BT 601 PPT

VIRUSES

Definition and meaning

Virology_BT601

Introductory lecture

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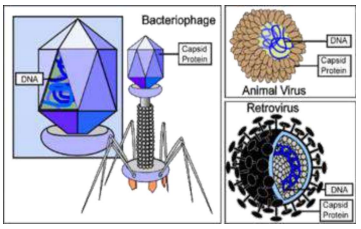
In this course



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

Virology

VIRUSES

Definition and meaning

Viruses

MEANING

- Latin word
- Meaning **poison** or **venom**
- Given by Beijerinck (1897)

Viruses

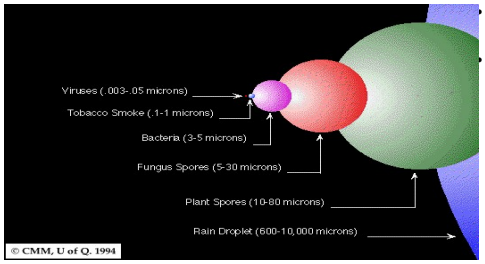
DEFINITION

- Viruses are non-cellular particles made up of genetic material and proteins that can invade living cells
- Obligate intracellular parasites

Viruses

INTERESTING FACT

- Submicroscopic
- Smaller than bacteria
- Smaller than the smallest cell



Viruses

TAKE HOME QUESTION!

Can you find the size range of viruses?

Virology

GENERAL PROPERTIES OF VIRUSES

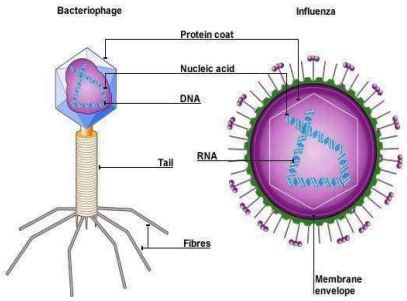
GENERAL PROPERTIES OF VIRUSES

VIRIONS

- Mature viruses
- Variable in size

VIRUS	SIZE
Foot and Mouth Disease virus	27nm
Pox virus	300nm

GENERAL PROPERTIES OF VIRUSES



STRUCTURE

1. Protein
2. Nucleic acid

Deoxyribonucleic Acid (DNA)

Ribonucliec Acid (RNA)

GENERAL PROPERTIES OF VIRUSES

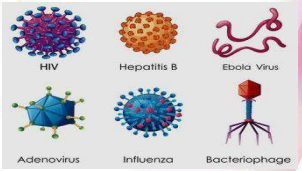
WHY VIRUSES NEED HOST?

- No enzymes of their own
- No metabolism
- Require host machinery

GENERAL PROPERTIES OF VIRUSES

COMPLEX VIRUSES

- Additionally contain:
  - Lipid
  - Polysaccharides
  - Trace elements

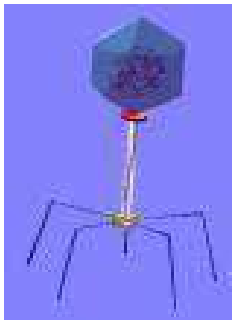


Virology

GENERAL PROPERTIES OF VIRUSES

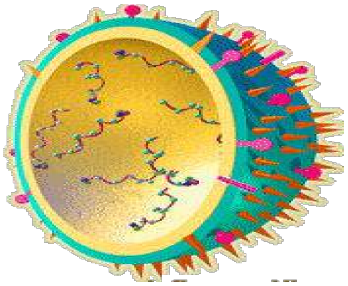
GENERAL PROPERTIES OF VIRUSES

- Non living structures
- Non-cellular
- Contain a protein coat called the capsid
- Have a nucleic acid core containing DNA or RNA (one or the other - not both)
- Capable of reproducing only when inside a HOST cell



GENERAL PROPERTIES OF VIRUSES

- Some viruses are enclosed in an protective envelope
- Some viruses may have spikes to help attach to the host cell
- Most viruses infect only specific host cells



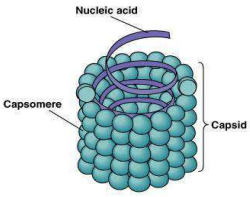
GENERAL PROPERTIES OF VIRUSES

MORPHOLOGY

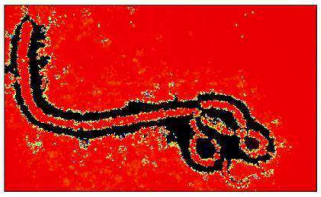
- Viruses come in a variety of shapes
- Some may be helical shape like the Ebola virus
- Some may be polyhedral shapes like the influenza virus
- Others have more complex shapes like bacteriophages

