

Virology_BT601

Introductory lecture

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PhD in Molecular Biology and Biotechnology

Specialization: Molecular Virology; Genetics

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Recommended Books

1. Principles of Molecular Virology

Sixth Edition

Alan J. Cann

Department of Biology, University of Leicester, Leicester, UK

2. Principles of Virology

Fourth Edition

Jane Flint et al. 2015

Department of Molecular Biology Princeton University Princeton,
New Jersey

In this course

What are Viruses?

Are Viruses Alive?

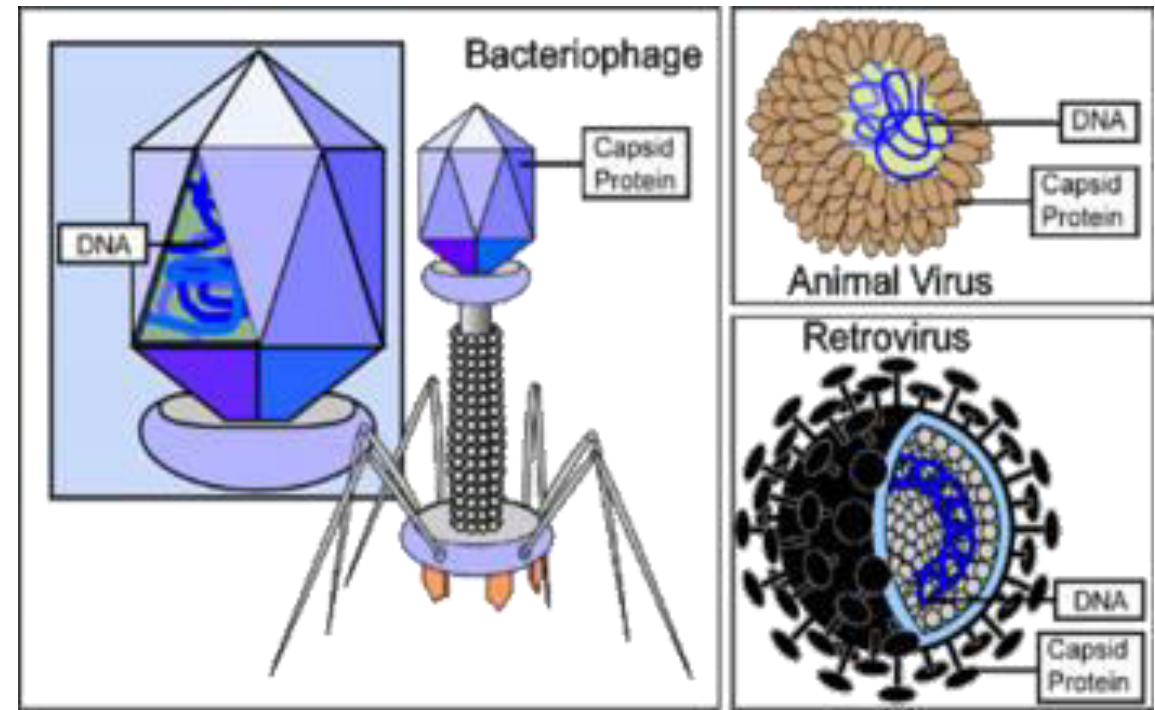
The History of Virology

Study of virus structure and composition

Reproduction and phases of virus replication

Virus-host interaction

The importance of viruses in biology and diseases



In this course

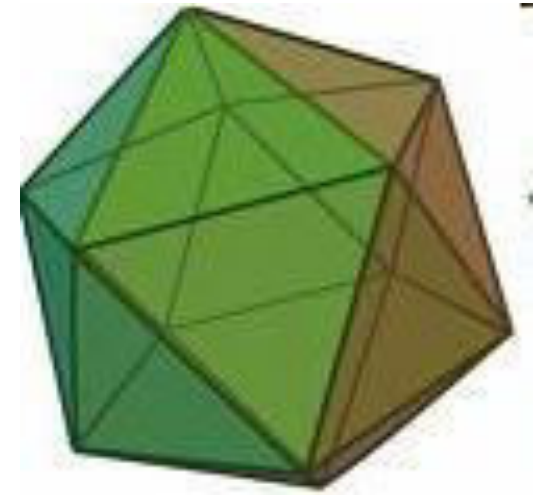
How viruses are different from other biological organisms

The molecular basis of how viruses work

Explain how the development of virology led us to our present understanding of viruses

Be able to discuss how technology has influenced the study of viruses in recent years

Discuss representative examples of viruses genomes to illustrate the range of genetic diversity seen in viruses



Why We Study Viruses

1. Viruses are everywhere
2. Causing diseases
 -  Human being  Animals  Plants
3. Generally can infect every living things including insects, bacteria and even other viruses
4. Viruses can be beneficial (**You've heard the Bad, here's the Good**)
5. Can cross species boundaries

More Insight

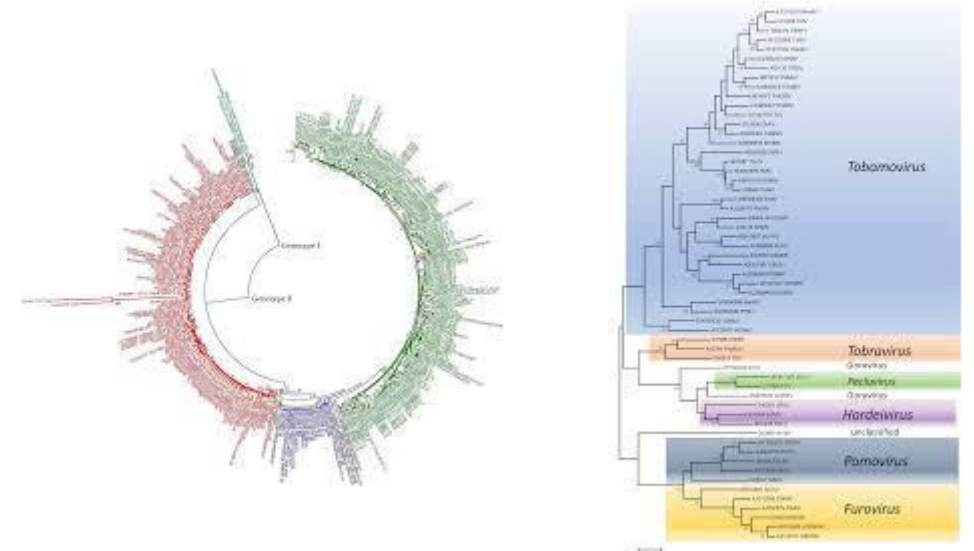
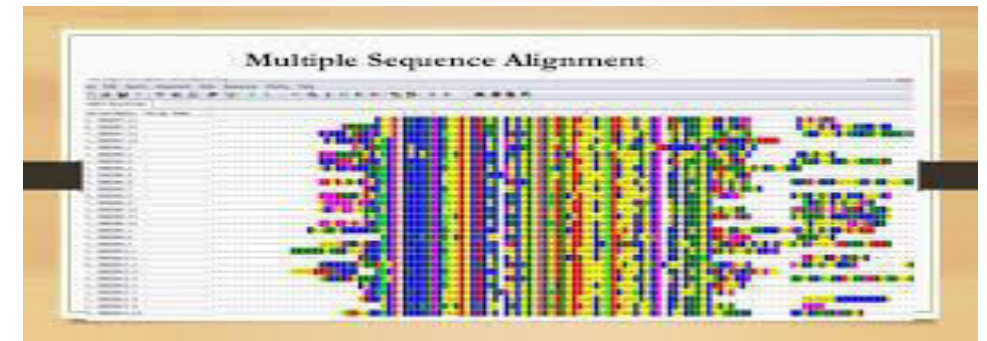
1. Replication of viruses
2. Discuss how drugs which block these processes can be used to combat virus infections
3. Mechanisms by which cells express the information stored in genes
4. Describe the genome coding strategies of different virus groups
5. Discuss the similarities and the differences between virus infections of plants and of animals

Immune responses

1. Explain how the immune responses to viruses enables the body to resist infection, and how viruses respond to this pressure
2. link between virus infection and disease
3. Research and Development on Vaccines
4. Planning and Implementation of Vaccination Programs
5. Surveillance of Disease Incidence and Adverse Events Before and After Implementation of Vaccination

Sequencing viral genome

1. What can virus sequences tell us
2. Phylogenetic Analysis
3. New viruses
4. Genotyping and sub-genotyping



What are VIRUSES

In this lecture we will look at the What are of viruses

At the end of this lecture

Student must have fully understood:

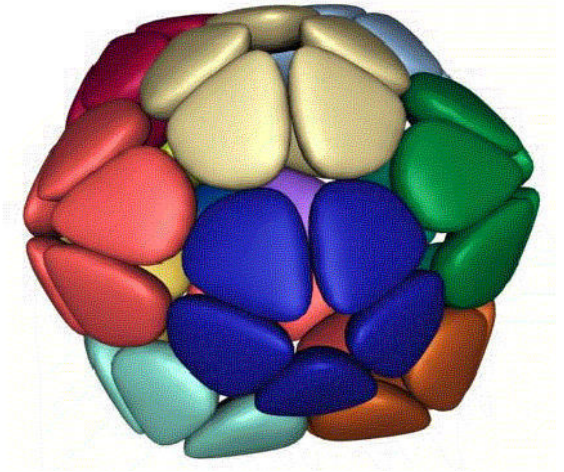
- Basic of Viruses

What are Viruses

- Virology is the study of viruses
- Are Viruses Living or Non-living?

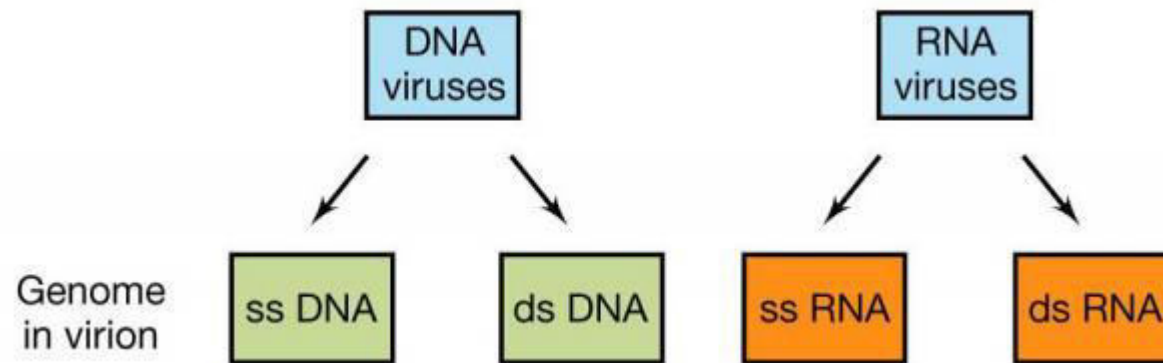
What are Viruses?

- A virus is a noncellular particle made up of genetic material and protein that can invade living cells
- Obligate intracellular parasites
- Too small to be seen by optical microscopes
- Size typically 20-400 nM: rod shaped or spherical
- Replicate inside host cells using the cell's enzyme systems



What are Viruses

- ❑ They have nucleic acid genomes, either DNA or RNA
- However, only one type of nucleic acid is found in the virion of any particular type of virus
- Single-stranded (ss), double-stranded (ds), or in the hepadnaviruses, partially double-stranded
- Coding capacity: ranging from few proteins to >200 proteins



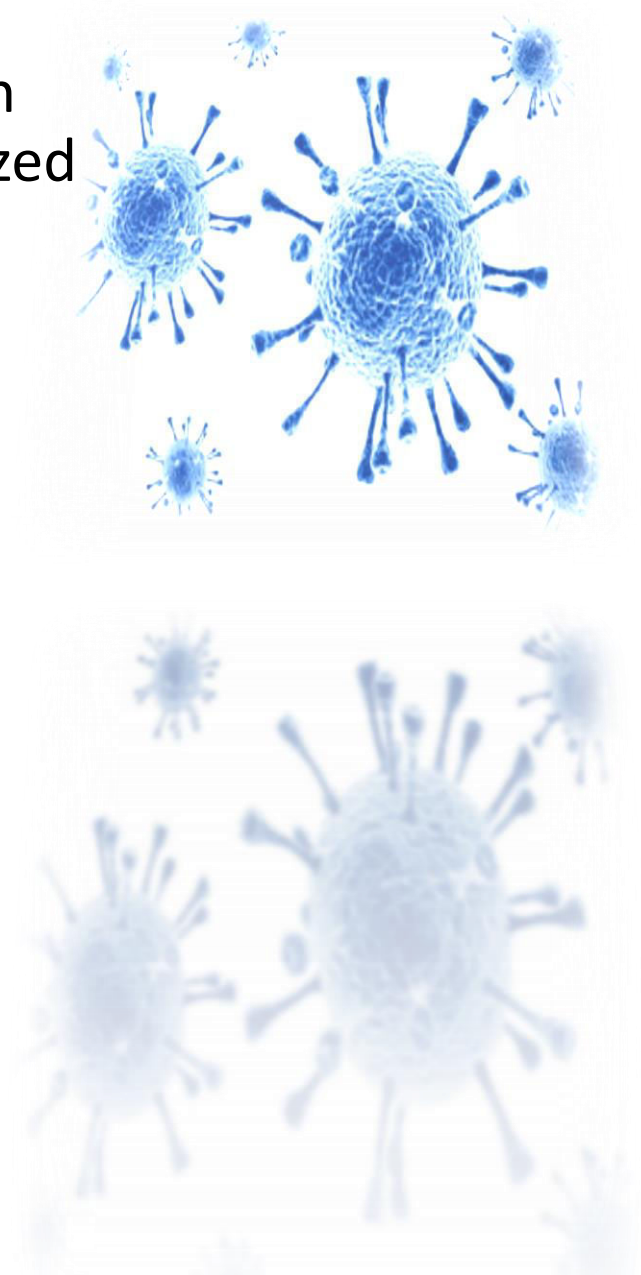
What are Viruses

- Unlike all living organisms, viruses do not 'grow' or undergo division but are produced from the assembly of pre-formed newly synthesized components within the host cell

- ☐ Host range - wide

- ☐ Viruses infect all types of living cells - animals, plants & bacteria

- animals
- plants
- microorganisms, including bacteria and archaea



What are Viruses

Viruses are smaller than bacteria: *common mistake*

The largest virus known (currently Pithovirus sibericum) is 1,200 nm long, while the smallest bacteria (e.g., Mycoplasma) are only 200-300 nm long.

The largest virus genome (Pandoravirus, 2.8 Mbp—million base pairs—approximately 2,500 genes)

is twenty times as big as

Smallest bacterial genome (Tremblaya princeps, at 139 kbp—thousand base pairs—and with only 120 protein coding genes)

Although it is still shorter than the smallest eukaryotic genome (parasitic protozoan Encephalitozoon, 2.3 Mbp)

General Characteristics of Viruses

In this lecture we will look at the General Characteristics of viruses

At the end of this lecture

Student must have fully understood:

- Basic of Viruses

General Characteristics of Viruses

- Viruses are found everywhere
- They are **acellular**, that is, they contain no cytoplasm or cellular organelles
- Metabolically inert
- Obligatory intracellular parasite
- They carry out no metabolism on their own and must replicate using the host cell's metabolic machinery

General Characteristics of Viruses

- In other words, viruses don't grow and divide
- New viral components are synthesized and assembled within the infected host cell
- Viruses can infect animals, plants, and even other microorganisms
- Totally dependent on their host cell for replication, they cannot be grown in synthetic culture media

General Characteristics of Viruses

- Viruses are infectious agents with both living and nonliving characteristics
- Living characteristics of viruses include the ability to reproduce – but only in living host cells – and the ability to mutate
- ❖ Nonliving characteristics include the fact that
 - not cells
 - have no cytoplasm
 - cellular organelles,
 - carry out no metabolism
 - therefore must replicate using the host cell's metabolic machinery
- They have a nucleic acid core containing DNA or RNA but not both
- Contain a protein coat called the capsid

History of VIRUSES

In this lecture we will look at the History of viruses

At the end of this lecture

Student must have fully understood:

- Early (1796–1930)
- Middle (1930–1954)
- Late (1957–1980)
- Recent (1980–)

History of VIRUSES

Early (1796–1930)

❖ 1796: (Jenner)

Cowpox virus used to vaccinate against smallpox

❖ Antony van Leeuwenhoek, a Dutch merchant, constructed the first simple microscopes and with these identified bacteria as the “**animalcules**” he saw in his specimens

❖ Robert Koch and Louis Pasteur in the 1880s jointly proposed the “germ theory” of disease that the significance of these organisms became apparent

❖ Koch’s postulates: Koch defined four famous criteria

1. The agent must be present in every case of the disease.
2. The agent must be isolated from the host and grown in vitro.
3. The disease must be reproduced when a pure culture of the agent is inoculated into a healthy susceptible host.
4. The same agent must be recovered once again from the experimentally infected host

❖ 1885: (Pasteur)

Rabies vaccine

❖ 1892:(Ivanovsky)

Description of filterable infectious agent

History of VIRUSES

Continue.....

- ❖ 1898: (Martinus Beijerinck)

Concept of the virus as a contagious element Plant virus, tobacco mosaic virus (TMV)

- ❖ 1898: (Freidrich Loeffler and Paul Frosch)

Animal virus (FMDV)

- ❖ 1901: (Reed et al)

Human virus (yellow fever virus)

- ❖ 1908: (Ellerman, Bang)

Leukemia-causing virus

- ❖ 1909: (Landsteiner, Popper)

Poliovirus

- ❖ 1911: (Rous)

Solid tumor virus (RSV)

- ❖ 1915–1917: (Twort, d'Hérelle)

were the first to recognize viruses that infect bacteria, which d'Herelle called bacteriophages (“eaters of bacteria”).

History of VIRUSES

Middle (1930–1954)

❖ 1931: (Woodruff, Goodpasture)

Virus propagation in embryonated chicken eggs

❖ 1933: (Smith et al.)

Human influenza virus

❖ 1938: (Theiler)

Yellow fever vaccine

❖ 1948: (Enders, Weller, Robbins) Poliovirus reproduction in nonneuronal cell cultures

❖ 1952: (Hershey, Chase)

Poliovirus plaque assay (Dulbecco) Viral genome is nucleic acid

❖ 1954: (Salk)

Polio vaccine

History of VIRUSES

Late (1957–1980)

- ❖ 1957: (Fraenkel-Conrat, Williams) In vitro assembly of virus (TMV)
 - (Isaacs, Lindemann) Interferon
- ❖ 1963: (Blumberg)
Hepatitis B virus
- ❖ 1978: (Sanger)
Viral genomes sequenced
- ❖ 1979: WHO declares smallpox eradicated

History of VIRUSES

Recent (1980–)

❖ 1983 (zur Hausen)

HPV cause cervical cancer

❖ 1983: (Barré-Sinoussi, Montagnier)

Discovery of the AIDS virus (HIV)

❖ 1989: (Houghton et al.)

Hepatitis C virus

❖ 2005: (Chisari, Rice, Wakita) Hepatitis C virus propagation in cultured cells

- (Palese, Tumpey, Taubenberger: Reconstruction and sequencing of the 1918 influenza virus genome

❖ 2006: (Fire, Mello): Gene silencing by double-stranded RNA, an antiviral response

❖ 2006: A vaccine protecting against the two cancer causing strains of human papillomavirus (HPV)

Classification of Viruses

In this lecture, we will look into viral classification, factors considered in classifying viruses

At the end of this lecture

Student must have fully understood:

- International Committee on Taxonomy of Viruses (ICTV)
- Factors considered in viral classification

Classification of Viruses

Taxonomy is the science of classification, very dynamic and substitution and subject to change on the basis of available data.

International Committee on Taxonomy of Viruses (ICTV)

The ICTV web site (<http://ictv.global>)

- Provides access to the current taxonomy database in online and downloadable formats
- Maintains a complete history of virus taxa back to the first release in 1971.
- Provide a comprehensive description of all virus taxa covering
 - virus structure
 - genome structure
 - biology
 - phylogenetics

Classification of Viruses

ICTV was established in 1966 as the International Committee on Nomenclature of Viruses (ICNV)

renamed

International Committee on Taxonomy of Viruses in 1977

Six subcommittees

Covers a different group of viruses

Develop the demarcation criteria

Demarcation criteria are based upon the genetic and biological properties

International Committee on Taxonomy of Viruses (ICTV)

The official objectives of the ICTV are:

- To develop an internationally agreed taxonomy for viruses
- To develop internationally agreed names for virus taxa, including species and sub-viral agents
- To communicate taxonomic decisions to all users of virus names, in particular the international community of virologists, by publications and via the Internet
- To maintain an index of virus names
- To maintain an ICTV database on the Internet, that records the data that characterize each named viral taxon, together with the common names of each taxon in all major languages

Rules for taxa

Orders

An order is a group of families sharing certain common characters. **Written in italics and the first letter is capitalized.** An order name must be a single word ending in the suffix *-virales*.

Families

A family is a group of genera. **Written in italics and the first letter is capitalized.** A family name must be a single word ending in the suffix *-viridae*.

Subfamilies

A subfamily is a group of genera sharing certain common characters. **Written in italics and the first letter is capitalized.** A subfamily name must be a single word ending in the suffix *-virinae*.

Genera

A virus genus is a group of related species that share some significant properties and often only differ in host range and virulence. **Written in italics and the first letter is capitalized.** A genus name must be a single word ending in the suffix *-virus*.

Species

Species names often take the form of *[Disease] virus*, particularly for higher plants and animals. **Written in italics and the first letter is capitalized.**

International Committee on Taxonomy of Viruses (ICTV)

- Beginning in 2017 the tenth ICTV report will be published online on the ICTV website

9 orders,

131 families,

46 subfamilies,

803 genera,

4,853 species

The Baltimore classification

In this lecture, we will look into viral classification

At the end of this lecture

Student must have fully understood:

- Baltimore classification
- Seven groups

The Baltimore classification

This classifies according to the viral mRNA synthesis

David Baltimore ([Nobel prize winner](#))

- **Seven groups** depending on a combination of their nucleic acid (DNA or RNA)
- Strandedness ([single-stranded or double-stranded](#))
- Sense
- Method of replication

The Baltimore classification

I: dsDNA viruses (e.g. Adenoviruses, Herpesviruses, Poxviruses)

II: ssDNA viruses (+ strand or "sense") DNA (e.g. Parvoviruses)

III: dsRNA viruses (e.g. Reoviruses)

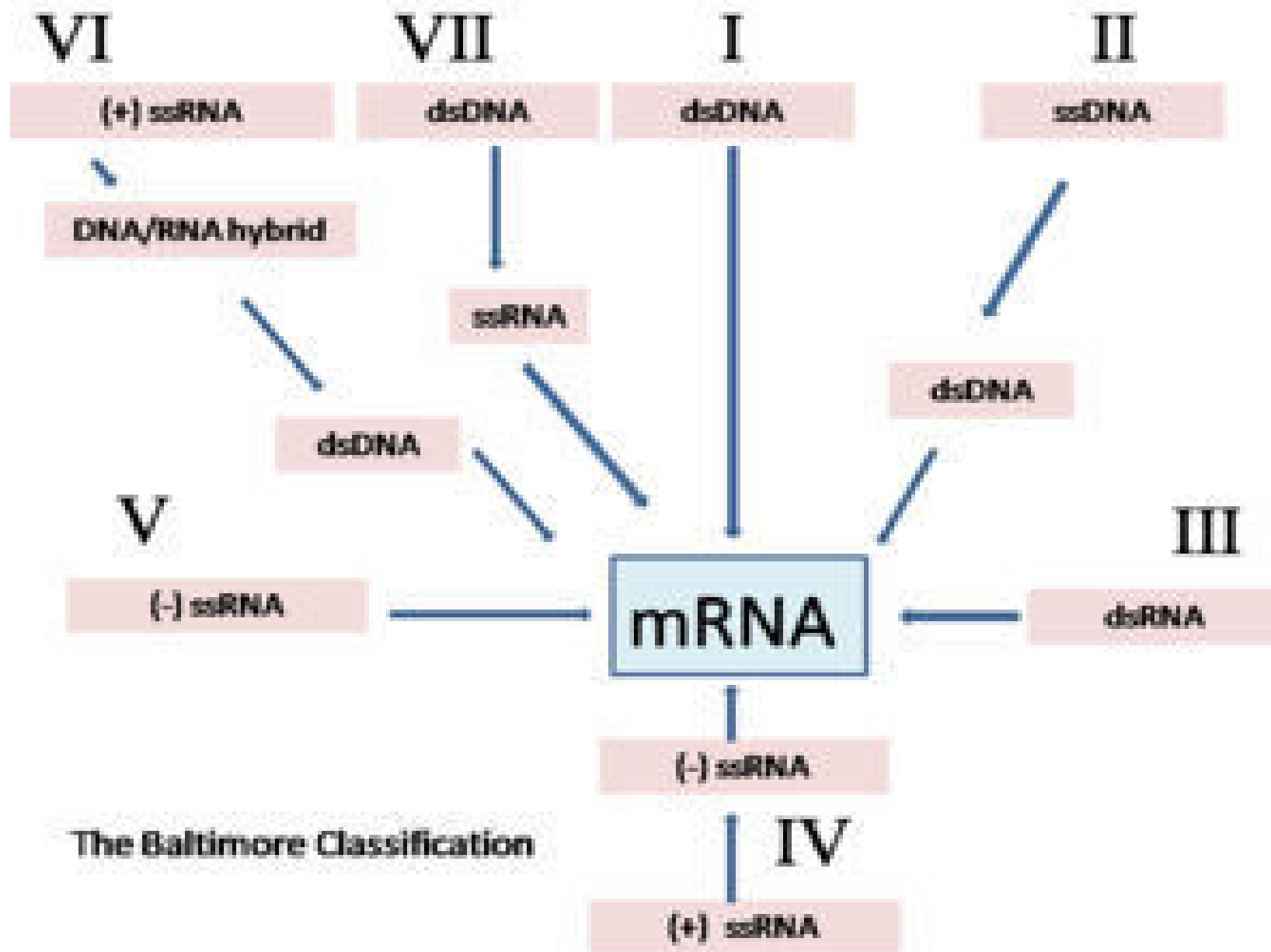
IV: (+)ssRNA viruses (+ strand or sense) RNA (e.g. Picornaviruses, Togaviruses)

V: (–)ssRNA viruses (– strand or antisense) RNA (e.g. Orthomyxoviruses, Rhabdoviruses)

VI: ssRNA-RT viruses (+ strand or sense) RNA with DNA intermediate in life-cycle (e.g. Retroviruses)

VII: dsDNA-RT viruses DNA with RNA intermediate in life-cycle (e.g. Hepadnaviruses)

The Baltimore classification



Classification of DNA Viruses

In this lecture we will look at the classification of Parvoviridae

At the end of this lecture

Student must have fully understood:

- Sub-family
- Nucleic acid nature
- Common associated diseases

Classification of DNA Viruses

DNA viruses contain DNA genome and infect mostly animal host. There are 6 major families

1. Parvoviridae

2. Papoviridae

3. Adenoviridae

4. Herpesviridae

5. Poxviridae

6. Hepadnaviridae

Classification of DNA Viruses

Parvoviridae

Taxonomic structure of the family Parvoviridae

Subfamily Parvovirinae

Genus *Parvovirus*

Genus *Erythrovirus*

Genus *Dependovirus*

Genus *Amdovirus*

Genus *Bocavirus*

Genus *Tetraparovirus*

Genus *Copipavirus*

Genus *Protopavirus*

Subfamily Densovirinae

Genus *Iteravirus*

Genus *Brevidensovirus*

Genus *Ambidensovirus*

Genus *Hepamdensovirus*

Genus *Penstyldensovirus*

>75 species

13 genera

2 subfamilies

Parvoviridae

- They are very small DNA viruses
- Single stranded DNA (ssDNA)
- Particle size of about 18-22nm in diameter with icosahedral symmetry of 32 capsomers
- They are non-enveloped
- **Parvovirinae:** Infect vertebrates
- **Densovirinae:** Infect invertebrates

Parvoviridae



- *Dependovirus*: replicate only in the presence of helper adenoviruses or herpesviruses
- Strains are designated adenoassociated viruses (AAV)
- *Densovirinae* viruses are typically named for their insect hosts
- B19 is most commonly associated with the disease erythema infectiosum (EI), also known as **fifth disease**
- Fifth disease is a mild childhood illness characterized by a facial rash, known as slapped cheek
- B19 is extremely contagious, and infection occurs worldwide

Classification of DNA Viruses

In this lecture we will look at the classification of Papovaviridae

At the end of this lecture

Student must have fully understood:

- Sub-family
- Nucleic acid nature
- Common associated diseases

Classification of DNA Viruses

1. Parvoviridae

2. Papoviridae

3. Adenoviridae

4. Herpesviridae

5. Poxviridae

6. Hepadnaviridae

Classification of DNA Viruses

Papovaviridae

Papovaviridae is divided into two subfamilies,

- ✓ Papillomavirinae
- ✓ Polyomavirinae

Genus Papillomavirus

Human papillomaviruses (HPV 1-62)

Shope papilloma virus of rabbits

Genus Polyomavirus

Polyoma virus of mice

Simian virus 40 (SV40)

JC and BK viruses

Papovaviridae

- double-stranded DNA
- Icosahedral, fibres at vertices
- 45-55nm diam
- no envelope
- no. of particle polypeptides: Approx. 5
- These two genera differ in several aspects:
 - 1) capsid size (Polyoma = 40 nm, Papilloma = 55 nm)
 - 2) length of genome (Polyoma = 5 kb, Papilloma = 8 kb)

Papovaviridae

- Human papillomavirus (HPV) is a viral infection: contagious (skin to skin contact)
- More than 100 HPV types have been identified
- Infect the squamous epithelia of skin and mucosa and usually cause benign papillomas or warts
- [Centers for Disease Control and Prevention \(CDC\)](#): HPV is the most common sexually transmitted infection that affects both men and women
- BK virus may cause mild respiratory disease
- JC virus can affect the respiratory system, the kidneys, or the brain
- Simian vacuolating virus 40 (SV40), which can infect humans, rodents, and monkeys
- SV40 infection in humans may lead to the growth of malignant tumours

Classification of DNA Viruses

In this lecture we will look at the classification of Adenoviridae

At the end of this lecture

Student must have fully understood:

- Sub-family
- Serotypes
- Common associated diseases

Classification of DNA Viruses

1. Parvoviridae

2. Papoviridae

3. Adenoviridae

4. Herpesviridae

5. Poxviridae

6. Hepadnaviridae

Classification of DNA Viruses

Adenoviridae

Member genres include

Genus Atadenovirus; type species: Ovine atadenovirus D

Genus Aviadenovirus; type species: Fowl aviadenovirus A

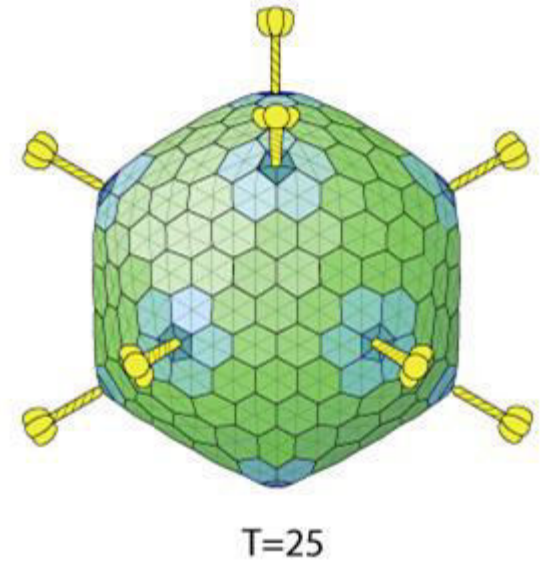
Genus Ichtadenovirus; type species: Sturgeon ichtadenovirus A

Genus Mastadenovirus (including all human adenoviruses); type species:
Human mastadenovirus C

Genus Siadenovirus; type species: Frog siadenovirus A

Adenoviridae

- The virions of this family are nonenveloped
- 70–90 nm in diameter
- capsid with a pseudo T=25 icosahedral symmetry
- The capsid shell consists of 720 hexon subunits arranged as 240 trimers and 12 vertex penton capsomers
- Adenoviruses possess a linear dsDNA genome
- Carry 30 to 40 genes



Adenoviridae

- Adenoviruses were first isolated in 1935 from human adenoid tissues
- 52 distinct antigenic types have been isolated from humans and many other types from animals
- 7 distinct species A-G
- All human serotypes are included in a single genus within the family Adenoviridae
- Reservoir: humans
- Distribution: worldwide distribution
- Mode of transmission: virus is transmitted via the fecal-oral route
- Incubation period: 3 to 10 days

Adenoviridae

- Most of the recent isolates are from AIDS patients
- Infections are common in children and world wide prevalence
- Adenoviruses infect and replicate in the epithelial cells of the:
 - pharynx,
 - conjunctiva,
 - urinary bladder
 - small intestine
- Adenovirus cause infections in
 - ❖ Respiratory tract
 - ❖ Eye,
 - ❖ Urinary bladder,
 - ❖ Intestines
 - ❖ More than one type of virus may cause clinically different diseases

Classification of DNA Viruses

In this lecture we will look at the classification of Herpesviruses

At the end of this lecture

Student must have fully understood:

- Sub-family
- Serotypes
- Common associated diseases

Classification of DNA Viruses

1. Parvoviridae

2. Papoviridae

3. Adenoviridae

4. Herpesviridae

5. Poxviridae

6. Hepadnaviridae

Classification of DNA Viruses

Herpesviridae

- The name was derived from the Greek word “Herpes”, “herpetos” meaning creeping, or crawling creature
- First isolated from the blue wildebeest in 1960 by veterinary scientist **Walter Plowright**
- Herpesviridae is a large family of DNA viruses that cause diseases in animals, including humans
- ❑ Three sub families in this family
 - a. Herpes Simplex Virus group called **Alpha Herpesvirinae**.
 - b. Cytomegalovirus group called **Beta Herpesvirinae**.
 - c. Lymphoproliferative Virus group called **Gamma Herpesvirinae**

Herpesviridae

Subfamily: Alphaherpesvirinae

Genus: Iltovirus, Mardivirus , Scutavirus , Simplexvirus , Varicellovirus

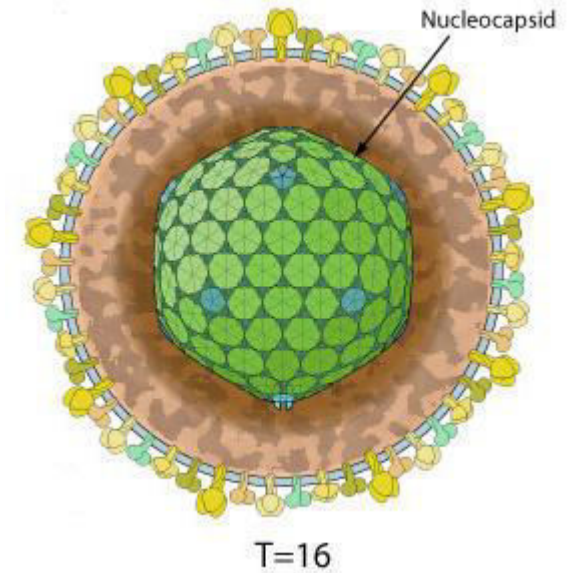
Subfamily: Betaherpesvirinae

Genus: Cytomegalovirus, Muromegalovirus, Roseolovirus, Proboscivirus

Subfamily: Gammaherpesvirinae

Genus: Lymphocryptovirus, Rhadinovirus, Macavirus, Percavirus

- Linear dsDNA genome of 120-240 kb
- Encode for 70 to 200 genes
- The virion is 120 – 200 mim in diameter and consists of 4 structural components
- T=16 icosahedral symmetry



Herpesviridae

- Herpes viruses are major causes of morbidity and mortality worldwide, responsible for a broad range of diseases in humans and animals
- ❖ There are 100 known herpes viruses; only eight infect humans
- Herpesviruses are those affecting humans, namely **herpes simplex virus type 1 (HSV-1)** and **herpes simplex virus type 2 (HSV-2)**
 - **HSV-1** is transmitted orally and is responsible for cold sores and fever blisters, typically occurring around the mouth,
 - **HSV-2** is transmitted sexually and is the main cause of the condition known as genital herpes
 - SV-2 infections have also been associated with the development of cervical cancer
- Both viruses are highly contagious
- ❖ Varicella Zoster Virus (VZV/HHV-3) is an acute infection that leads to varicella, or chicken pox

Herpesviridae

Epstein-Barr virus (EBV/HHV-4) is common and widespread amongst human populations

- Infects human B-lymphocytes and epithelial cells
- HHV-4 typically spreads through bodily fluids, particularly saliva, sharing utensils, toothbrushes

Cytomegalovirus (CMV/HHV-5) is another member of the herpes family

- 50%-70% of all adults are infected as well as 50% of all children
- Common manifestation is gastrointestinal upsets

Human Herpesvirus type 8 (KSHV/HHV-8) also known as Kaposi's sarcoma, manifests as a connective tissue cancer

- Complications from HHV 1, 2, 3, 4, 5, 6, 7, 8, are predominately seen if the immune system is compromised as a result of drug treatment, or suppression

Classification of DNA Viruses

In this lecture we will look at the classification of Poxviridae

At the end of this lecture

Student must have fully understood:

- Sub-family
- Serotypes
- Common associated diseases

Classification of DNA Viruses

1. Parvoviridae

2. Papoviridae

3. Adenoviridae

4. Herpesviridae

5. Poxviridae

6. Hepadnaviridae

Classification of DNA Viruses

Poxviridae

- Humans, vertebrates, and arthropods serve as natural host
- 69 species in this family, divided among 28 genera
- ❖ 2 subfamilies

Subfamily Chordopoxvirinae

Avipoxvirus, Capripoxvirus, Cervidpoxvirus, Crocodylipoxvirus, Leporipoxvirus, Molluscipoxvirus, Orthopoxvirus, Parapoxvirus, Suipoxvirus, Yatapoxvirus,

Subfamily Entomopoxvirinae (Pox viruses infecting insects)

Alphaentomopoxvirus, Betaentomopoxvirus, Gammaentomopoxvirus,

Poxviridae

- Poxviruses are the largest and most complex of all viruses
- Throughout human history, Poxviruses have held great clinical significance
- An infectious disease caused by one of two virus variants, variola major and variola minor
- Smallpox: World Health Organization, 300-500 million deaths resulted from smallpox alone in the 20th century
- Human smallpox has been eradicated since 1977
- Four genera of poxviruses may infect humans:
orthopoxvirus, parapoxvirus, yatapoxvirus, molluscipoxvirus
- Exist throughout the world and cause disease in humans and many other types of animals

Classification of DNA Viruses

- Poxviruses are brick or oval-shaped viruses
- 220-450 nm long and 140-260 nm wide
- Large double-stranded DNA genomes
- dsDNA genome of 130-375kb
- Monkeypox is restricted to West Africa
- Cowpox virus is restricted to Europe and western parts of the former USSR

Entomopoxvirinae

- The species of the genus Alphaentomopoxvirus infect beetles
- The species of the genus Betaentomopoxvirus infect butterflies, moths, grasshoppers and locusts
- The species of the genus Gammaentomopoxvirus infect flies and mosquitoes

Classification of DNA Viruses

In this lecture we will look at the classification of Hepadnaviridae

At the end of this lecture

Student must have fully understood:

- Sub-family
- Common associated diseases

Classification of DNA Viruses

1. Parvoviridae

2. Papoviridae

3. Adenoviridae

4. Herpesviridae

5. Poxviridae

6. Hepadnaviridae

Classification of DNA Viruses

Hepadnaviridae

- Humans, apes, and birds serve as natural hosts
- *Hepa* = liver and *dna* = deoxyribonucleic acid
- There are currently 7 species in this family, divided among 2 genera
- Its best-known member is the **Hepatitis B virus**
- All of the known hepadnaviruses are hepatotropic
- Diseases associated with this family include:
 - liver infections, such as hepatitis
 - hepatocellular carcinomas (chronic infections)
 - cirrhosis

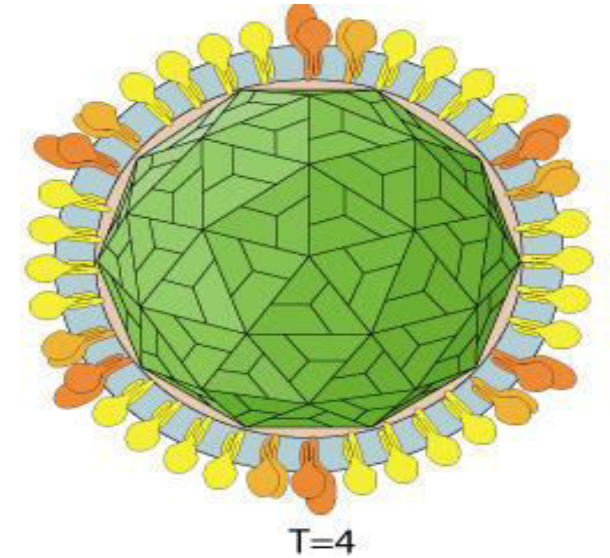
Hepadnaviridae

- Partially dsDNA circular genome, about 3.2 kb in size. Encodes for 7 proteins
- The hepadnaviruses include **three** viruses of mammals and **two** viruses of birds
- ❖ **Genus Orthohepadnavirus**
 - Hepatitis B (HBV)
 - Ground Squirrel Hepatitis B
 - Woodchuck Hepatitis B
- ❖ **Genus Avihepadnavirus**
 - Duck Hepatitis B
 - Heron Hepatitis B

Classification of DNA Viruses

Hepadnaviruses are spherical, occasionally pleomorphic
40–48 nm in diameter

Icosahedric capsid with a T=4 symmetry



The *hepadnaviruses* are exquisitely host specific

The only known natural hosts of hepatitis B virus are humans, but chimpanzees and gibbons may also be infected experimentally

Transmission of hepatitis B virus has also been reported in African monkeys, rhesus and woolly monkeys

Hepatitis B

- Viral infection
- It is one of five main hepatitis viruses: A, B, C, D, and E.
- Attacks the liver and can cause both acute and chronic disease
- Transmitted through contact with the blood or other body fluids of an infected person
- Estimated 257 million people are living with hepatitis B virus infection
- In 2015, hepatitis B resulted in 887 000 deaths
- Mild to severe symptoms

Duck hepatitis B virus

- Duck viral hepatitis is an acute, highly contagious, viral disease of young ducklings
- Short incubation period
- Sudden onset, high mortality, and characteristic liver lesions
- Economic importance in all duck-raising areas
- Three distinct types of duck hepatitis virus (DHV) have been isolated from diseased ducklings
- Mortality may be as high as 95% in ducklings



Classification of RNA Viruses

In this lecture we will look at the classification of
Picornaviridae and Caliciviridae

At the end of this lecture

Student must have fully understood:

- About the genome
- Common associated diseases

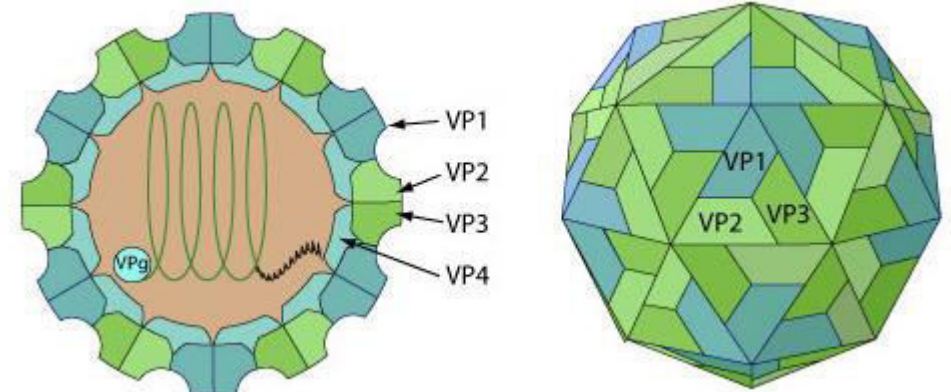
Classification of RNA Viruses

Picornaviridae

- PICO is prefix meaning (micro or small)
 - Positive-sense, non-segmented ssRNA with a poly(A) tail
 - The family comprises 35 genera containing 80 species
- (a) Enteroviruses — Enterovirus group (found in the intestine) e.g. Poliovirus
 - (b) Hepatoviruses — Hepatitis A virus group (affinity for liver)
 - (c) Cardiovirus — EMC Virus group (affecting the heart)
 - (d) Rhinovirus — Common cold virus (affecting Nose)
 - (e) Aphthovirus — Foot & Mouth disease virus (causing vesicles in the mouth)

