraemic phase through the placenta.
The damage to the fetus seems to involve all germ layers and results from rapid death of some cells and persistent viral infection in others.

Preconception Risks

0-12 weeks 100% risk of foetus being congenitally infected resulting in major congenital abnormalities.

Spontaneous abortion occurs in 20% of cases.

13-16 weeks Deafness and retinopathy 15%
After 16 weeks Normal development, slight risk of deafness and retinopathy

ii. Herpes Simplex Virus (HSV)

HSV is spread by contact, as the virus is shed in saliva, tears, genital and other secretions.

Primary infection is usually trivial or subclinical in most individuals. It is a disease mainly of very young children i.e. below 5 years. About 10% of the population acquires HSV infection through the genital route and the risk is concentrated in young adulthood. Following primary infection, 45% of orally infected individuals and 60% of patients with genital herpes will experience recurrences. The actual frequency of recurrences varies widely between individuals. The mean number of episodes per year is about 1.6.

During the primary infection, HSV spreads locally and a short-lived viraemia occurs, whereby the virus is disseminated in the body. Spread to the to craniospinal ganglia occurs. The virus then establishes latency in the craniospinal ganglia. The exact mechanism of latency is not known, it may be true latency where there is no viral replication or viral

persistence where there is a low level of viral replication. It is well known that many triggers can provoke a recurrence. These include physical or psychological stress, infection; especially pneumococcal and meningococcal, fever, irradiation; including sunlight, and menstruation.

HSV is involved in a variety of clinical manifestations which includes:

- Acute gingivostomatitis
- Herpes Labialis (cold sore)
- Ocular Herpes
- Herpes Genitalis
- Other forms of cutaneous herpes
- Meningitis
- Encephalitis
- Neonatal herpes