GENERAL PROPERTIES OF VIRUSES

Types of Viruses: Helical Viruses



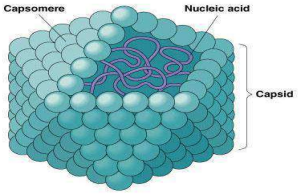
(a) A helical virus



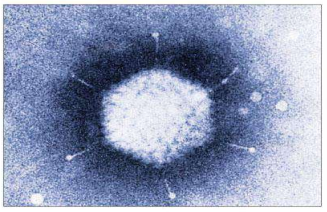
(b) Ebola virus

GENERAL PROPERTIES OF VIRUSES

Polyhedral Viruses



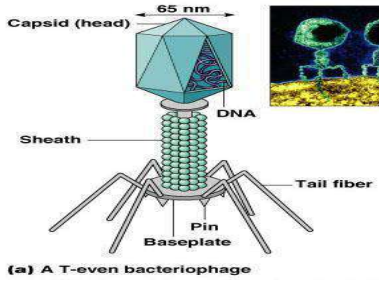
(a) A polyhedral virus



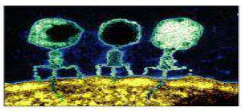
(b) A *Mastadenovirus*

GENERAL PROPERTIES OF VIRUSES

Complex Viruses



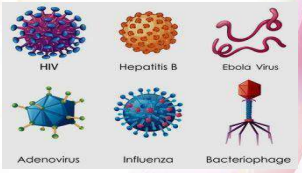
(a) A T-even bacteriophage



GENERAL PROPERTIES OF VIRUSES

COMPLEX VIRUSES

- Additionally contain:
  - Lipid
  - Polysaccharides
  - Trace elements



Virology

Discovery of viruses

Discovery of viruses

Ancient Times

- First written record
- From Egypt around 3700BC
- Signs of poliomyelitis in a priest

Discovery of viruses

Small pox

- Edward Jenner (1796)
- Smallpox vaccine using milder cowpox virus
- No knowledge of viral particles

Discovery of viruses

Iwanowski

- In 1892, Dmitri Iwanowski found out reason for disease in plants
- He thought the bacteria causing disease is small enough to pass the smallest filters
- He did not know the significance of his results

Discovery of viruses

The name "VIRUS"

- In 1898
- By Beijernick
- Studied filtered diseased plant juices and found the caused disease in healthy plants

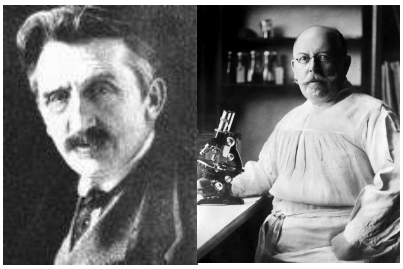
Discovery of viruses

Tobacco Mosaic Virus3

- Wendell Stanley (1935) discovered that:
- Viruses are composed of protein and nucleic acid
- Studied Diseased tobacco plants
- Discovered isolates of virus
- Won Nobel award

Discovery of viruses

Foot and Mouth disease



- In 1898
- Recognized by
- Fredrich Loeffler
- Pal Froscch
- Disease in cattle

Discovery of viruses

Poliomyelitis

- 1909
- Karl Landsteiner and Erwin Popper
- First human virus to be found

Discovery of viruses

Bacteriophages

- Fredrick Twort (1915) and Felix D'Herelle (1917)
- Recognized viruses that infect bacteria
- Bacteriophages (eaters of bacteria)

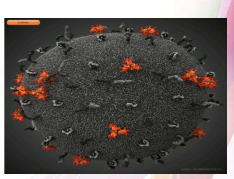
END slide

Virology

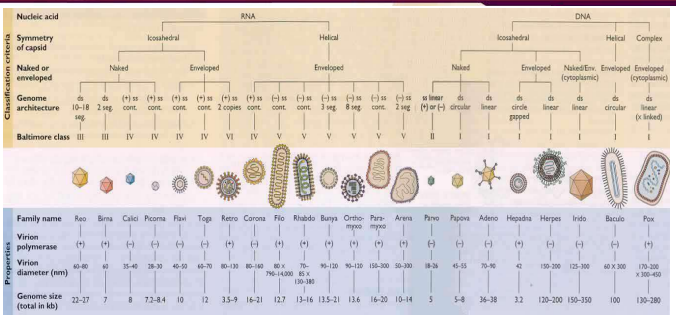
GENERAL CLASSIFICATION OF VIRUSES

Viral Classification

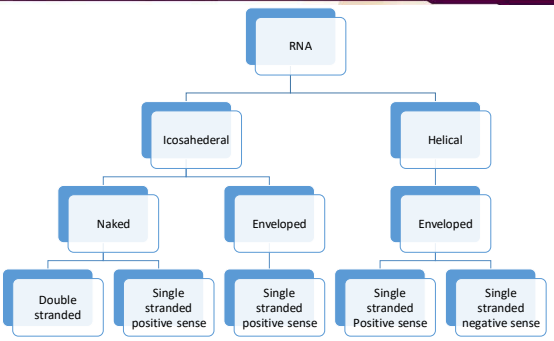
- Naming of organisms and categorizing them
- Based on a certain factors like phenotype, nucleic acid type, replication etc