Picornaviridae

- Picornaviruses are found in mammals and birds
- **Structure:** Non-enveloped, with an icosahedral capsid
- 30-32 nm virions comprising 60 protomers
- Genome 6.7-10.1 kb



Enteroviruses

- More than 60 known serotypes

Polio Virus

- Poliomyelitis commonly known as polio
- An infectious disease caused by polioviruses 1, 2, and 3
- Transmitted through the fecal-oral route
- Directly from person-to-person or indirectly through contaminated water sources
- Characterized by permanent paralysis due to spinal nerve damage and muscular wasting
- Particularly in young children
- India, Pakistan, and Nigeria have 98 percent of polio cases in the world

Classification of RNA Viruses

Caliciviridae

- Positive-sense, single-stranded and segmented RNA
- Not enveloped
- These group of viruses are similar to Picornaviridae but slightly larger (about 35-39nm) in diameter
- Seven species, divided among five genera
 - Vesivirus ([Vesicular exanthema of swine virus](#))
 - Lagovirus ([Rabbit hemorrhagic disease virus](#))
 - Norovirus ([Norwalk virus](#))
 - Sapovirus ([Sapporo virus](#))
 - Nebovirus ([Newbury-1 virus](#))

Caliciviridae

- Diseases associated with this family include

Feline calicivirus (respiratory disease)

Rabbit hemorrhagic disease virus (often-fatal hemorrhages)

Norwalk group of viruses (gastroenteritis)

- Caliciviruses naturally infect vertebrates, and have been found

humans

cattle

pigs

cats

chickens

reptiles

dolphins

amphibians

Classification of RNA Viruses

In this lecture we will look at the classification of
Reoviridae and Arboviridae

At the end of this lecture

Student must have fully understood:

- Genome and structure
- Common associated diseases

Classification of RNA Viruses

Reoviridae

- Reo = Respiratory enteric orphan
- Early recognized that these viruses causing respiratory and enteric infections
- Wide host range, including vertebrates, invertebrates, plants, protists and fungi include a total of 75 virus species
- Two subfamilies, *Spinareovirinae* and *Sedoreovirinae*
- 12 established genera: *Aquareovirus*, *Coltivirus*, *Cypovirus*, *Fijivirus*, *Idnoreovirus*, *Mycoreovirus*, *Orbivirus*, *Orthoreovirus*, *Oryzavirus*, *Phytoreovirus*, *Rotavirus*, and *Seadornavirus*
- Four of which—*Orthoreovirus*, *Coltivirus*, *Rotavirus*, and *Orbivirus* —can infect humans and animals
- Four other genera infect only plants and insects
- One infects fish

Reoviridae

Structure:

- The virus is an Icosahedral particles with diameter range of 60-80nm

Genome:

- Largest family of dsRNA viruses
- 9 genera of viruses having genomes composed of 9, 10, 11 or 12 segments of linear dsRNA
- 10 gene segments in three size classes (L, M, S)
- Total size ~23,500 base pairs

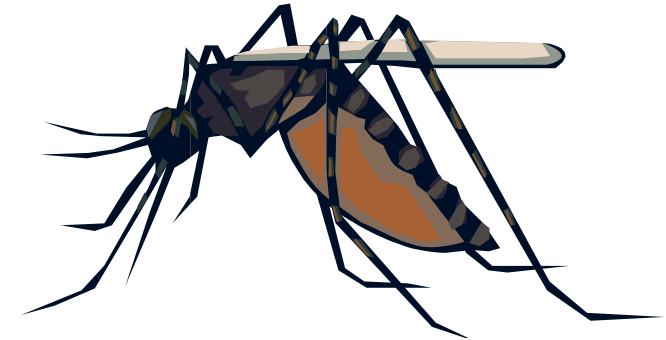
Disease:

- Reoviruses of human include rotaviruses, which cause infantile gastroenteritis and have distinctive wheel-shaped appearance
- Orbiviruses: Colorado tick fever virus of humans and blue-tongue of cattle and sheep

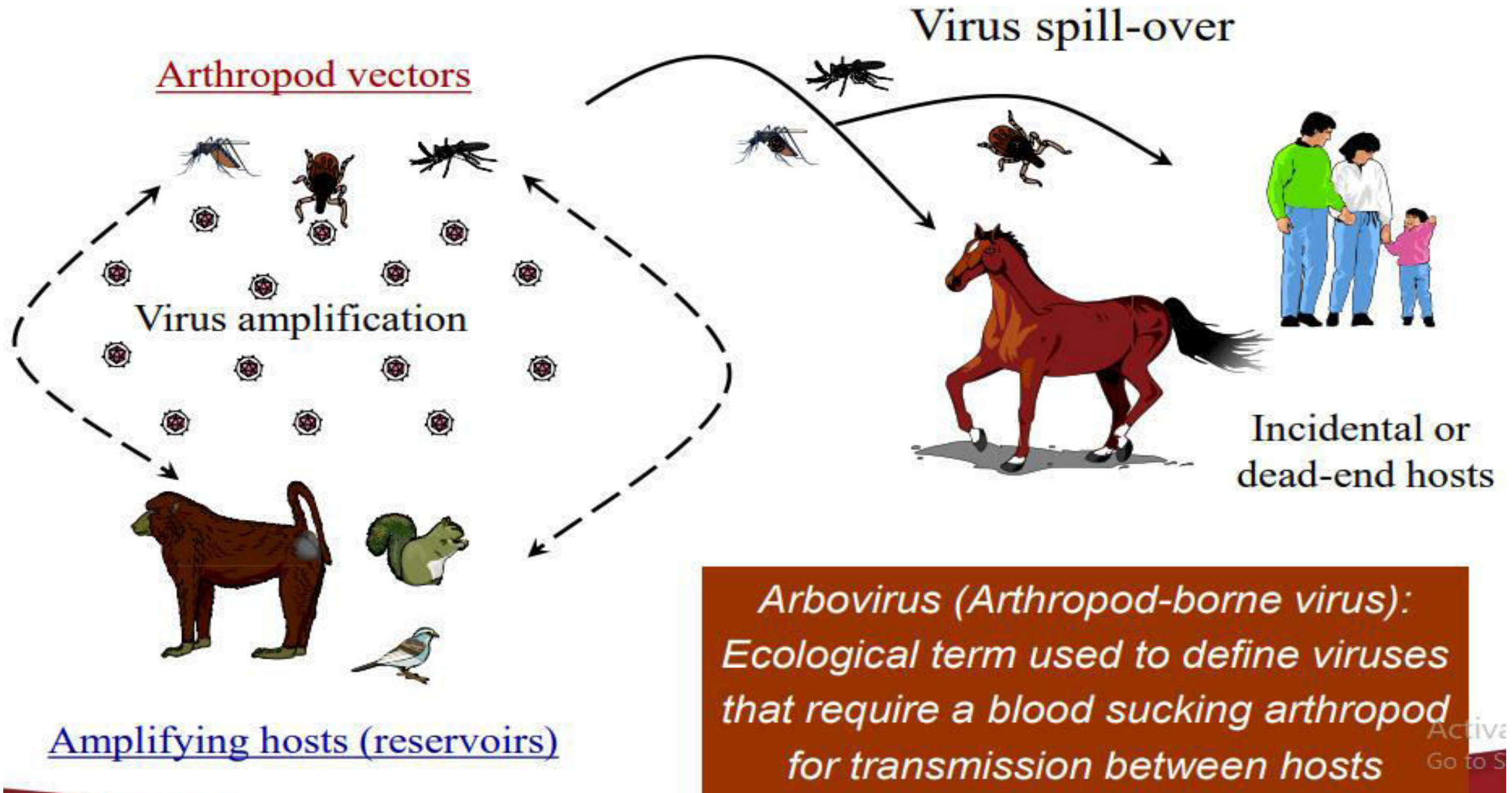
Classification of RNA Viruses

Arboviridae

- ARBO is derived from Arthropod ('AR') - Borne ('BO') viruses
- Arthropod-borne virus
- Transmitted to humans primarily through the bites of infected mosquitoes, ticks and sand flies
- Arboviruses can affect animals, humans, and plants
- Over 600 known arboviruses
- 130 of them are known human pathogens
- The incubation period - varies from virus to virus, but is usually limited between 2 and 15



Typical Arbovirus Transmission Cycle



Types of Arboviruses

- ❑ Five viral families that comprise the majority of arboviruses transmissible to humans and livestock including:
 - alphaviruses (Togaviridae)
 - flaviviruses (Flaviviridae)
 - rhabdoviruses (Rhabdoviridae)
 - bunyaviruses (Bunyaviridae)
 - reoviruses (Reoviridae)

Transmission: Arthropod Vectors

- ❑ The arboviruses spread mainly through insect bites
- ✓ **Mosquito (Most Common):** Japanese encephalitis, dengue, yellow fever, St. Louis encephalitis, EEE, WEE, etc.
- ✓ **Ticks:** Crimean-Congo haemorrhagic fever, various tick-borne encephalitides etc.
- ✓ **Sandflies:** Sicilian sandfly fever, Rift valley fever.



Classification of RNA Viruses

In this lecture we will look at the classification of
Togaviridae and Flaviviridae

At the end of this lecture

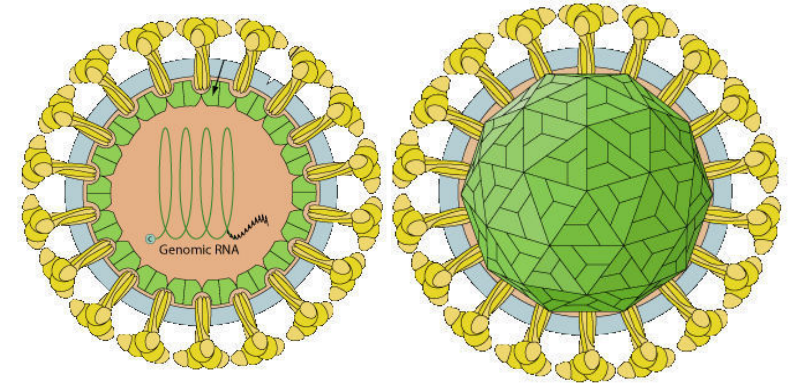
Student must have fully understood:

- Genome and structure
- Common associated diseases

Classification of RNA Viruses

Togaviridae

- ssRNA(+)
- Humans, mammals, birds, and mosquitoes serve as natural hosts
- 32 species divided among 2 genera
 - ❖ Alphavirus
 - ❖ Rubivirus
- Genome is linear, non-segmented
- 10–12 kb nucleotides long
- The virus is enveloped and forms spherical particles (65–70 nm diameter),
- Capsid within is icosahedral, constructed of 240 monomers, having a triangulation number of 4



Togaviridae

- **Alphavirus**: mainly arthropod borne viruses
 - large number of species: Humans, mammals, birds
 - Most **alphaviruses** are mosquito-borne and are pathogenic in their vertebrate hosts
 - ❖ encephalitic (such as Eastern equine encephalitis virus, Western equine encephalitis virus)
 - ❖ arthritic type (such as Chikungunya virus, Ross river virus)
- **Rubivirus**: Humans
 - Rubella virus: Rubella, common childhood disease
 - Rubella virus is transmitted by respiratory routes among humans

Classification of RNA Viruses

Flaviviridae

- The flaviviruses are a family of small enveloped viruses
- Positive-strand RNA genomes of approximately 9.0 to 13 kb
- Enveloped, 40–60 nm
- Non-segmented RNA
- Infect mammals and birds
- **Incubation period**: 2 – 15 days
- Many flaviviruses are host-specific: **hepatitis C virus (HCV)** in the genus, *Hepacivirus*

Flaviviridae

Majority of known members in the genus *Flavivirus* are arthropod borne

Mosquito associated diseases

- Yellow fever (see Yellow Fever fact sheet)
- Dengue fever
- West Nile virus
- Zika virus

Tick-borne diseases

- Tick-borne Encephalitis
- Alkhurma disease

Flaviviridae

- ❑ Four genera
 - **Genus Flavivirus:** Yellow fever virus, others include West Nile virus, Dengue Fever and Zika virus
 - **Genus Hepacivirus:** hepatitis C virus
 - **Genus Pegivirus** Pegivirus A, C, B
 - **Genus Pestivirus** Bovine virus diarrhea virus 1, swine fever virus—contains viruses infecting non-human mammals

Classification of RNA Viruses

In this lecture we will look at the classification of *Arenaviridae* and *Coronaviridae*

At the end of this lecture

Student must have fully understood:

- Genome and structure
- Common associated diseases

Classification of RNA Viruses

Arenaviridae

- Associated with rodent-transmitted diseases in humans
- Eight arenaviruses are known to cause human disease
- Single-stranded RNA viruses
- The genus *Arenavirus* includes 22 viral species
- 9 recently discovered
- *Lymphocytic choriomeningitis virus* (LCMV): causes inflammation covering the brain and spinal cord
- Lassa virus (LASV), Lujo virus (LUJV), Machupo virus (MACV), Sabia virus (SABV)

Arenaviridae

Two groups:

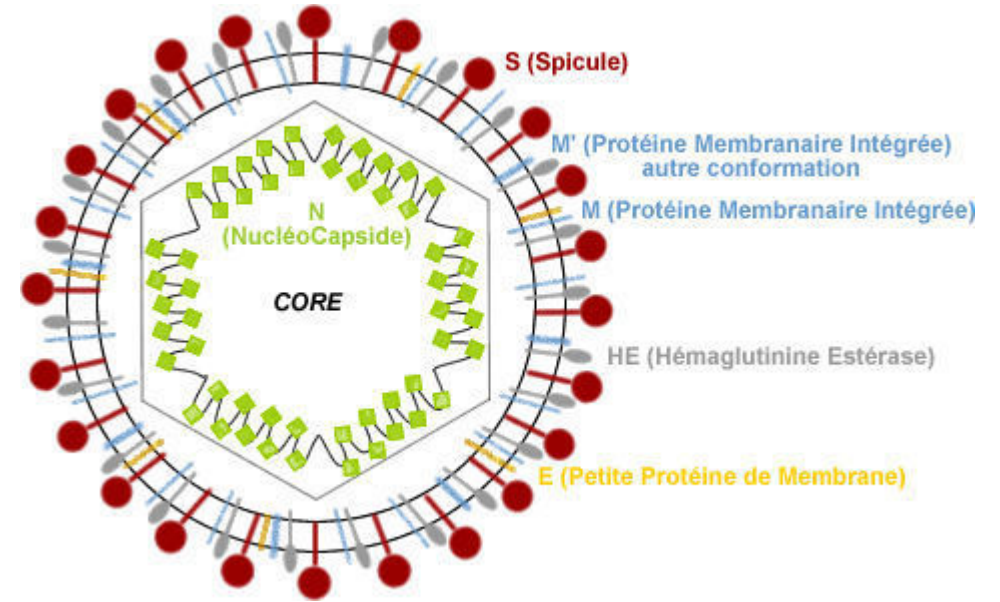
Old World and New World viruses

- ❖ Lassa fever (Old World)
- ❖ Bolivian and Venezuelan haemorrhagic fevers (New World)
- Negative-sense RNA viruses
- Segmented RNA genome that consists of two single-stranded ambisense RNAs
- L segment is about 7,5 kb and S segment 3,5 kb
- Enveloped, spherical
- Diameter from 60 to 300 nm

Classification of RNA Viruses

Coronaviridae

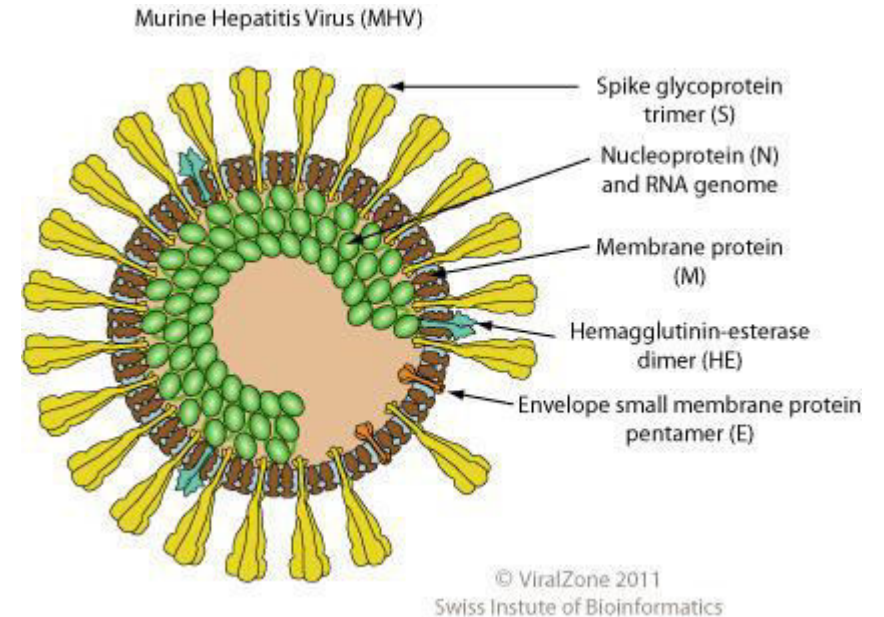
- Single-stranded, positive RNA viruses
- Genome is 26–32 kb in length
- ❖ largest of all RNA virus genomes
- ☐ Two subfamilies
 - ✓ Coronavirinae
 - ✓ Torovirinae
- Coronaviruses infect a wide range of mammals and birds
- Worldwide distributed
- Avian infectious bronchitis virus (IBV)



Coronaviridae

- Enveloped, spherical, about 120 nm in diameter
- **Genera:** Alphacoronavirus, Betacoronavirus, Gammacoronavirus, Torovirus, and Bafinivirus

- ☐ Three classes of vertebrates:
 - coronaviruses and toroviruses for mammals
 - coronaviruses for birds
 - bafiniviruses for fishes



Classification of RNA Viruses

In this lecture we will look at the classification of *Retroviridae* and *Bunyaviridae*

At the end of this lecture

Student must have fully understood:

- Genome and structure
- Common associated diseases

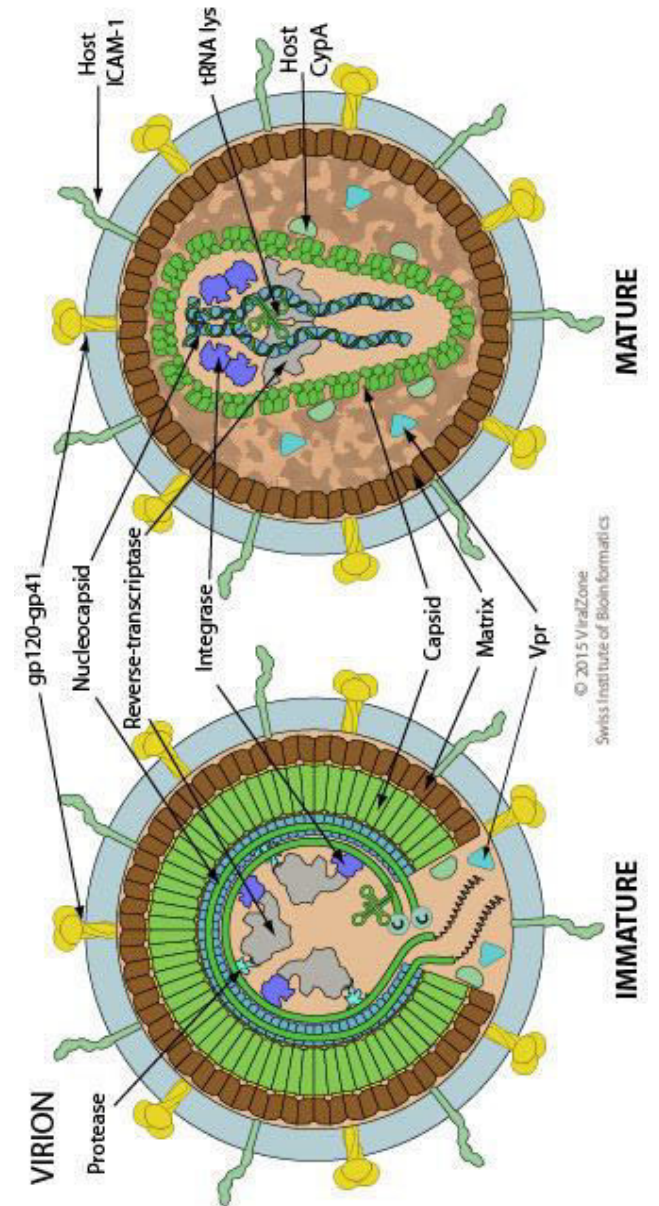
Classification of RNA Viruses

Retroviridae

- Single stranded(+) sense RNA genome 7-11 kb
- 5'cap, 3'poly(A) tail
- Retrovirus virions are 80-120 nm in diameter
- Inserts a copy of its genome into the DNA of a host cell that it invades, thus changing the genome of that cell
- Contains reverse transcriptase
- Retroviruses cause **tumour growth** and certain **cancers** in animals
- ✓ equine infectious anemia
- ✓ human T-cell lymphotropic virus type 1 (HTLV-1)
- ✓ **human immunodeficiency virus (HIV)**: causes acquired immunodeficiency syndrome (AIDS) in humans

Retroviridae

- ❑ Two subfamilies
 - ❖ **Orthoretrovirinae**
 - ✓ Genus Alpharetrovirus; Avian leukosis virus
 - ✓ Genus Betaretrovirus; Mouse mammary tumour virus
 - ✓ Genus Gammaretrovirus; Murine leukemia virus
 - ✓ Genus Deltaretrovirus; Bovine leukemia virus
 - ✓ Genus Epsilonretrovirus
 - ✓ Genus Lentivirus; immunodeficiency virus 1
 - ❖ **Spumaretrovirinae**
 - ✓ Genus Bovispumavirus, Equispumavirus, Felispumavirus, Prosimiispumavirus, Simiispumavirus
- Virions are spherical
- Enveloped and are 80–100 nm in diameter



Classification of RNA Viruses

Bunyaviridae

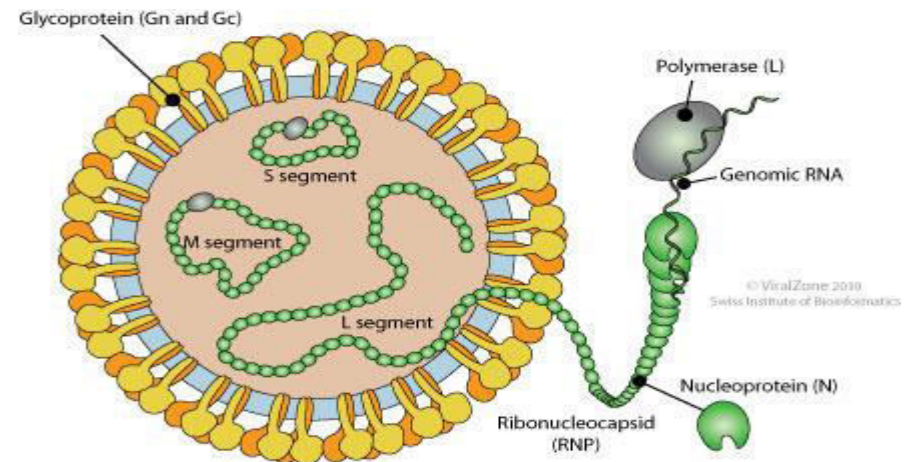
- Negative-sense single-stranded RNA viruses
- A majority of bunyaviruses are **vector-borne**
- Generally found in arthropods (mosquitos, tick, or sandfly) or rodents
- ❑ five genera viruses:

Orthobunyavirus, Phlebovirus, Nairovirus, Hantavirus, Tospovirus

- **Tospoviruses infect only plants**
- Handling some of these viruses in high containment (Biosafety Level 3 or even Biosafety level 4)
- **Crimean-Congo hemorrhagic fever virus, Rift Valley fever virus**

Bunyaviridae

- Enveloped, spherical
- Diameter from 80 to 120nm
- Segmented Negative-stranded RNA linear genome, L segment is between 6.8 and 12 kb, M segment between 3.2 and 4.9 kb and S segment between 1 and 3 kb



Classification of RNA Viruses

In this lecture we will look at the classification of viruses: **Orthomyxoviridae**

RNA viruses

At the end of this lecture

Student must have fully understood:

- characteristics of Orthomyxoviruses

Classification of RNA Viruses

Orthomyxoviridae

➤ RNA Viruses

☐ 7 genera

- Influenza virus A,
- Influenza virus B,
- Influenza virus C,
- Influenza virus D,
- Isavirus,
- Thogotovirus,
- Quarantavirus
- **First four genera** contain viruses that cause influenza in vertebrates, including birds, humans, and other mammals
- **Isaviruses** infect salmon
- **thogotoviruses** are arboviruses, infecting vertebrates and invertebrates, such as ticks and mosquitoes

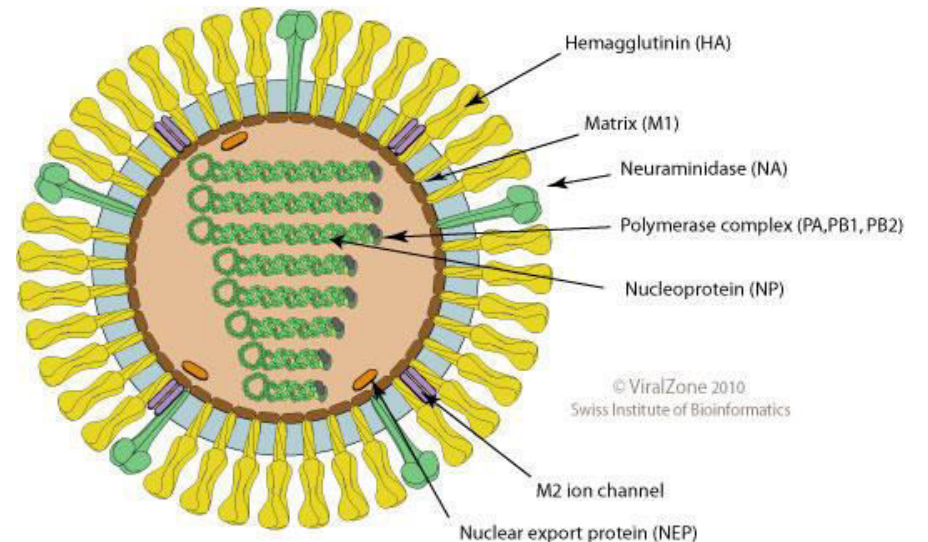
Orthomyxoviridae

- ✓ Influenza virus A infects humans, other mammals, and birds, and causes all flu pandemics
- ✓ Influenza virus B infects humans and seals
- ✓ Influenza virus C infects humans, pigs, and dogs
- ✓ Influenza virus D infects pigs and cattle

- ✓ Interspecies transmission has been documented including human infections with avian and swine influenza viruses

Orthomyxoviridae

- Negative-sense ssRNA virus and segmented
- Enveloped virions
- Usually rounded but can be filamentous.
- The virions are 80-120 nm in diameter
- Contains 8 segments coding for 11 proteins
- Genome total size is 13.5Kb



Classification of RNA Viruses

In this lecture we will look at the classification of viruses: *Paramyxoviridae*
RNA viruses

At the end of this lecture

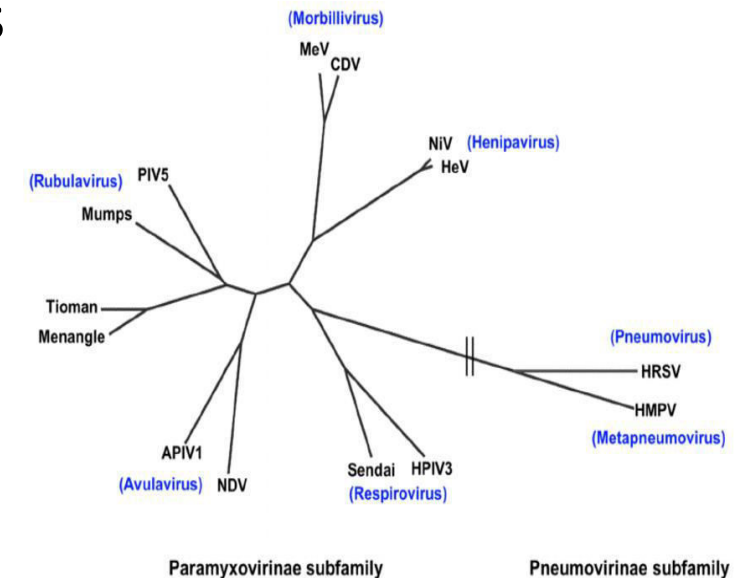
Student must have fully understood:

- characteristics of *Paramyxoviridae*
- Common disease cause by paramyxoviruses

Classification of RNA Viruses

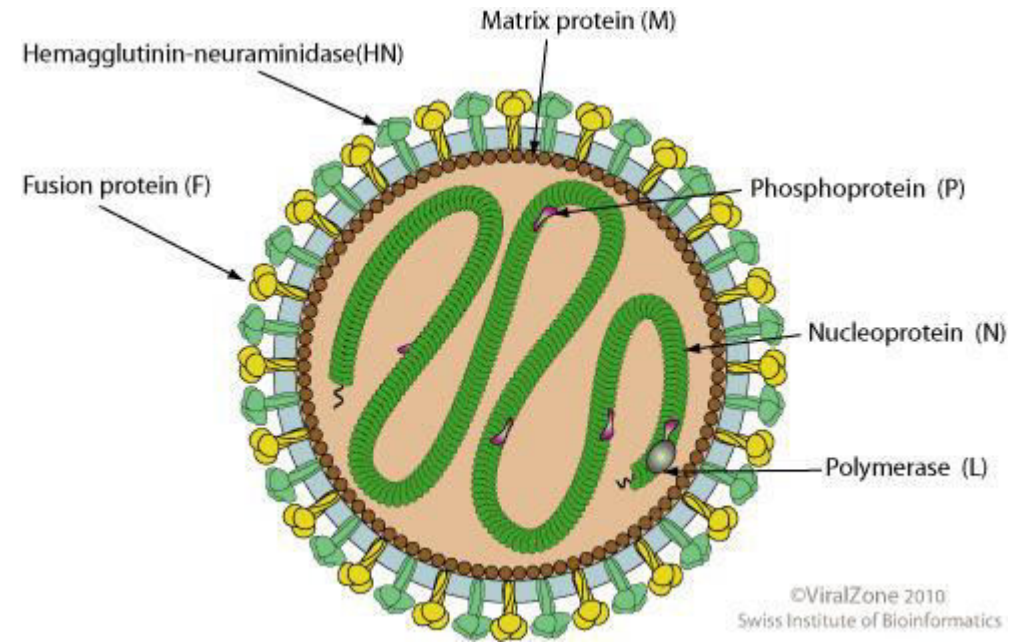
Paramyxoviridae

- Single-stranded, negative-sense RNA viruses
- Vertebrates serve as natural hosts
- 49 species in this family, divided among 7 genera
- **Avulavirus** Birds
- **Morbillivirus** Humans; dogs; cats; cetaceans
- **Aquaparamyxovirus** Fish
- **Henipavirus** Bats
- **Respirovirus** Rodents; humans
- **Rubulavirus** Humans; apes; pigs; dogs
- **Ferlavirus** Unknown



Paramyxoviridae

- Virions are enveloped and can be spherical or pleomorphic
- Diameter of about 150 nm
- Genome 15 kb in size.
- Encodes for eight proteins



Paramyxoviridae

- ❑ Human diseases are caused by paramyxoviruses
 - mumps
 - measles
 - respiratory syncytial virus (RSV), which is the major cause of bronchiolitis and pneumonia in infants and children
 - human parainfluenza viruses (HPIV) are the second most common causes of respiratory tract disease in infants and children
- ❑ Diseases in other animal species
 - canine distemper virus (dogs)
 - phocine distemper virus (seals)
 - cetacean morbillivirus (dolphins)
 - Newcastle disease virus (birds)
 - rinderpest virus (cattle)

Classification of RNA Viruses

In this lecture we will look at the classification of viruses: *Rhaboviridae and Toroviridae*

RNA viruses

At the end of this lecture

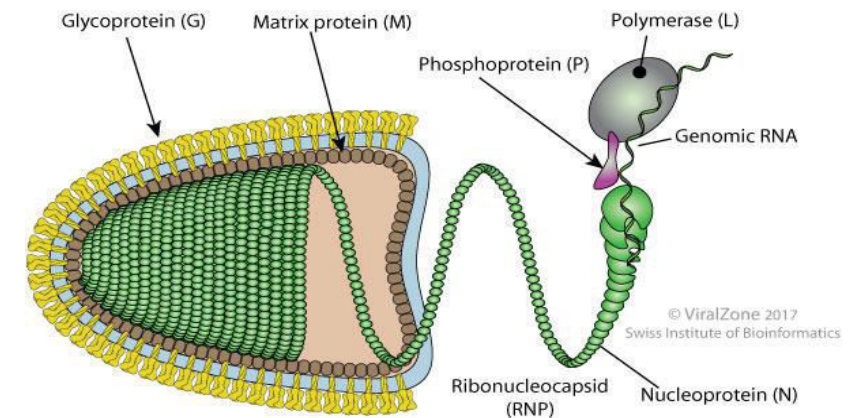
Student must have fully understood:

- characteristics of *Rhaboviridae and Toroviridae*

Classification of RNA Viruses

Rhabdoviridae

- Negative-sense, single-stranded RNA genomes of approximately 10–16 kb
- Virions are typically enveloped with bullet-shaped or bacilliform morphology
- 180 nm long and 75 nm wide
- Diverse group of over 150 viruses
- Vertebrates, invertebrates, plants serve as natural hosts
- 18 genera are recognized



Rhaboviridae

- Six different genera including:
- **Mammals:** *vesiculoviruses*, *ephemeroviruses* and *lyssaviruses*
- **Plants:** *cyto-* and *nucleorhabdoviruses*
- **Fish:** *novirhabdoviruses*
- Only the lyssaviruses and the vesiculoviruses are recognised as viral agents able to infect both animals and humans and cause clinical disease
- **lyssaviruses** include one of the most notable viral infections known to man
rabies
- Common symptoms of lyssavirus virus infections are neurological

Toroviridae

Toroviridae

- ssRNA(+)
- Toro is derived from the Latin word 'torus meaning lowest convex molding in the base of a column.
- Breda virus infecting cattle
- **Torovirus** infecting man
- They are pleomorphic, biconcave disk, rod- shaped viral particles
- 20 – 140nm diameter containing an elongated tubular capsid with helical symmetry

Evolution of Viruses

In this lecture we will look at the Evolution of viruses

At the end of this lecture

Student must have fully understood:

- How the viruses evolved?

Evolution of Viruses

- Viruses undergo evolution and natural selection, just like cell-based life, and most of them evolve rapidly
- Two viruses infect a cell at the same time, they may swap genetic material to make new, "mixed" viruses with unique properties
 - flu strains can arise this way
- RNA viruses have high mutation rates
- Evolution of drug resistance in HIV

Evolution of Viruses

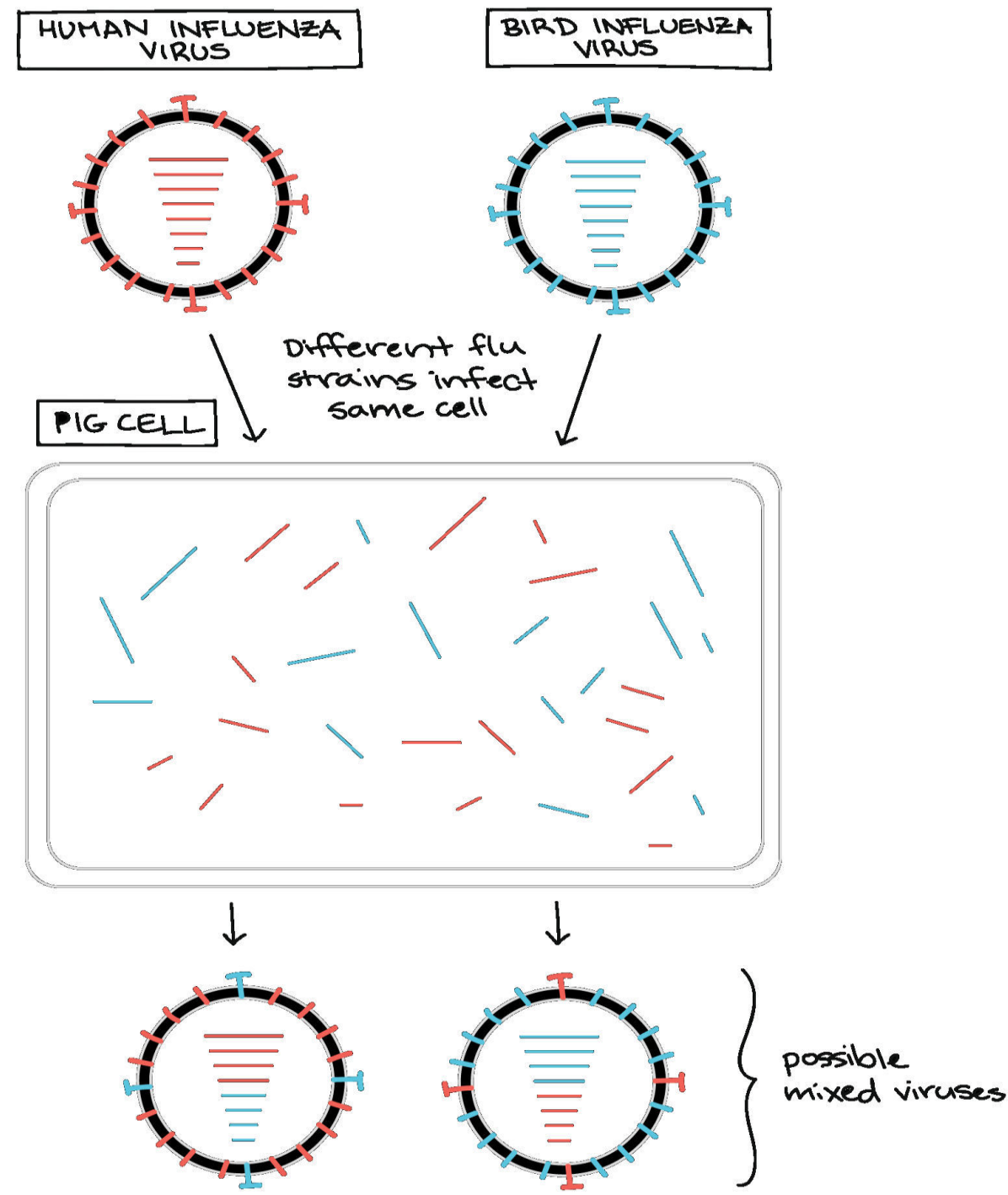
Genetic variation

- Genetic (heritable) differences in a population
- In viruses, variation comes from two main sources
 - **Recombination**: viruses swap chunks of genetic material (DNA or RNA)
 - **Random mutation**: a change occurs in the DNA or RNA sequence of a virus

Evolution of Viruses

How viruses swap DNA and RNA?

- Influenza ("flu") viruses are masters of reassortment
- Eight RNA segments, each carrying one or a few genes
- When two influenza viruses infect the same cell at the same time, some of the new viruses made inside of the cell may have a mix of segments (e.g., segments 1-4 from strain A and segments 5-8 from strain B)
- This kind of swap is common for influenza viruses in nature



Evolution of Viruses

Viral mutations

A permanent change in the genetic material

- Some viruses have very high mutation rates – RNA Viruses
 - **DNA polymerases:** which has proofreading
 - **RNA polymerase:** which don't proofread - many more mistakes

Evolution of Viruses

HIV drug resistance?

- Causes **acquired immune deficiency syndrome (AIDS)**
- RNA virus
- high mutation rate and evolves rapidly
- leading to the emergence of drug-resistant strains

Evolution of Viruses

HIV drug resistance?

- drugs can block the replication of HIV by inhibiting key viral enzymes - **reverse transcriptase inhibitor**
- reduce a patient's viral levels
- drug keeps the enzyme from doing its job of copying the RNA genome of HIV into DNA - **nevirapine**
- HIV virus can't permanently infect a cell
- genetic change that alters the drug's binding site on the enzyme
- drug is no longer able to latch on and inhibit enzyme activity

Evolution of Viruses

Hypothesis

- **Progressive Hypothesis:** Viruses originated from the evolution of mobile genetic elements that gained the ability to move between cells
- **Regressive Hypothesis:** Viruses evolved from more complex free-living organisms that lost genetic material as they adapted to a parasitic approach to reproduction
- **Virus-First Hypothesis:** Viruses existed before cells as self replicating units that eventually became more complex and may have led to the rise of the first cells. Also known as the coevolution hypothesis.

Virion, Viroid, Prion

In this lecture we will look at the various terms

At the end of this lecture

Student must have fully understood:

Describe virion and their unique characteristics

Describe viroid and their unique characteristics

Describe prions and their unique characteristics

Virion, Viroid, Prion

Virus

- A biological viruses' code is composed of nucleic acids called DNA or RNA encased in a protective shell
 - Flu virus
 - HIV
 - NDV
- Wide host range

Virion

- If it is found extracellular, the virus is called a virion

Viroid, Prion

Viroid

- Infectious agent is composed of just a piece of single-stranded circular RNA, without that protective shell, we call this a viroid
 - An infectious RNA molecule
- RNA in a viroid coils around itself to become double-stranded for strength
- Not believed to interact in any meaningful way with mammals
- Causing diseases in plants, like potatoes

Prion

Prion

- We believed that any infectious particle must contain DNA or RNA
- In 1982, Stanley Prusiner, studying scrapie discovered that the disease was caused by proteinaceous infectious particles, or prions
 - Infectious protein particles
 - Can be transmitted
- A prion is a misfolded rogue form of a normal protein (PrPc) found in the cell

A fatal, degenerative disease in sheep

Prion

- Once prions enter the brain, they force the normal cellular proteins to begin folding into abnormal shapes
- Cause various forms of transmissible spongiform encephalopathy (TSE)
- TSE is a rare degenerative disorder that affects the brain and nervous system
- Accumulation of rogue proteins causes the brain tissue to become sponge-like
- killing brain cells and forming holes in the tissue, leading to brain damage, loss of motor coordination, and dementia.

Prion

- TSEs in humans include kuru, fatal familial insomnia and Creutzfeldt-Jakob disease
- TSEs in animals include
 - mad cow disease (cattle)
 - scrapie (in sheep and goats)
 - chronic wasting disease (deer)
- ❑ TSEs can be transmitted between animals and from animals to humans by eating contaminated meat or animal feed.