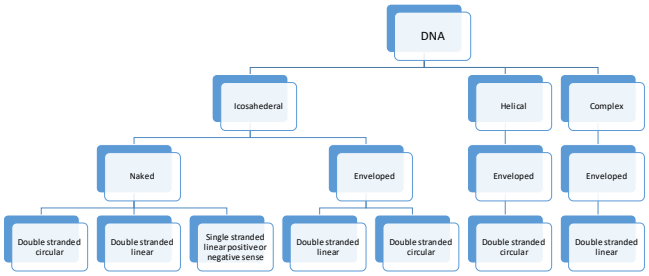
Viral Classification



Viral Classification



Viral Classification



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)

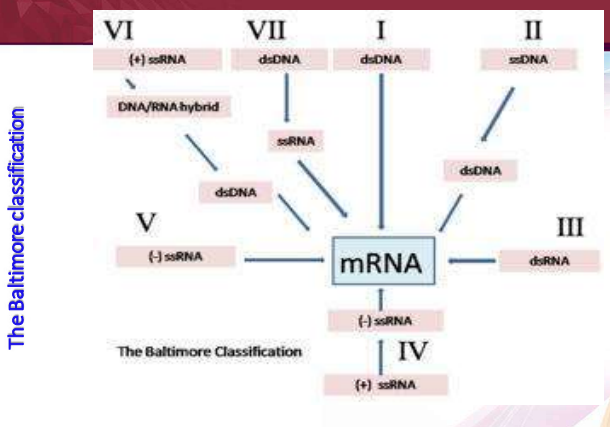
III: dsRNA viruses (e.g. Reoviruses)

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)



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

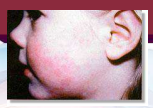
Genus *Iteravirus*
Genus *Brevidensovirus*
Genus *Ambidensovirus*
Genus *Hepamdensovirus*
Genus *Penstyldensovirus*

>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 genuses include

Genus Atadenovirus; type species: Ovine atadenovirus D

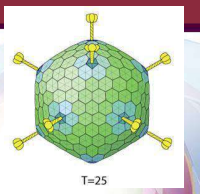
Genus Aviadenovirus; type species: Fowl aviadenovirus A

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. Lymphnproliferative Virus group called Gamma Herpesvirinae

Herpesviridae

Subfamily: Alphaherpesvirinae

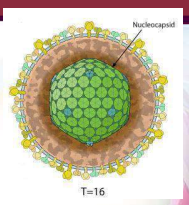
Genus: Iltovirus, Mardivirus , Scutavirus , Simplexvirus , Varicellovirus

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

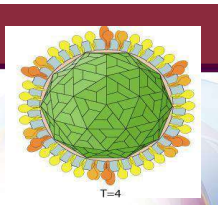
Hepadnaviridae

- 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 hepadnaviurses 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
- Ttransmitted 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

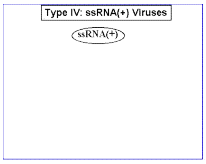
Virology

CLASSIFICATION OF POSITIVE SENSE RNA VIRUSES

Classification of RNA viruses

RNA VIRUS

- Ribonucleic acid as genome
- Single stranded or double stranded
- Belongs to Group III, Group IV or Group V of Baltimore Classification system



Classification of RNA viruses

DOUBLE STRANDED RNA VIRUSES

FAMILY	GENUS	TYPE SPECIES
Birnaviridae	Avibirnavirus Aquabirnavirus	Infectious bursal disease virus Infectious pancreatic necrosis virus
Reoviridae	Orthoreovirus Orbivirus Rotavirus Coltivirus Aquareovirus	Mammalian orthoreovirus Bluetonue virus-1 Rotavirus A Colorado tick fever virus Aquareovirus A

Classification of RNA viruses

SINGLE STRANDED RNA VIRUSES

FAMILY	GENUS	TYPE SPECIES
Arteriviridae	Arterivirus	Equine arterivirus
Astroviridae	Astrovirus	Human astrovirus-1
Calciviiridae	Vesivirus Lagovirus Hepatitis E like virus	Swine vesicular exanthema virus Hepatitis E virus
Coronaviridae	Coronavirus Torovirus	Infectious bronchitis virus Equine torovirus
Flaviviridae	Flavivirus Hepacivirus pestivirus	Yellow fever virus Hepatitis C virus Bovine viral diarrhea
Picornaviridae	Enterovirus Rhinovirus Hepatovirus	Poliovirus Human rhinovirus A Hepatitis A virus
Togaviridae	Alphavirus Rubivirus	Sindbis virus Rubella virus

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 repiratory 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 60-8Onm

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:

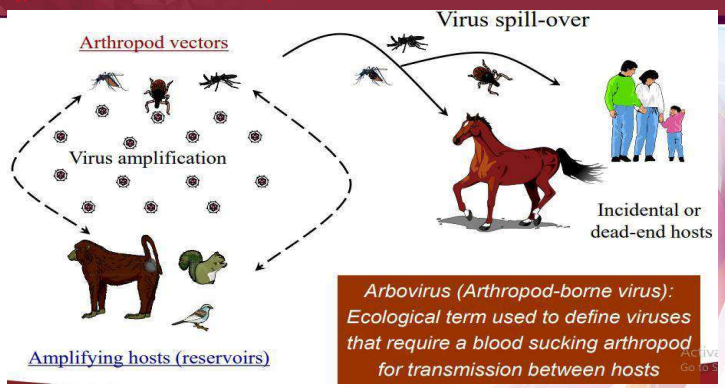
- Reoviruses of human include rotaviruses, which cause infantile gastroenteritis and have distinctive wheel-shaped appearance
- Orbiviruses: Colorado tick fever virus of humans and blue-tongue of cattle and sheep