Virus structure

In this lecture we will look at the structure of viruses

At the end of this lecture

Student must have fully understood:

Virus structure

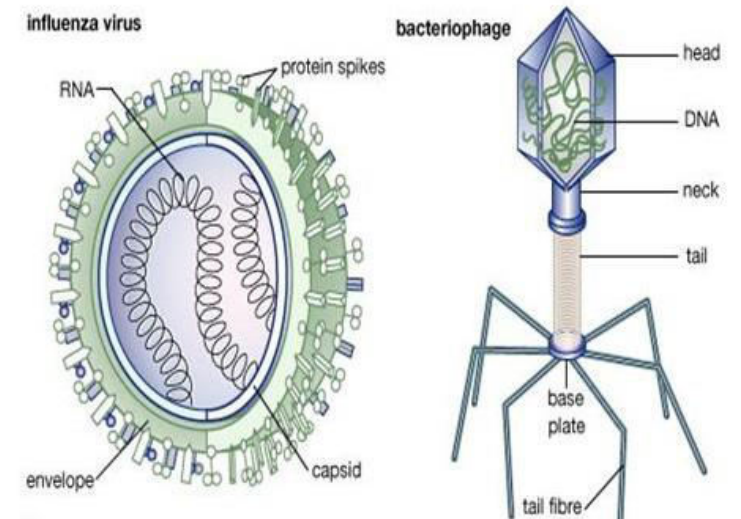
- Genetic materials
- Capsid
- Envelope

Virus structure

- Basic rules of virus architecture, structure, and assembly are the same for all families
- Some structures are much more complex than others, and require complex assembly and disassembly

Nucleic acid

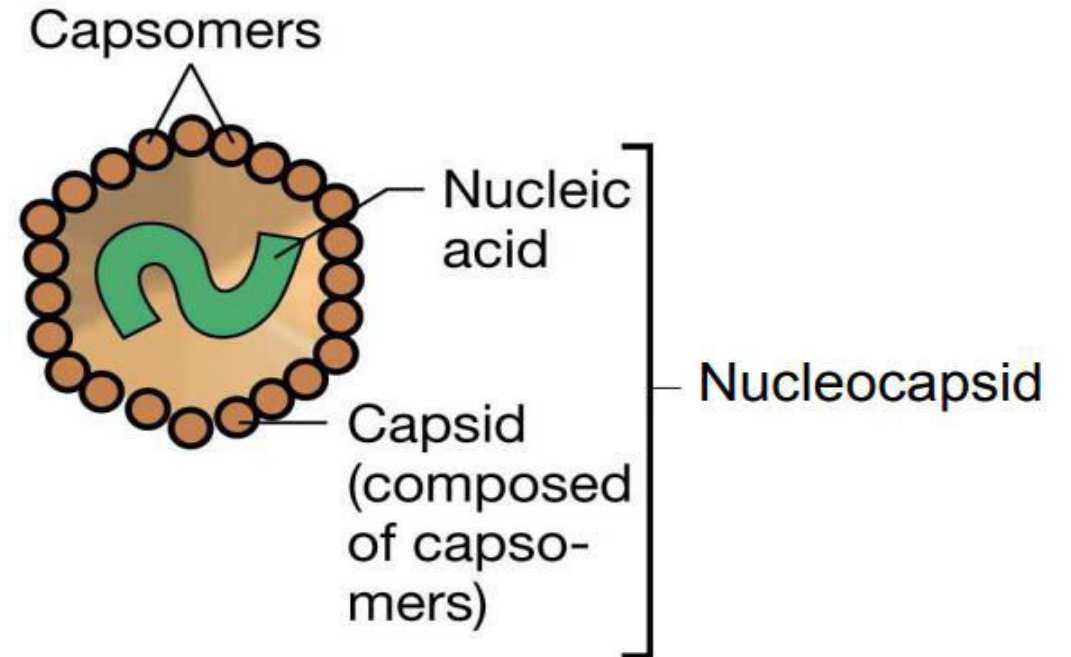
- A virus can have either DNA or RNA—but never both
- Nucleic acid of a virus can be single-stranded or double-stranded
- Can be linear or circular
- Influenza virus: the nucleic acid is in several separate segments



Virus structure

Capsid

- The nucleic acid of a virus is protected by a protein coat called the **capsid**
- Each capsid is composed of protein subunits called **capsomeres**
- The arrangement of capsomeres is characteristic of a particular type of virus



Virus structure

Capsid

The capsid (coat) protein is the basic unit of structure; functions that may be fulfilled by the capsid protein are to:

- Protect viral nucleic acid
- Interact specifically with the viral nucleic acid for packaging
- Interact with vector for specific transmission
- Interact with host receptors for entry to cell
- Allow for release of nucleic acid upon entry into new cell
- Assist in processes of viral and/or host gene regulation

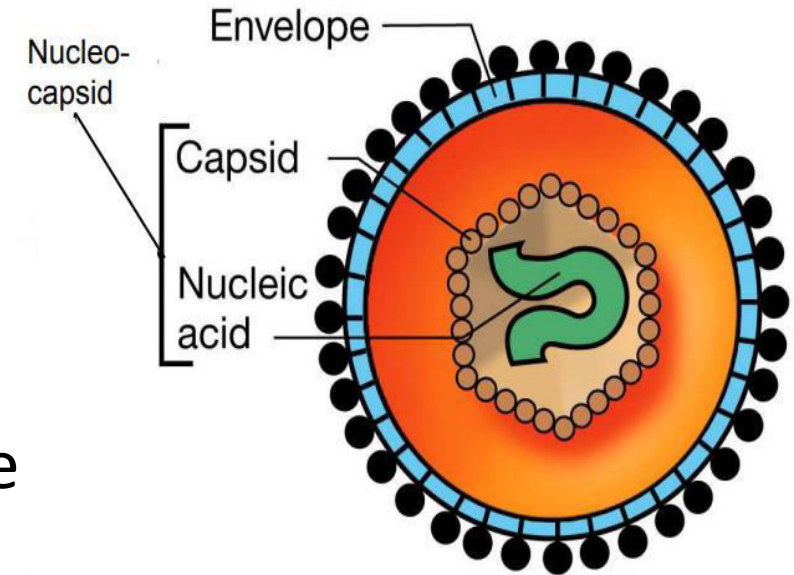
Virus structure

Envelope

- In some viruses, the capsid is covered by an **envelope**
- lipids, proteins, and carbohydrates

■ Spikes

- Projection from the surface of the envelope
- Viruses attach to host cells
- Influenza virus to clump red blood cells is associated with spikes
- Viruses whose capsids are not covered by an envelope are known as **nonenveloped viruses**



Structure of virus capsids

In this lecture we will look at the structure of viruses

At the end of this lecture

Student must have fully understood:

Structure of virus capsids

icosahedral, enveloped, complex or helical

- Capsid function

Structure of virus capsids

Capsid architecture

- Viruses may be classified into several different morphological types on the basis of their capsid architecture.
- The structure of these capsids has been revealed by electron microscopy and a technique called X-ray crystallography

Structure of virus capsids

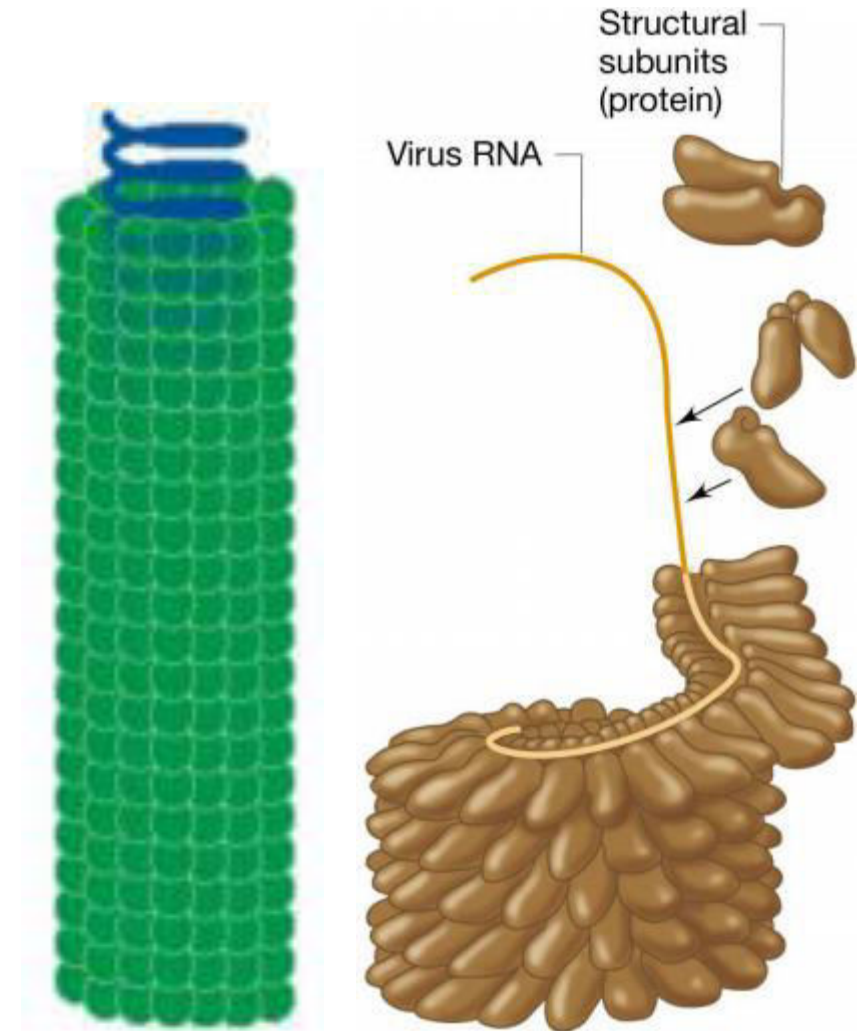
Capsid Function: To protect the fragile nucleic acid genome from

- Physical damage - Shearing of the nucleic acid by mechanical forces.
- Chemical damage - UV irradiation (from sunlight) leading to chemical modification causing mutations.
- Enzyme damage - Nucleases derived from dead or leaky cells or deliberately secreted as a defense against infection.
- Packaging of the viral genome
- Interactions with the host cell. Capsid proteins of naked viruses mediate attachment and virus entry.
- Stimulation of the host immune system. Capsid proteins of naked viruses are often the major virus antigens

Structure of virus capsids

Helical Viruses

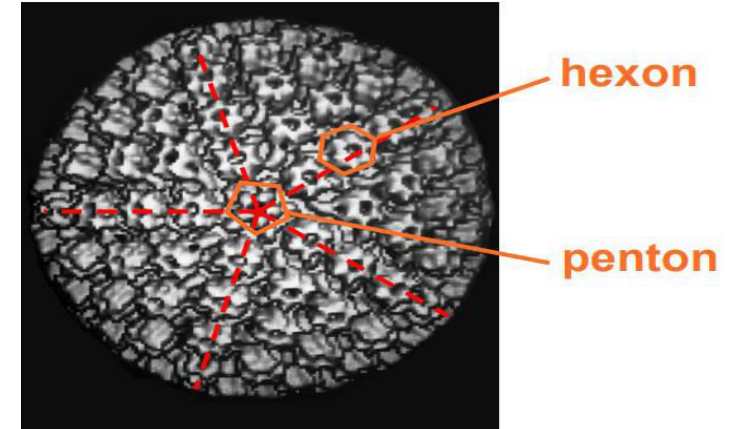
- Long rods that may be rigid or flexible
- Viral nucleic acid is found within a hollow, cylindrical capsid that has a helical structure
- 20-30nm wide, length variable, 300nm
- tobacco mosaic virus (TMV)



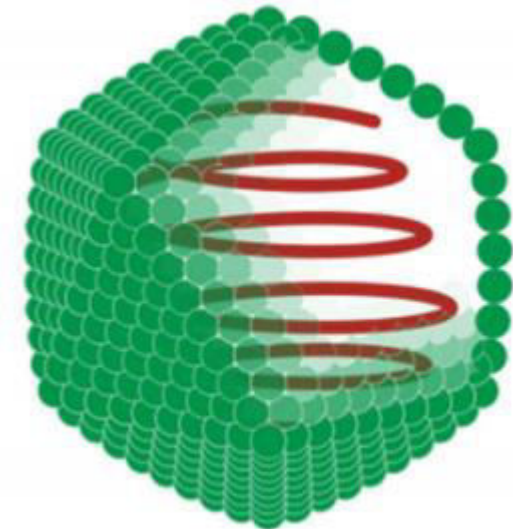
Structure of virus capsids

Icosahedral

- Spherical in shape
- Many animal, plant, and bacterial viruses are polyhedral, or many-sided, viruses
- The icosahedron is made up of equilateral triangles fused together in a spherical shape
- poliovirus, rhinovirus, and adenovirus



HSV-1 capsid

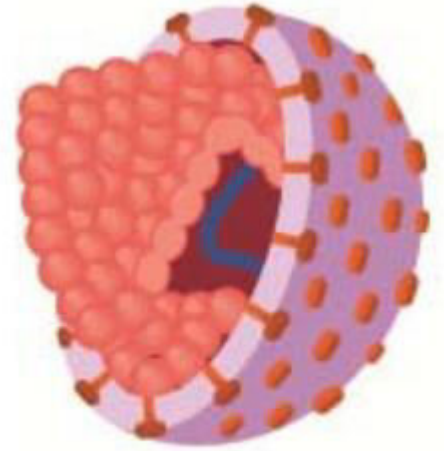


Icosahedral

Structure of virus capsids

Enveloped Viruses

- Capsid of some viruses is covered by an envelope.
- Enveloped viruses are roughly spherical.
- When helical or polyhedral viruses are enclosed by envelopes,
 - Called enveloped helical or enveloped polyhedral viruses.
- An example of an enveloped helical virus is the influenza virus, HCV, HIV

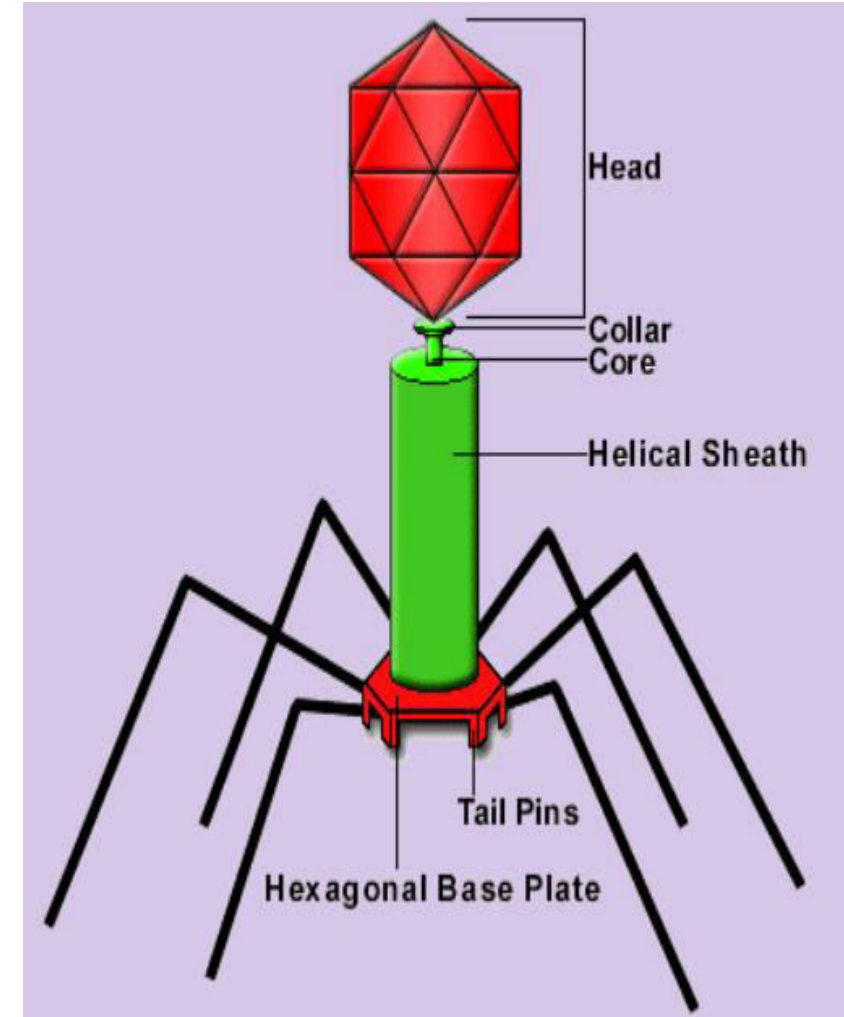


Envelope
Hepatitis C

Structure of virus capsids

Complex Viruses

- Bacterial viruses, have complicated structures and are called complex viruses
- Bacteriophage
- bacteriophages have capsids to which additional structures are attached
- The capsid (head) is polyhedral and that the tail sheath is helical



Bacteriophages

In this lecture we will look at the Bacteriophage

At the end of this lecture

Student must have fully understood:

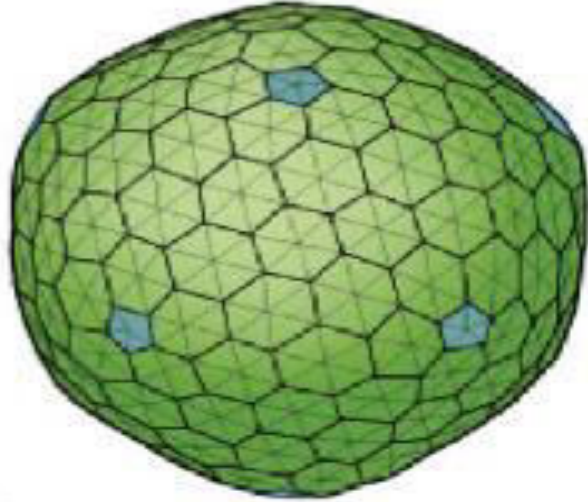
Virus attacking bacteria

Structure

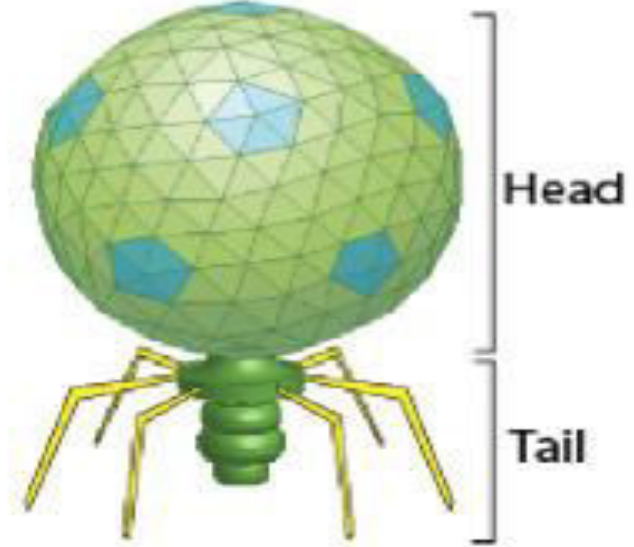
Bacteriophages

- Simply phage
- Infect bacteria
- Bacterial machinery system for its own multiplication and development
- Capsid of a bacteriophage can be icosahedral, filamentous, or head-tail in shape
 - Spherical phages such as X174 (ssDNA)
 - Filamentous phages such as M13(ssDNA)
 - T-even phages such as T2, T4 and T6 that infect E.coli
 - Temperate phages such as λ

Bacteriophages



Icosahedral phage
(Corticovirus)

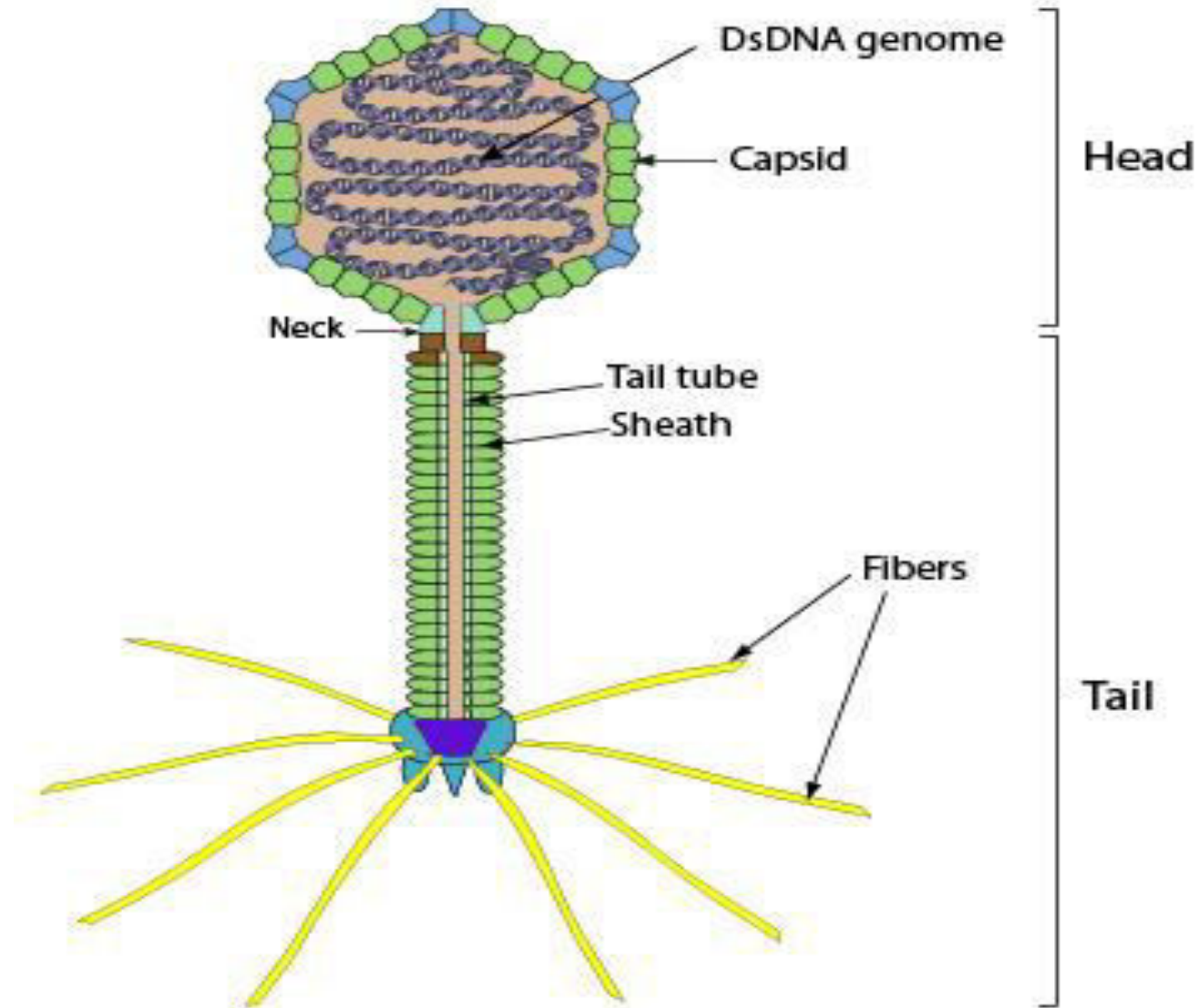


Head-tail phage
(T7)



Filamentous phage
(Inovirus)

Basic structure of Bacteriophages



Bacteriophages

- Either DNA or RNA
- Contain as few as four genes or as many as several hundred

lifecycle of the phage

lytic life cycle

- In which they burst and kill their host cells

lysogenic lifecycle

- Don't kill the host cell

Bacteriophages: lytic cycle

In this lecture we will look at the Bacteriophage lytic cycle

At the end of this lecture

Student must have fully understood:

Steps involved

Bacteriophages: lytic cycle

- Viral DNA exists as a separate free floating molecule
- Replicates separately from the host bacterial DNA
- Cause cell lysis
- kills the host bacterium
- The lytic cycle, also commonly referred to as the reproductive cycle

Bacteriophages: lytic cycle

□ Five-stage cycle

Attachment:

- Proteins in the "tail" of the phage bind to a specific receptor on the surface of the bacterial cell
- Once attached, weak chemical bonds are formed
- helps the virus adhere to the host cell

Entry:

- The phage injects its genome into the cytoplasm of the bacterium
- Phage releases enzymes that weaken the cell wall
- Empty capsid or virus body remains in the bacterial cell

Bacteriophages: lytic cycle

DNA copying and protein synthesis:

- Phage DNA is copied, and phage genes are expressed to make proteins, such as capsid proteins
- Phage uses the host nucleotides to replicate its own DNA
- Uses the host ribosomes, enzymes, and amino acids to synthesize its own enzymes and proteins

Assembly of new phage:

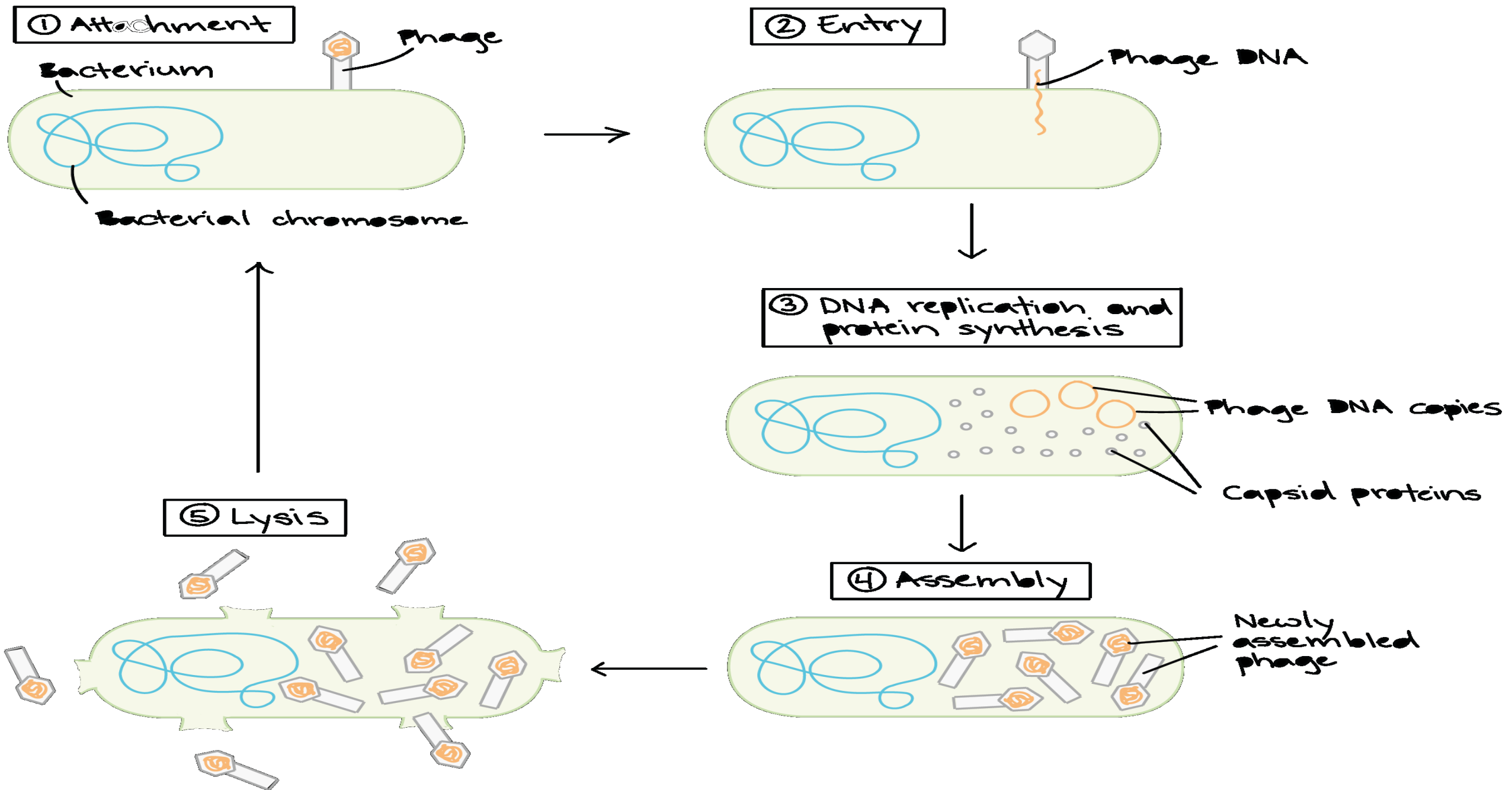
- Many viral components made in the host cell
- Assembled into complete viruses

Bacteriophages: lytic cycle

Lysis:

- The viruses are released from the host cell after assembly of the new viral particle
- Phage produces an enzyme that breaks down the bacterial cell wall
- Allows entry of fluid
- New phage cells (about 100-200)
- Fluid that cause cell lysis
- The phages released are free to infect more host cells and continue with the process

the LYTIC CYCLE



Bacteriophages: lysogenic cycle

In this lecture we will look at the Bacteriophage lysogenic cycle

At the end of this lecture

Student must have fully understood:

Steps involved

Bacteriophages: lysogenic cycle

- Phage to reproduce without killing its host
- First two steps (**attachment and DNA injection**) occur just as they do for the lytic cycle
- It recombines with a particular region of the bacterial chromosome.
- Phage DNA to be integrated into the chromosome

Bacteriophages: lysogenic cycle

□ Five-stage cycle

I. Attachment:

- Proteins in the "tail" of the phage bind to a specific receptor on the surface of the bacterial cell
- Once attached, weak chemical bonds are formed
- helps the virus adhere to the host cell

II. Entry:

- The phage injects its genome into the cytoplasm of the bacterium
- Phage releases enzymes that weaken the cell wall
- Empty capsid or virus body remains in the bacterial cell

Bacteriophages: lysogenic cycle

III. Integration of Viral genome to the host genome:

- After the entry of viral genome
- Integrated into the host genome
- Prophage

IV. Integration of Viral genome to the host genome:

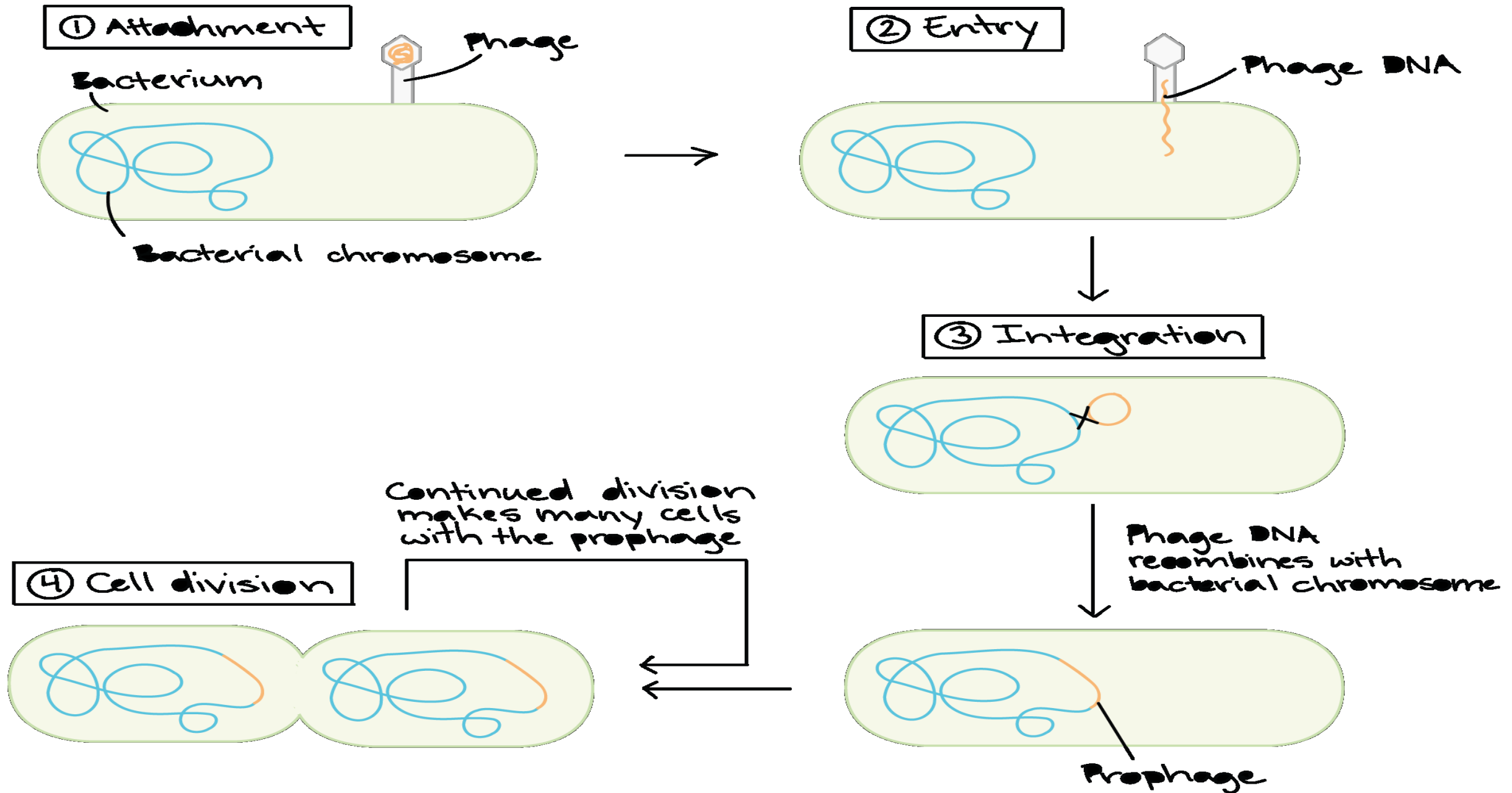
- Viral genome replicates along with bacterial genome
- Host cell DNA Polymerase copies viral genome
- Pass to the daughter cells

Bacteriophages: lysogenic cycle

Induction of lytic cycle:

- Occasionally, viral genome detaches and release into the bacterial cytoplasm
- The dissociation is call induction

the LYSOGENIC CYCLE



Virus Infection

In this lecture we will look at the Virus Infection

At the end of this lecture

Student must have fully understood:

How do animal viruses infect cells?

Virus Infection

- **Animal viruses**, like other viruses, depend on host cells to complete their life cycle
- In order to reproduce
- Recognition is must
- Bind to receptors on the host cell membrane
- ❖ **Receptor:**
 - A protein molecules, that receives signals for a cell
 - Cell surface receptors
 - Bind to ligands and cause responses in the immune system
 - Various immune cells like B cells, T cells, NK cells, monocytes and stem cells

Virus Infection

- I. Viral attachment or adsorption to the host cell
- II. Viral entry into a cell
- III. Un-coating
- IV. Viral replication within the host cell
- V. Viral assembly and maturation
- VI. Viral release from the host cell

I. Viral attachment or adsorption to the host cell

Interaction of a unique protein on the surface of the virus with a highly specific receptor site on the surface of the cell

- **Receptor** on cell membrane
- **Attachment proteins** on the surface of viruses

Virus	Viral Attachment Molecule	Likely Cellular Receptor	Target Cell Type
rabies virus (Rhabdoviridae)	glycoprotein	Acetylcholine receptor	neuron
FIV (Retroviridae)	gp120	CD9, leukocyte	T cell, macrophage
pseudorabies virus Herpesviridae	gC	Heparin sulfate proteoglycans	many cell types
influenza A virus Orthomyxoviridae	Hemagglutinin (HA)	Sialic-acid containing glycoproteins	respiratory epithelium

II. Viral entry into a cell

- **Endocytosis:** engulfment by the host cell
- **Membrane fusion:** viral envelope with the host cell membrane

- A. Enveloped viruses entering by fusion
- B. Enveloped viruses entering by endocytosis
- C. Naked viruses entering by capsid rearrangement
- D. Naked viruses entering by endocytosis

II. Viral entry into a cell

A. Enveloped viruses entering by fusion

- envelope virus fuses directly to host plasma membrane, and the nucleocapsid is deposited into cytoplasm
- ✓ paramyxoviruses (e.g. mumps and measles)

B. Enveloped viruses entering by endocytosis

- Viruses engulfed by receptor-mediated endocytosis to form coated vesicles, which fuse with lysosomes
- Lysosomal enzymes may help with un-coating

C. Naked viruses entering by capsid rearrangement

- Viruses undergo a major change in capsid structure on adsorption to plasma membrane,
- Nucleic acids are released into the cytoplasm

D. Naked viruses entering by endocytosis

Virus Infection

In this lecture we will look at the Virus Infection

At the end of this lecture

Student must have fully understood:

How do animal viruses infect cells?

Virus Infection

- I. Viral attachment or adsorption to the host cell
- II. Viral entry into a cell
- III. Un-coating
- IV. Viral replication within the host cell
- V. Viral assembly and maturation
- VI. Viral release from the host cell

III. Un-coating

- Uncoating occurs concomitantly with or shortly after penetration
- Released of genome from the capsid after penetration
- Infectivity in the parental virus is lost at this point
- Uncoating is done enzymatically (from lysosome)
- Occurs in the cytoplasm of the host cell

IV. Viral replication within the host cell

- Complex process
- Process depends on the nucleic acid type
 - **DNA virus replication**: replicate in the nucleus (with the exception of the poxviruses)
 - **RNA virus replication**: replicate in the cytoplasm of the host cell (with the exception of the orthomyxoviruses and retroviruses)

V. Viral assembly and maturation

- Once new viral genomes and proteins have been produced, they are assembled into new virions
- **Naked Viruses:** Maturation consists of two main processes:
 - the assembly of the capsid
 - its association with the nucleic acid
- Maturation occurs at the site of nucleic acid replication
- Mature viruses forming inclusion body
- **Enveloped viruses:** capsid must first be assembled around the nucleic acid to form the nucleocapsid, which is then surrounded by the envelope

VI. Viral release from the host cell

Viral exit methods include budding, exocytosis, and cell lysis

I. Enveloped viruses released by budding

- Viral-encoded envelope glycoproteins are incorporated into the host cell membranes by the Golgi apparatus
- The release of influenza virus particles from cells requires the activity of a virion enzyme: a neuraminidase

II. Enveloped viruses released by exocytosis

- is the process by which cells release particles from within the cell into the extracellular space
- vesicles containing substances fuse with the plasma membrane
- vesicles containing the virus are secreted/excreted out of the infected cell

Positive-Strand RNA Viruses Replication

In this lecture we will look at the Virus Replications

At the end of this lecture

Student must have fully understood:

How do (+) stranded RNA viruses replicated?

Positive-Strand RNA Viruses Replication

- Positive sense, negative sense, double stranded viruses, and retroviruses are RNA viruses with different modes of replication
- The (+) strand RNA viruses are the most plentiful on this planet
- Families Arteriviridae , Astroviridae , Caliciviridae , Coronaviridae , Flaviviridae , Hepeviridae , Nodaviridae , Picornaviridae , and Togaviridae include viruses that infect vertebrates

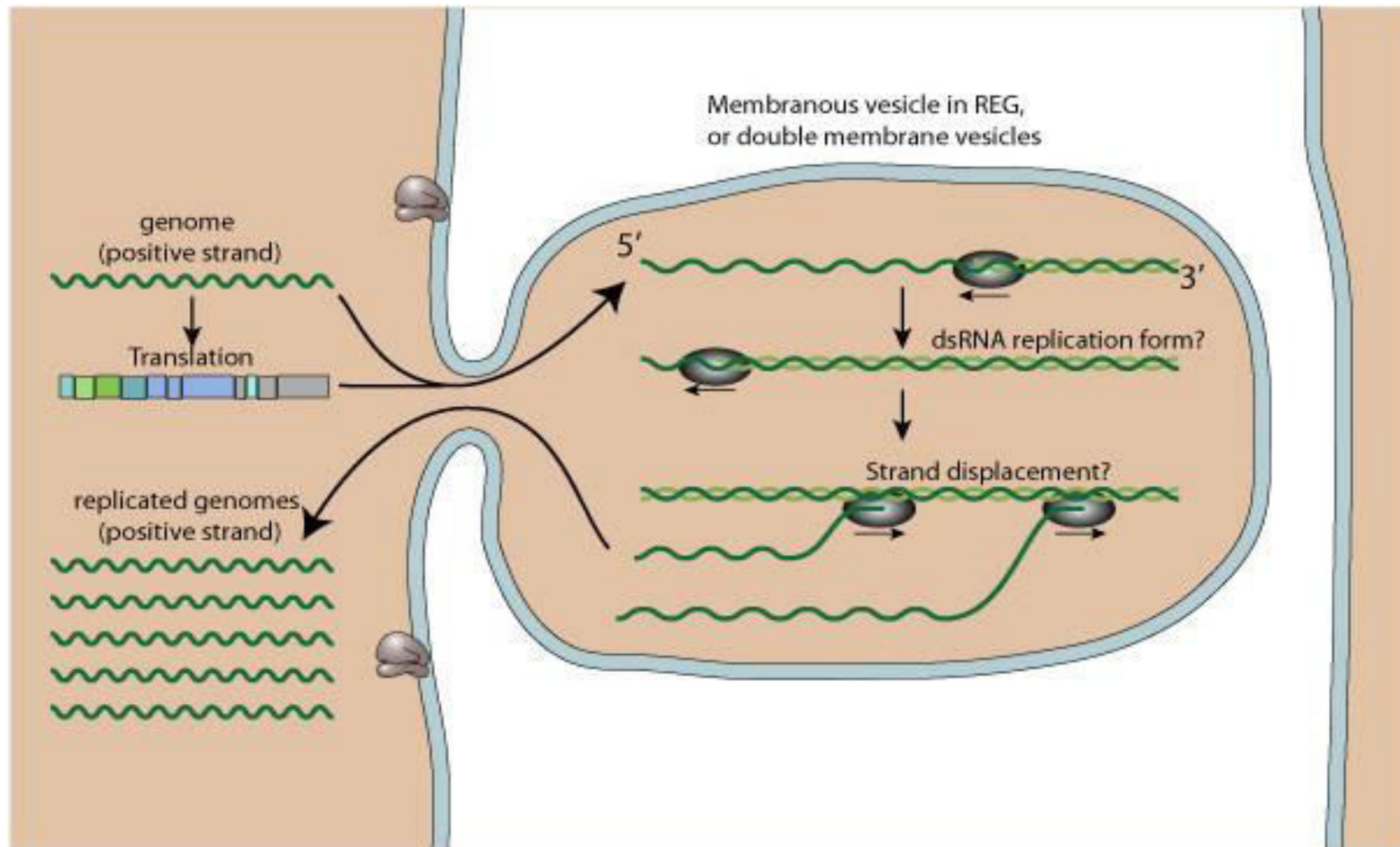
Positive-Strand RNA Viruses Replication

- Viral replication usually takes place in the cytoplasm
- Act as both genomic and mRNA
- The (+) strand RNA genomes usually can be translated directly into protein by host ribosomes
- All positive-sense ssRNA virus genomes encode **RNA-dependent RNA polymerase**
- A viral protein that synthesizes RNA from an RNA template.
- Host cell proteins recruited by positive-sense ssRNA viruses during replication include RNA-binding proteins, chaperone proteins, and membrane remodeling and lipid synthesis proteins
- Which collectively participate in exploiting the cell's secretory pathway for viral replication

Positive-Strand RNA Viruses Replication

The genome is replicated in two steps

- (+) strand genome is first copied into a full length (-) strand
- All synthesize through a double stranded intermediate - **RI - replication intermediate**
- Used as a template to make more (+) ssRNA
- (+) ssRNA serve as a template for the transcription of proteins



Negative-Strand RNA Viruses Replication

In this lecture we will look at the NS RNA Viruses Replication

At the end of this lecture

Student must have fully understood:

A brief review on Mechanism of NSV replication

Negative-Strand RNA Viruses Replication

- Also known as an antisense-strand RNA virus
- Negative-strand (NS) RNA viruses encompass some of the most significant human and agricultural pathogens
- Influenza, measles, mumps, Ebola viruses
- Produce frequent epidemics of disease and high mortality outbreaks by transmission from animal reservoirs
- Two main groups based on their genomic RNA
 - non-segmented NS (NNS) RNA viruses
 - segmented NS (SNS) RNA viruses

Negative-Strand RNA Viruses Replication

NNS RNA viruses comprise four families:

- *Rhabdoviridae* (rabies virus)
- *Paramyxoviridae* (measles viruses)
- *Filoviridae* (Ebola and Marburg viruses)
- *Bornaviridae* (Borna disease virus)

SNS RNA viruses comprise three families:

- *Arenaviridae* (Machupo virus (MACV))
- *Bunyaviridae* (Rift Valley fever virus)
- *Orthomyxoviridae* (influenza A virus)

Negative-Strand RNA Viruses Replication

- Negative strand RNA viruses have a unique mechanism of replication
- Their genome is a single strand RNA that has to be transcribed as soon as the virus enters the host in order to carry out viral replication

Steps Involve in Replication Process

The life cycle of NSV has a number of steps

- Virus enters the cell by receptor mediated endocytosis and releases its negative RNA into the cytoplasm
- The viral replication process begins at 3' end and forms a complete positive sense RNA using negative sense genomic template

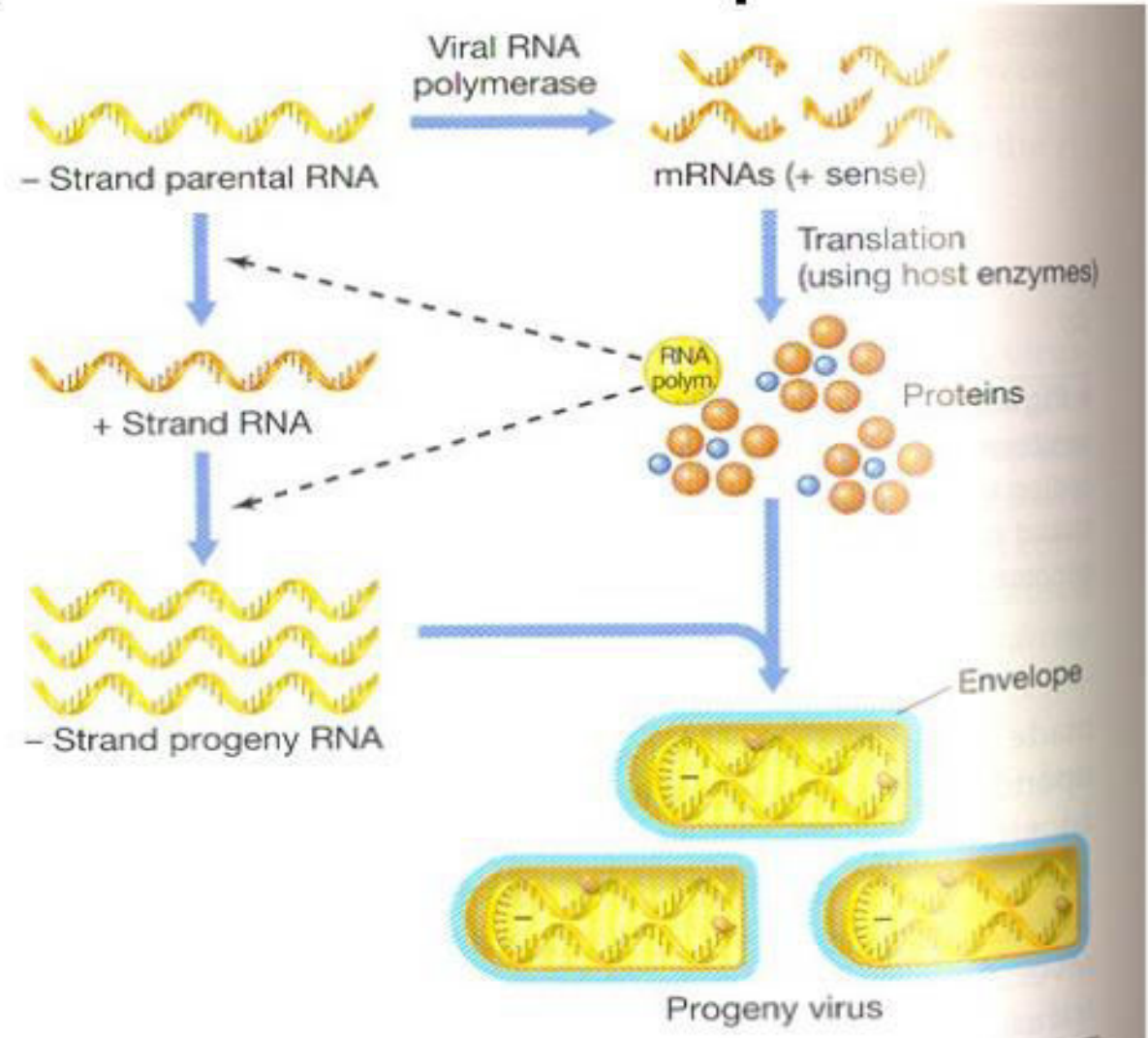
Negative-Strand RNA Viruses Replication

- The virus uses its own RNA replicase, also known as RNA-dependent RNA polymerase (RdRp), to form positive RNA template strands through complementary base pairing
- The virus first infects the host cell by binding to the host cell receptor through a viral surface glycoprotein
- The fusion of the glycoprotein viral membrane with the plasma membrane of the host cell in an acidic environment allows for the release of viral ribonucleoprotein (RNP) complexes into the cytoplasm
- Most NSV replicate in the cytoplasm of infected cells

Negative-Strand RNA Viruses Replication

Newly synthesized RNP complexes are then assembled with viral structural proteins at the plasma membrane or at membranes of the Golgi apparatus. This is all followed by the release of the newly synthesized viruses.

(-)RNA Virus Replication



Double Stranded RNA Viruses Replication

In this lecture we will look at the Double Stranded RNA Viruses Replication

At the end of this lecture

Student must have fully understood:

A Brief Overview on Mechanism of dsRNA viruses Replications

Double stranded RNA Viruses Replication

- Double-stranded RNA viruses are known in all major groups of organisms, from bacteria and fungi to animals and plants
- They represent a diverse group of viruses that vary widely in host range (humans, animals, plants, fungi and bacteria)
- dsRNA viruses are all non-enveloped and possess icosahedral capsids
- They have segmented genomes, and two families of dsRNA viruses infect humans
- **Example:** Poxvirus, Herpes Virus

Double stranded RNA Viruses Replication

- The dsRNA viruses are classified into five major groups
- With the exception of members of the *Totiviridae*, all the dsRNA viruses have multiple segments of dsRNA in their genomes.

Members of this group include the rotaviruses, renowned globally as the most common cause of gastroenteritis in young children, and picobirnaviruses, renowned worldwide as the most commonly occurring virus in fecal samples of both humans and animals with or without signs of diarrhea

Double Stranded RNA Viruses Replication

The virion (genomic) RNA is double stranded and so cannot function as mRNA; thus these viruses also need to package an RNA polymerase to make their mRNA after infection of the host cell

Example: Rotaviruses (belong to reovirus family)

Location of Replication

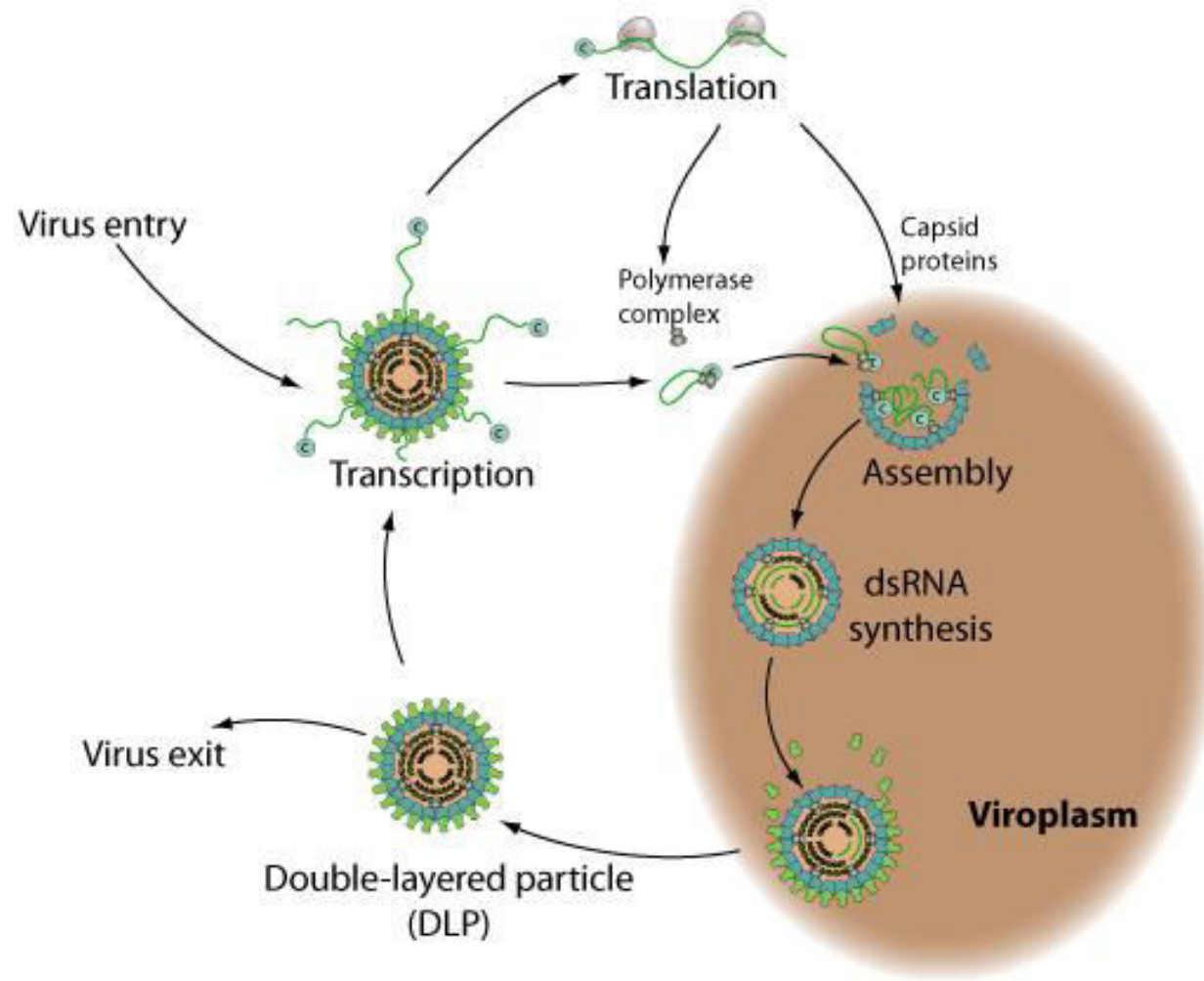
- Host cell cytoplasm, replication/transcription occurs in capsids for most dsRNA viruses
- In double stranded RNA virus Replication process occurs in the host cytoplasm and converts ss-mRNA to ds-genomic RNA

Double stranded RNA Viruses Replication Mechanism

- In a double stranded RNA form, **retroviruses** infect a host cell with their genome, and then are reverse transcribed into double stranded DNA, with the DNA then integrated into the host cell genome
- When integrated into a host genome, a retrovirus is hard to detect and can lay dormant for prolonged periods, having no discernible effect on the host.
- Upon infection, the genomic dsRNA is transcribed in mRNAs that will both serve for translation and/or replication.

Double stranded RNA Viruses Replication Mechanism

Double-stranded RNA virus replication



Double stranded RNA Viruses Replication Mechanism

- mRNAs translation produces the proteins necessary to ensure replication and encapsidation
- Replication occurs in host cytoplasm and converts ss-mRNA to ds-genomic RNA
- dsRNA is a kind of molecule that cells do not produce, and eukaryotic hosts have various antiviral systems that detect and inactivate dsRNA
- To circumvent this defense, many dsRNA viruses are replicating their RNA inside icosahedral capsids
- The RNA polymerases are situated at the fivefold axis of symmetry and produces mRNAs that are extruded from the particle.
- The genomic dsRNA never enters the cytoplasm, thereby is concealed from cellular dsRNA sensors.

dsRNA Virus Replication Cycle

- RNA replication occurs in the cytoplasm for all dsRNA viruses that have been investigated
- Transcription, defined as the synthesis of viral (+)-strands from a dsRNA template, takes place within viral particles or core particles
- The new (+)-strands are generally extruded from the viral particles
- These (+)-strands are then translated to make viral proteins
- It is then the same (+)-strands that are packaged to make new particles or sub viral particles.
- Once the new particles or cores have formed, (-)-strand synthesis on the (+)- strand template (replication) completes the formation of new dsRNA. In systems where the virus is destined for export, addition of new layers of protein and/or membrane completes the virus reproduction cycle.

DNA Viruses Replication

In this lecture we will look at the DNA Viruses Replication

At the end of this lecture

Student must have fully understood:

A Brief Overview on Mechanism of DNA viruses Replications

DNA Viruses Replication

- Some of the most well-known viruses, such as those that cause herpes, smallpox, hepatitis and warts, have a **DNA based genome**
- DNA replication occurs in the nucleus
- Many human parvoviruses are satellite viruses that require co-infection by another DNA virus – either an Adenovirus, or a Herpesvirus – in order to replicate
- These Adeno-Associated viruses (AAV) have been developed as gene therapy vectors
- On the other hand, the bocaviruses (which cause respiratory infections) and Parvovirus B-19 are capable of replication in the absence of a helper virus.

Several methods of replication can be recognized.

➤ A. Adenoviruses

- ✓ Adenoviruses show asymmetric replication, which initiates at the 3' end of one of the strands using a protein primer.
- ✓ The growing strand displaces the preexisting strand of the same polarity and builds a complete duplex molecule.
- ✓ The displaced strand in turn replicates in a similar manner after generating a panhandle structure by pairing the inverted terminal repetitions.

➤ B. Herpesviruses

- Herpesviruses have linear genomes with terminal repeats.
- On reaching the nucleus, the terminal ends undergo limited exonucleotic digestion and then pair to form circles.
- Replication is thought to take place via a rolling circle mechanism, where concatemers are formed. During maturation, unit-length molecules are cut from the concatemers.

➤ C. Papovaviruses

- The DNA of papovaviruses are circular and the replication is bidirectional and symmetrical, via cyclic intermediates.

➤ **D. Parvoviruses**

- ✓ The replication of single stranded parvoviruses is initiated when +ve and -ve stranded DNA from different parvovirus particles come together to form a double stranded DNA molecule from which transcription and replication takes place.

➤ **E. Poxviruses**

- The striking feature of poxvirus DNA is that the two complementary strands are joined.
- The replicative intermediates, present in the cytoplasm, are special concatemers containing pairs of genomes connected either head to head or tail to tail.

➤ **F. Hepadnaviruses**

- Hepatitis B virus employs reverse transcription for replication.
- The genome consists of a partially double-stranded circular DNA with a complete negative strand and an incomplete positive strand.
- Upon entering the cell, the positive strand is completed and transcribed. RNA transcripts are in turn reverse-transcribed into DNA by a viral enzyme in several steps, following closely the model of retroviruses, including a jump of the nascent positive strand from one direct repeat (DR) to another.

Double stranded RNA Viruses Replication Mechanism

- Replication requires expression of at least one virus protein, sometimes many
- DNA is always synthesized 5' - 3' via semiconservative replication
- Replication initiates at a defined origin using a primer
- The host provides other proteins
- Simple viruses conserve genetic information
 - always hijack more host proteins
- Complex viruses encode many, but not all proteins required for replication

Requirements for DNA Replication

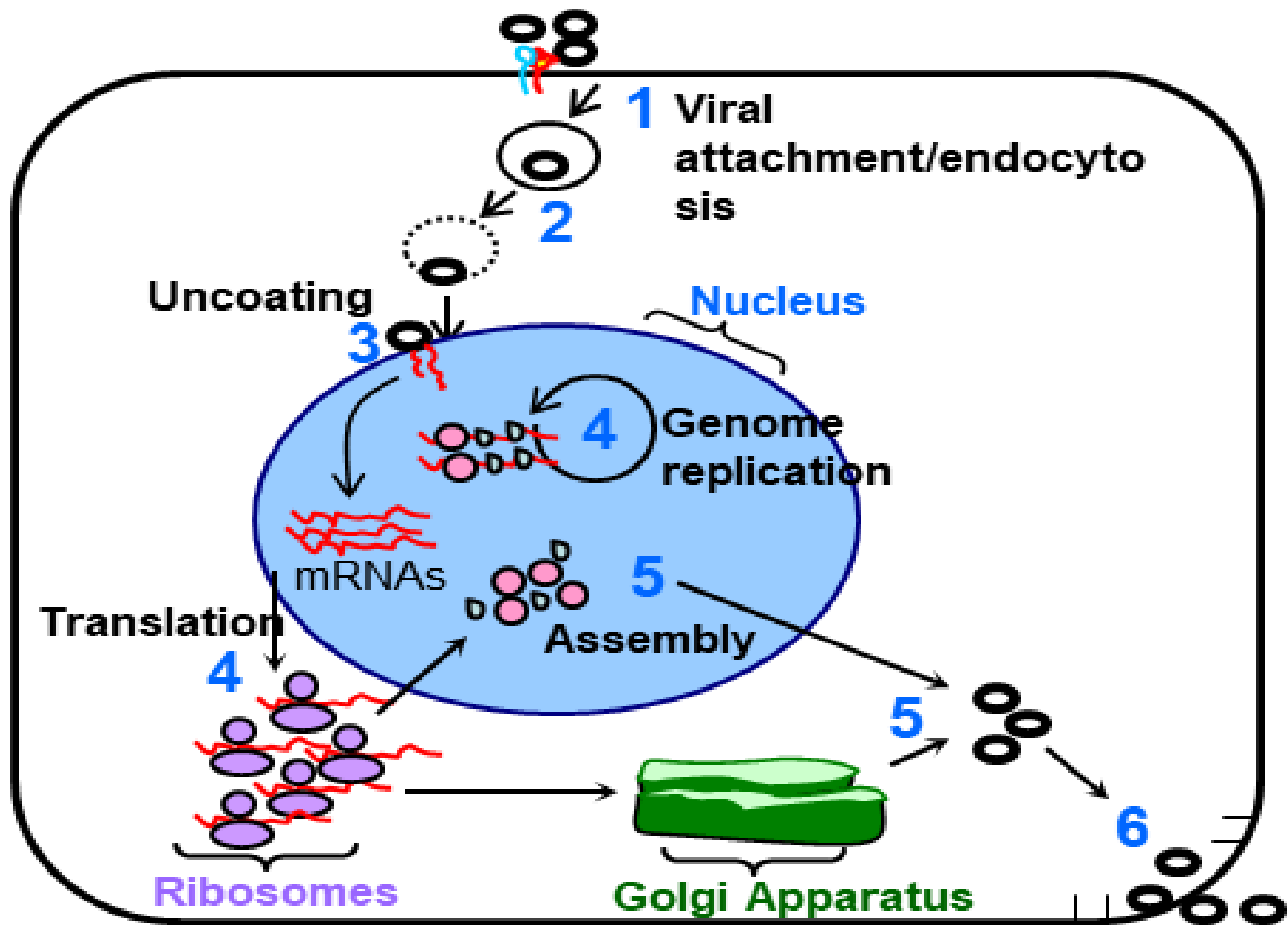
- Ori recognition for initiation
 - binding to an AT-rich DNA segment
- Priming of DNA synthesis
 - RNA - Okazaki fragments
 - DNA - hairpin structures
 - protein - covalently attached to 5' end
- Elongation
- Termination

DNA Virus Replication

- Small DNA viruses do not encode an entire replication system
 - encode proteins that orchestrate the host
 - Papillomaviridae, Polyomaviridae, Parvoviridae
- Large DNA viruses encode most of their own replication systems
 - Herpesviridae, Adenoviridae, Poxviridae

Virus Encoded Proteins

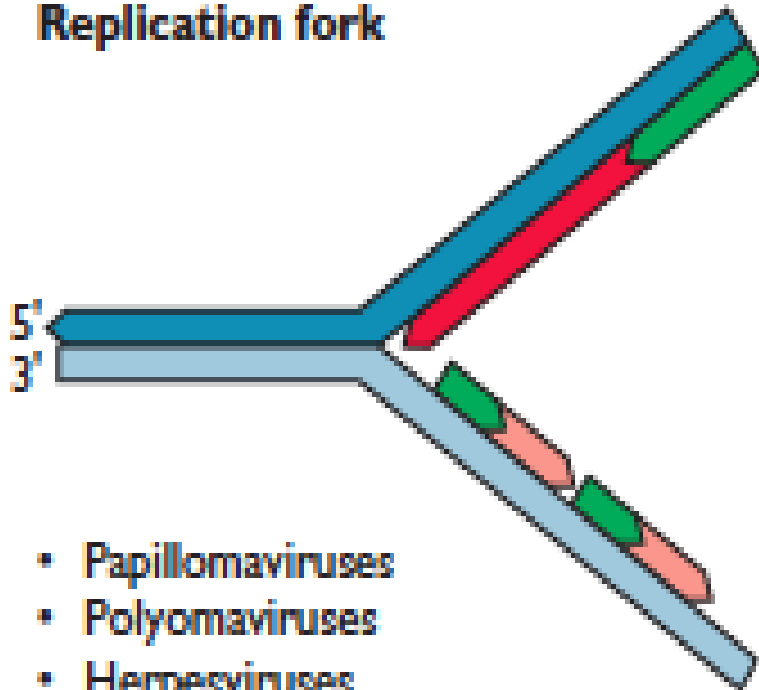
- ❖ Origin Binding Protein, Helicases and Primase
- ❖ DNA polymerase and accessory proteins
- ❖ Exonucleases
- ❖ Enzymes of nucleic acid metabolism (thymidine kinase, ribonucleotide reductase, dUTPase)



Two Basic Modes of Replication

Leading

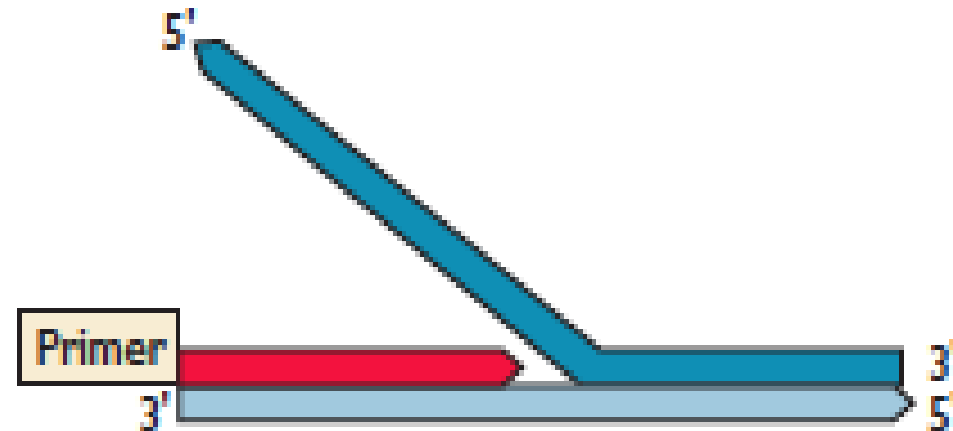
Replication fork



- Papillomaviruses
- Polyomaviruses
- Herpesviruses
- Retroviral proviruses

Lagging

Strand displacement (primer)



- Adenoviruses (protein)
- Parvoviruses (DNA hairpin)
- Poxviruses (DNA hairpin)

Virus Specimen Collection

In this lecture we will look at the Virus specimen collection

At the end of this lecture

Student must have fully understood:

Methods to collect samples from various sources

Virus Specimen Collection

- Sample Collection, such as handling, labeling, processing, aliquoting, storage, and transportation, may affect the results of the study
- If case sample are handled differently from controls samples, differential misclassification may occur
- Each specimen must be correctly labeled.
- ❖ Label information should include:
 - patient name, case number, date of birth, age, sex, date specimen was collected, source of specimen, and the test requested.

Virus transport medium (VTM)

- It is recommended for the collection of viral samples
- This medium contains antibiotics and an antifungal agent to minimize bacterial and yeast contamination and cannot be used for mycoplasma detection.
- MRT can be stored at room temperature but once inoculated, the tubes must be refrigerated.
- Contains only buffers and salt.
- Doesn't contain any nutritional ingredients such as carbon, nitrogen, and organic growth factors so as to prevent microbial multiplication.
- Addition of antibiotics and other substances like glycerol may be added for transporting specimens.

UTM™ Viral Transport Media

- UTM™, Universal Transport Medium, is a room temperature stable viral transport medium for collection, transport, maintenance and long term freeze storage of Viruses
- UTM™ has been used successfully for Rapid Antigen Testing, DFA, Viral Culture and for Molecular-based Assays.

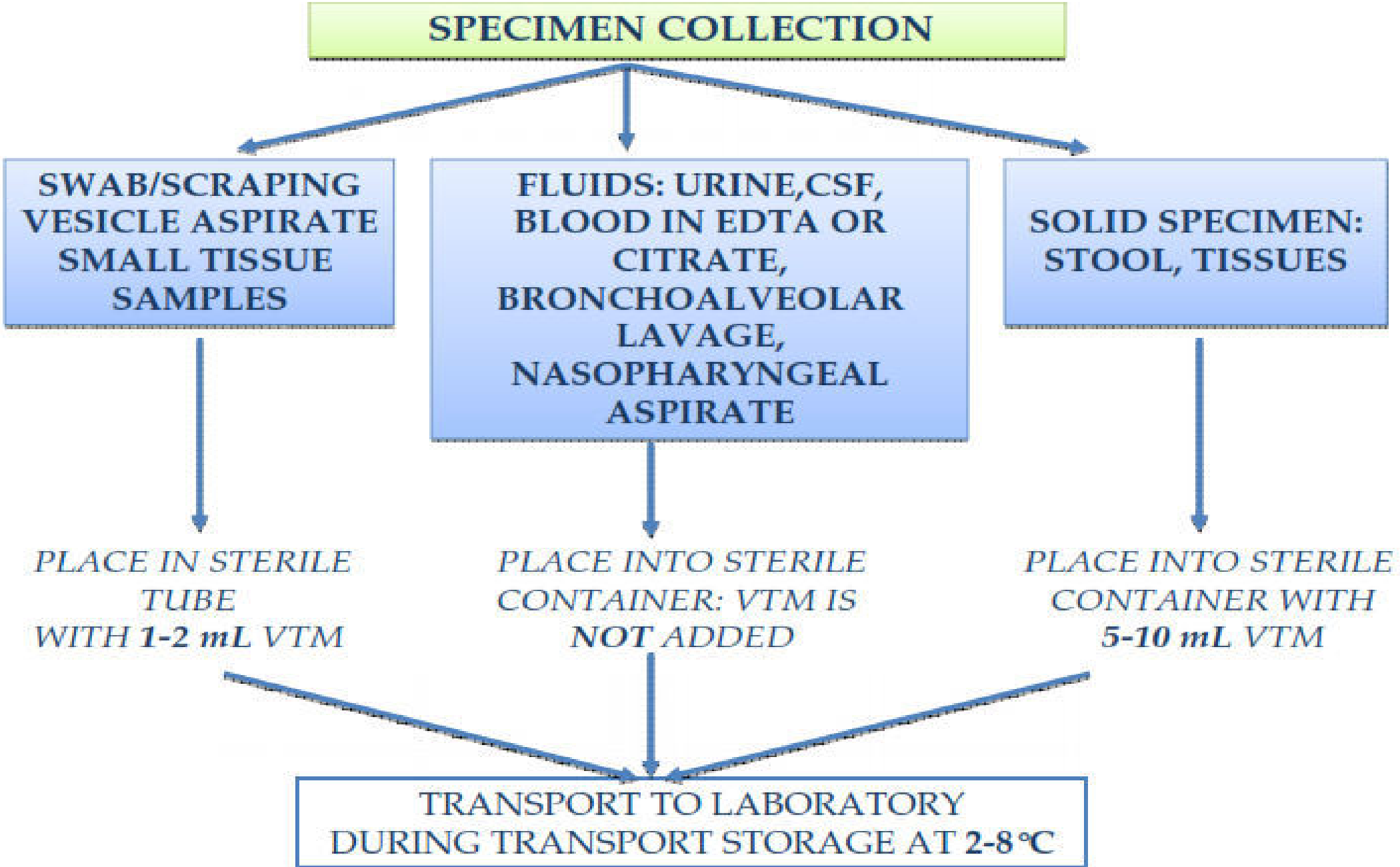


Virus transport medium (VTM)

Basic concepts for specimen collection

- Collect the specimen early in the acute phase of infection,
- Use appropriate collection devices and collect a sufficient quantity of material
- Collect an appropriate specimen
- Label the specimen and complete the requisition
- Minimize transport time and keep specimens for virus culture cold in transport

SPECIMEN COLLECTION



```
graph TD; A[SPECIMEN COLLECTION] --> B[SWAB/SCRAPING<br/>VESICLE ASPIRATE<br/>SMALL TISSUE<br/>SAMPLES]; A --> C[FLUIDS: URINE, CSF,<br/>BLOOD IN EDTA OR<br/>CITRATE,<br/>BRONCHOALVEOLAR<br/>LAVAGE,<br/>NASOPHARYNGEAL<br/>ASPIRATE]; A --> D[SOLID SPECIMEN:<br/>STOOL, TISSUES]; B --> E[PLACE IN STERILE<br/>TUBE<br/>WITH 1-2 mL VTM]; C --> F[PLACE INTO STERILE<br/>CONTAINER: VTM IS<br/>NOT ADDED]; D --> G[PLACE INTO STERILE<br/>CONTAINER WITH<br/>5-10 mL VTM]; E --> H[TRANSPORT TO LABORATORY<br/>DURING TRANSPORT STORAGE AT 2-8°C]; F --> H; G --> H;
```

**SWAB/SCRAPING
VESICLE ASPIRATE
SMALL TISSUE
SAMPLES**

*PLACE IN STERILE
TUBE
WITH 1-2 mL VTM*

**FLUIDS: URINE, CSF,
BLOOD IN EDTA OR
CITRATE,
BRONCHOALVEOLAR
LAVAGE,
NASOPHARYNGEAL
ASPIRATE**

*PLACE INTO STERILE
CONTAINER: VTM IS
NOT ADDED*

**SOLID SPECIMEN:
STOOL, TISSUES**

*PLACE INTO STERILE
CONTAINER WITH
5-10 mL VTM*

**TRANSPORT TO LABORATORY
DURING TRANSPORT STORAGE AT 2-8°C**

SWABS

➤ For diagnosis of viral infections

Swabs should be:

- made of rayon
 - Should not be made of cotton or calcium alginate
- Swab's shaft should be:
 - made of plastics or metal
 - Should not be made of wood



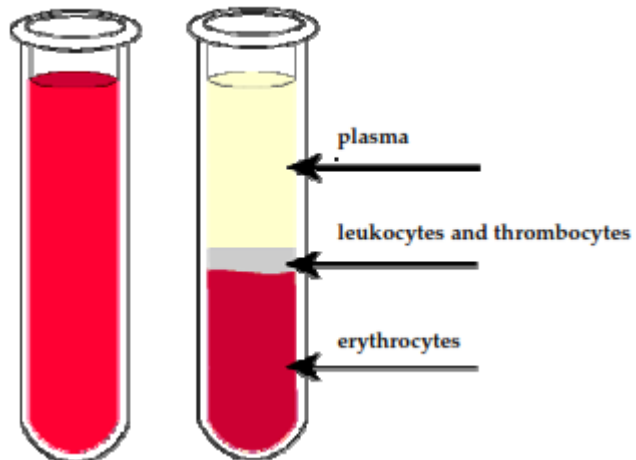
Collection of specimen

Blood

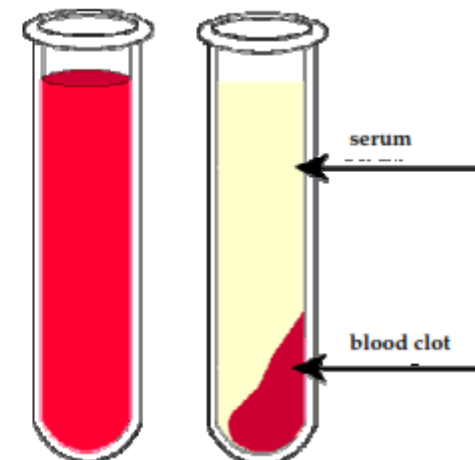
- by venepuncture or through venal catheter
- Blood is taken for viral or serology diagnosis

For viral diagnosis

- - whole blood with EDTA, sodium-citrate or heparin
- - “buffy coat” (Leukocytes or Thrombocytes) or plasma



For serology - serum



Collection of specimen

Saliva

Saliva specimens have high sensitivity and specificity in the detection of respiratory viruses

- 1 swab from the bottom of the mouth
- 1 swab from the area around Stenson's ducts
- Place in tube with VTM

Urine

- 10-20 ml, middle stream (sterile container)

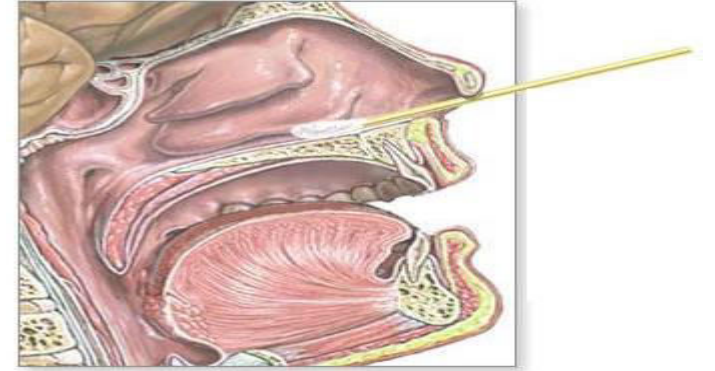
Specimen from upper respiratory tract

Nasopharyngeal swab

through nostrile to nasopharincs place in VTM

Nasopharyngeal wash

- pouring of saline by syringe through nostrile to nasopharings
aspirating of saline with respiratory secretions place in VTM



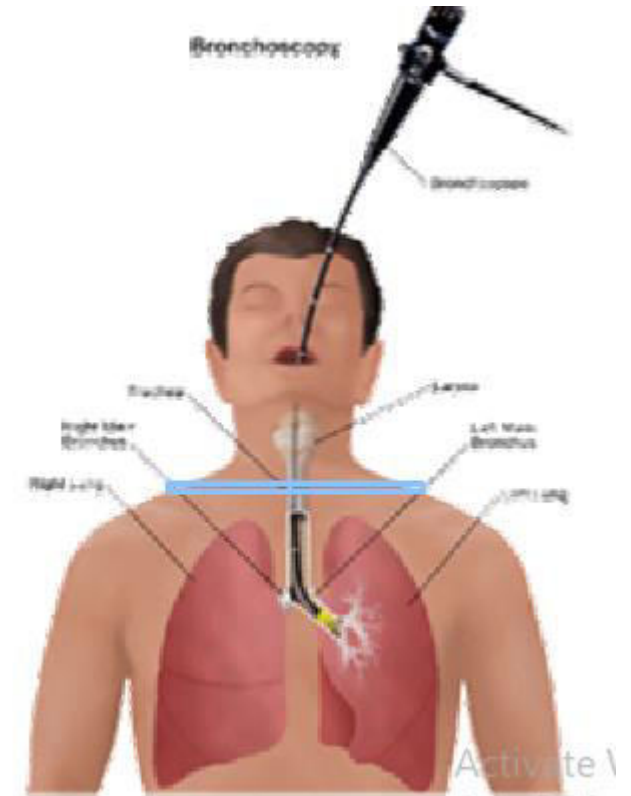
Specimen from lower respiratory tract

Bronchoalveolar lavage (BAL)

- patient in anesthesia, placement of bronchoscope
- 8-10 mL in sterile container
- no VTM

Sputum

- Not useful for viral detection



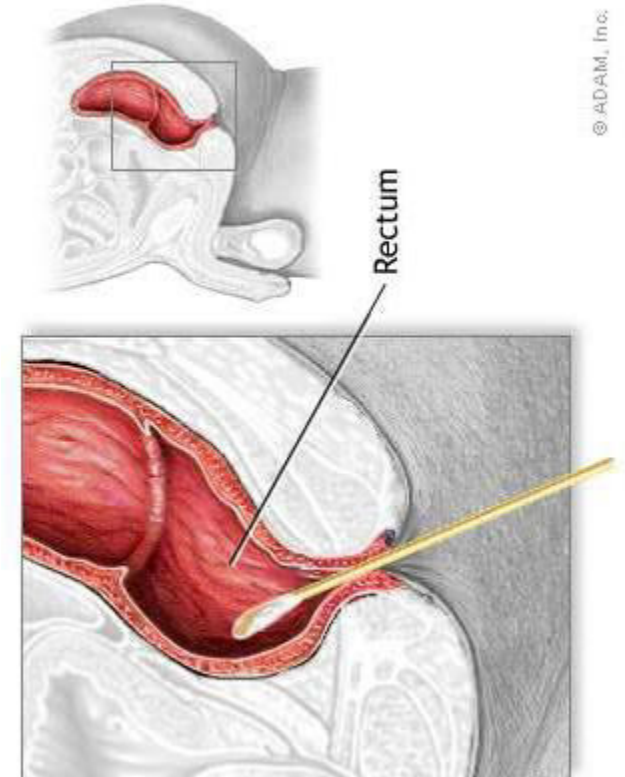
Specimen from gastrointestinal tract

Feces

2-4 g of feces (sterile container)
place in VTM (8-10 ml)

Rectal swab

place in VTM



Specimen from skin

Swab from the lesion

- imbue with sterile physiologic solution
- place in the test-tube in VTM

Vesicle swab

- cleaning with sterile saline; piercing with sterile instrument
- 1 swab for the fluid
- 1 swab for the material from the bottom of the vesicle
- both swabs in VTM

Vesicle aspirate

- piercing with sterile needle
- aspiration of fluid with syringe
- washing the syringe with VTM

Effect of physical and chemical factors on viruses

➤ Inactivation of virus represents permanent loss of contamination.

Temperature: - viruses are stable on low temperature (keeping)

- - viruses with envelope are heat sensitive

Drying: most of the viruses are sensitive Irradiation (UV, X-ray)

- inactivation of viruses

Chemical agents

- organic solvent (chloroform) – inactivation of the viruses
- with envelope
- oxido-reduct. agents (formaldehyde, chlorine, iodine) – inactivation

pH low pH - viruses without envelope are mostly stable

SPECIMEN TRANSPORT

- During transport specimen should be:
- protected from breaking
- protected from light
- At adequate temperature:
- 48 h at +4°C (refrigerator, wet ice) more than 48 h at -70°C (dry ice)
must not be frozen at -20°C!



Cultivation of Viruses

In this lecture we will look at the Virus cultivation

At the end of this lecture

Student must have fully understood:

The methods used for the cultivation of Viruses

Cultivation of Viruses

- Viruses do not fall in the category of unicellular microorganism
- They are obligate intracellular parasites and lack the machinery necessary for protein and nucleic acid synthesis
- They depend on the host machinery for their growth and survival
- Unlike other microorganism, complex processes are involved in their multiplication
- Outside of the host cells, viruses are inactive, however, inside living cells, viruses show some of the characteristics of living things

Cultivation of Viruses

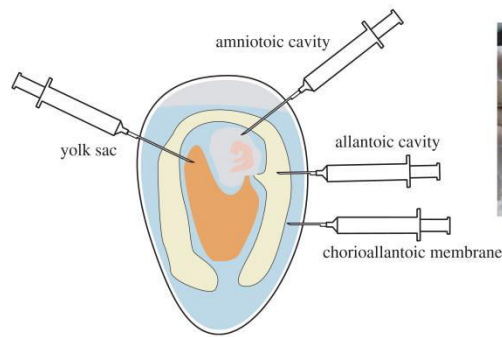
- Since the viruses are obligate intracellular parasites, they cannot be grown on any inanimate culture medium
- Viruses can be cultivated within suitable hosts, such as a living cell
- ❖ The primary purposes of viral cultivation are:
 - I. To isolate and identify viruses in clinical specimens
 - II. To prepare viruses for vaccines
 - III. And to do detailed research on viral structure, multiplication cycles, genetics, and effects on host cells

Cultivation of Viruses

- Viruses not only need living cells to grow in but also they are specific about the type of cell they infect and grow in
- There is no universal cell that will support all viruses
- ❖ Viruses tend to be host specific; therefore:
 - human viruses grow best in cells of human origin,
 - bovine viruses in bovine cells,
 - canine viruses in canine cells,
 - while some viruses will not grow in vitro at all
- Therefore in the laboratory the suspected virus must be grown in a culture method known to support its growth

Methods for Cultivation of Virus

- Generally three methods are employed for the virus cultivation
 - Inoculation of virus into animals
 - Inoculation of virus into embryonated eggs
 - Tissue culture



Methods for Cultivation of Virus

- Animals are used for studying viruses which do not grow in cell cultures or eggs, and for testing vaccines
- Eggs support a fairly wide range of animal and human viruses – hence their importance in the diagnostic service
- Cell cultures; different types of cell lines will support different types of viruses

Virus Inoculation in Animal

In this lecture we will look at the Virus Inoculation in Animal

At the end of this lecture

Student must have fully understood:

How viruses are inoculated into living Animals for cultivation

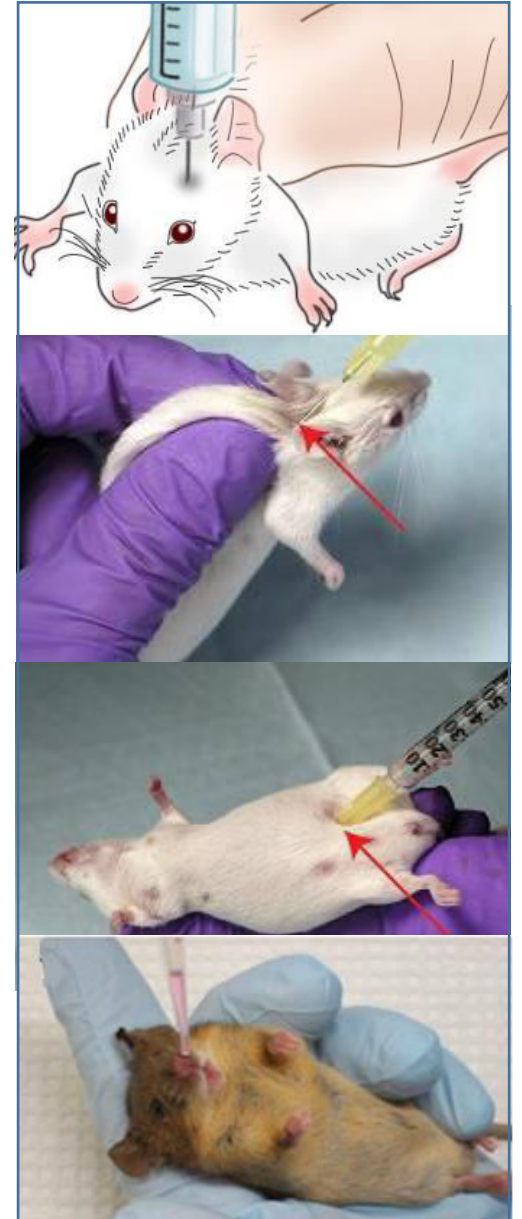
Virus Inoculation in Animal

- Laboratory animals play an essential role in studies of viral pathogenesis
- Live animals such as monkeys, mice, rabbits, guinea pigs, ferrets are widely used for cultivating virus
- Mice are the most widely employed animals in virology



Virus Inoculation in Animal

- The different routes of inoculation in mice are:
 - intracerebral
 - subcutaneous
 - intraperitoneal
- or intranasal
- After the animal is inoculated with the virus suspension, the animal is:
 - observed for signs of disease
 - visible lesions
 - or is killed so that infected tissues can be examined for virus



Advantages and disadvantages of animal inoculation

Advantages

- Production of antibodies can be identified
- Diagnosis , pathogenesis and clinical symptoms are determined. [?]
Primary isolation of certain viruses
- Mice provide a reliable model for studying viral replication
- Used for the study of immune responses, epidemiology and oncogenesis

Advantages and disadvantages of animal inoculation

Disadvantages

- Expensive and difficulties in maintenance of animals
- Difficulty in choosing of animals for particular virus
- Some human viruses cannot be grown in animals or can be grown but do not cause diseases
- Mice do not provide models for vaccine development.