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

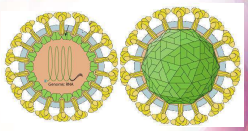
Student must have fully understood:

- 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 to13 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:

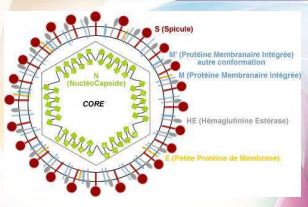
Old World and New World viruses

- 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

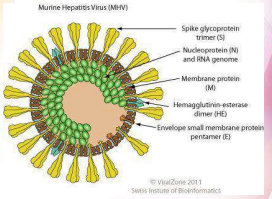
Coronaviridae

- 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

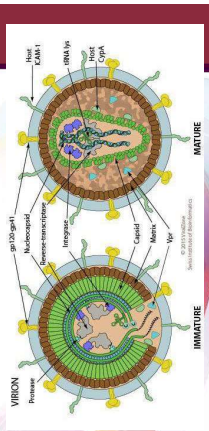
Classification of RNA Viruses

Retroviridae

- Single standed(+) sense RNA genome 7-11 kb
- 5'cap, 3'poly(A) tail
- Retrovirus virionsare 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

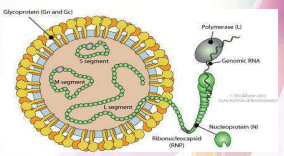
- Negative-sense single-stranded RNA viruses
- A majority of bunyaviruses are vector-borne
- Generally found in arthropods (mosquitos, tick, or sandfly) or rodents
- five genera viruses:

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,
  - Quaranjavirus
  - 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

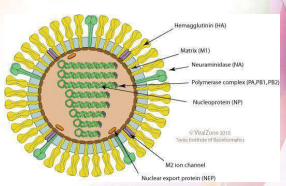
Orthomyxoviridae

- ✓ 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:

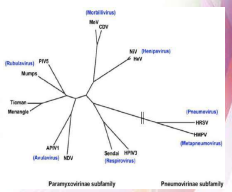
- characteristics of *Paramyxoviridae*
- Common disease cause by paramyxoviruses

Classification of RNA Viruses

Paramyxoviridae

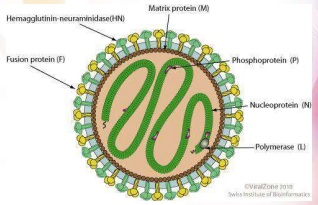
- Single-stranded, negative-sense RNA viruses
- Vertebrates serve as natural hosts
- 49 species in this family, divided among 7 genera

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

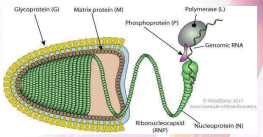
Student must have fully understood:

- characteristics of *Rhaboviridae and Toroviridae*

Classification of RNA Viruses

Rhaboviridae

- 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, vertebrates, 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 melding 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

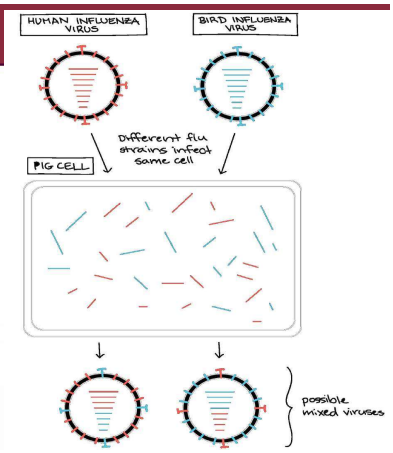
Genetic variation

- 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

A fatal, degenerative disease in sheep

- 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

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

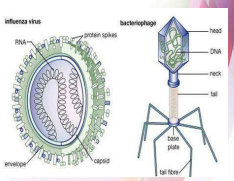
- Genetic materials
- Capsid
- Envelope

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 dissassembly

Nucleic acid

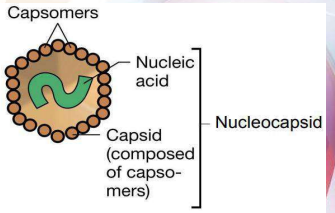
- A virus can have either DNA or RNA—but never both
- Nucleic acid of a virus can be single-stranded or double-stranded
- Can be linear or circular
- Influenza virus: the nucleic acid is in several separate segments



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

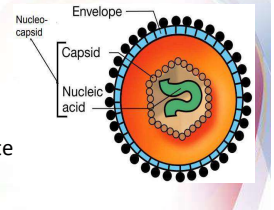
The capsid (coat) protein is the basic unit of structure; functions that may be fulfilled by the capsid protein are to:

- 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 associate with spikes
- Viruses whose capsids are not covered by an envelope are known as nonenveloped viruses

Virology

STRUCTURE AND FORMATION OF VIRAL PARTICLES

Structure and Formation of Viral particles

Viral Capsids and Envelopes

Structure and Formation of Viral particles

CAPSID

Virus particles contain the viral genome packaged in a protein coat called the capsid.