Embryonated Eggs

In this lecture we will look at the Emryonated Hen Eggs

At the end of this lecture

Student must have fully understood:

How viruses are inoculated and cultivated in chicken embryonated eggs

Virus Inoculation in Embryonated Eggs

- Goodpasture and Burnet in 1931 first used the embryonated hen's egg for the cultivation of virus
- The process of cultivation of viruses in embryonated eggs depends on the type of egg being used
- Eggs provide a suitable means for:
 - A. the primary isolation and identification of viruses
 - B. the maintenance of stock cultures
 - C. and the production of vaccines

Virus Inoculation in Embryonated Eggs

Terms most often refer to eggs

- Embryonated: having an embryo
 - Unembryonated: not having an embryo
 - De-embryonated: having lost an embryo
-
- Chicken, duck, and turkey eggs are the most common choices for inoculation
 - The egg used for cultivation must be sterile and the shell should be intact and healthy
 - Rigorous sterile techniques must be used to prevent contamination by bacteria and fungi from the air and the outer surface of the shell

Advantages of Virus Inoculation in Embryonated Eggs

- An embryo is an early developmental stage of animals marked by rapid differentiation of cells
- Birds undergo their embryonic period within the closed protective case of an egg, which makes an incubating bird egg a nearly perfect system for viral propagation
- It is an intact and self-supporting unit, complete with its own sterile environment and nourishment
- It furnishes several embryonic tissues that readily support viral multiplication
- Defense mechanisms are not involved in embryonated eggs
- Cost- much less, Maintenance-easier, Less labor and Readily available

Virus Inoculation in Embryonated Eggs

- The egg must be injected through the shell, usually by drilling a hole or making a small window
- The viral suspension or suspected virus- containing fluid is injected into the fluid of the egg
- The exact tissue that is inoculated is guided by the type of virus being cultivated and the goals of the experiment



Detection of Viral Growth

- Viruses multiplying in embryos may or may not cause effects visible to the naked eye
- The signs of viral growth include:
 - ✓ Death of the embryo
 - ✓ Defects in embryonic development
 - ✓ and localized areas of damage in the membranes, resulting in discrete opaque spots called pocks



Detection of Viral Growth

- If a virus does not produce obvious changes in the developing embryonic tissue, virologists have other methods of detection
- Embryonic fluids and tissues can be prepared for direct examination with an electron microscope
- Certain viruses can also be detected by:
 - their ability to agglutinate red blood cells
 - or by their reaction with an antibody of known specificity

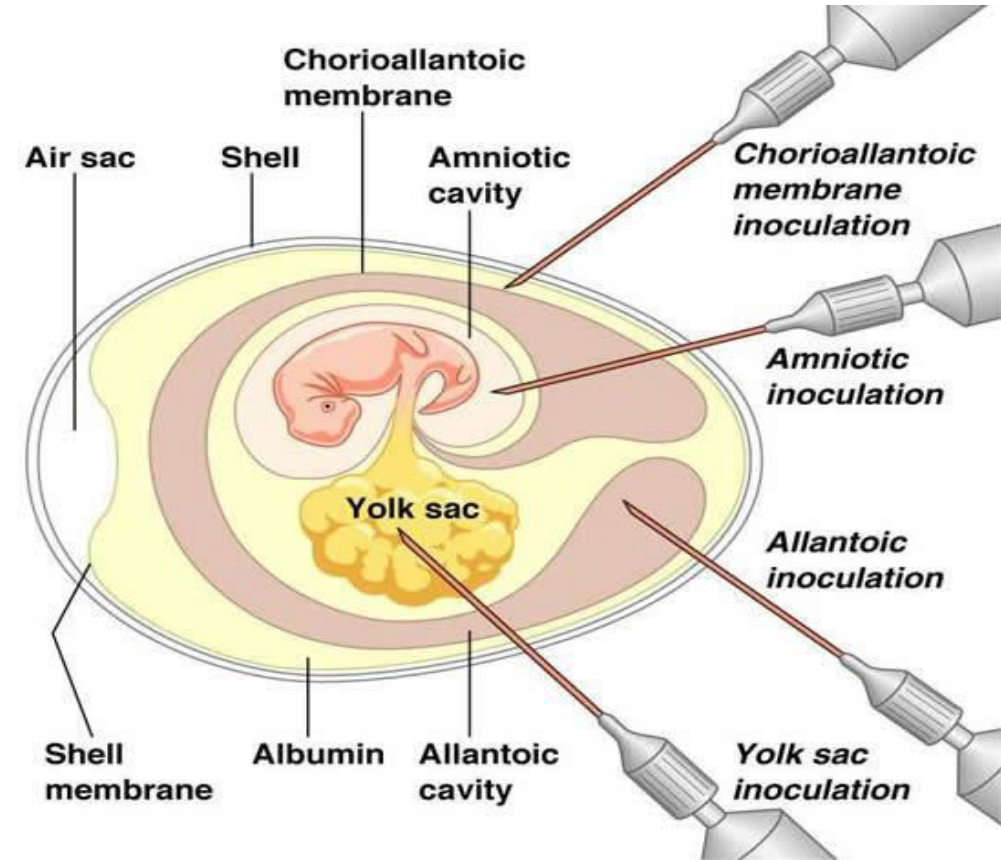
Parts of Embryonated Egg

- The yolk sac is the source of nourishment for the developing Embryo
- As the embryo develops, the yolk sac decreases in size until it is completely absorbed into the digestive system of the mature embryo
- The amnion is a thin membrane that encloses the embryo and Protects it from physical damage
- It also serves as an exchange system and is best seen in the younger embryos

Routes of Viral Inoculation

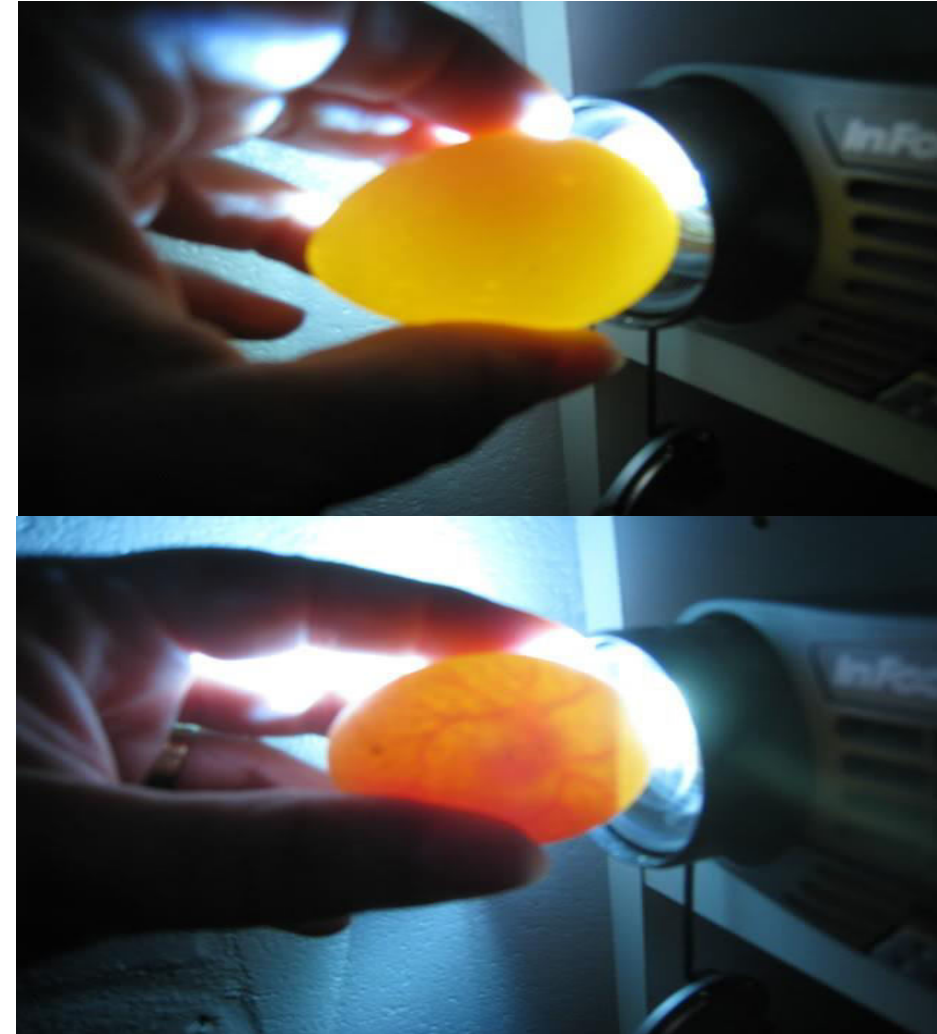
- An embryonated egg offers various sites for the cultivation of viruses
- The different sites of viral inoculation in embryonated eggs are:

1. Chorioallantoic membrane(CAM)
2. Amniotic Cavity
3. Allantoic Cavity
4. Yolk sac



Candling of Egg

- Candling is the process of holding a strong light above or below the egg to observe the embryo
- A candling lamp consists of a strong electric bulb covered by a plastic or aluminum container that has a handle and an aperture



Propagation in Cell Culture

In this lecture we will look at the Virus propagation in cell culture

At the end of this lecture

Student must have fully understood:

How viruses are inoculated and cultivated in cell culture and different types of cell culture

Propagation in Cell Culture

- Prior to the advent of cell culture, animal viruses could be propagated only on whole animals or embryonated chicken eggs
- Cell cultures have replaced embryonated eggs as the preferred type of growth medium for many viruses
- Cell culture consists of cells grown in culture media in the laboratory
- These cultures can be propagated and handled like bacterial cultures; they are more convenient to work with than whole animals or embryonated eggs

Propagation in Cell Culture

- Cell cultures have replaced embryonated eggs as preferred type of growth medium for many viruses.
- Cell culture consists of cells grown in culture media in the laboratory.

There are three types of tissue culture:

- 1) Organ culture
- 2) Explant culture
- 3) Cell culture

Propagation in Cell Culture

Cell culture

- The essential constituents of the growth medium are physiologic amounts of essential amino acids and vitamins , salts , glucose and a buffering system generally consisting of bicarbonate in equilibrium with atmosphere containing about 5% carbon dioxide.
- Tissues are dissociated into the components of cells by the action of proteolytic enzymes such as trypsin and mechanical shaking.
- This is the type of culture routinely employed for growing viruses.

Propagation in Cell Culture

- Cell monolayers are most commonly used for culture of viruses.
- There are three categories, namely
 - (1) Primary
 - (2) Semi continuous
 - (3) Continuous cell cultures
- ❖ Primary cultures:
 - ✓ Viable cell suspensions may be obtained by dissociating tissues or organs, e.g. human amnion, with trypsin, collagenase or other enzymes

Propagation in Cell Culture

- Semi continuous cell cultures are established with the successful subculture of primary cell monolayers.
 - Continuous cultures are produced either by transformation (spontaneous or engineered) of cell strains in vitro, or by culture of cells taken from tumors
- ❖ e.g
- Hela (human cervical carcinoma)
 - human rhabdomyosarcoma cell line (RD cells).

Propagation in Cell Culture

The following continuous cell lines are commonly used:

- Hela and HEp2 are used for cultivation of HSV, adenovirus, poliovirus and some coxsackie viruses
- Vero cells will also support growth of these viruses and are used with BHK21 cells for growth of arboviruses
- RK13 cells and BHK21 cells for isolation and propagation of rubella virus .
- RD cells for the isolation of coxsackie A virus

Primary Culture

- Primary cell cultures are widely used for the isolation of animal viruses and cultivation of viruses for vaccine production.
- The culture vessel is incubated at 37°C for a few days to get a primary culture.
- A small volume of cell suspension is aseptically transferred to a culture flask or petri dish containing nutrient medium , with the help of pipette.

Secondary cell culture

- Secondary cell cultures are used for the isolation of wide group of animal viruses and growing fastidious viruses.
- Some secondary cultures are used for vaccine production.
- Examples : Human embryonic kidney cells and skin fibroblast cells.
- As secondary cell cultures can be maintained and subcultured for 20-50 times , they are called semi-continuous cells.
- The cell culture established from primary cell culture are called secondary culture or sub-culture.

Propagation in Cell Culture

- These cell lines may be maintained by serial subcultivation or stored in the cold (-70°C) for use when necessary.
- Some cell lines are now permitted to be used for vaccine manufacture, for example : Vero cells for rabies vaccine.

Advantages of cell culture

Relative ease, broad spectrum, cheaper and sensitivity

Disadvantage of cell culture

- The process requires trained technicians with experience in working on a full time basis.
- State health laboratories and hospital laboratories do not isolate and identify viruses in clinical work.
- Tissue or serum for analysis is sent to central laboratories to identify virus.

Hemagglutination assay

In this lecture we will look at the hemagglutination assay

At the end of this lecture

Student must have fully understood:

Hemagglutination assay for viruses detection in clinical or cultured samples

Haemagglutination Assay (HA)

The **hemagglutination assay** was developed in 1941–42 by American virologist George H. as methods for quantitating the relative concentration of viruses, bacteria.

The hemagglutination assay is a method for titering viruses and some bacteria based on their ability to attach to molecules present on the surface of red blood cells.

HA apply the process of **hemagglutination**, in which **sialic acid receptors** on the surface of red blood cells (RBCs) bind to the hemagglutinin glycoprotein found on the surface of viruses or bacteria and create a network, or **lattice structure**, of interconnected RBC's and virus particles.

The agglutinated lattice maintains the RBC's in a suspended distribution, typically viewed as a diffuse reddish solution.

Titer: The maximum dilution that gives visible agglutination

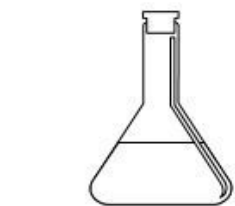
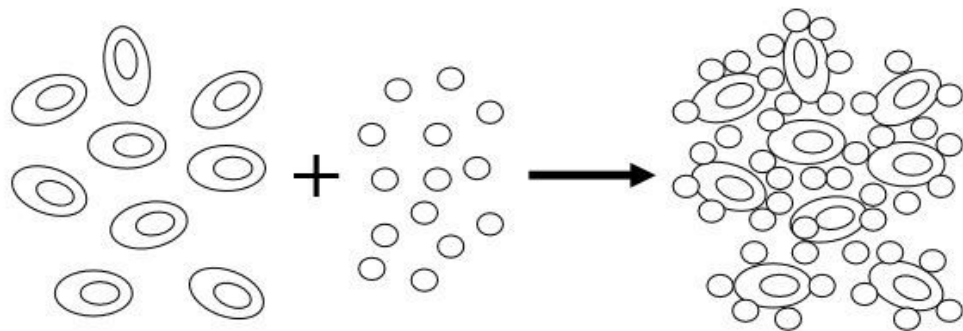
Haemagglutination Assay (HA)

The formation of the lattice depends on the concentrations of the virus, and when the relative virus concentration is too low, the RBC's are not constrained by the lattice and settle to the bottom of the well.

Hemagglutination is observed in the presence of staphylococci, vibrios, and other bacterial species, similar to the mechanism viruses use to cause agglutination of erythrocytes.

The RBC's used in HA and HI assays are typically from chickens, turkeys, horses, guinea pigs, or humans depending on the selectivity of the targeted virus or bacterium and the associated surface receptors on the RBC.

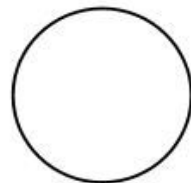
Hemagglutination (HA) Test



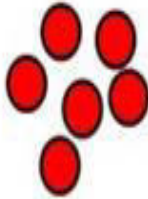
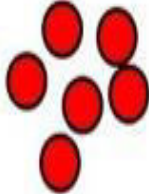
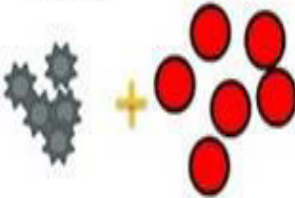
RBC Suspension



HA Virus



Settling Pattern

Components	Interaction	Microtiter Results
RBCs 		No Reaction 
Virus + RBCs 		Hemagglutination 

Materials Required

- Certified Biological Safety Cabinet
- Centrifuge
- Microscope (optional)
- U- or V-bottom microtiter plates
- Pipettors with tips (sterile ART, nonsterile)

Single channel (calibrated) – 25-200ul

Multichannel (calibrated) – 25-200ul

- PBS (Phosphate buffer saline)
- RBCs (10%)

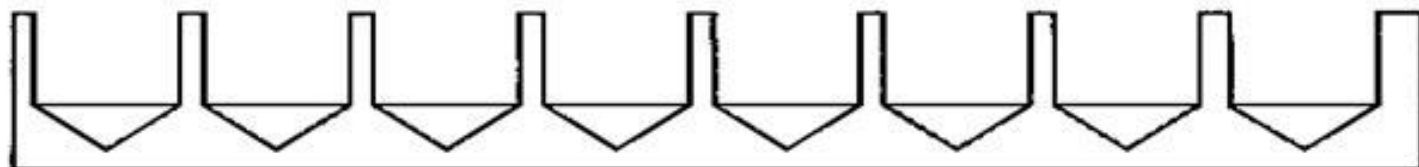
RBCs Preparation

1. 4 ml of turkey blood is pipetted into a 15 ml PBS.
2. Spin in centrifuge at 800 rpm for 10 minutes.
3. Discard the supernatant without disturbing the blood cells.
4. Add 12 ml PBS and mix by inverting – do not vortex.
5. Spin at 800 rpm for 5 minutes and repeat wash two more times.
6. Discard supernatant after final wash and add enough PBS to make a 10% solution of red blood cells. This solution is useable for one week.

Viral Dilution and Assay

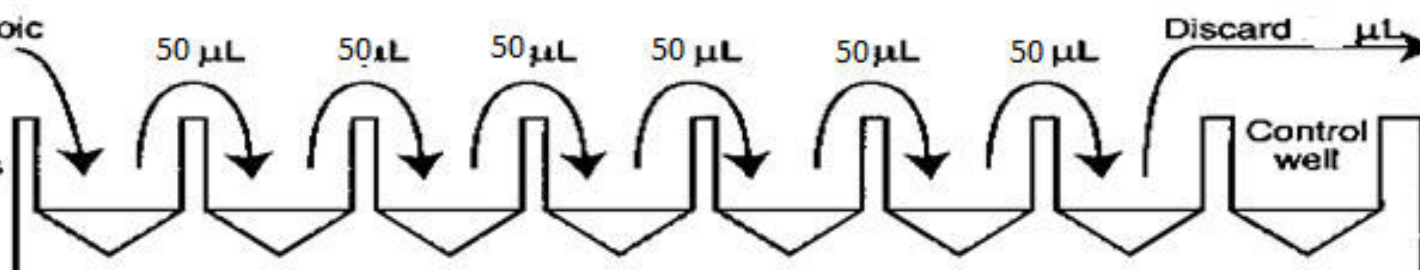
1. A round-bottomed 96-well U, V shaped plate is preferred for this assay. Flat-bottomed plates will also work, but need to be placed at an incline to develop.
2. To each well, add 50 μ l PBS.
3. In the first column, add 50 μ l of allantoic fluid sample.
4. Mix each well and transfer 50 μ l to the next well on its right. Repeat mixing and transferring 50 μ l down the length of the plate. Discard 50 μ l from the last well into a bleach solution.
5. Add 50 μ l of 10% red blood cell working solution to each well. Mix gently.
6. Leave at room temperature for 30-60 minutes to develop.
7. **HA negative:** A sharp button of red blood cells at the bottom of the V-bottom well.
8. **HA positive:** A clumping of red blood cells, no button or a very a small button of red blood cells at the bottom of the V-bottom well.
9. The virus's HA titer is a simple number of the highest dilution factor that produced a positive reading.

Place 50 μL
PBS in
each well

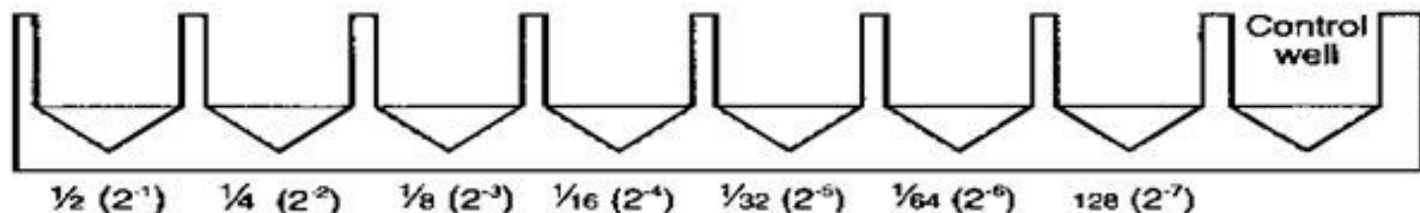


Add 50 μL allantoic
fluid to first well

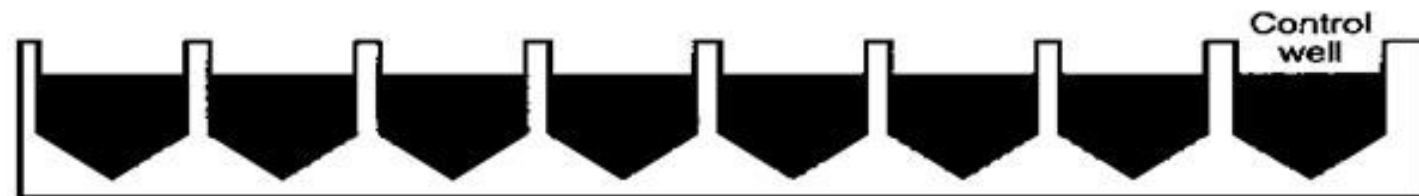
Make 2-fold
serial dilutions
of allantoic
fluid



Each well
now contains
diluted
allantoic fluid
(except
control well)

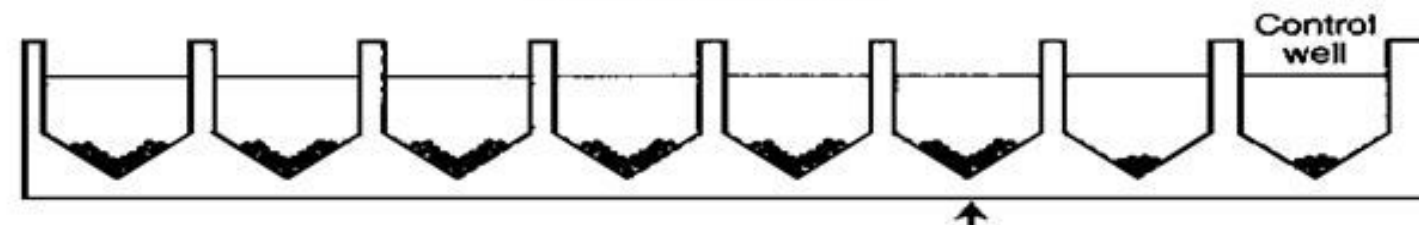


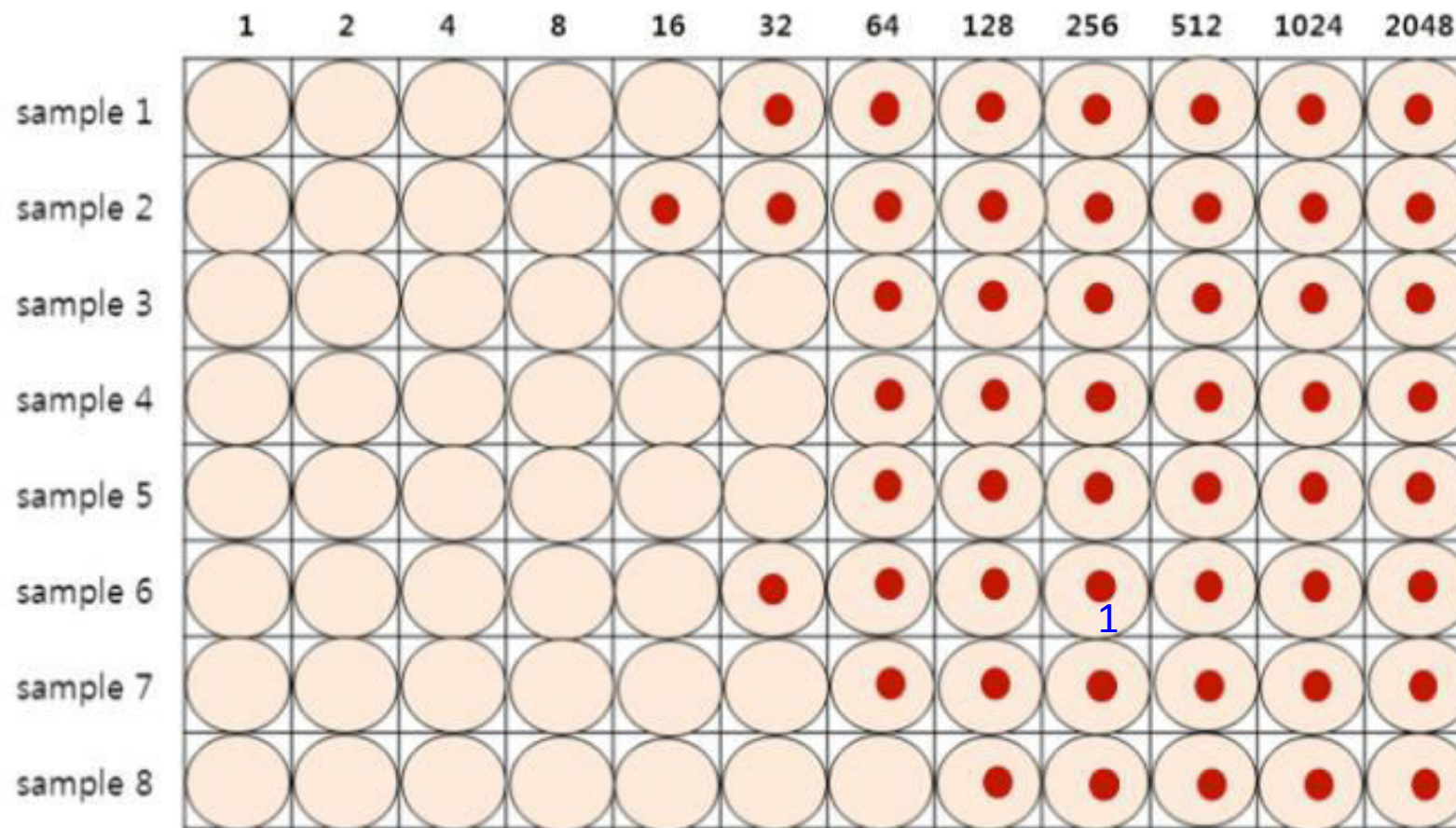
Add 50 μL
of 10% red
blood cells
to each well



Leave for 45 minutes

Read
settling
patterns





1 HAU = highest dilution giving complete HA
 Number of HAUs/50 ul = reciprocal of highest dilution
 e.g. an endpoint of 1:16 = 16 HAU/50 ul

HAU titer			
Sample1	16	Sample5	32
Sample2	8	Sample6	16
Sample3	32	Sample7	32
Sample4	32	Sample8	64

Hemagglutination Inhibition assay

In this lecture we will look at the hemagglutination inhibition assay

At the end of this lecture

Student must have fully understood:

Hemagglutination inhibition assay for specific antibody against viruses in serum samples

Haemagglutination Inhibition Assay (HIA or HI)

The basis of the HAI assay is that antibodies to that particular virus (for example-influenza virus) will prevent attachment of the virus to RBC.

Therefore hemagglutination is inhibited when antibodies are present.

Developed by American virologist George Hirst in 1941–42 as methods for quantifying the relative concentration of viruses, bacteria, or antibodies.

HAI Titer: The highest dilution of serum (Ab) that prevents hemagglutination is called the HAI titer of the serum.

Exact opposite of the hemagglutination titer.

Haemagglutination Inhibition Assay (HIA or HI)

Hemagglutination Inhibition Assay (HIA) is a procedure used to identify certain viruses that can cause haemagglutination.

Certain viruses such as Rubella, Herpes zoster and Influenza are composed of a protein envelope that is recognised to bind to erythrocytes in mammalian and avian species.

This binding causes the formation of a lattice which can be seen as agglutination.

The efficiency of haemagglutinin binding is dependent upon the type of linkage that connects the RBC to the receptor molecule of the virus, as well as the type of virus and host species.

Haemagglutination Inhibition Assay (HIA or HI)

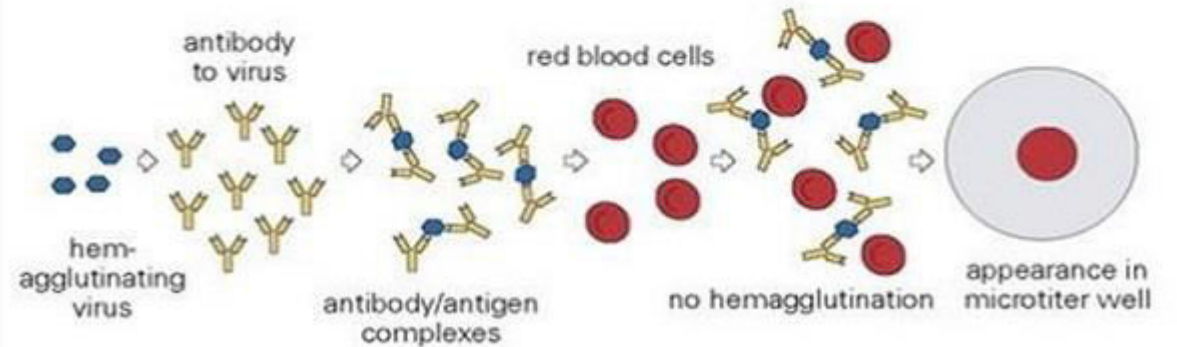
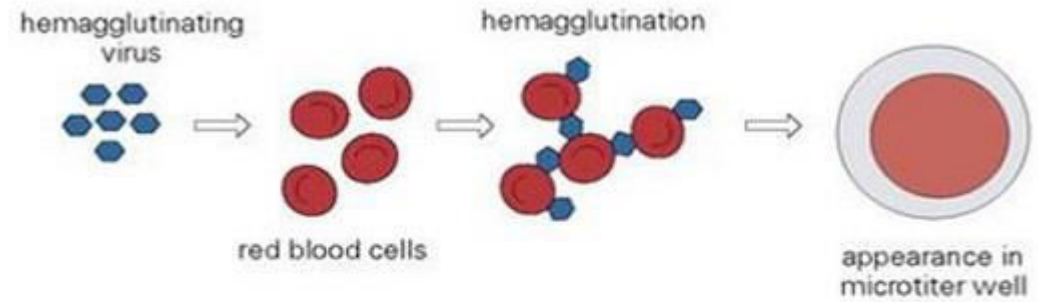
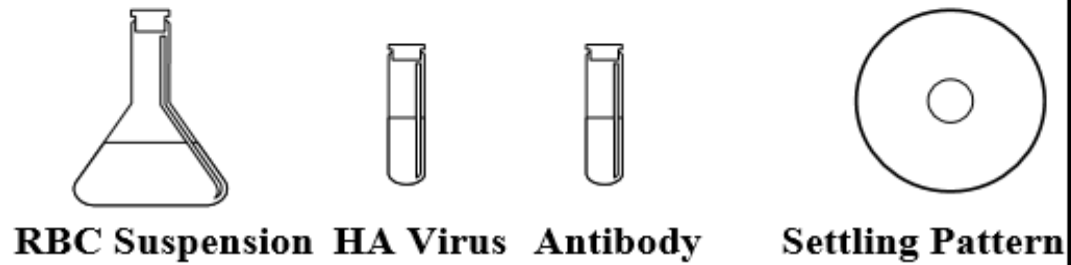
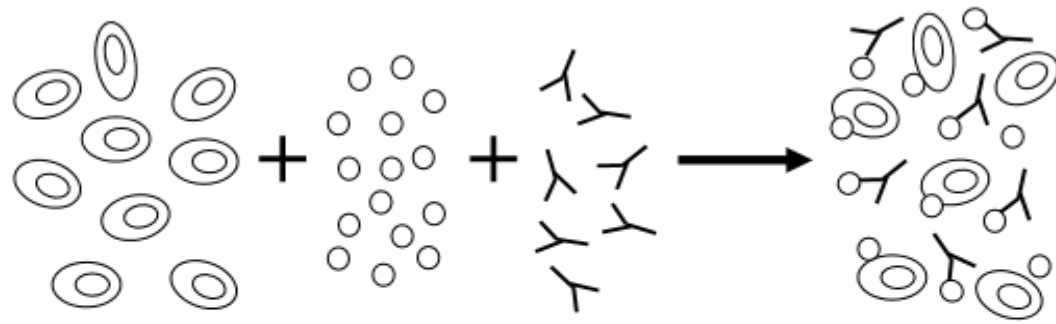
The Hemagglutination Inhibition Assay can be used to prevent RBCs from binding to viruses through the addition of specific antibodies.

Antibodies for either the hosts RBC or Virus receptor can be added to a patients sera to prevent attachment, with the antibodies binding to the receptor sites instead.

Viruses such as Rubella and Influenza are highly specific to the antibodies used within HAI which makes the use of this method highly effective for diagnosis.

Other viruses such as Flaviviruses have been found to cross react to other related viruses which make the use of the HAI test less sensitive and specific.

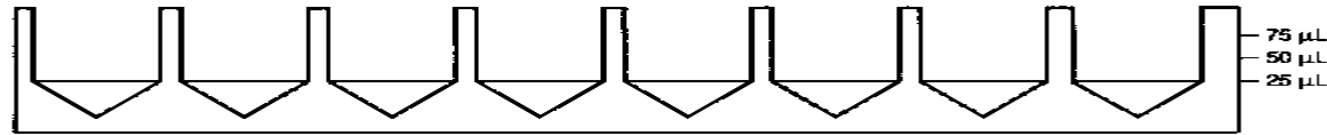
Hemagglutination-Inhibition (HI) Test



Haemagglutination Inhibition Assay (HIA or HI)

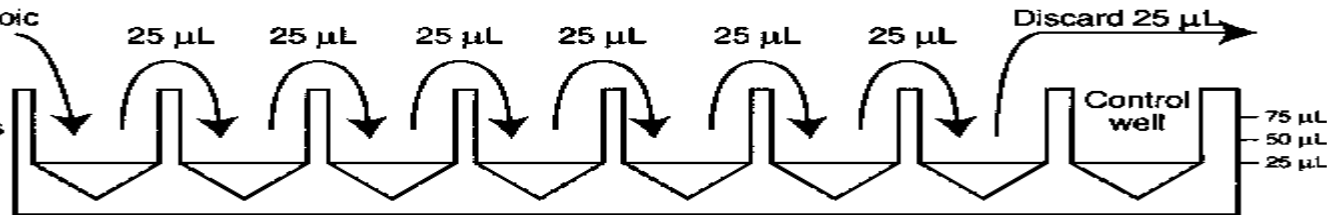
1. Dispense 25 μ L of PBS into each well of the 96 microwell plate.
2. Shake each serum sample and dispense 25 μ L into the first well
3. Use a multichannel pipette to make two-fold serial dilutions along the row until the second last well from the end. The last well is the serum control. Do not dilute this well.
4. Add 25 μ L of the 4HA dilution of antigen to each well excluding the control wells in the last column.
5. Gently tap the sides of the microwell plates to mix the reagents. Cover plates with a lid. Allow to stand for 30 minutes at room temperature.
6. Add 25 μ L of 1 percent washed red blood cells to each well including the control wells in the last column.
7. Gently tap the sides of the microwell plates to mix the reagents. Cover the plates with a lid. Allow to stand at room temperature for 45 minutes.
8. Read the settling patterns for each serum sample. Read the control serum well first then read the patterns in the other wells.
11. Record the pattern observed in each well on a microwell plate recording sheet. Determine the endpoint. This is the point where there is complete inhibition of haemagglutination.

1. Place 25 μL PBS in each well

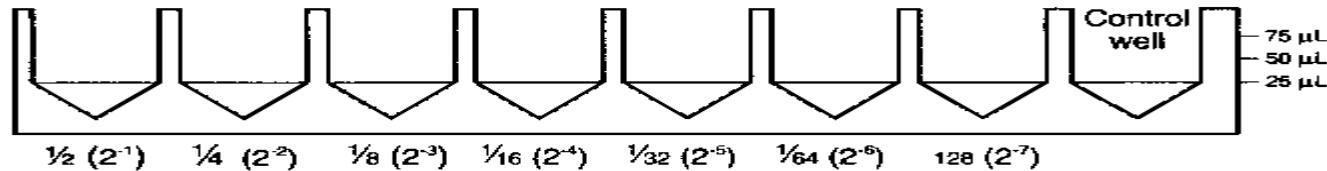


2. Add 25 μL allantoic fluid to first well

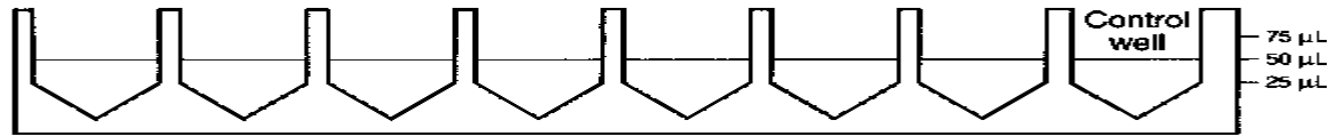
2. Make 2-fold serial dilutions of allantoic fluid



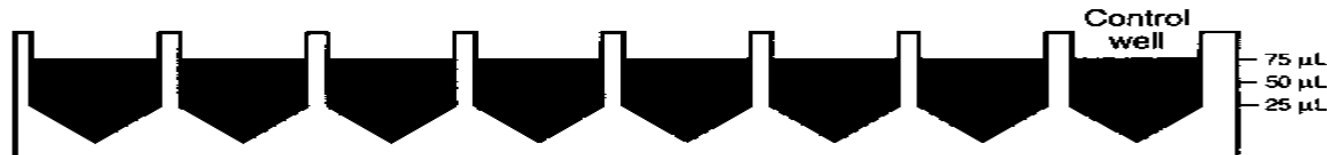
3. Each well now contains diluted allantoic fluid (except control well)



4. Add 25 μL PBS to each well

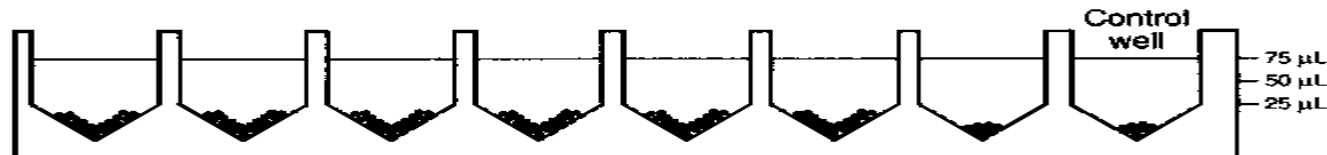


5. Add 25 μL of 1% red blood cells to each well

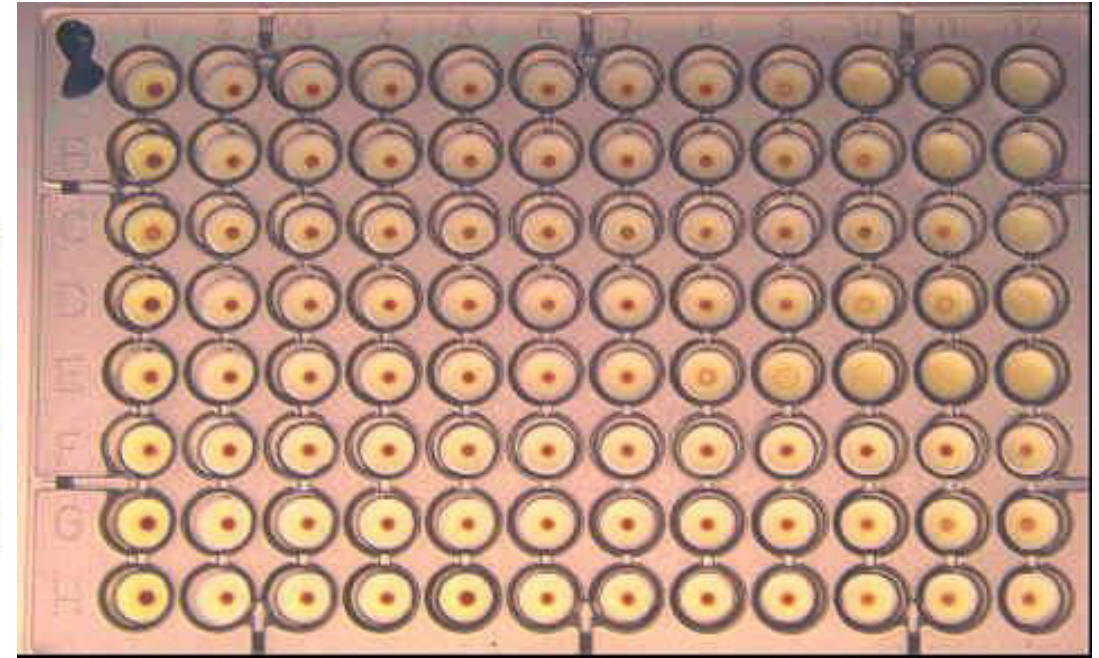
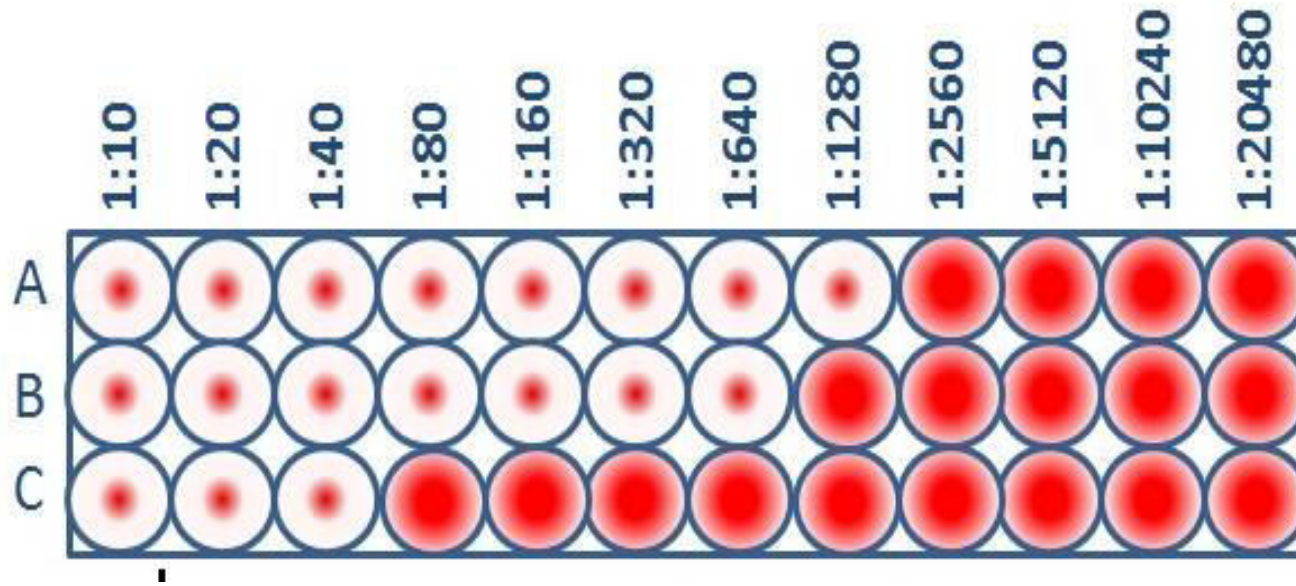


Leave for 45 minutes

6. Read settling patterns



The last well to show complete haemagglutination contains 1 haemagglutinating unit in 25 μL . This well contains a 1 in 64 dilution. Therefore the original allantoic fluid contained 64 haemagglutinating units in each 25 μL .



This virus sample has an HAI titer of 1280, which means that the greatest dilution of antibody that still blocked hemagglutination from occurring was at 1280 dilution.

1 HIU = highest dilution giving complete inhibition

Number of HIUs/25 μ l = reciprocal of highest dilution

e.g. an endpoint of 1:8 = 8 HIU/50 μ l

At this dilution, the antibodies were still capable of recognizing and binding to the antigens on the virus.

Enzyme-Linked Immunosorbent Assay (ELISA)

In this lecture we will look at the Enzyme-Linked Immunosorbent Assay (ELISA)

At the end of this lecture

Student must have fully understood:

What is ELISA and its principles, types, advantages and disadvantages and short briefed Direct ELISA and its procedure.

Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA (enzyme-linked immunosorbent assay) is a immunological or plate-based assay technique designed for detecting and quantifying substances such as peptides, proteins, antibodies and hormones.

Other names, such as enzyme immunoassay (EIA), are also used to describe the same technology.

Antibodies are made in response to infection and so an antibody ELISA can indicate whether or not an animal has been in contact with a certain viruses.

Some other examples include: pregnancy tests, and measurement of cytokines or soluble receptors in cell supernatant or serum.

Enzyme-Linked Immunosorbent Assay (ELISA)

- Enzyme Linked Immunosorbent Assay (ELISA)
- Term Was Coined By Engvall and Pearlmann in 1971
- Different Types
 - ❖ Direct
 - ❖ Indirect
 - ❖ Sandwich
 - ❖ Competitive ELISA
- Can Be Used To Detect Both Antibody and Antigen
- Very Sensitive
- Relies on Monoclonal Abs

Enzyme-Linked Immunosorbent Assay (ELISA)

- In an ELISA, an antigen must be immobilized to a solid surface and then complexed with an antibody that is linked to an enzyme.
- Detection is accomplished by assessing the conjugated enzyme activity via incubation with a substrate to produce a measureable product.
- The most crucial element of the detection strategy is a highly specific antibody-antigen interaction.
- ELISAs are typically performed in 96-well (or 384-well) polystyrene plates, which will passively bind antibodies and proteins.
- It is this binding and immobilization of reagents that makes ELISAs so easy to design and perform.
- Having the reactants of the ELISA immobilized to the microplate surface makes it easy to separate bound from non-bound material during the assay.
- This ability to wash away nonspecifically bound materials makes the ELISA a powerful tool for measuring specific analytes within a crude preparation.

Advantages and disadvantages of ELISA

❖ Advantages

- **High sensitivity and specificity**: it is common for ELISAs to detect antigens at the picogram level in a very specific manner due to the use of antibodies.
- **High throughput**: commercial ELISA kits are normally available in a 96-well plate format.
- But the assay can be easily adapted to 384-well plates.
- **Easy to perform**: protocols are easy to follow and involve little hands-on time.
- **Quantitative**: it can determine the concentration of antigen in a sample.
- **Possibility to test various sample types**: serum, plasma, cellular and tissue extracts, urine, and saliva among others.

Advantages and disadvantages of ELISA

❖ Disadvantage

- **Temporary readouts:** detection is based on enzyme/substrate reactions and therefore readout must be obtained in a short time span
- **Limited antigen information:** information limited to amount or presence of the antigen in the sample

Advantages and disadvantages of ELISA

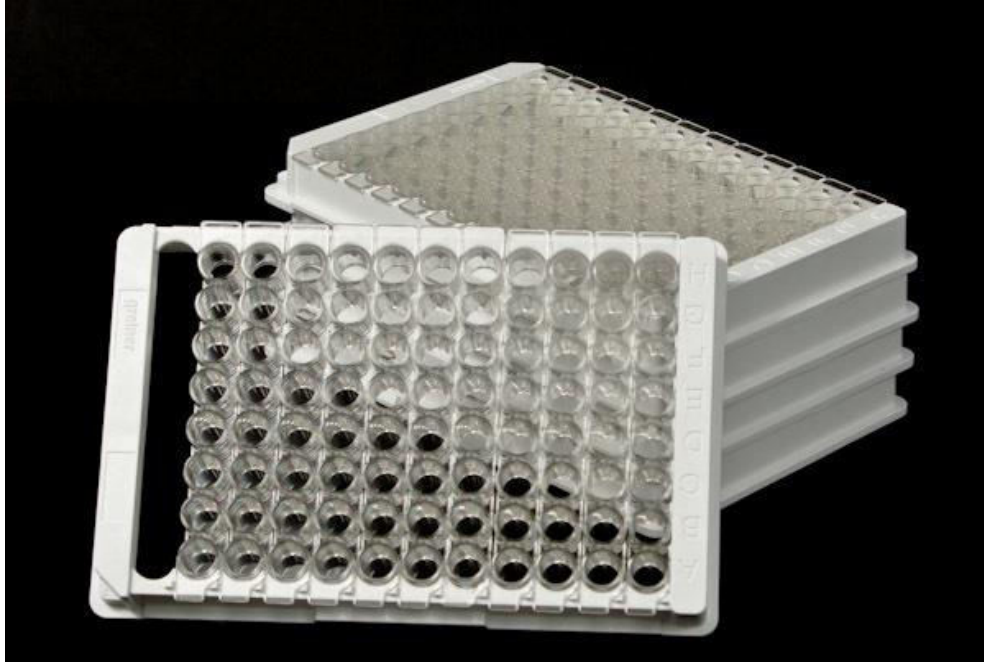
Review or introduce the following key points about antibodies and antigens:

- Antigens are foreign proteins which cause the immune system to generate antibodies.
- Specific antibodies are produced for each antigen. They bind like a lock and key.
- There are different antibodies produced by the human immune system (IgG, IgM, IgE, etc)
- The part of the antigen that binds with the antibody is referred to as the epitope.
- Some antigens have multiple epitopes, so they are recognized by different antibodies
- Scientists have developed diagnostic assays that utilize the unique and specific binding properties of antibodies and antigens.
- Primary antibodies recognize the original antigen we are testing against.
- Secondary antibodies recognize the first (primary) antibody.
- Using both increases sensitivity.

General ELISA Procedure

- Unless you are using a kit with a plate that is pre-coated with antibody, an ELISA begins with a coating step, in which the first layer, consisting of a target antigen or antibody, is adsorbed onto a 96-well polystyrene plate.
- This is followed by a blocking step in which all unbound sites are coated with a blocking agent.
- Following a series of washes, the plate is incubated with enzyme-conjugated antibody.
- Another series of washes removes all unbound antibody. A substrate is then added, producing a colorimetric signal.
- Finally, the plate is read

General ELISA Procedure



Blocking Reagents

- ❖ Blocking Reagent 10% FBS in PBS
- ❖ Alternatively 1% BSA (Immunoassay Grade)
- ❖ Filter To Remove Particulates
- ❖ Plate Is Brought To Room temperature
- ❖ Add 200 μ L per well Blocking Buffer
- ❖ Wait For 2 Hours At Room temperature

Enzyme and Substrate

- Appropriate substrates
- **3,3',5,5'-Tetramethylbenzidine** or **TMB**
- **2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)** or **ABTS**
- In ELISA, **horseradish peroxidase (HRP)** and **alkaline phosphatase (AP)** are usually used.

Direct ELISA

- For direct detection, an antigen coated to a multi-well plate is detected by an antibody that has been directly conjugated to an enzyme.
- This detection method is a good option if there is no commercially available ELISA kits for your target protein.

☐ Advantages

- Quick because only one antibody and fewer steps are used.
- Cross-reactivity of secondary antibody is eliminated

☐ Disadvantages

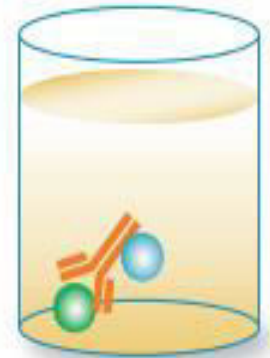
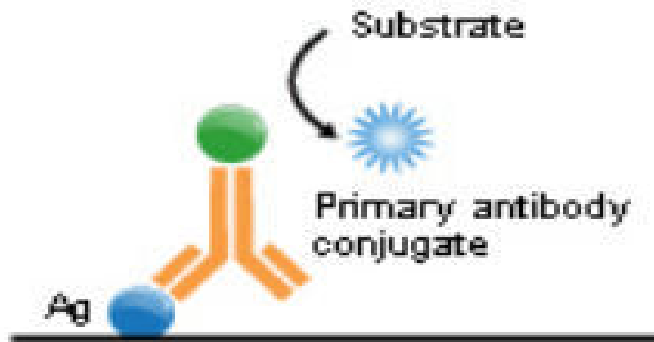
- Immunoreactivity of the primary antibody might be adversely affected by labeling with enzymes or tags.
- Labeling primary antibodies for each specific ELISA system is time-consuming and expensive.
- No flexibility in choice of primary antibody label from one experiment to another.
- Minimal signal amplification.

Direct ELISA

The steps of direct ELISA follows the mechanism below:

- A buffered solution of the antigen to be tested for is added to each well of a microtiter plate, where it is given time to adhere to the plastic through charge interactions.
- A solution of nonreacting protein, such as bovine serum albumin or casein, is added to well (usually 96-well plates) in order to cover any plastic surface in the well which remains uncoated by the antigen.
- The primary antibody with an attached (conjugated) enzyme is added, which binds specifically to the test antigen coating the well.
- A substrate for this enzyme is then added. Often, this substrate changes color upon reaction with the enzyme.
- The higher the concentration of the primary antibody present in the serum, the stronger the color change. Often, a spectrometer is used to give quantitative values for color strength.

- Direct ELISA detection is much faster than other ELISA techniques as fewer steps are required.
- The assay is also less prone to error since fewer reagents and steps are needed, i.e. no potentially cross-reacting secondary antibody needed.



Indirect - Enzyme-Linked Immunosorbent Assay (ELISA)

In this lecture we will look at the In-direct Enzyme-Linked Immunosorbent Assay (ELISA)

At the end of this lecture

Student must have fully understood:

Procedure and principle of indirect ELISA

Indirect - ELISA

Antibody can be detected or quantitatively determined by indirect ELISA.

In this technique, antigen is coated on the microtiter well. Serum or some other sample containing primary antibody is added to the microtiter well and allowed to react with the coated antigen.

Any free primary antibody is washed away and the bound antibody to the antigen is detected by adding an enzyme conjugated secondary antibody that binds to the primary antibody.

Unbound secondary antibody is then washed away and a specific substrate for the enzyme is added.

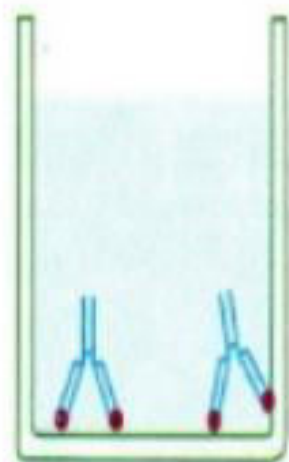
Enzyme hydrolyzes the substrate to form colored products.

The amount of colored end product is measured by spectrophotometric plate readers that can measure the absorbance of all the wells of 96-well plate.



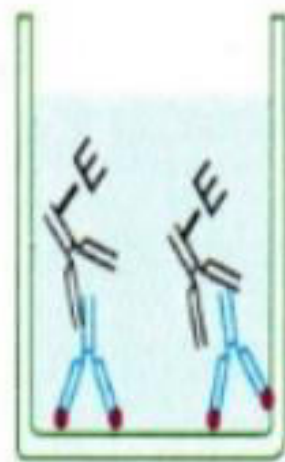
Antigen-coated well

Wash
→



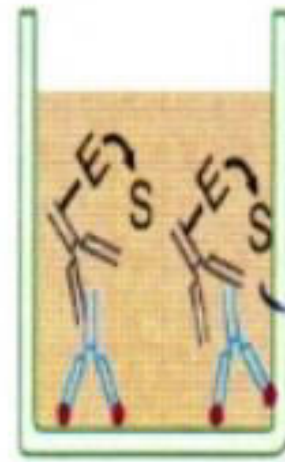
Specific antibody binds to antigen

Wash
→



Enzyme-linked antibody binds to specific antibody

Wash
→



Substrate is added and converted by enzyme into coloured product, the rate of colour formation is proportional to the amount of specific antibody

Procedure of Indirect ELISA

- Coat the micro titer plate wells with antigen.
- Block all unbound sites to prevent false positive results.
- Add sample containing antibody (e.g. rabbit monoclonal antibody) to the wells and incubate the plate at 37°C.
- Wash the plate, so that unbound antibody is removed.
- Add secondary antibody conjugated to an enzyme (e.g. anti- mouse IgG).
- Wash the plate, so that unbound enzyme-linked antibodies are removed.
- Add substrate which is converted by the enzyme to produce a colored product.
- Reaction of a substrate with the enzyme to produce a colored product.

Advantages

- Increased sensitivity, since more than one labeled antibody is bound per primary antibody.
- A wide variety of labeled secondary antibodies are available commercially.
- Maximum immunoreactivity of the primary antibody is retained because it is not labeled.
- Versatile because many primary antibodies can be made in one species and the same labeled secondary antibody can be used for detection.
- Flexibility, since different primary detection antibodies can be used with a single labeled secondary antibody.
- Cost savings, since fewer labeled antibodies are required.
- Different visualization markers can be used with the same primary antibody.

Disadvantages

- ❖ Cross-reactivity might occur with the secondary antibody, resulting in nonspecific signal.
- ❖ An extra incubation step is required in the procedure.

Sandwich - Enzyme-Linked Immunosorbent Assay (ELISA)

In this lecture we will look at the sandwich Enzyme-Linked Immunosorbent Assay (ELISA)

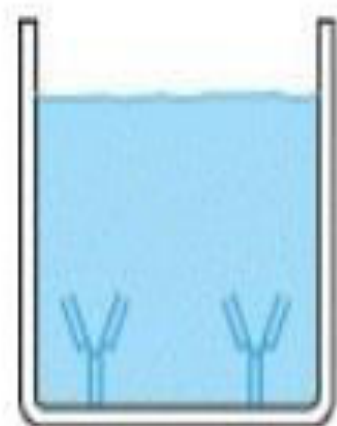
At the end of this lecture

Student must have fully understood:

Procedure and principle of sandwich - ELISA

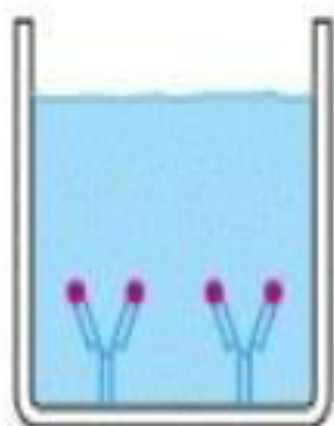
Sandwich - ELISA

- Sandwich ELISAs typically require the use of matched antibody pairs, where each antibody is specific for a different, non-overlapping part (epitope) of the antigen molecule.
- A first antibody (known as capture antibody) is coated to the wells.
- The sample solution is then added to the well. A second antibody (known as detection antibody) follows this step in order to measure the concentration of the sample
- Antigen can be detected by sandwich ELISA.
- In this technique, antibody is coated on the microtiter well.
- A sample containing antigen is added to the well and allowed to react with the antibody attached to the well, forming antigen-antibody complex.
- After the well is washed, a second enzyme-linked antibody specific for a different epitope on the antigen is added and allowed to react with the bound antigen.
- Then after unbound secondary antibody is removed by washing.
- Finally substrate is added to the plate which is hydrolyzed by enzyme to form colored products.



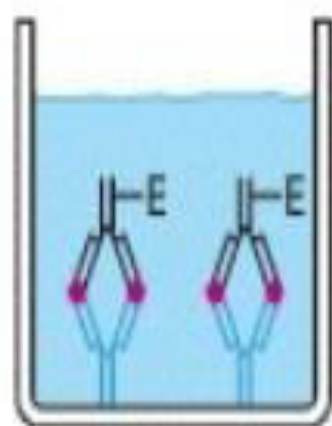
Monoclonal
antibody-
coated well

Wash



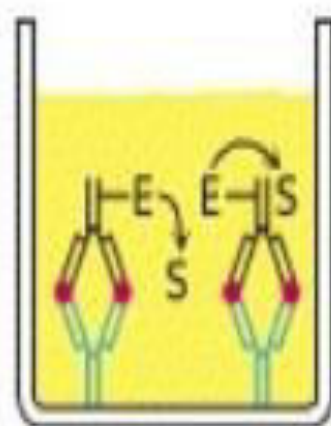
Antigen binds
to antibody

Wash



A second monoclonal
antibody, linked to
enzyme, binds to
immobilized antigen

Wash



Substrate is added and
converted by enzyme into
colored product; the rate
of color formation is
proportional to the
amount of antigen

Procedure of sandwich ELISA

- Prepare a surface to which a known quantity of antibody is bound.
- Add the antigen-containing sample to the plate and incubate the plate at 37°C.
- Wash the plate, so that unbound antigen is removed.
- Add the enzyme-linked antibodies which are also specific to the antigen and then incubate at 37°C.
- Wash the plate, so that unbound enzyme-linked antibodies are removed.
- Add substrate which is converted by the enzyme to produce a colored product.
- Reaction of a substrate with the enzyme to produce a colored product.

Advantages

- High specificity, since two antibodies are used the antigen is specifically captured and detected.
- Suitable for complex samples, since the antigen does not require purification prior to measurement.
- Flexibility and sensitivity, since both direct and indirect detection methods can be used.

competitive - Enzyme-Linked Immunosorbent Assay (ELISA)

In this lecture we will look at the competitive Enzyme-Linked Immunosorbent Assay (ELISA)

At the end of this lecture

Student must have fully understood:

Procedure and principle of competitive - ELISA

competitive - ELISA

- Also known as **inhibition ELISA** or **competitive immunoassay**
- Is perhaps the most complex of all the ELISA techniques
- The central event of competitive ELISA is a competitive binding process executed by original antigen (sample antigen) and add-in antigen
- This assay measures the concentration of an antigen by detection of signal interference
- The sample antigen competes with a reference antigen for binding to a specific amount of labeled antibody
- The reference antigen is pre-coated on a multi-well plate
- The sample is pre-incubated with labeled antibody and added to the wells.
- Depending on the amount of antigen in the sample, more or less free antibodies will be available to bind the reference antigen
- This means the more antigen there is in the sample, the less reference antigen will be detected and the weaker the signal

Procedure of competitive ELISA

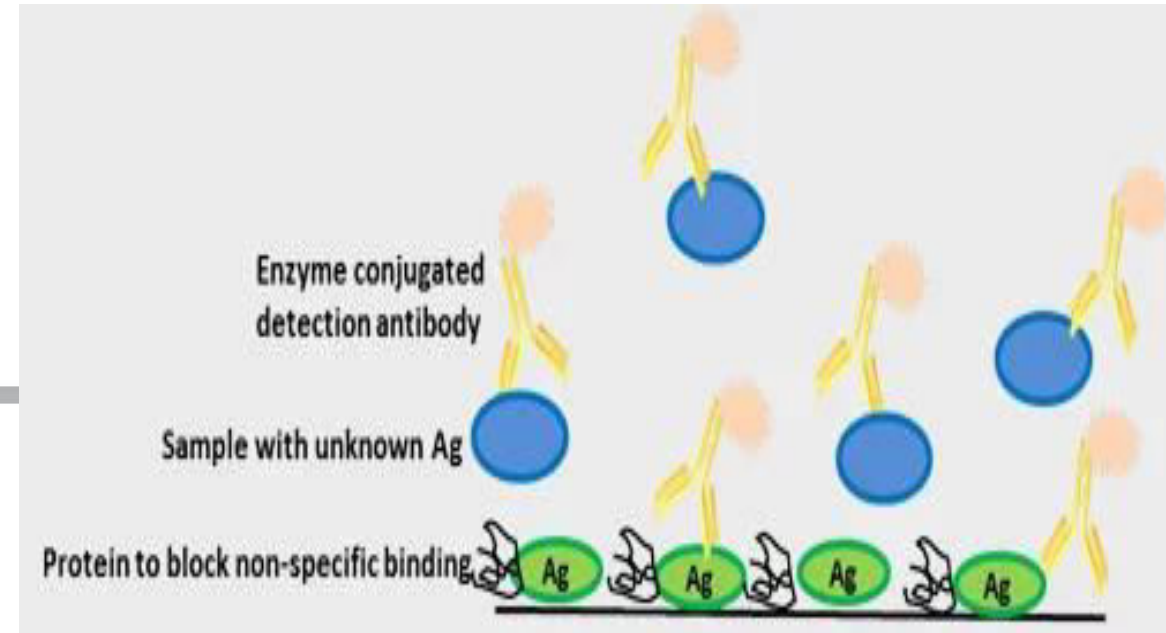
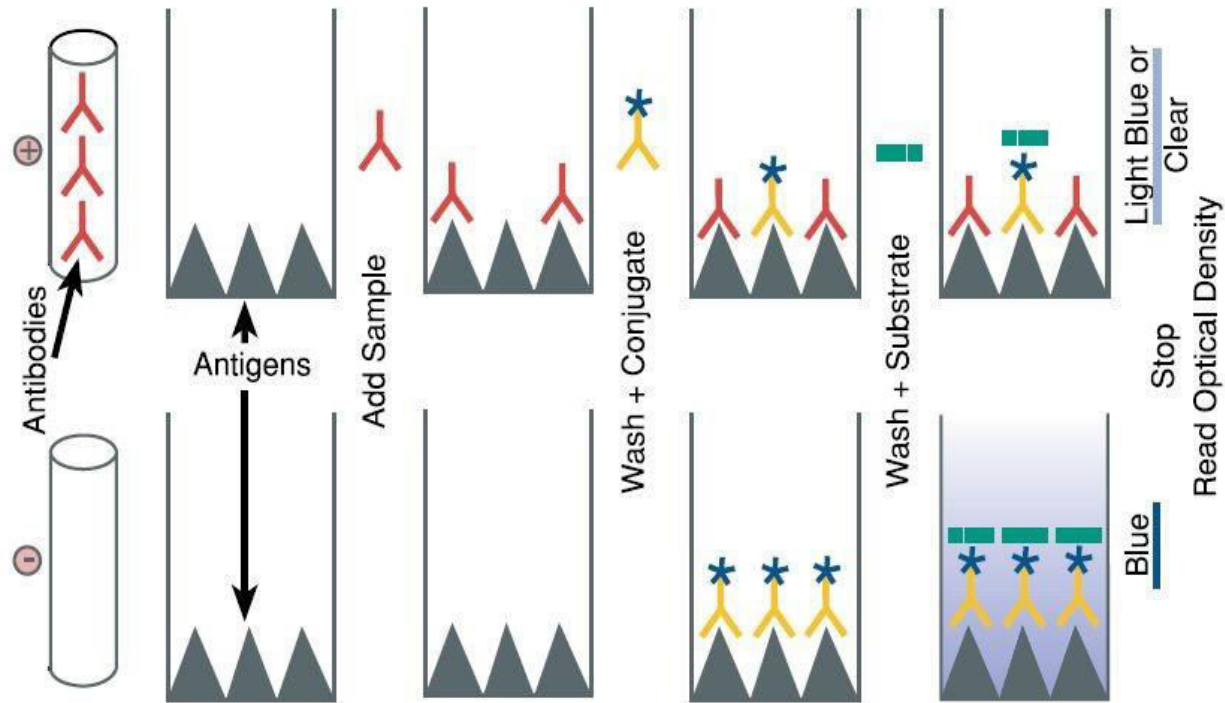
The steps competitive ELISA are:

- Unlabeled antibody is incubated in the presence of its antigen (sample).
- These bound antibody/antigen complexes are then added to an antigen-coated well.
- The plate is washed, so unbound antibodies are removed. (The more antigen in the sample, the more Ag-Ab complexes are formed and so there are less unbound antibodies available to bind to the antigen in the well, hence "competition".)
- The secondary antibody, specific to the primary antibody, is added. This second antibody is coupled to the enzyme.
- A substrate is added, and remaining enzymes elicit a chromogenic or fluorescent signal.
- The reaction is stopped to prevent eventual saturation of the signal.

Procedure of competitive ELISA

- Some competitive ELISA kits include enzyme-linked antigen rather than enzyme-linked antibody.
- The labeled antigen competes for primary antibody binding sites with the sample antigen (unlabeled).
- The less antigen in the sample, the more labeled antigen is retained in the well and the stronger the signal.

Procedure of competitive ELISA



Competitive ELISA

Advantages

- The main advantage of the competitive ELISA is that no sample processing is required and crude or impure samples can be used.
- Less sensitive to sample dilution and sample matrix effects than the sandwich ELISA.
- Less variability between duplicate samples and between assays.
- Maximum flexibility in experimental setup since it can be based on direct, indirect or sandwich ELISAs.

Disadvantages

- Because each ELISA technique can be adapted to a competitive format, the same limitations as described for each ELISA technique above would be applicable to the respective competitive/inhibition ELISA technique.

When you typically use it

- This ELISA technique is commonly used when only one antibody is available for the antigen of interest. It is also suitable for detecting small antigens that cannot be bound by two different antibodies such as in the sandwich ELISA technique.

reverse transcriptase PCR (RT-PCR)

In this lecture we will look at the reverse transcriptase PCR (RT-PCR)

At the end of this lecture

Student must have fully understood:

Procedure of reverse transcriptase PCR (RT-PCR),
convert the RNA into cDNA for PCR

reverse transcriptase PCR (RT-PCR)

- The polymerase chain reaction (PCR) is a cyclical enzyme-driven amplification technique for copying a chain of DNA into billions of new copies.
- The purpose of PCR is to make a very tiny amount of very distinct genetic material detectable and to use some form of technology to detect what would otherwise be too little material to see in the first place.
- PCR can be used to detect some viruses.
- These viruses have genes or a genome that is made of DNA.
- But many viruses don't store their genetic code as DNA, they use RNA as the plan from which they make more of themselves and their viral proteins.

reverse transcriptase PCR (RT-PCR)

- DNA viruses include the adenoviruses, herpesviruses, HIV (an example which also has RNA stages), polyomaviruses, bocaviruses, parvoviruses, papillomaviruses, poxviruses, megaviruses and many others.
- RNA viruses include influenza viruses, parainfluenza viruses, rhinoviruses, enteroviruses, cosavirus, klasseviruses, parechoviruses, respiratory syncytial virus, coronaviruses, zika virus, dengue viruses, Ebola virus, human metapneumovirus and many others.
- PCR won't detect RNA without modification of the method.

reverse transcriptase PCR (RT-PCR)

- PCR is based around the use of a heat stable DNA-dependent DNA polymerase enzyme.
- For the PCR to work, we need to first make virus RNA genes or genomes into DNA so that the main enzyme can use it and duplicate it and make enough of it to detect
- To make a DNA copy of the RNA, we need to add in another enzyme and another, preceding, step to the PCR process.
- That enzyme, the reverse transcriptase (RT) is added in a step called reverse transcription (also RT).
- The addition of the RT step changes the naming of the technique from PCR to RT-PCR.
- RT-PCR is not to be confused with **real-time PCR** which is shortened to rtPCR

reverse transcriptase PCR (RT-PCR)

- The synthesis of DNA from an RNA template, via reverse transcription, produces **complementary DNA (cDNA)**.
- Reverse transcriptases (RTs) use an RNA template and a primer complementary to the 3' end of the RNA to direct the synthesis of the first strand cDNA, which can be used directly as a template for the Polymerase Chain Reaction (PCR).
- This combination of reverse transcription and PCR (RT-PCR) allows the detection of low abundance RNAs in a sample, and production of the corresponding cDNA, thereby facilitating the cloning of low copy genes.
- Alternatively, the first-strand cDNA can be made double-stranded using DNA Polymerase I and DNA Ligase.

Primers used in RT-PCR

- ❑ The oligo (dT)18

- ❑ Random hexamer primers

- ❑ Gene Specific

- The oligo (dT)18 primer anneals selectively to the poly(A) tail of mRNA (Can be converted the mRNA into cDNA only)
- Random hexamer primers do not require the presence of the poly(A) tail, therefore, they can be used for transcription of the 5'-end regions of mRNA or cDNA synthesis of RNA species lacking a poly(A) tail (e.g., microRNAs) (Can be used to covert all types of RNA in to cDNA).
- Gene-specific primers may also be used.

Procedure of RT-PCR

- Transfer 1 to 5 µg your prepared total RNA (10 µL) into a sterile tube.
- 1 µL oligo dT
- 1 µL sterile water
- Heat the mixture to 65°C for 5 minutes.
- Quickly chill the sample on ice for 2 minutes.
- Microcentrifuge briefly to bring the solution to the bottom of the tube.
- Leave the RNA Mixture on ice while preparing the Reaction Mixture.
- Reaction Mixture - Add the following to the new and separate tube:
 - 4 µL 10X RT buffer
 - 2 µL 10 mM dNTPs
 - 1 µL reverse transcriptase
 - 1 µL Rnase inhibitor

Procedure of RT-PCR

- Incubate sample at 42°C for 50 minutes.
- Heat inactivate the enzyme at 65°C to 70°C for 15 minutes.
- Place the sample on ice for 5 minutes. Mix gently and do a quick spin to collect the mixture in the tube.
- Add the following to the reaction:
 - 1 µL RNase H (keep on ice the entire time it is out of the -20°C)
- Incubate sample at 37°C for 20 minutes.
- Microcentrifuge sample briefly to bring solution to bottom of the tube.
- Keep the sample on ice any time it is out from now forward.
- You can freeze this reaction in the -20°C once it is done or go on to the PCR Amplification

Note: All RT-PCR are carried out through available commercial kits, this is not a manual process.

e.g. Thermo scientific

Invitrogene

Real time PCR (qRT-PCR)

In this lecture we will look at the Real time (RT-PCR) or quantitative real time PCR (qRT-PCR)

At the end of this lecture

Student must have fully understood:

Procedure of quantitative real time PCR (qRT-PCR) for viruses detection

Real time PCR (qRT-PCR)

- The introduction of real-time quantitative PCR technology has revolutionized the field of molecular diagnostics and has enabled the shift of molecular diagnostics toward a high-throughput, automated technology with lower turnaround times
- Real-time quantitative PCR allows the sensitive, specific and reproducible quantitation of nucleic acids.
- The employment of polymerase chain reaction (PCR) techniques for virus detection and quantification offers the advantages of high sensitivity and reproducibility, combined with an extremely broad dynamic range.
- Compared with traditional PCR assays, diagnostic assays based upon real-time PCR technology have increased speed and dynamic range.
- They enable quantitative analysis of gene copies and have the potential for increased specificity when nucleic acid probes are used.
- Optimized real-time PCR assays can also be highly sensitive, detecting as few as 1-10 copies of a target gene in a nucleic acid sample.

Real time PCR (qRT-PCR)

- Real-time PCR is a variation of the standard PCR technique that is commonly used to quantify DNA or RNA in a sample.
- Using sequence-specific primers, the number of copies of a particular DNA or RNA sequence can be determined.
- By measuring the amount of amplified product at each stage during the PCR cycle, quantification is possible.
- If a particular sequence (DNA or RNA) is abundant in the sample, amplification is observed in earlier cycles; if the sequence is scarce, amplification is observed in later cycles.

Real time PCR (qRT-PCR)

Quantification of amplified product is obtained using

- fluorescent probes
- fluorescent DNA-binding dyes
- real-time PCR instruments that measure fluorescence while performing the thermal cycling needed for the PCR reaction

Quantitative polymérase Chain réaction (qPCR)

- Quantitative PCR utilizes polymerase chain reaction chemistry to amplify viral DNA or RNA to produce high enough concentrations for detection and quantification by fluorescence.
- Quantitative detection can be achieved using a wide variety of fluorescence detection strategies, including sequence specific probes or universal probes such as SYBR Green dye.
- Several detection chemistries are available: double-stranded DNA-intercalating agents (DNA-binding dyes [e.g., SYBR[®] Green 1]), hydrolysis probes (e.g., TaqMan[®] probes), dual hybridization probes, molecular beacons and Scorpion probes.
- SYBR Green dye binds to all double-stranded DNA produced during the reaction.
- Real-time qPCR takes around 1–4 hours and can provide quantitative results containing too few viruses to be analyzed by other methods.

Probe

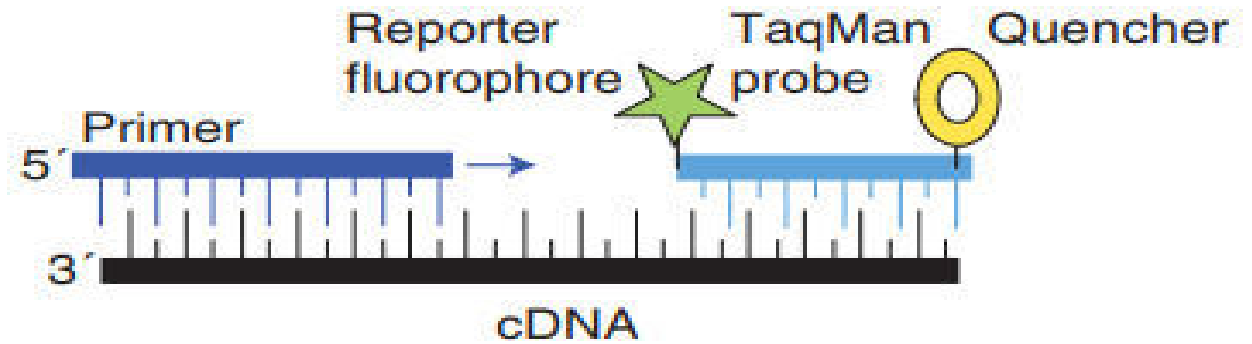
- Dual-labeled oligonucleotide fluorogenic probes allowed the elimination of post-PCR processing for the analysis of probe degradation
- Probe has a reporter fluorescent dye at the 5' end and a quencher dye attached to the 3' end
- Probe is intact, the close proximity of the quencher significantly decreases the fluorescence emitted by the reporter dye
- A fluorescence signal is only emitted upon cleavage of the probe, based on the fluorescence resonance energy transfer (FRET) principle

TaqMan Probe

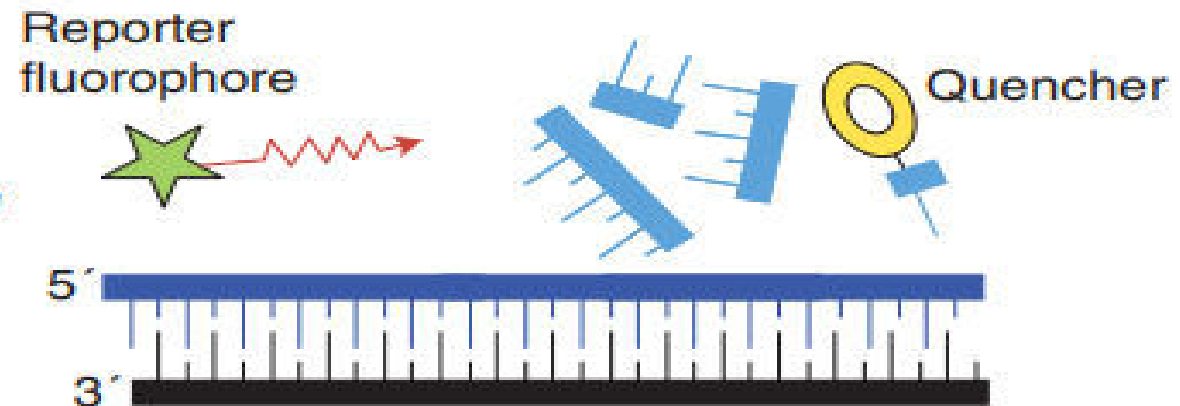
- In the real-time quantitative TaqMan[®] assay a fluorogenic nonextendable 'TaqMan' probe is used.
- The probe has a fluorescent reporter dye attached to its 5' end and a quencher dye at its 3' terminus.
- If the target sequence is present, the fluorogenic probe anneals downstream from one of the primer sites and is cleaved by the 5' nuclease activity of the Taq polymerase enzyme during the extension phase of the PCR.
- Whilst the probe is intact, FRET occurs and the fluorescence emission of the reporter dye is absorbed by the quenching dye.
- Cleavage of the probe by Taq polymerase during PCR separates the reporter and quencher dyes, thereby increasing the fluorescence from the former.

When the TaqMan probe is intact, the reporter and quencher stay close to each other, which prevents the emission of any fluorescence

The primer and TaqMan probe anneal to the complementary DNA strand following denaturation



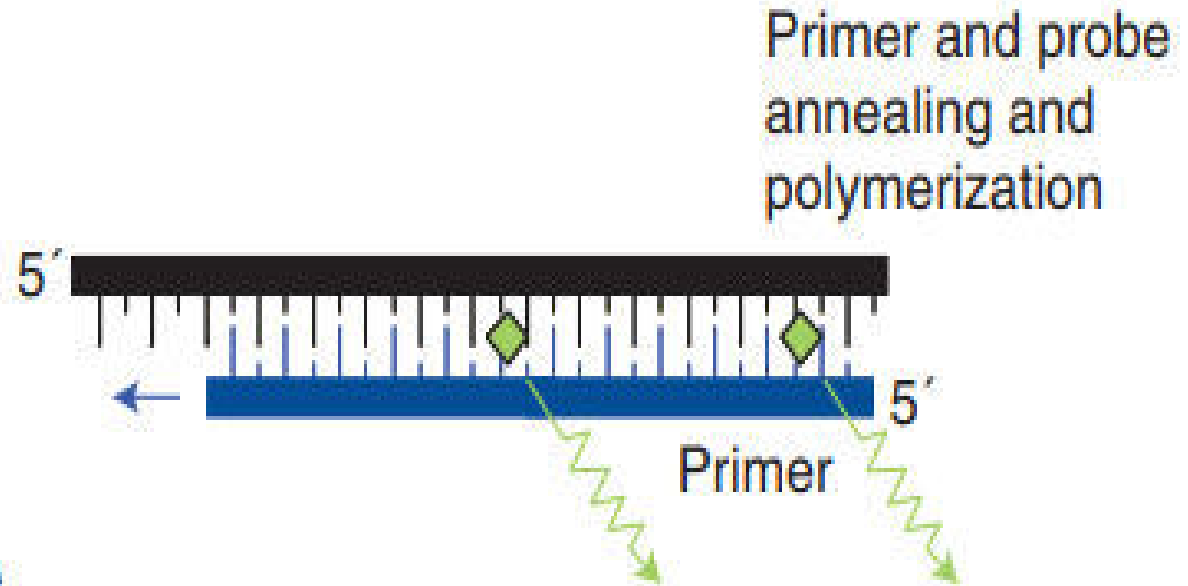
After hybridization and during the extension phase, the 5' endonuclease activity of the Taq DNA polymerase cleaves the probe, which separates reporter and quencher dyes and fluorescence is detected



dsDNA-intercalating agents (DNA-binding dyes)

- SYBR Green 1 is a nonsequence-specific fluorogenic minor groove DNA-binding dye that intercalates into dsDNA (it does not bind to single-stranded DNA)
- SYBR Green 1 exhibits little fluorescence when unbound in solution but emits a strong fluorescent signal upon binding to dsDNA
- An increase in the fluorescence signal occurs during polymerization and this decreases when DNA is denatured.
- Fluorescent measurements are performed at the end of the elongation step of each PCR cycle to monitor the increasing amount of amplified DNA.
- The advantage of this technique is that it is relatively cheap as it can be used with any pair of primers for any target.
- However, as the presence of any dsDNA generates fluorescence, specificity of this assay is greatly decreased due to amplification of nonspecific PCR products and primer-dimers

Unbound SYBR® Green 1 DNA-binding dye in solution exhibits very little fluorescence. During primer extension and polymerization, SYBR® Green 1 molecules become intercalated within the double-stranded DNA product, resulting in an increase in detected fluorescence



Unbound SYBR® Green 1 dye molecules

qRT-PCR

- The threshold cycle (Ct) is defined as the fractional PCR cycle number at which the reporter fluorescence is greater than the minimal detection level (i.e., the threshold).
- The Ct is a basic principle of real-time PCR and is an essential component in producing accurate and reproducible data.
- Two different methods are commonly used to quantify the results obtained by real-time PCR – the standard-curve or absolute quantitation method and the relative quantitation also known as the comparative threshold method (2-Ct method).
- In real-time quantitative PCR, normalization to a housekeeping gene is the accepted method to correct for intersample variations in different experiments (i.e., minor discrepancies in the amounts of input RNA and minor differences in PCR efficiency).
- Great care should be taken when choosing which real-time instrument to buy and it is important to match the instruments capabilities with laboratory needs.

Plaque Assay

In this lecture we will look at the Plaque Assay for detection of viruses

At the end of this lecture

Student must have fully understood:

Mechanism, process and use of Plaque assay for detecting Viruses

Plaque Assay

The plaque assay can be used to purify a clonal population of virus or to determine viral titer as plaque-forming units per ml (pfu/ml) so that known amounts of virus can be used to infect cells during subsequent work.

PURPOSE

- Primarily used to determine the number of infectious viral particles in a sample as plaque-forming units (pfu/mL).
- This assay is the most widely used technique for the isolation of virus and its purification, and to optimize the viral titers.
- **Examples:** For measuring concentration of
 - Polioviruses
 - Newcastle disease virus(NDV)
 - West Nile virus (WNV),
 - Dengue virus (DENGv),
 - Chikungunya virus (CHIKV) or
 - Japanese encephalitis virus (JPEV).

Plaque Assay

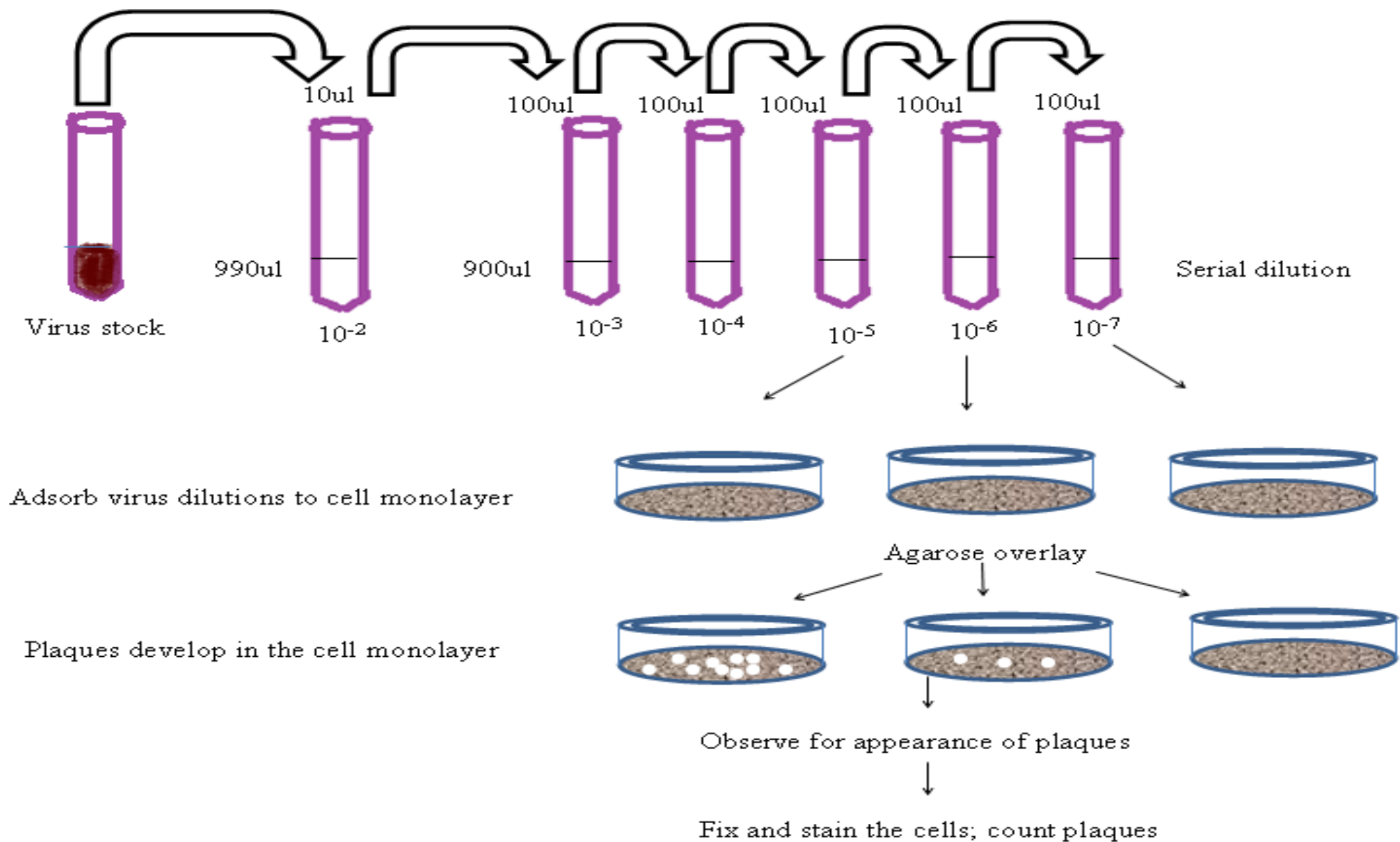
- **Plaque:** an area of cells in a monolayer which display a cytopathic effect, e.g. appearing round and darker than other cells under the microscope, or as white spots when visualized by eye; the plaque center may lack cells due to virus-induced lysis
- **Plaque-forming unit (PFU):** a virus or group of viruses which cause a plaque.
- The plaque assay is originally a virological assay employed to count and measure the infectivity level of the bacteriophages.
- later, it was applied to measure and count the mammalian viruses as well.
- This technique was first developed to calculate the titers of bacteriophage stocks.
- Renato Dulbecco modified this procedure in 1952 for use in animal virology, and it has since been used for reliable determination of the titers of many different viruses.

Basic Principle of Plaque Assay

- The basis of plaque assay is to measure the ability of a single infectious virus to form a “plaque” on a concurrent monolayer culture cells.
- A plaque is developed as a part of infection of one cell by a single virus particle that is followed by the replication of that virus, and finally, the death of the cell.
- The newly replicated virus particles will later infect and then kill surrounding cells.
- The plaques formed can then be counted and the viral titer calculated
- Plaque assays can be carried out in 24-well cell cluster plates or cell culture plates

Plaque Assay Protocol

- To perform a plaque assay, 10-fold dilutions of a virus stock are prepared, and 0.1 ml aliquots are inoculated onto susceptible cell monolayers.
- After an incubation period, to allow virus to attach to cells, the monolayers are covered with a nutrient medium containing a substance, usually agar, that causes the formation of a gel.
- When the plates are incubated, the original infected cells release viral progeny.
- The spread of the new viruses is restricted to neighboring cells by the gel.
- Consequently, each infectious particle produces a circular zone of infected cells called a plaque.
- Eventually the plaque becomes large enough to be visible to the naked eye.
- Dyes that stain living cells are often used to enhance the contrast between the living cells and the plaques.
- Only viruses that cause visible damage of cells can be assayed in this way.