Structure and Formation of Viral particles

ENVELOPE

Surrounding the capsid

composed of lipid bilayer that contains viral proteins

derived from the host cell membranes.

Structure and Formation of Viral particles

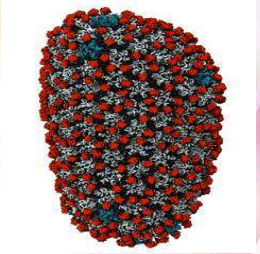
FUNCTIONS

The capsid and envelope play many roles in

viral infection, including virus attachment to cells, entry into cells, release of the capsid contents into the cells, and packaging of newly formed viral particles. The capsid and envelope are also responsible for transfer of the viral genetic material from one cell to another. These structures also determine the stability characteristics of the virus particle, such as resistance to chemical or physical inactivation.

Virology

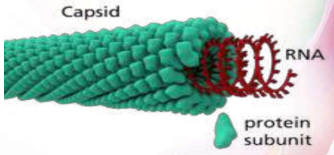
VIRAL CAPSID



Viral Capsid

MEANING

- Protein shell of virus
- Made up of protein sub-units (protomers)
- Encloses genome



Viral Capsid

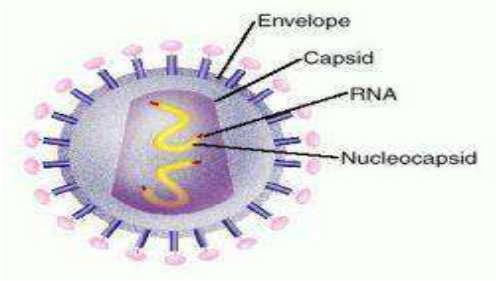
FUNCTION

- Protection of genome
- Prevent digestion by cellular enzymes
- In cytoplasm Helps to introduce genome in host cell
- Bind to cell surface

Viral Capsid

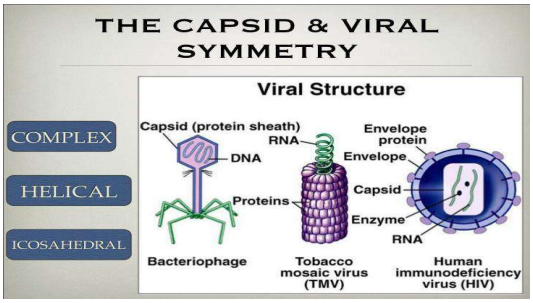
NUCLEOCAPSID

Genome plus capsid Collective term



Viral Capsid

TYPES



Virology

BACTERIOPHAGES

Introduction

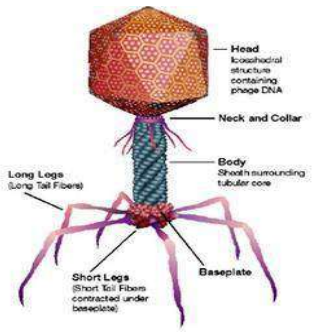
Bacteriophages

INTRODUCTION

- Viruses which infect and replicate in the bacterial cell are called Bacteriophages
- Morphologically diverse
- Some filamentous with helical symmetry
- Others icosahedral