Plaque Assay Protocol

1. Seed 1×10^6 cells each well in two 6-well plates. Allow cells to grow overnight. Cells should be 60-80% confluent the day of infection.
2. Label 6 sterile microcentrifuge tubes, and prepared a serial 1:10 dilutions of the virus stock.
3. Remove medium in the 6-well plates using a sterile Pasteur pipette.
4. Add 100 microliter of the 10^{-1} to 10^{-6} dilution to one of the wells. Repeat for the second plate.
5. Incubate at room temperature for 1 hour to allow the virus to infect the cells.
6. Melt 10 ml of sterile 2% agarose in H_2O (autoclaved) and keep the agarose molten in $37^\circ C$ water bath. Prewarm culture media to $37^\circ C$.
7. Carefully remove the virus without dislodging cells.
8. Mix 1:1 warm culture media and 2% agarose.

9- Gently add 1.5 ml of the 1% agarose to overlay cells in each well. Leave cells at room temperature for at least 20 minutes. Don't disturb the plates until the agarose overlay has set.

10- Add 1.5 ml of the culture medium to each well.

11- Incubate the cells at room temperature or 27 °C for 4-5 days.

12- Remove culture medium, add 1 ml of 0.03% neutral red in PBS to each well. Incubate at room temperature or 27 °C for 2-3 hours.

13- Remove neutral red/PBS, invert the dishes, and leave them in dark at room temperature overnight. Plaques will appear as small clear area against a red or pink background.

14- Count the plaques on each well and determine the virus titer:

virus titer (pfu/ml) = number of plaques * (1 ml / 0.1 ml) / fold of dilution



–Each plaque represents 1
PFU (Plaque Forming Unit)

TCID50 (50% Tissue Culture Infectious Dose)

In this lecture we will look at the TCID50 (50% Tissue Culture Infectious Dose) and cytopathic affect

At the end of this lecture

Student must have fully understood:

Mechanism, process and use of TCID50 (50% Tissue Culture Infectious Dose)

TCID₅₀ (50% Tissue Culture Infectious Dose)

- The TCID₅₀ (Median Tissue Culture Infectious Dose) is one of the methods used when verifying viral titer.
- TCID₅₀ signifies the concentration at which 50% of the cells are infected when a test tube or well plate upon which cells have been cultured is inoculated with a diluted solution of viral fluid.
- Amount of a pathogenic agent that will produce pathological change in 50% of cell cultures inoculated.
- Expressed as TCID₅₀/ml.

TCID₅₀ (50% Tissue Culture Infections Dose)

- The procedure is performed to determine the infectious titer of any virus which can cause cytopathic effects (CPE) in tissue culture over a reasonable period of 5 to 20 days while cells in culture remain viable.
- This procedure is performed to quantify how much infectious virus is in a preparation.
- Not all virus types cause CPE in tissue culture, and the cell line and virus must be carefully matched in order to see a cytopathic effect.
- The TCID₅₀ is determined in replicate cultures of serial dilutions of the virus sample.
- The titer of the virus stock is expressed as the TCID which can be calculated using a statistical Excel program and is more accurate than a negative end-point.

TCID50 (50% Tissue Culture Infections Dose)

- This assay may be more common in clinical research applications where the lethal dose of virus must be determined or if the virus does not form plaques.
- When used in the context of tissue culture, host cells are plated and serial dilutions of the virus are added.
- After incubation, the percentage of cell death (i.e. infected cells) is manually observed and recorded for each virus dilution, and results are used to mathematically calculate a TCID50 result.
- Two methods commonly used to calculate TCID50 (can also be used to calculate other types of 50% endpoint such EC50, IC50, and LD50) are:
 - ❖ Spearman-Kärber
 - ❖ Reed-Muench method

Cytopathic effects (CPE)

- Cytopathic effect or cytopathogenic effect (abbreviated CPE) refers to structural changes in host cells that are caused by viral invasion.
- The infecting virus causes lysis of the host cell or when the cell dies without lysis due to an inability to reproduce
- Both of these effects occur due to CPEs.
- If a virus causes these morphological changes in the host cell, it is said to be cytopathogenic
- Common examples of CPE include rounding of the infected cell, fusion with adjacent cells to form syncytia, and the appearance of nuclear or cytoplasmic inclusion bodies.
- CPEs are important aspects of a viral infection in diagnostics.

CPE: Common types

Total destruction

- Total destruction of the host cell monolayer is the most severe type of CPE.
- To observe this process, cells are seeded on a glass surface and a confluent monolayer of host cell is formed.
- Then, the viral infection is introduced. All cells in the monolayer shrink rapidly, become dense in a process known as pyknosis, and detach from the glass within three days.
- This form of CPE is typically seen with enteroviruses

CPE: Common types

Subtotal destruction

- Subtotal destruction of the host cell monolayer is less severe than total destruction.
- Similarly to total destruction, this CPE is observed by seeding a confluent monolayer of host cell on a glass surface then introducing a viral infection.
- Subtotal destruction characteristically shows detachment of some but not all the cells in the monolayer.
- It is commonly observed with some togaviruses, some picornaviruses, and some types of paramyxoviruses

CPE: Common types

Focal degeneration

- Focal degeneration causes a localized attack of the host cell monolayer.
- Although this type of CPE may eventually affect the entire tissue, the initial stages and spreading occur at localized viral centers known as foci.
- Focal degeneration is due to direct cell-to-cell transfer of the virus rather than diffusion through the extracellular medium.
- This different mode of transfer differentiates it from total and subtotal destruction and causes the characteristic localized effects.
- Initially, host cells become enlarged, rounded, and refractile.
- Eventually, the host cells detach from the surface.
- The spreading of the virus occurs concentrically, so that the cells lifting off are surrounded by enlarged, rounded cells which are surrounded by healthy tissue.
- This type of CPE is characteristic of herpesviruses and poxviruses

Epidemiology I

In this lecture we will look at the Epidemiology of viral infection

At the end of this lecture

Student must have fully understood:

Epidemiology of viral infections, Stages of the viral disease, Transmission ways of viruses

Epidemiology of viral infection

- Epidemiology is the study of the determinants, dynamics, and distribution of diseases in populations
- Fundamental to the understanding of the occurrence of viral diseases is delineation of the mechanisms whereby viruses are spread
- how they cause disease
- how viruses survive in nature
- how they evolve and how this potentially alters properties such as virulence
- how diseases caused by viruses continue to emerge and reemerge
- how new viral diseases arise

Viral Disease

- The relative susceptibility of a person and the severity of the disease depend on factors such as:
 - the nature of exposure;
 - the immune status,
 - age, and general health of the person;
 - the viral dose and
 - the genetics of the virus and the host

- **new variant or viral strain** would cause a viral outbreak (new influenza virus strain).
- **acute A hepatitis** is more symptomatic in adulthood than in childhood.
- **Infants** are especially prone to more serious presentation of Paramyxovirus respiratory infections and gastroenteritis.
- However, children generally do not mount as severe an immunopathological response as adults, and some infections (Varicella caused by VZV a Herpesvirus) are more benign in children.
- The **elderly** are especially susceptible to new viral infections and reactivation of latent viruses.

Stages of the viral disease

- The initial period before the characteristic symptoms of a disease is termed the incubation period.
- During that period, the virus is replicating but has not reached the target tissue or induced sufficient damage to cause disease.
- The incubation period is relatively short if the infection of primary site produces the characteristic symptoms of the disease; e.g., 1-2 days for influenza.
- This incubation period is longer for viral systematic infections such as
 - poliomyelitis (5-20 days),
 - measles (9-12 days),
 - rubella (17-20 days),
 - hepatitis (15-40 days for hepatitis A and days for hepatitis B),
 - rabies (days),
 - AIDS for Acquired Immunodeficiency Syndrome cause by HIVs (1-13 years).

Types of viral infections

➤ Viral infections are usually self-limiting. Sometimes, however, the virus persists for long periods of time in the host.

➤ Long-term virus-host interaction may take several forms:

1- Chronic infections

2- Latent infections

3- Inapparent or subclinical infections

Transmission ways of viruses

Viruses are transmitted

- by direct contact,
- sexual intercourses,
- injection with contaminated fluids or blood,
- use of contaminated injection materials (syringes, catheters), or prosthetic devices,
- and the transplantation of noncontrolled contaminated organ,
- more often by respiratory and oral-fecal routes.
- They may also be transmitted by a maternal-neonatal transmission during foetal live or the delivery.

Transmission ways of viruses

- **Animals** can also act as vectors that spread viral infection to other animals and humans.
- They can also be reservoir for the virus that maintain and amplify the virus in environment.
- Viral infections that are shared by animals or insects and humans are called **zoonosis**.
- **Arthropods** include mosquitos, ticks can act as vectors for Togavirus, Flavivirus, Bunyavirus and Reovirus.
- These viruses are often referred to as arboviruses because they are arthropod borne viruses, such as Yellow fever virus, Dengue viruses, Rift Valley fever virus.

Epidemiology II

In this lecture we will look at the Epidemiology of viral infection

At the end of this lecture

Student must have fully understood:

Terms and concepts used in epidemiology

Terms and concepts used in epidemiology

- **Endemic:** The term endemic (enzootic) disease refers to the presence of several or continuous chains of transmission that result in the continuous occurrence of disease in a population
- **Epidemic** (epizootic) disease refers to peaks in disease incidence that exceed the endemic baseline or expected incidence of disease
- **Pandemic** (panzootic) disease refers to a large-scale epidemic involving spread of disease across continents or even worldwide, such as that recently associated with H1N1 influenza virus and, previously, with canine parvovirus, amongst many other examples

Terms and concepts used in epidemiology

- *Incubation period* refers to the interval between infection and the onset of clinical sign
- *Period of contagiousness* (infectious period) refers to the time during which an infected animal sheds virus. This period varies depending on the disease concerned
- *Seroepidemiology* simply denotes the use of serological data as the basis of epidemiological investigation, as determined by diagnostic serological techniques
- *Molecular epidemiology* denotes the use of molecular biological data as the basis of epidemiological investigation
- Real time (quantitative) PCR assays and nucleotide sequence data are increasingly used for such studies, as they facilitate rapid detection of viruses and direct genetic comparison of individual virus strains

Calculations of Rates and Proportions

- The comparison of disease experience in different populations is expressed in the form of rates and proportions.
- Multipliers (eg, rates per 10n) are used to provide rates that are manageable whole numbers—the most common rate multiplier used is 100,000—that is, the given rate is expressed per 100,000 of the given population per unit of time.
- Four rates or proportions are most widely used to describe disease occurrence in populations:
 - ❖ *incidence*
 - ❖ *prevalence*
 - ❖ *morbidity rate*
 - ❖ *mortality rate*

Incidence

- The cumulative incidence, sometimes termed attack rate when used during a disease outbreak, is a measure of the occurrence of new cases of infection or disease in a population in a given time period
 - for example, a month or a year—and is especially useful for describing acute diseases of short duration

- For acute infections, several parameters determine the incidence of infection or disease in a population, including
 - (1) the percentage of susceptible animals
 - (2) the percentage of susceptible animals that are infected
 - (3) the percentage of infected animals that suffer disease
 - (4) the contact rate for those diseases transmitted by contact, which is affected by animal housing density, housing time, and related factors

Prevalence

- The incidence of chronic viral disease is often difficult to measure, especially when the onset is insidious and most animals are sub clinically infected.
 - For such diseases it is customary to determine the prevalence—that is, the ratio at a particular point in time, of the number of cases currently present in the population divided by the number of animals in the population
 - It is a snapshot of the occurrence of infection or disease at a given time and, hence, a proportion rather than a rate.
 - Prevalence is thus a function of the incidence and the duration of the disease
-
- Seroprevalence relates to the occurrence of antibodies to a particular virus in a population

Morbidity and Mortality Rates

- The morbidity rate is the percentage of animals in a population that develop clinical signs attributable to a particular virus over a defined period of time.
- Mortality from a disease can be categorized in two ways:
 - the cause- specific mortality rate (the number of deaths from the disease in a given year, divided by the total population at mid-year), often expressed per 100,000 population,
 - or the case-fatality rate (the percentage of animals with a particular disease that die from the disease within a defined time period)

Epidemiology III

In this lecture we will look at the Epidemiology of viral infection

At the end of this lecture

Student must have fully understood:

Types of epidemiologic investigation

Types of epidemiologic investigation

- Case-control, cohort, cross-sectional, and long-term herd studies provide the conceptual frameworks within which
 - relationships between risk factors and the incidence and prevalence of disease,
 - safety and efficacy of vaccines,
 - therapeutic value of vaccines and drugs can be determined

- The latter two relationships can also be assessed with randomized controlled trials

Case-Control Studies

- Case-control studies are typically retrospective—that is, investigation starts after the disease episode has occurred.
- In human disease epidemiology, this is the most common type of study, often used to identify risk factors for a disease whose causative agent has not been identified.
- Advantages of retrospective studies are that they make use of existing data and are relatively inexpensive to carry out.
- Case-control studies do not require the creation of new data or records, they do require careful selection of the control group, which is sometimes matched to the case (subject) group.
- The unit of interest might be individual animals or groups of animals such as herds/flocks but, because the necessary records are generally not available in most animal disease outbreaks

Cohort Studies

- Cohort studies are prospective or longitudinal and involve comparisons of the incidence of disease or infection in risk-factor positive (exposed) and risk-factor negative (control) animals or herds.
- Examples of risk factors include vaccination history, biosecurity practices, and distance to the neighboring herds.
- This type of study requires the creation of new data and records. It also requires careful selection of the control group, which should be designed to be as similar as possible to the exposed group, except for the risk factor(s) being studied.
- Cohort studies do not lend themselves to quick analysis, because groups must be followed until disease is observed, often for long periods of time

Cross-Sectional Studies

- When risk factors for a specific viral disease are unknown
- A cross-sectional study can be carried out relatively quickly using antibody or organism detection methods for the virus and a questionnaire to obtain data on risk factors
- Crosssectional studies provide data on the prevalence of the particular disease/infection in a population in a specific area at a given time
- Allow assessment of the relationship between risk factors that don't hange over time and of the infection with the virus of interest

Long-Term Herd Studies

- Long-term herd studies, using cross-sectional or longitudinal designs, are another kind of epidemiologic investigation that can provide unique information about the presence and continued activity (or lack of activity) of a given virus in an area.
- They can also be designed to provide information on the efficacy of vaccines or therapeutic drugs.
- Despite automation of diagnostic methods and computerization of data files, such studies are still expensive and labor-intensive.
- When used for evaluating vaccines or therapeutic agents, long-term herd studies have the advantage that they include all the variables attributable to the entire husbandry system

Morbidity and Mortality Rates

➤ The

Virus Transmission

In this lecture we will look at the Virus Transmission

At the end of this lecture

Student must have fully understood:

Virus transmission may be horizontal or vertical

Virus Transmission

- Viruses survive in nature only if they can be transmitted from one host to another, whether of the same or different species
- Transmission cycles require virus entry into the body, replication, and shedding, with subsequent spread to another host
- Virus transmission may be **horizontal or vertical**.
- **Vertical transmission** describes transmission from dam to offspring.
- **Horizontal transmission** that is, between animals within the population at risk, and can occur via direct contact, indirect contact, or by a common vehicle;
- they may be air-borne,
- vector-borne,
- or iatrogenic

Horizontal Transmission

Direct-Contact Transmission

- Direct-contact transmission involves actual physical contact (eg, licking, rubbing, or biting) between an infected animal and a susceptible animal.
- This category also includes sexual contact, which, for example, is important in the transmission of some herpesviruses and retroviruses such as human immunodeficiency virus (HIV)

Indirect-Contact Transmission

- Indirect-contact transmission occurs via fomites, such as shared eating containers, bedding, dander, restraint devices, vehicles, clothing, improperly sterilized surgical equipment, or improperly sterilized syringes or needles
- For example, equine arteritis virus that is shed in the semen of carrier stallions can be spread on fomites or contaminated bedding to cohoused horses without any direct sexual contact

Horizontal Transmission

Common-Vehicle Transmission

- Common-vehicle transmission includes fecal contamination of food and water supplies (fecal-oral transmission) and virus-contaminated meat or bone products

Air-Borne Transmission

- Air-borne transmission, which results in infection of the respiratory tract, occurs via droplets and droplet nuclei (aerosols) emitted from infected animals during coughing or sneezing (eg, influenza) or from environmental sources such as dander or dust from bedding (eg, Marek's disease).
- Large droplets settle quickly, but microdroplets evaporate, forming droplet nuclei (less than 5 μm in diameter) that remain suspended in the air for extended periods

Horizontal Transmission

Arthropod-Borne Transmission

- Arthropod-borne transmission involves the bites of arthropod vectors
- mosquitoes transmit equine encephalitis viruses;
- ticks transmit African swine fever virus;
- Culicoides spp. transmit bluetongue and African horse sickness viruses

Nosocomial Transmission

- Nosocomial transmission occurs while an animal is in a veterinary hospital or clinic
- During the peak of the canine parvovirus epidemic in the 1980s, many puppies became infected in veterinary hospitals and clinics
- Feline and canine respiratory and enteric viral infections are also acquired nosocomially with considerable frequency in animal shelters

Influenza Viruses

In this lecture we will look at the Influenza viruses

At the end of this lecture

Student must have fully understood:

Influenza virus types, Virus transmission

Influenza Viruses

- Influenza, commonly known as the flu, is an infectious disease caused by an influenza virus
- There are four types of influenza viruses: A, B, C and D
- Three of the four types of influenza viruses affect humans: Type A, Type B, and Type C
- Type D has not been known to infect humans, but is believed to have the potential to do so
- Human influenza A and B viruses cause seasonal epidemics of disease almost every winter
- Influenza type C infections generally cause a mild respiratory illness and are not thought to cause epidemics

Influenza A viruses

- Influenza A viruses are divided into subtypes based on two proteins on the surface of the virus:
 - hemagglutinin (H)
 - neuraminidase (N)
- There are 18 different hemagglutinin subtypes and 11 different neuraminidase subtypes
- Wild aquatic birds are the natural hosts for a large variety of influenza A.
- Occasionally, viruses are transmitted to other species and may then cause devastating outbreaks in domestic poultry or give rise to human influenza pandemics

Influenza A viruses

- The type A viruses are the most virulent human pathogens among the four influenza types and cause the severest disease.
- The influenza A virus can be subdivided into different serotypes based on the antibody response to these viruse
- The serotypes that have been confirmed in humans, ordered by the number of known human pandemic deaths, are:
 - H1N1, which caused Spanish Flu in 1918, and Swine Flu in 2009
 - H2N2, which caused Asian Flu in 1957
 - H3N2, which caused Hong Kong Flu in 1968
 - H5N1, which caused Bird Flu in 2004
 - H7N7, which has unusual zoonotic potential
 - H1N2, endemic in humans, pigs and birds
 - H9N2
 - H7N2
 - H7N3
 - H10N7
 - H7N9, responsible for an ongoing epidemic in China
 - H6N1, which only infected one person, who recovered

Influenza virus B

- Influenza B almost exclusively infects humans and is less common than influenza A
- The only other animals known to be susceptible to influenza B infection are the seal and the ferret
- This type of influenza mutates at a rate 2–3 times slower than type A and consequently is less genetically diverse, with only one influenza B serotype
- As a result of this lack of antigenic diversity, a degree of immunity to influenza B is usually acquired at an early age

Influenza virus C and D

- Influenza C virus, which infects humans, dogs and pigs, sometimes causing both severe illness and local epidemics
- Influenza C is less common than the other types and usually only causes mild disease in children
- Influenza D virus, which infects pigs and cattle.
- The virus has the potential to infect humans, although no such cases have been observed yet
- This virus is currently not associated with any major epidemics

Swine Flu

In this lecture we will look at the Swine Flu

At the end of this lecture

Student must have fully understood:

Types, outbreaks from 2009 to 2019

Swine Flu

- H1N1 flu is also known as swine flu.
- It's called swine flu because in the past, the people who caught it had direct contact with pigs.
- In the spring of 2009, scientists recognized a particular strain of flu virus known as H1N1.
- This virus is actually a combination of viruses from pigs, birds and humans.
- During the 2009-10 flu season, H1N1 caused the respiratory infection in humans that was commonly referred to as swine flu
- World Health Organization declared the flu caused by H1N1 to be a global pandemic

Swine Flu

- It is an orthomyxovirus that contains the glycoproteins haemagglutinin and neuraminidase
- They are described as H1N1, H1N2 etc depending on the type of H or N antigens they express with metabolic synergy
- Haemagglutinin causes red blood cells to clump together and binds the virus to the infected cell
- Neuraminidase is a type of glycoside hydrolase enzyme which helps to move the virus particles through the infected cell and assist in budding from the host cells

Swine Flu

- This novel virus spread worldwide and had caused about 17,000 deaths by the start of 2010.
- On August 10, 2010, the World Health Organization declared the H1N1 influenza pandemic over, saying worldwide flu activity had returned to typical seasonal patterns
- Like other seasonal flu viruses, common symptoms of swine flu (H1N1) develop between one and three days after you've been infected and can include:
 - Fever, which is usually high, but is sometimes absent
 - Cough
 - Runny or stuffy nose
 - Sore throat
 - Body aches
 - Headache
 - Chills
 - Fatigue or tiredness, which can be extreme
 - Diarrhea and vomiting occasionally, but more commonly seen than with other strains of flu

Swine Flu

2009 A(H1N1) pandemic

- In the 2009 flu pandemic, the virus isolated from patients in the United States was found to be made up of genetic elements from four different flu viruses
 - North American swine influenza, North American avian influenza, human influenza, and swine influenza virus typically found in Asia and Europe
- This new strain appears to be a result of reassortment of human influenza and swine influenza viruses, in all four different strains of subtype H1N1
- On October 25, 2009, U.S. President Barack Obama officially declared H1N1 a national emergency
- The March 21, 2010 worldwide update, by the U.N.'s World Health Organization (WHO), states that "213 countries and overseas territories/communities have reported laboratory confirmed cases of pandemic influenza H1N1 2009, including at least 16,931 deaths"

Swine Flu

2015 India outbreak

- Swine flu was reported in India in early 2015. The disease affected more than 31,000 people and claimed over 1,900 lives

2017 Maldives outbreak

- Maldives reported Swine flu in early 2017
- 501 people were tested for the disease; 185 (37%) of those tested were positive for the disease
- 4 people from these 185 died due to this disease

2017 Myanmar outbreak

- Myanmar reported H1N1 in late July 2017.
- As of 27 July, 30 confirmed cases and 6 people had died

Swine Flu

2017–2018 Pakistan outbreak

- Pakistan reported H1N1 cases mostly arising from the city of Multan, with deaths resulting from the epidemic reaching 42.
- There have also been confirmed cases in cities of Gujranwala and Lahore

2019 Outbreak in Malta

- An outbreak of swine flu in the European Union member state was reported in mid-January 2019, with the island's main state hospital overcrowded within a week.
- The outbreak is ongoing

2019 Outbreak in Morocco

- Many deaths due to H1N1 have been recorded in the month of January 2019 in Morocco
- The outbreak is ongoing
- As of February 4th, 11 deaths have been reported in various regions of Morocco

HCV (Hepatitis C Virus)

In this lecture we will look at the **HCV (Hepatitis C Virus)**

At the end of this lecture

Student must have fully understood:

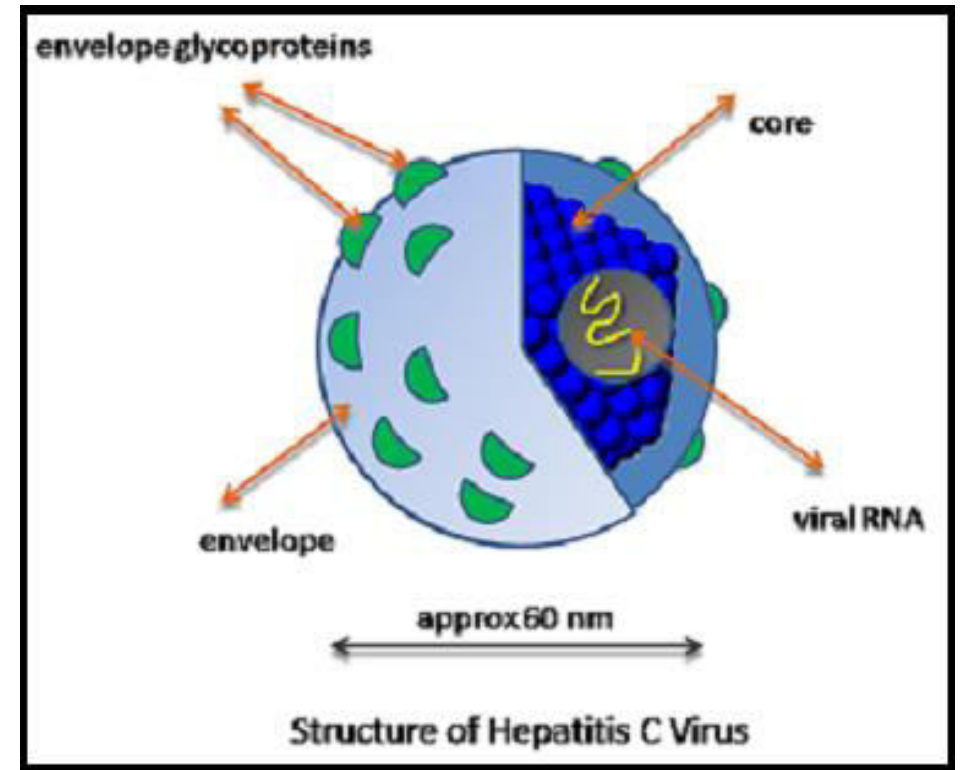
Structure, Mode of transmission, Mechanism,
Causes, Symptoms Diagnosis and Treatment of HCV

HCV (Hepatitis C Virus)

- Hepatitis C virus (HCV) is an enveloped, single-stranded RNA virus, belonging to the **Flaviviridae family**.
- The hepatitis C virus is a **disease of the liver**.
- In hepatitis C, the major damage is not actually done by the virus itself. Rather, the major damage caused to the liver is actually **due to the inflammation** that the body creates trying to fight off the virus. When the body starts to fight the disease, it causes damage to the cells and eventually 'fibrosis' where the liver cells are replaced by tough, but non-functional fibrous tissue.
- Worldwide 20%-90% of patients with thalassaemia are seropositive for anti-HCV antibodies
- Chronic HCV infection is more common in patients who had a large number of blood transfusions before 1990.

Structure of HCV (Hepatitis C Virus)

- The hepatitis C virus particle consists of a lipid membrane envelope that is 55 to 65 nm in diameter.
- Two viral envelope glycoproteins, **E1 and E2**, are embedded in the lipid envelope.
- They take part in viral attachment and entry into the cell.
- Within the envelope is an icosahedral core that is 33 to 40 nm in diameter. Inside the core is the RNA material of the virus



HCV Replication Cycle

Seven steps of the viral life cycle

1-Attachment: the viral particle, surrounded with lipoproteins, binds the target cells by interacting with several receptors

2- Entry: following attachment, the virus enters through clathrin-mediated Endocytosis

3-Uncoating: the cellular and viral membranes fuse and the capsid is disorganized with a process triggered by the low pH of the endosome

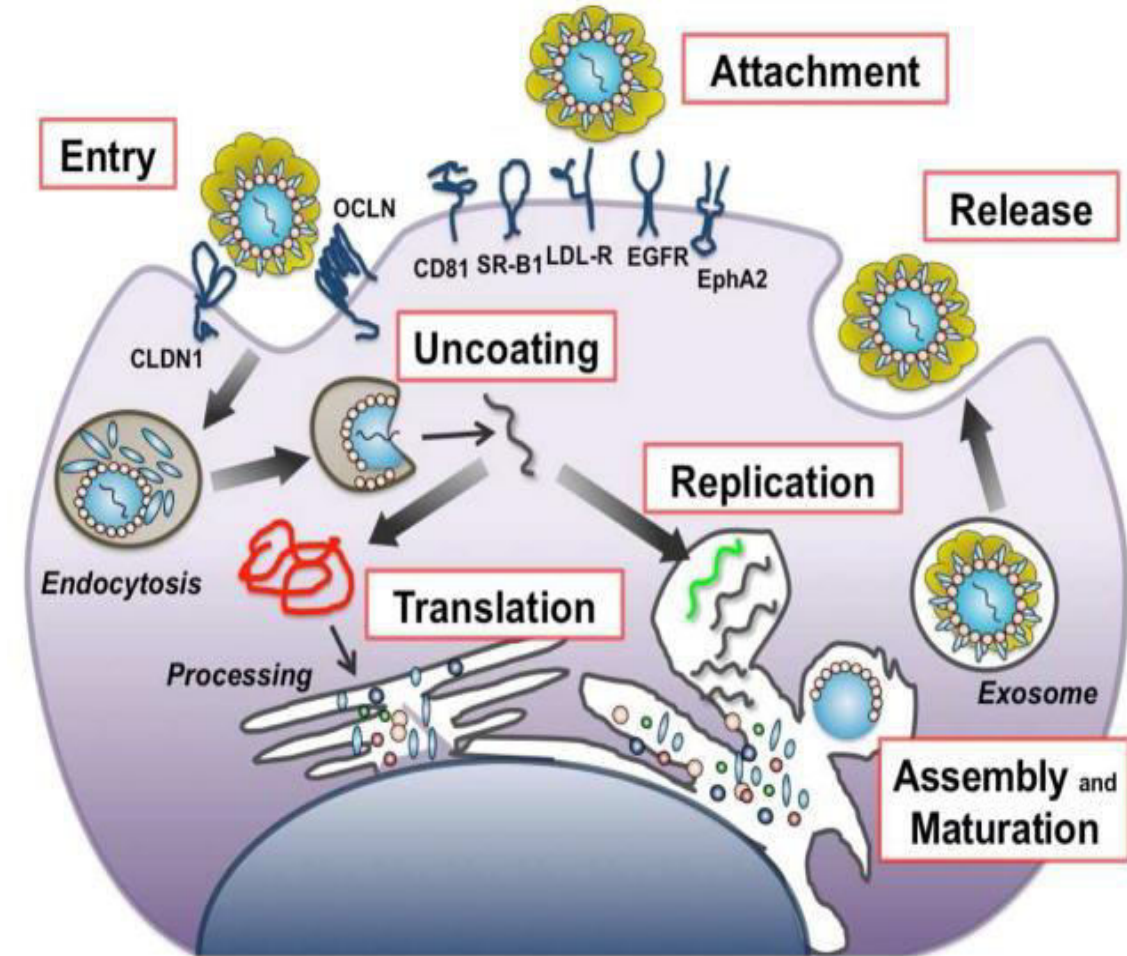
4-Translation: the genomic RNA is directly translated in a polyprotein precursor

5-Replication: the non-structural proteins and some host factors form a replication complex that synthesized multiple copies of the HCV RNA genome via a minus-strand replicative intermediate

HCV Replication Cycle

6- Assembly and maturation: packaging of viral progeny takes place in the endoplasmic reticulum from which the virion acquires the envelope with E1 and E2 glycoproteins. Maturation and association with endogenous lipoproteins to form lipoviral particles immediately follow;

7- Release: virions are released from the cells most likely by exocytosis or transmitted to other cells via a cell-free mechanism.



Transmission of HCV

➤ HCV is transmitted from one person to another principally by parenteral route.

The major routes of transmission are:

- ❖ Injection drug use,
- ❖ blood transfusion, and
- ❖ unsafe therapeutic injections.

Other routes of transmissions include;

- ❖ healthcare related procedures (occupational exposures like needle stick injuries),
 - ❖ tattooing,
 - ❖ perinatal transmission, and sexual transmission
- Transmission through occupational, perinatal, and sexual routes is less efficient as compared to transmission through large or repeated percutaneous exposures.

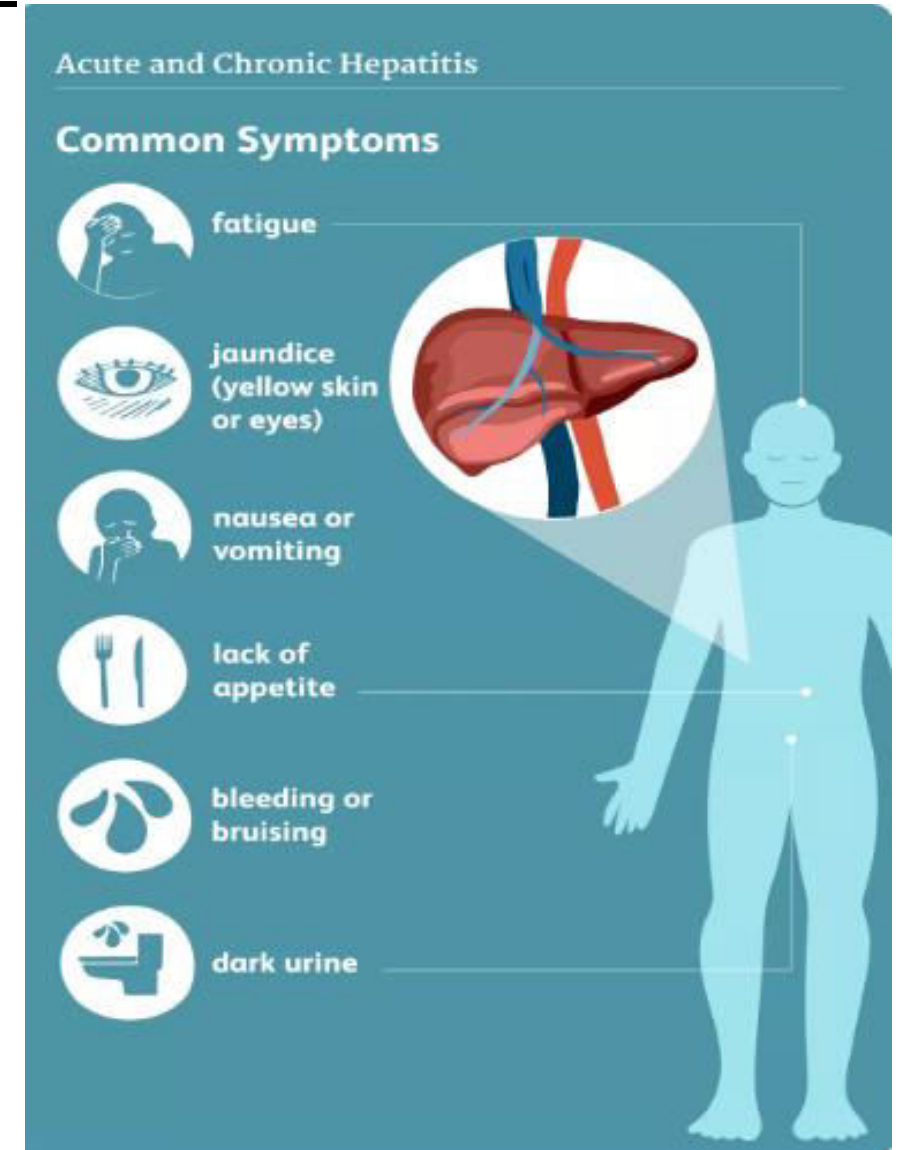
Diagnosis, Prevention and Treatment

Diagnosis of HCV

➤ HCV infection is diagnosed in 2 steps:

❖ **Screening for anti-HCV antibodies** with a serological test identifies people who have been infected with the virus.

❖ If the test is positive for anti-HCV antibodies, **a nucleic acid test for HCV ribonucleic acid (RNA)** is needed to confirm chronic infection because about 30% of people infected with HCV spontaneously clear the infection by a strong immune response without the need for treatment. Although no longer infected, they will still test positive for anti-HCV antibodies.



Symptoms & Treatment of HCV

HCV Symptoms:

- The incubation period for hepatitis C is 2 weeks to 6 months.
- Following initial infection, approximately 80% of people do not exhibit any symptoms.
- Those who are acutely symptomatic may exhibit fever, fatigue, decreased appetite, nausea, vomiting, abdominal pain, dark urine, grey colored feces, joint pain and jaundice (yellowing of skin and the whites of the eyes).

Treatment

Hepatitis C is treated with **anti-viral medications** with the objective of having no hepatitis C virus detected in the body after completion of a 12-week course of treatment.

The type of anti-viral medication used will depend on:

- Hepatitis C genotype
- Degree of liver damage
- Previous treatment for hepatitis C.
- Treatment for chronic hepatitis C usually involved taking **two main medicines:**
 - **Pegylated interferon**— a medication given by injection that encourages the immune system to attack the virus
 - **Ribavirin**— an antiviral medication that stops the virus replicating.

HIV (Human Immunodeficiency Virus)

In this lecture we will look at the **HIV (Human Immunodeficiency Virus)**

At the end of this lecture

Student must have fully understood:

Structure, Mode of transmission, Mechanism,
Causes, Symptoms and Diagnosis of HIV

HIV (Human Immunodeficiency Virus)

HIV (human immunodeficiency virus) is a virus that damages the cells in your immune system and weakens your ability to fight everyday infections and disease.

H= Infects only *Human Beings*

I= Immunodeficiency Virus *weakens the immune system* and increases the risk of infection

V= *Virus* that affects the body and eventually overcomes the body's immune system

- HIV belongs to a special class of viruses known as *retroviruses* where they are placed in the subgroup *lentiviruses*
- HIV Invades the helper *T cells (CD4 cells)* in the body of the host (defense mechanism of a person) to replicate itself.
- It causes the *acquired immunodeficiency syndrome*, a condition in humans in which progressive failure of the immune system allows life-threatening opportunistic infections and cancers to thrive

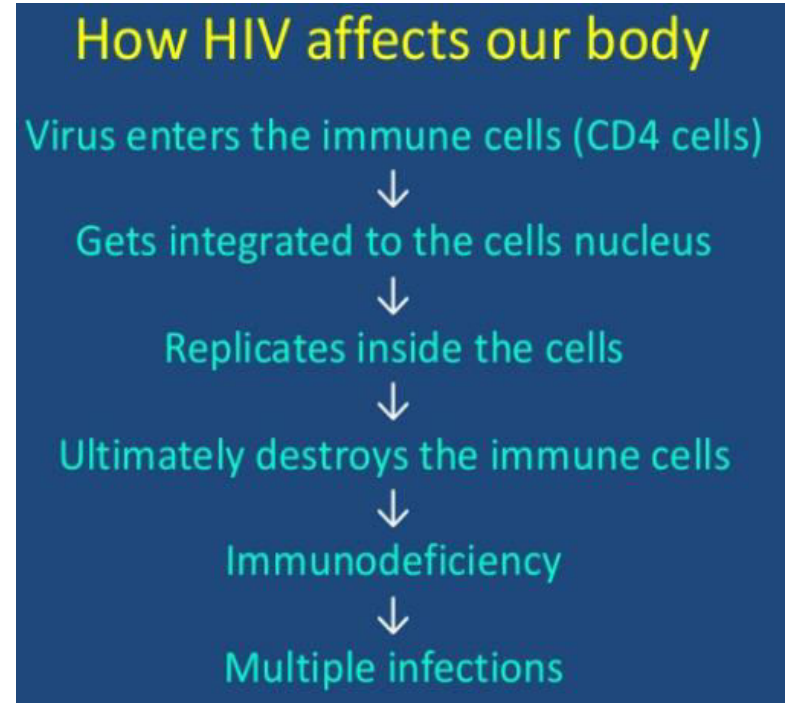
Clinical Manifestations

Four stages of HIV Infection:

- I- Initial infection
- II- Asymptomatic Carrier State
- III- AIDS-related Complex (ARC)
- IV- AIDS

AIDS

- Acquired Immunodeficiency Syndrome
- HIV is the Virus that causes AIDS
- AIDS (acquired immune deficiency syndrome) is the final stage of HIV disease, which causes severe damage to the immune system.
- Disease limits the body's ability to fight infection due to markedly reduced helper T cells.
- Patients have a very weak immune system (defense mechanism).
- Patients predisposed to multiple opportunistic infections leading to death.

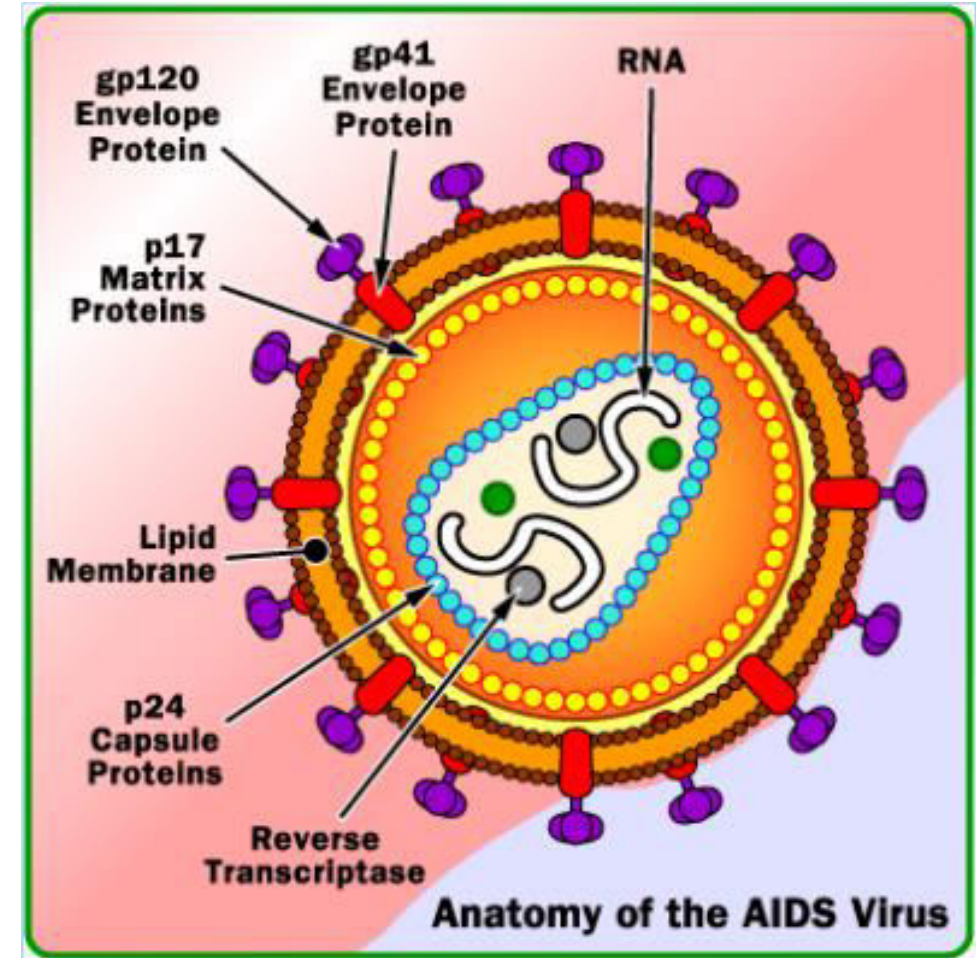


Structure of HIV Viral genome

- Two viral strands of RNA found in core surrounded by protein outer coat.
- Outer envelope contains a lipid matrix within which specific viral glycoproteins are imbedded.
- These knob-like structures responsible for
- binding to target cell.

Mode of Transmittance of HIV

HIV is spread when blood, semen, or vaginal fluids from an infected person enter another person's body, usually through sexual contact, from sharing needles when injecting drugs, or From mother to baby during birth.



Diagnosis, Prevention and Treatment

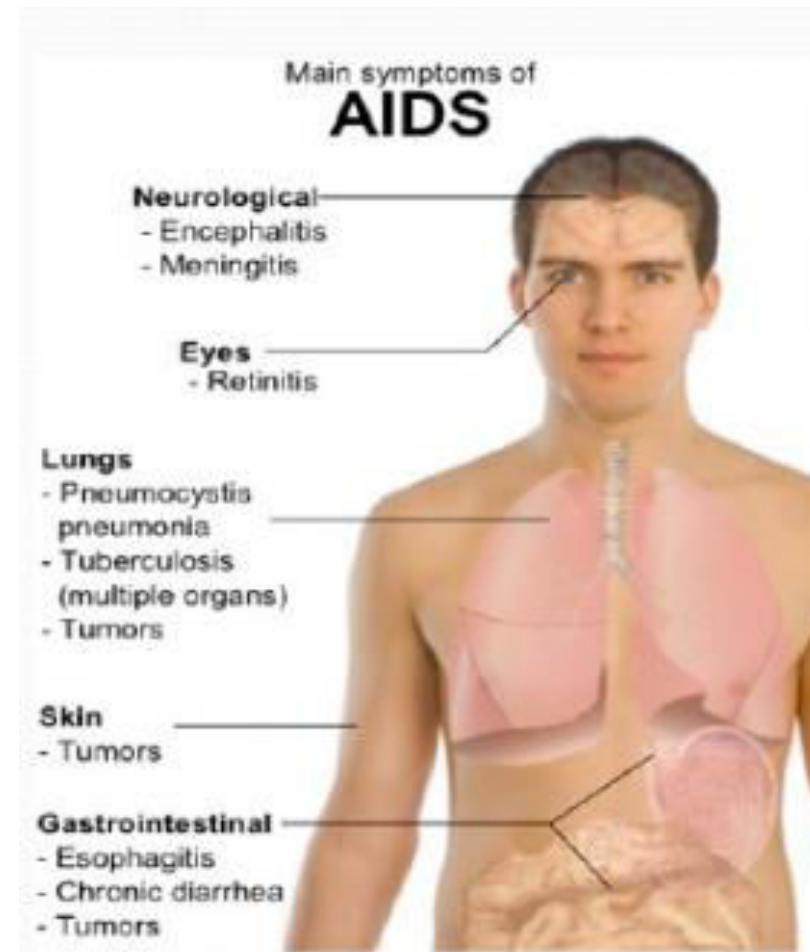
Lab Diagnosis of HIV

Direct Tests

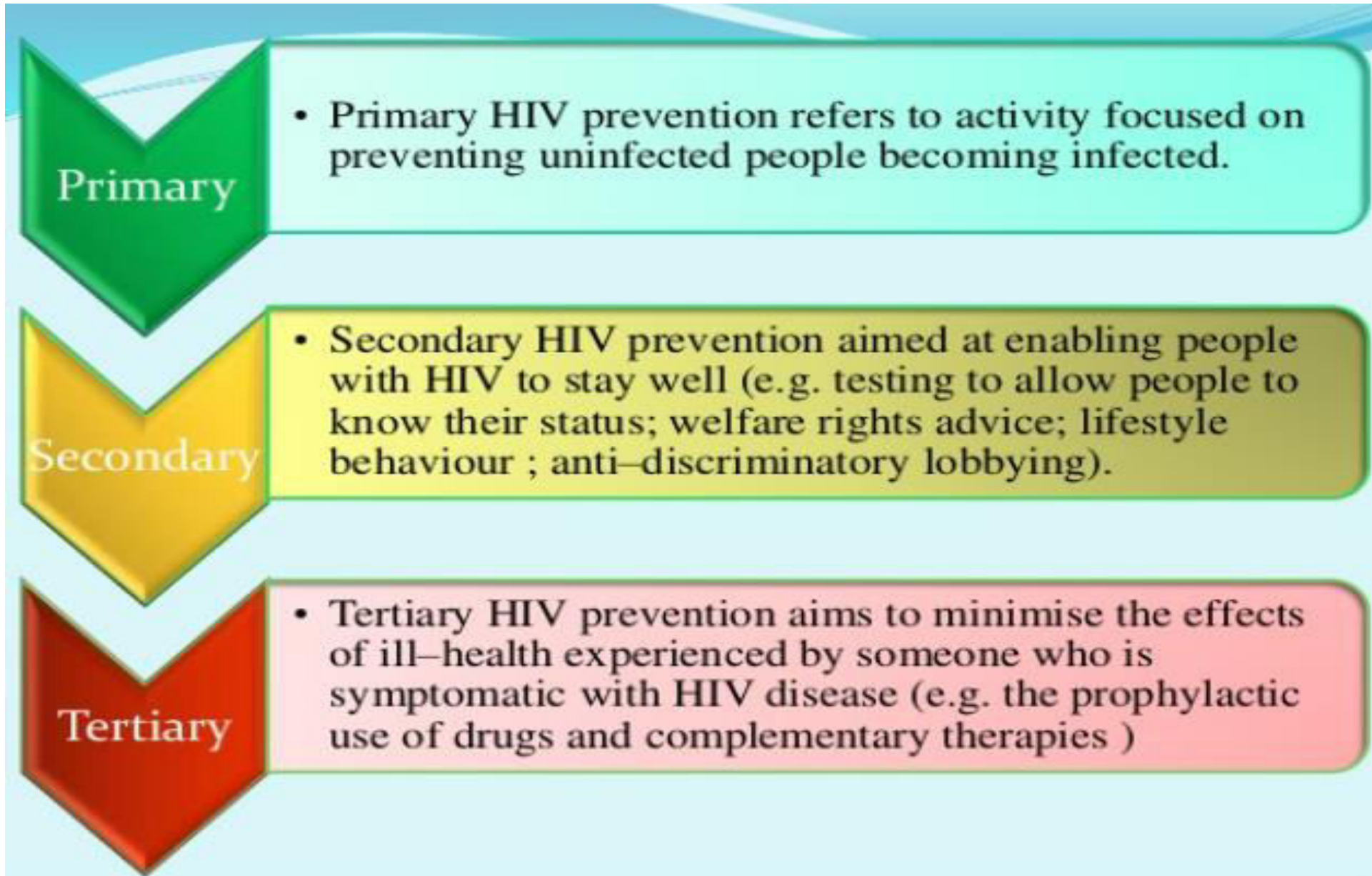
- ELISA (enzyme-linked-immunosorbent-serological assay)
- Recombinant DNA Techniques
- Viral Isolation in culture
- PCR

Indirect Tests

- CD4 counts
- Lymphopenia
- Lymphnode biopsy



HIV Prevention



Measles

In this lecture we will look at the **Measles**

At the end of this lecture

Student must have fully understood:

Structure, Mode of transmission, Mechanism,
Causes, Symptoms Diagnosis and Treatment of
Measles Viruses

Measles

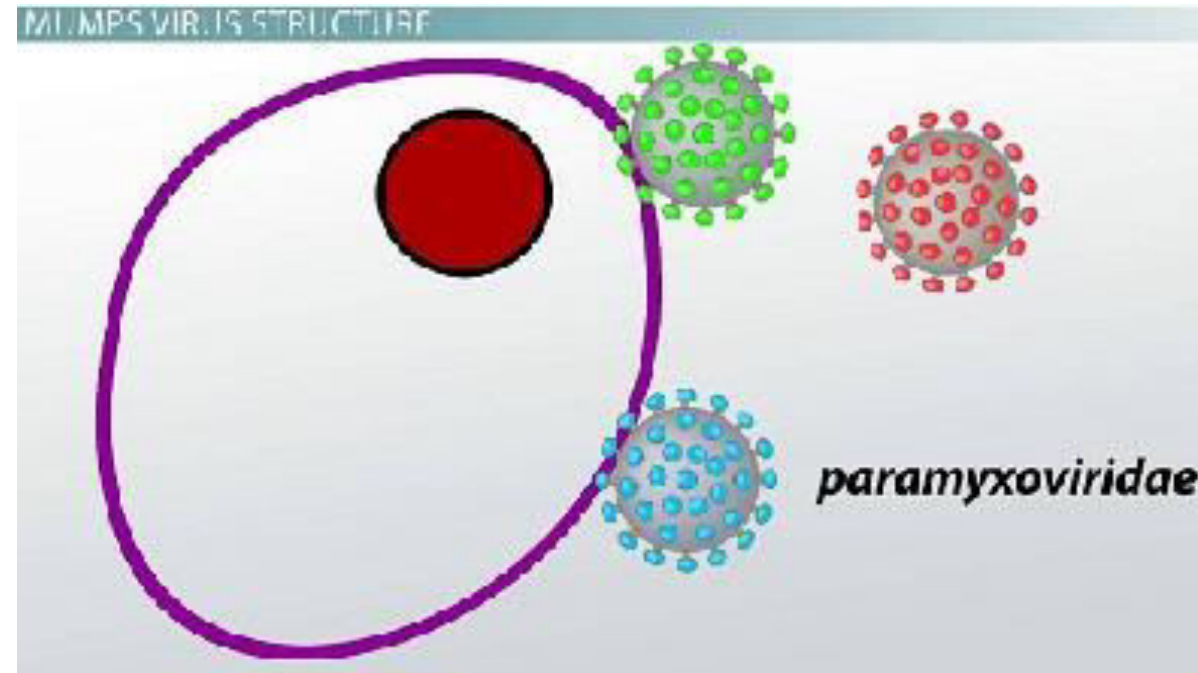
- **Measles** is a highly contagious infectious disease caused by the **measles** virus.
- It is a childhood infection caused by Virus.
- Also called **rubeola**
- Measles can be serious and even fatal for small children. While death rates have been falling worldwide as more children receive the measles vaccine, than 100,000 people a year, most under the age of 5.
- Measles causes a red, blotchy rash that usually appears first on the face and behind the ears, then spreads downward to the chest and back and finally to the feet.



Structure of Measles Virus

Etiology

- The cause of Measles is an RNA Virus of the genus **Morbillivirus** in the family **Paramyxoviridae**
- It is round, like a ball, and has an envelope on the outside. When it leaves the host cell, the **measles virus** steals part of the cell's membrane to make the envelope, which can then help hide the **virus** from the host's immune system.



Virulence Factors

1. Portal of entry- Through Respiratory mucus membrane.

- It first infects the respiratory mucosa, spreads through the lymphatics and bloodstream, and can then infect the conjunctiva, respiratory tract, urinary tract, GI tract, endothelial cells, and the central nervous system

2. Attachment

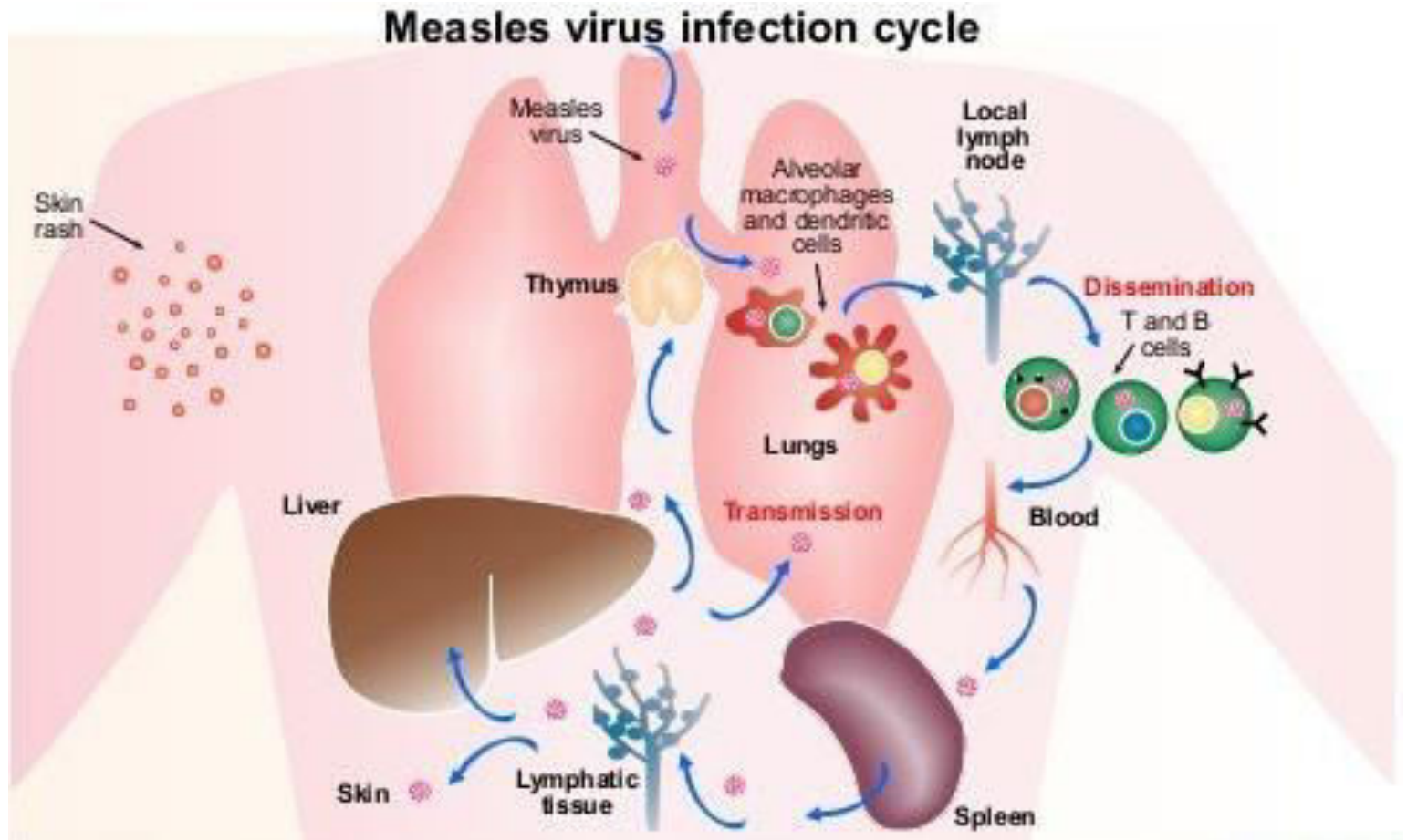
- Hemagglutinin
 - Hemagglutinin is an integral membrane protein found on the surface of the measles virus.
 - Hemagglutinin binds to CD46, a glycoprotein found on the surface of most cells.

3. Evade the immune response

- The damage, as well as the control of the disease, is most probably caused by the immune system.
 - Immunosuppression:
 - The measles virus blocks T_H proliferation response to IL-2.

Measles Replication Cycle

4- Destruction of Tissue a serious febrile illness. The maculopapular rash, which starts at the hairline and spreads over the whole body, is caused by immune T-cells targeted to the infected endothelial cells of the small blood vessels. T-cell deficient individuals do not have the rash, but do have uncontrolled disease which usually results in death.



Transmission of Measles

- Measles transmission is primarily **person to person** via large respiratory droplets.
- **Airborne transmission** via aerosolized droplet
- Measles is highly communicable, with >90% among susceptible persons. Measles may be transmitted from 4 days prior to 4 days after rash onset. Maximum communicability occurs from onset of symptoms through the first 3-4 days of rash.

Reservoir

- Measles is a **human** disease. There is no known animal reservoir, and an asymptomatic carrier state has not been documented.

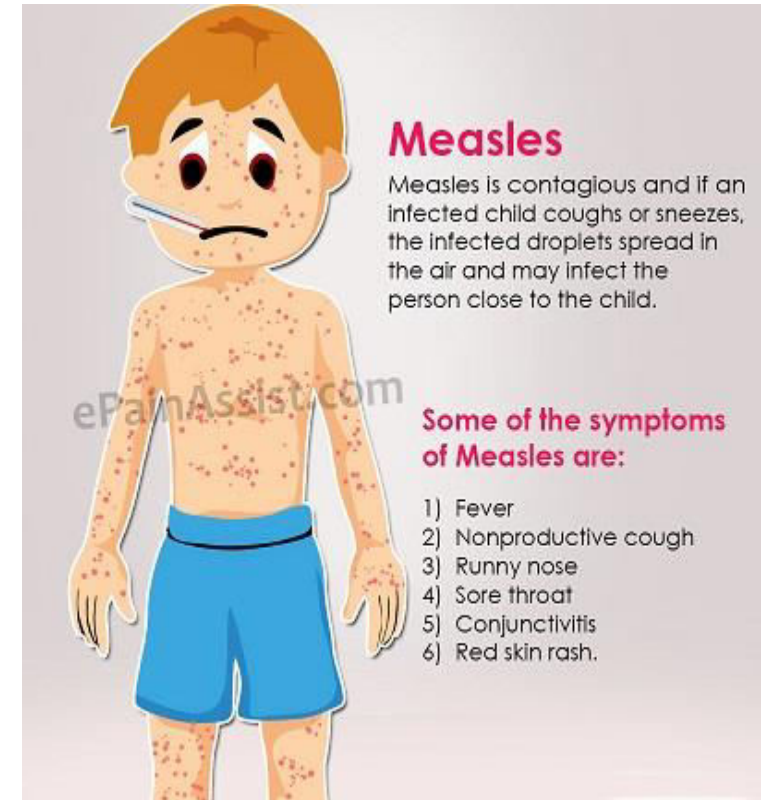
Symptoms & Treatment of Measles

Measles Symptoms:

➤ It is an acute viral infection characterized by a final stage with a maculopapular rash erupting successively over the neck and face, trunk, arms, and legs, and accompanied by a high fever.

Treatment

- There is no specific antiviral treatment
- Complications may require antibiotic treatment
- Treatment for the symptoms includes plenty of fluids and paracetamol for the fever.
- Aspirin should not be given to children under 12 years of age unless specifically recommended by a doctor.



Polio Virus

In this lecture we will look at the **Brief Introduction of Polio**

At the end of this lecture

Student must have fully understood:

Poliovirus infection- including symptoms, causes treatment and prevention

Polio

Poliomyelitis or polio is an infection **caused by the polio virus**. It is a serious disease affecting a person's brain and spinal cord. Infection with the poliovirus can lead to a life-threatening muscular paralysis of the body.

- **Poliovirus**, the **causative agent** of poliomyelitis, is a human enterovirus and member of the family of **Picornaviridae**.
- Three serotypes are: PV1, PV2 and PV3.
- The term "**Poliomyelitis**" derives from the ancient Greek word **Polio's** means "grey" and **myelos** meaning "marrow" referring to the grey matter of the spinal cord. The suffix itis denotes inflammation, i.e. **inflammation of the spinal cord's grey matter**.
- A severe infection can extend into the brain stream and even higher center resulting in Polio encephalitis and apnea.

Clinical Features

Three Forms of Polio Infection

- Sub-clinical Infections
- Non-paralytic Infections
- Paralytic Infections
- **Sub-clinical**- most common polio infection, accounts for 95% of all cases. Sub-clinical infected individuals typically are asymptomatic and does not affect the central nervous system.
- **Non-paralytic**- causes mild symptoms and does not result in paralysis. it does affect the central nervous system. Makes up 1-5 % of all polio cases
- **Paralytic**- rarest and most serious form of polio, causes full or partial paralysis in affected individuals. **Three types of paralysis that can occur: spinal polio** (affects the spine), **bubbar polio** (affects the brainstem, & **bulbospinal polio** (affects both the spine and brainstem). Occurs in 0.1-2% cases

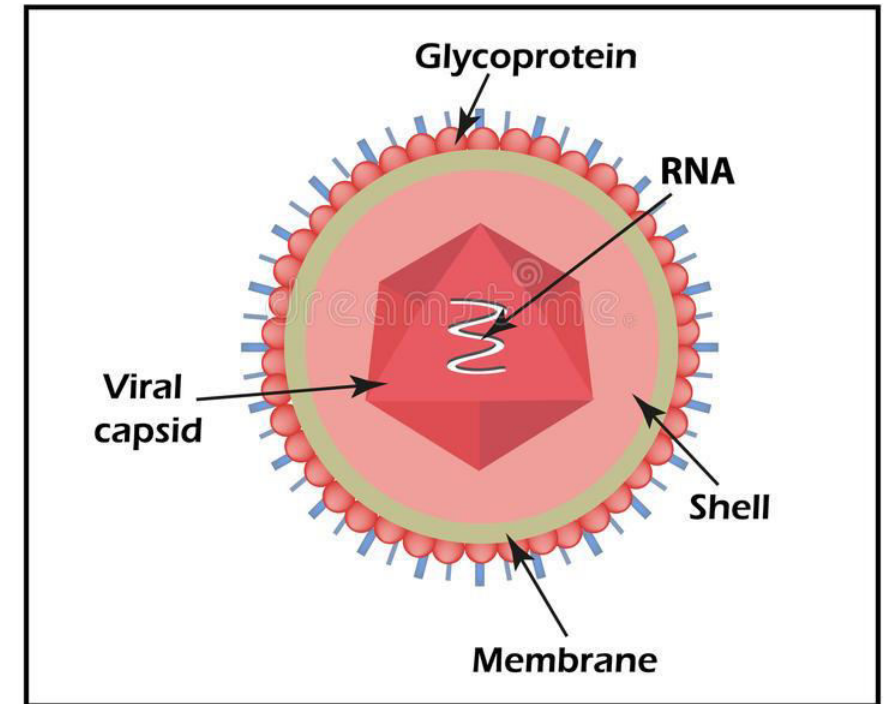
Structure & Transmission of Poliovirus

- Picornaviruses are small, about 300 angstroms in diameter
- and are comprised of an icosahedral protein coat and a single-stranded positive sense RNA genome.

Mode of Transmittance of Poliovirus

Transmission Person-to-person spread of poliovirus via the **fecal-oral route** is the most important route of transmission, although the **oral-oral route** may account for some cases.

Structure of Polio



Diagnosis, Prevention and Treatment

Diagnosis and Tests:

The health care provider may find:-

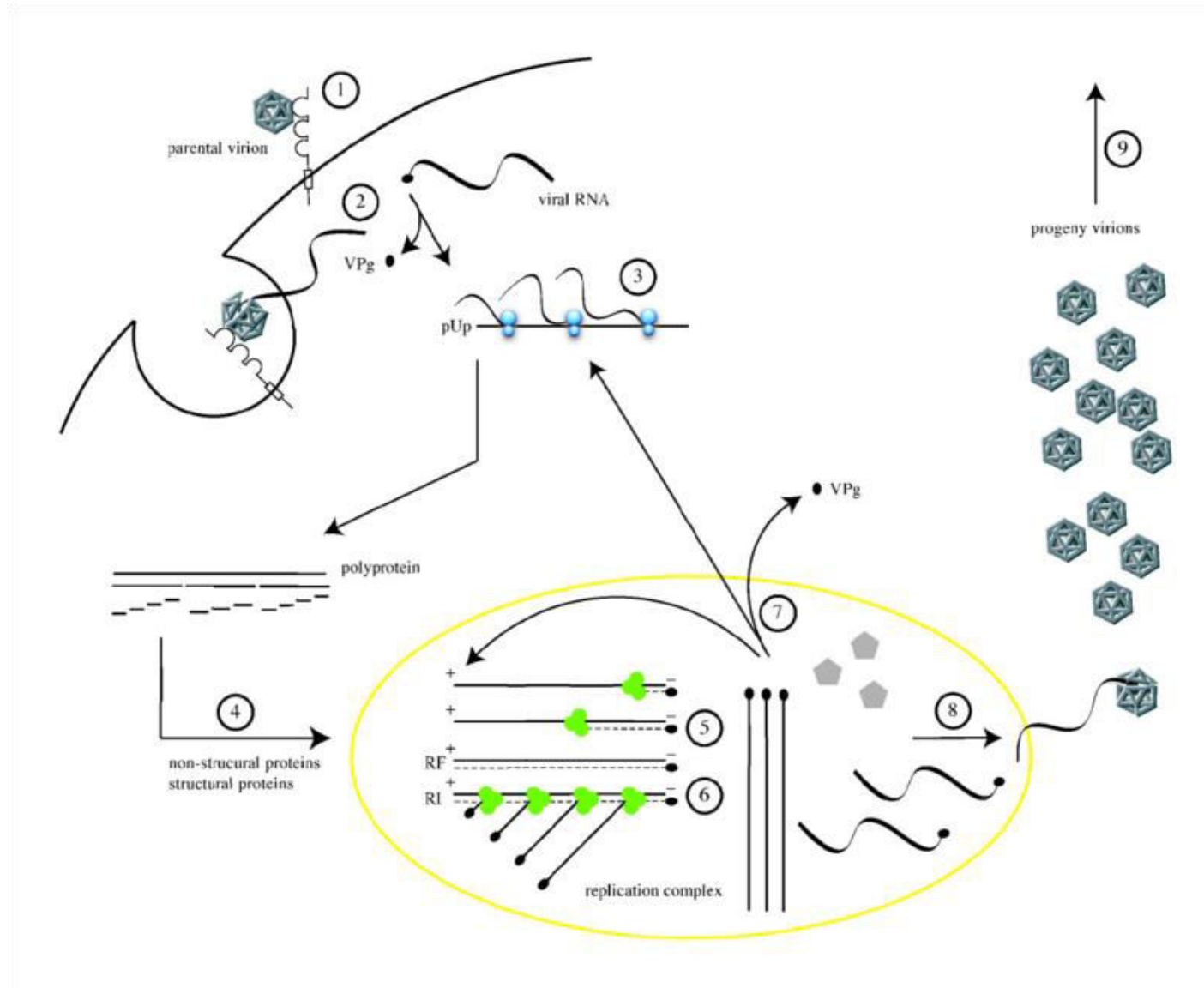
Abnormal reflexes, Back stiffness, Difficulty in lifting the head or legs when lying flat or the back stiff neck, trouble bending the neck.

Tests includes-

Cultures of throat washing, stools or spinal fluid, spinal tap and examination of the spinal fluid (CSF exam.) using PCR.

Test for levels of antibodies to the Polio virus.

Lifecycle of Poliovirus



Rabies Virus

In this lecture we will look at the **Rabies**

At the end of this lecture

Student must have fully understood:

Mechanism, Causes, Symptoms and Diagnosis &
Treatment of Rabies

Rabies

Rabies is an acute, progressive encephalomyelitis or highly fatal viral disease.

- It is an Epizootic disorder.
- Highly fatal viral disease of CNS.

History

- The first written record of Rabies is in the Mesopotamian civilization (1930 BC), which dictates that the owner of a dog showing symptoms of Rabies should take preventive measure against bites.

Causative Agents

- It is caused by the Neurotropic RNA Virus belongs to **Rhabdoviridae type I** (Lyssavirus type I) is bullet shaped virus.

Clinical Features

➤ Mainly neurologic

❖ Early signs (non-specific)

- Fever, headache, weakness, achy muscles

❖ Late signs

i-Incoordination, confusion, strange Behavior

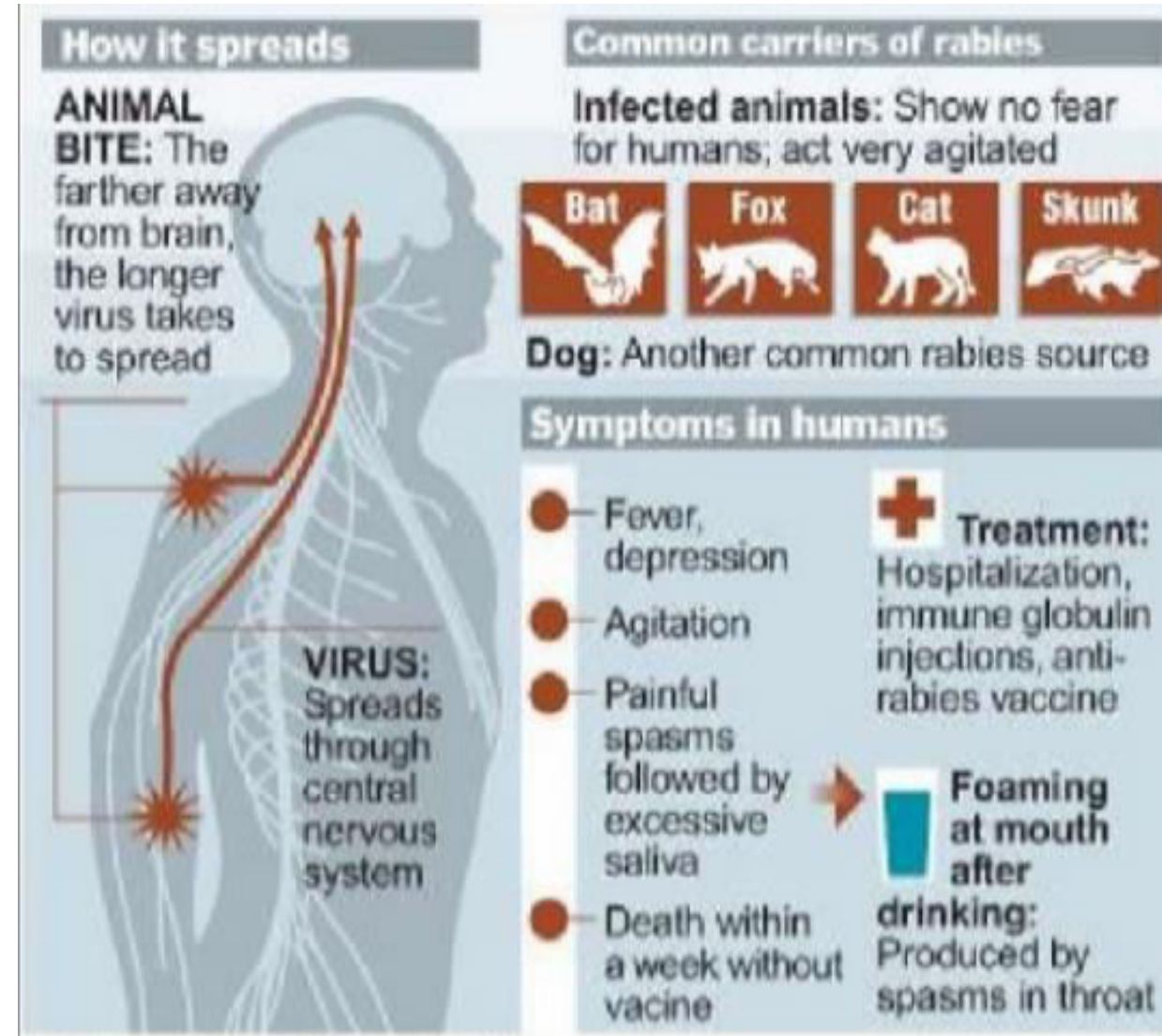
ii-Attacking and biting moving at stationary objects

iii-Salivation (can't swallow, like choking)

iv-Hydrophobia, Photophobia, Aerophobia

v-Paralysis, Seizures

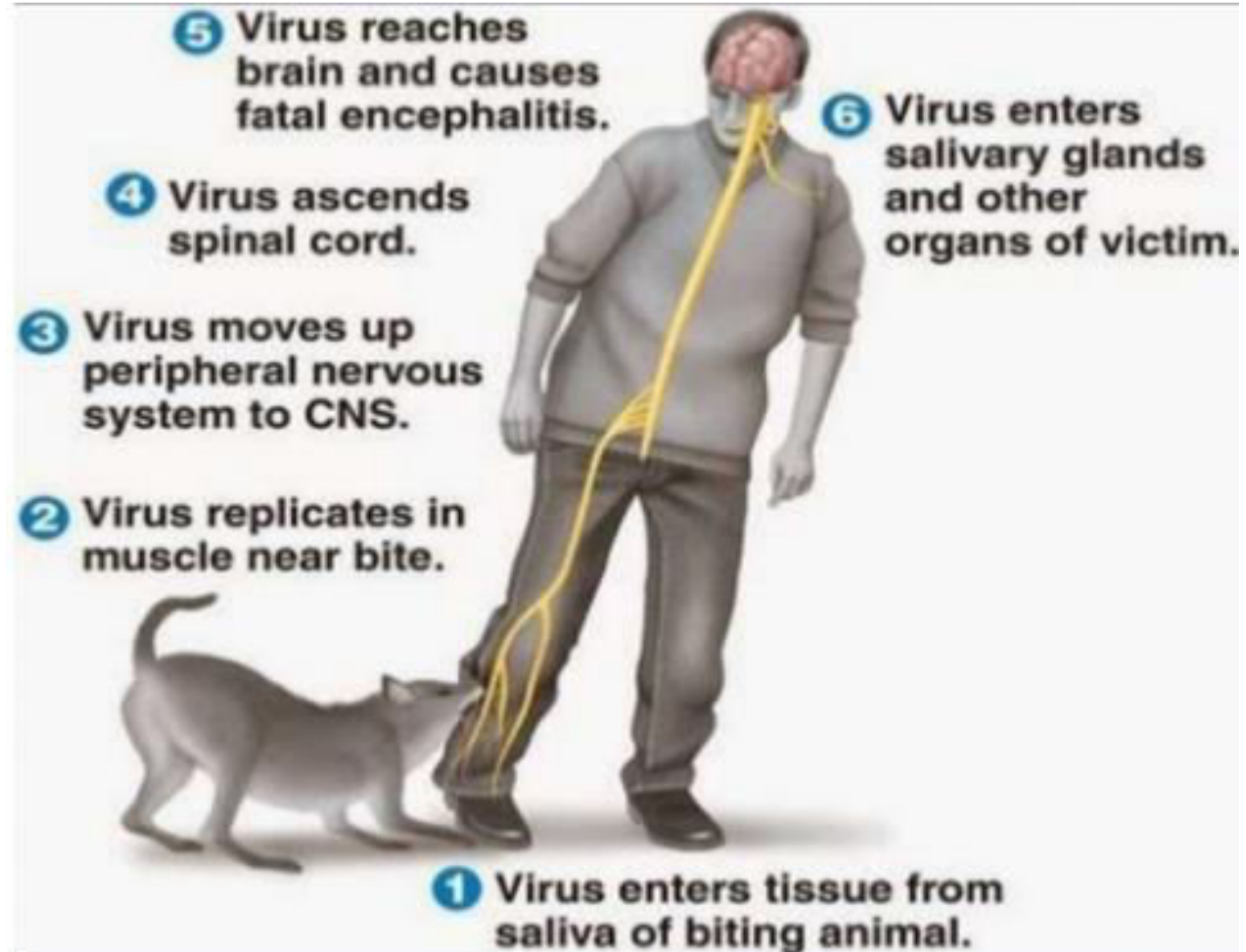
➤ Death within 2 weeks of showing signs



Pathogenesis

Stages of Rabies Infection

- Non Specific encephalitis
- Acute neurological encephalitis
- Coma
- Death



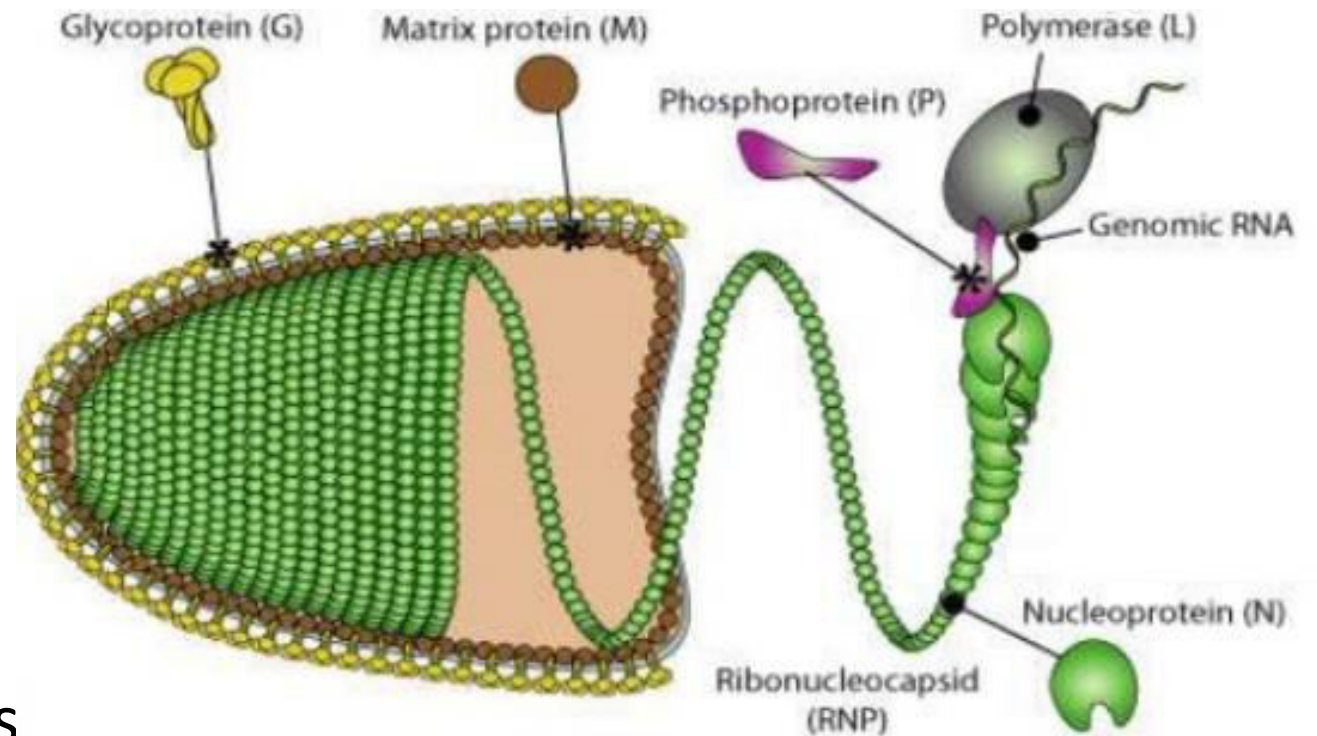
Morphology & Mode of transmission

Morphology

- Size is 180 nm
- 75 nm, bullet shaped ,
- ss RNA virus
- N proteins + RNA = nucleoprotein complex

Mode of Transmittance

- Most common modes rabies virus transmission between animals occurs as a result of a bite from a rabid animal any contact of saliva with mucous membranes(eyes, nose, mouth) or a wound
- Can also be transmitted by drinking raw milk from rabies infected animals organ transplant (human to human) kidney transfer , cornea
- Animal bites, Licks, Aerosols, Person to Person



Diagnosis, Prevention and Treatment

INCUBATION PERIOD:

- Normally 3 - 8 weeks
- May be short that is 4 days or may be prolonged for years

Diagnosis

- ❖ History
- ❖ Signs & Symptoms
- ❖ Clinical Examination
- ❖ Detection of antigen by taking skin biopsy using immunofluorescence
- ❖ Virus isolation from saliva and athersecretions
- ❖ CSF analysis and CT scan
- ❖ ELIS
- ❖ RT PCR
- ❖ DFA Testing
- ❖ Negri Bodies

Diagnosis, Prevention and Treatment

Control Measures

- ❖ Notification
- ❖ Isolation
- ❖ Disinfection
- ❖ Immunization

Vaccines for Immunization

- Nervous Tissue Vaccine (NTV)
- Duck Embryo Vaccine (DEV)
- **Cell Culture Vaccines**
 - ❖ Human diploid Cell Vaccine
 - ❖ Second generation tissue culture Vaccine
- ❖ **Passive Immunization**

Horse Anti Rabies Serum

Human rabies immunoglobulin (HRIG)

Newcastle disease

In this lecture we will look at the **Newcastle disease**

At the end of this lecture

Student must have fully understood:

Newcastle disease in birds, transmission, structure,
and morbidity and mortality rates

Newcastle disease

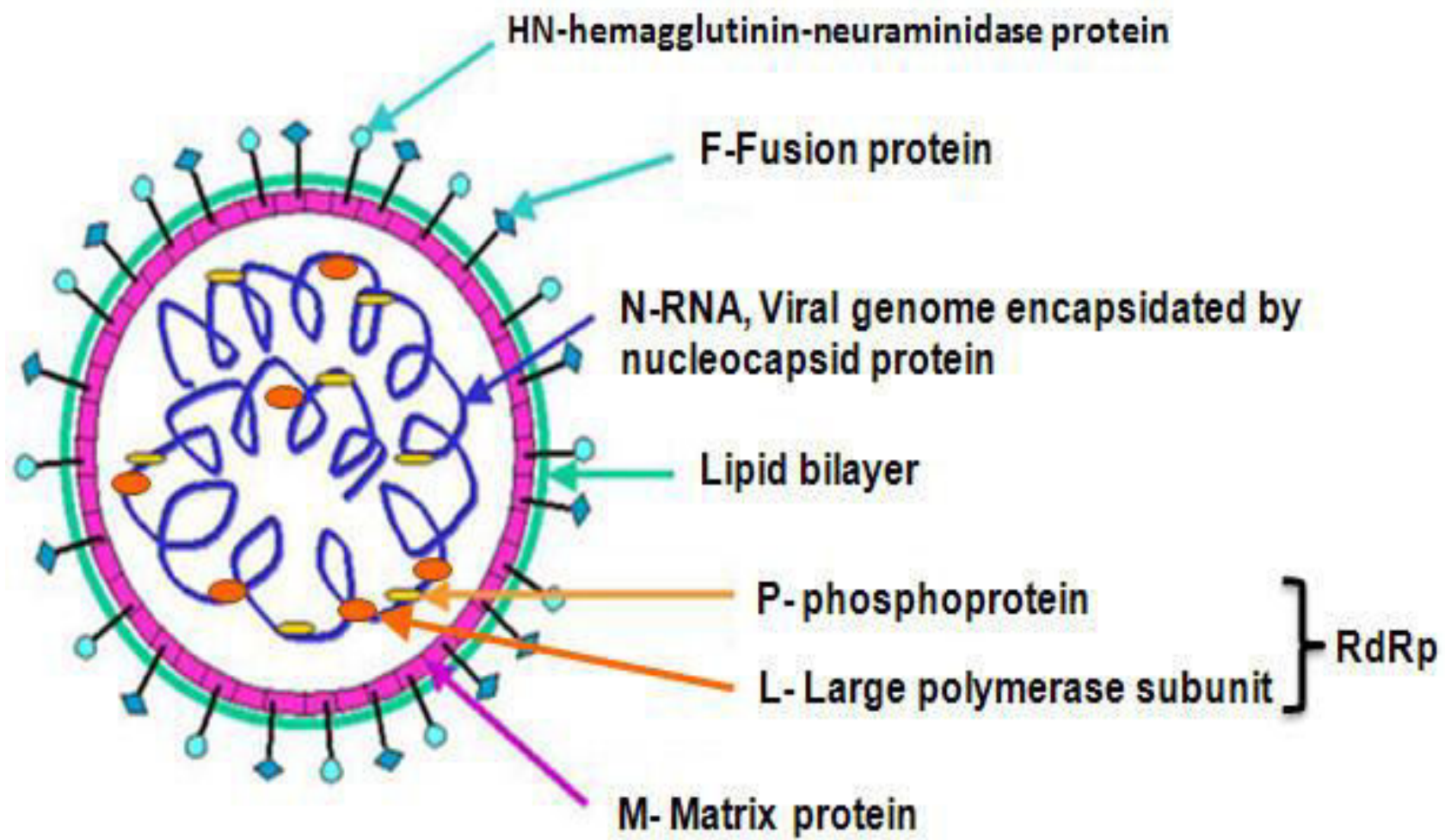
- First identified in Newcastle in 1926, (Java)
- ND is contagious and fatal viral disease affecting most species of birds (chickens, turkeys, pigeons, parrots, ducks, geese, quails) and human.
- Considered the most serious poultry disease worldwide
- Respiratory tract and multi-organ systemic disease with a near 100% mortality rate
- So rapidly acting that birds may die without showing any clinical signs
- Endemic to Asia, the Middle East and Africa – where H5N1 Avian Influenza is also established
- Occurs in Europe, Australia and North America

Newcastle disease Virus Characteristics

Taxonomy of the NDV:

- Family: Paramyxoviridae
- Subfamily: Paramyxovirinae
- Genus: Avulavirus

- The virus is enveloped, roughly spherical, with a diameter around 100-300 nm.
- Enveloped virus (containing lipid, CHO & protein).
- The genome is segmented & single stranded negative sense RNA consisting of 15,186 nucleotides.
- Two specific virus proteins (hemagglutinin-neuraminidase & fusion protein) are the main proteins found in the outer coat of the virus.
- Replication occurs in the cytoplasm of the host cell.
- Affected species; birds & human.
- Morbidity; Up to 100% & Mortality; 90%.



Newcastle disease

Strains of NDV classified according to their pathogenicity into:

- Velogenic – highly lethal to all life history stages, cause severe intestinal and/or neurologic disease resulting in high mortality

Neurotropic (Beache's form)

Viscerotropic (Doyle's form)

- Mesogenic (Beaudett's form)– deadly to embryos and younger birds, cause respiratory or nervous signs with moderate mortality.
- Lentogenic (Hitchner form)– mild or asymptomatic respiratory infection, cause mild or inapparent respiratory disease.
- Asymptomatic enteric NDV.
- Exotic Newcastle Disease = Velogenic & Mesogenic

Transmission

- Spread primarily via bodily discharges of birds
- Infected birds droppings
- Secretions from the nose, mouth and eyes
- Dissemination by contaminated animals and humans to susceptible birds
- Infected carriers (e.g., parrots) capable of shedding virus for > 1 year

Clinical Signs and Symptoms

- Signs vary with species and virulence
- Greenish, dark watery diarrhea
- Respiratory symptoms.
- Nervous signs.
- Digestive symptoms.
- Drop in egg production with thin, rough-shelled eggs.
- Swelling of tissues around eyes and in the neck.
- Sudden death.
- Surviving birds may have neurological or reproductive damage
- In human;(Mild conjunctivitis, influenza-like symptoms and laryngitis).