Bacteriophages

Parts



1. Head
2. Tail

Bacteriophages

Genome

- Inside head
- DNA/RNA

Bacteriophages

END

Replication

- Attachment
- Penetration
- Uncoating
- Biosynthesis
- Release

Virology

Relication of T-Phages

T-phages

Replication

- Attachment
- Penetration
- Uncoating
- Biosynthesis
- Release

T-phages

Attachment

Specific receptors

Techoic acid

Lipids

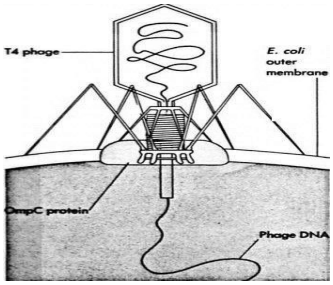
Proteins

flagella

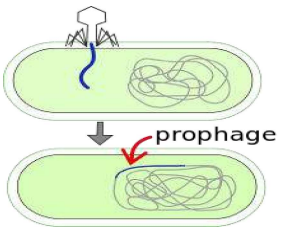
On specific bacterial hosts

T-phages

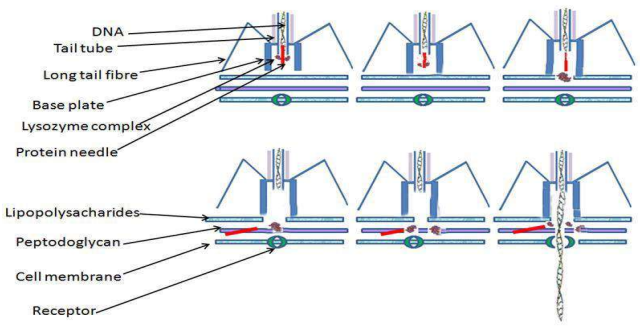
Penetration



- Leave capsid outside

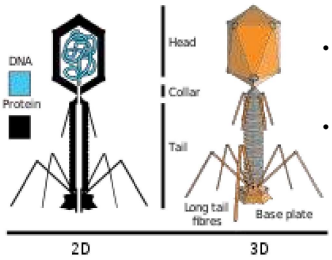


T-phages



T-phages

Biosynthesis



- Bacterial ribosomes translate viral proteins
- Proteins modify bacterial RNA polymerase
- Proteins for new virions

T-phages

Assembly

- T4: helper proteins required
- Base plates assembled first
- Tails built afterwards
- Heads constructed separately
- Whole process takes 15 minutes

T-phages

Release

- Lysis
  - ❖Tailed phages
  - ❖endolysin
- Extrusion
  - ❖enveloped
- Budding
- Secretion

END

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

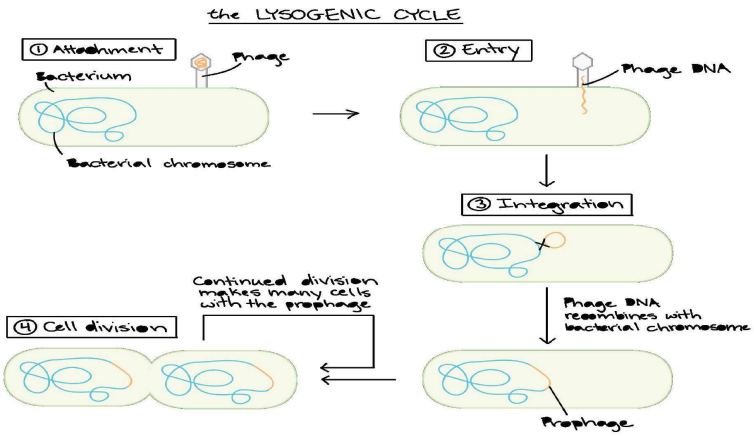
IV. Integration of Viral genome to the host genome:

- 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



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 call 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

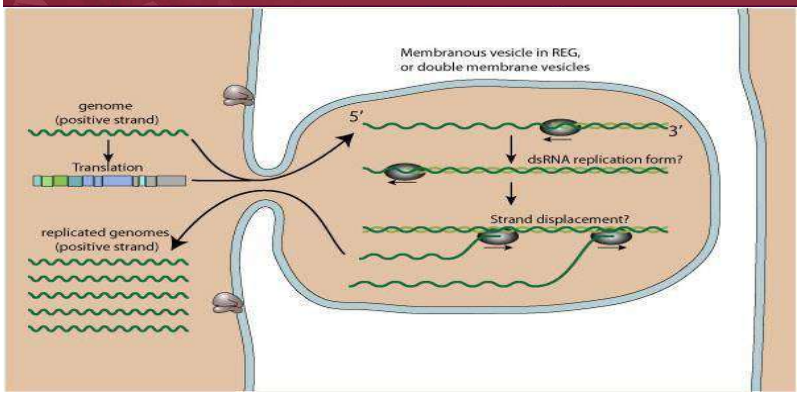
Positive-Strand RNA Viruses Replication

- 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

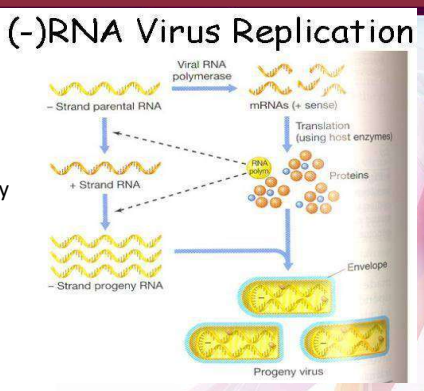
- Virus enters the cell by receptor mediated endocytosis and releases its negative RNA into the cytoplasm
- The viral replication process begins at 3' end and forms a complete positive sense RNA using negative sense genomic template

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.



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)

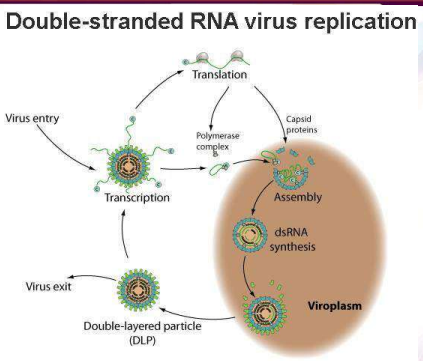
Location of Replication

- 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 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

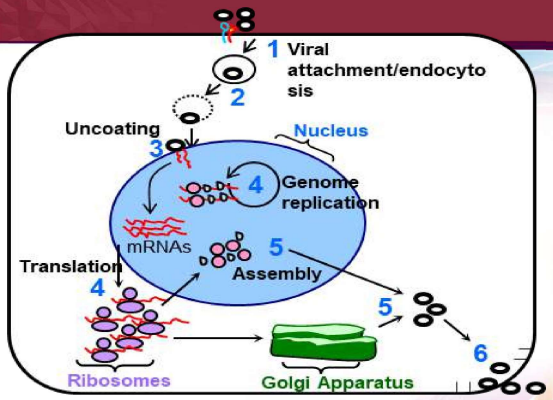
-encode proteins that orchestrate the host
-Papillomaviridae, Polyomaviridae, Parvoviridae

- Large DNA viruses encode most of their own replication systems

-Herpesviridae, Adenoviridae, Poxviridae

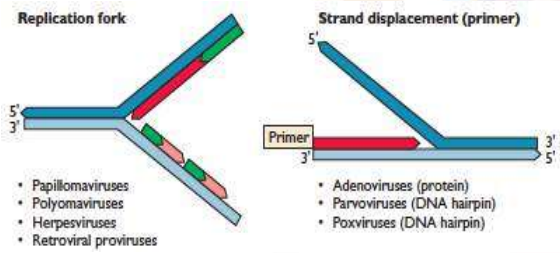
Virus Encoded Proteins

- ❖ 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



Legging

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.

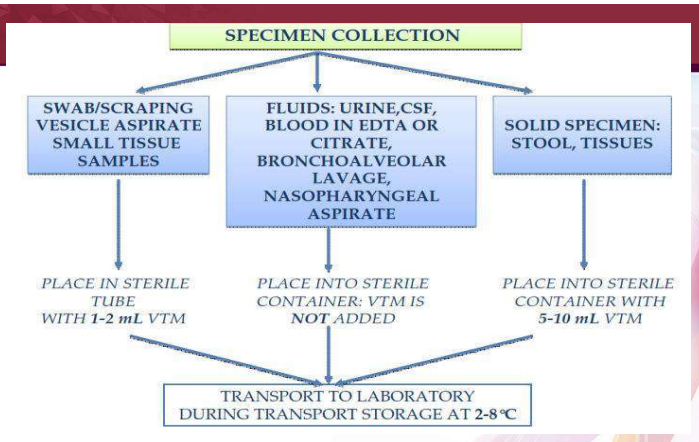
UTM™ Viral Transport Media

- 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



SWABS

- For diagnosis of viral infections

Swabs should be:

- made of reyon
  - 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

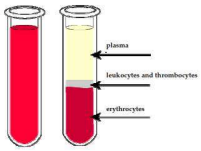
Collection of specimen

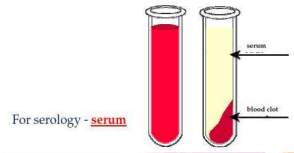
Blood

- 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





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

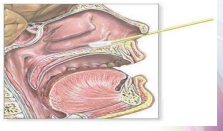
Urine

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

Specimen from upper respiratory tract

Nasopharingeal swab

through nostrile to nasopharincs place in VTM



Nasopharingeal wash

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

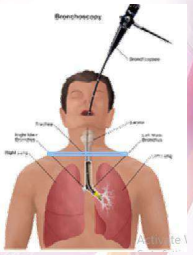
Specimen from lower respiratory tract

Bronchoalveolar lavage (BAL)

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

Sputum

- Not useful for viral detection



Specimen from gastrointestinal tract

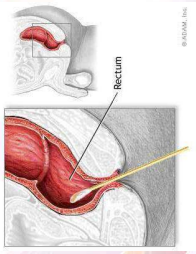
Feces

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 phisical 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:

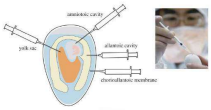
I. To isolate and identify viruses in clinical specimens
II. To prepare viruses for vaccines
III. And to do detailed research on viral structure, multiplication cycles, genetics, and effects on host cells

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:

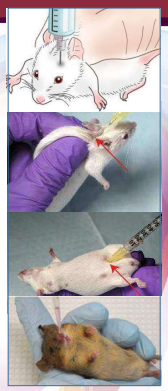
How viruses are inoculated into living Animals for cultivation

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, epidemology and oncogenesis

Advantages and disadvantages of animal inoculation

Disadvantages

- Expensive and difficulties in maintaince 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

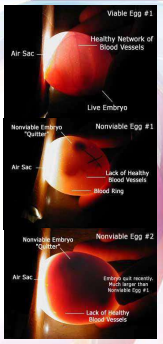
- An embryo is an early developmental stage of animals marked by rapid differentiation of cells
- Birds undergo their embryonic period within the closed protective case of an egg, which makes an incubating bird egg a nearly perfect system for viral propagation
- It is an intact and self-supporting unit, 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

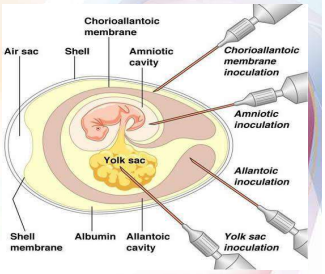
Parts of Embryonated Egg

- 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

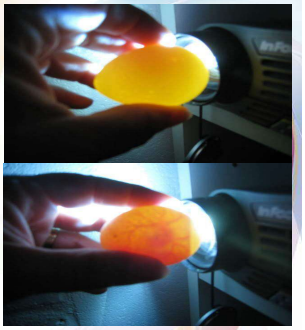
- An embryonated egg offers various sites for the cultivation of viruses
- The different sites of viral inoculation in embryonated eggs are:

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.
- The 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^{o}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^{o}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.

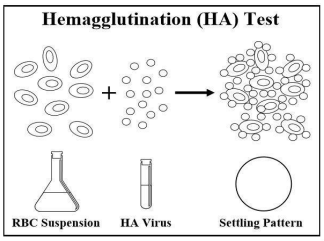
Titer: The maximum dilution that gives visible agglutination

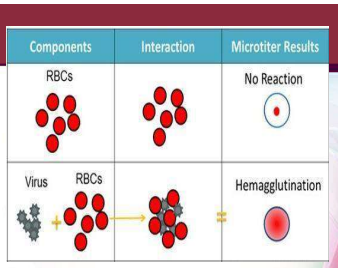
Haemagglutination Assay (HA)

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.





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

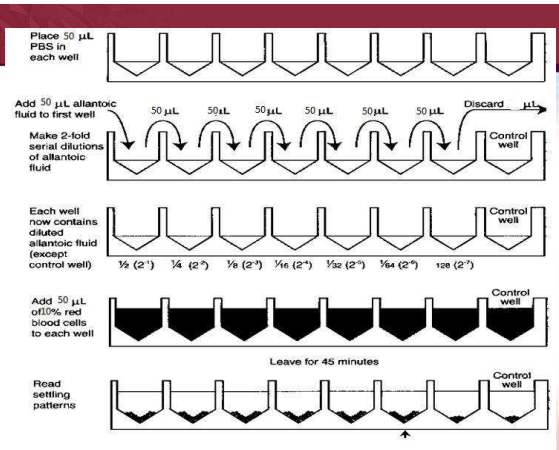
- PBS (Phosphate buffer saline)
- RBCs (10%)

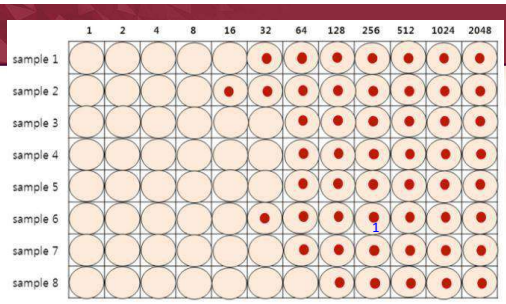
RBCs Preparation

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 µl PBS.
3. In the first column, add 50 µl of allantoic fluid sample.
4. Mix each well and transfer 50 µl to the next well on its right. Repeat mixing and transferring 50 µl down the length of the plate. Discard 50 µl from the last well into a bleach solution.
5. Add 50 µl 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.





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 haemagglutin 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 specie.

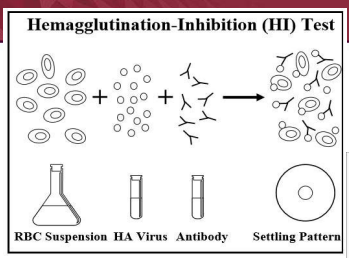
Haemagglutination Inhibition Assay (HIA or HI)

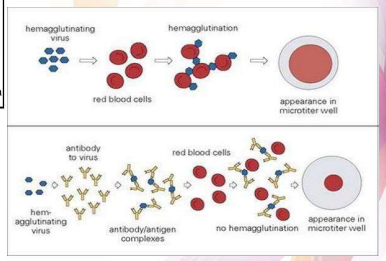
The Hemagglutination Inhibition Assay can be used to prevent RBCs from binding to viruses through the addition of specific antibodies.

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 Infleunza are highly specific to the antibodies used within HAI which makes the use of this method highly effective for diagnosis.

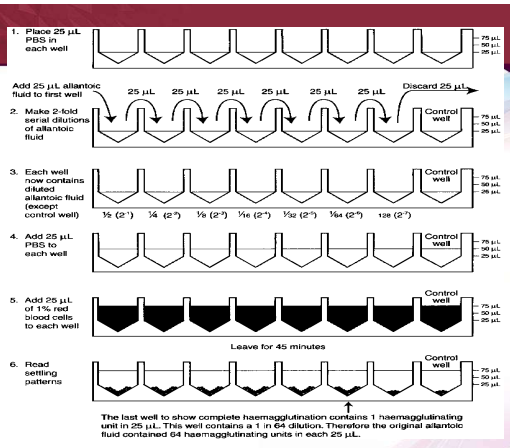
Other viruses such as Flaviviruses have been found to cross react to other related viruses which make the use of the HAI test less sensitive and specific.

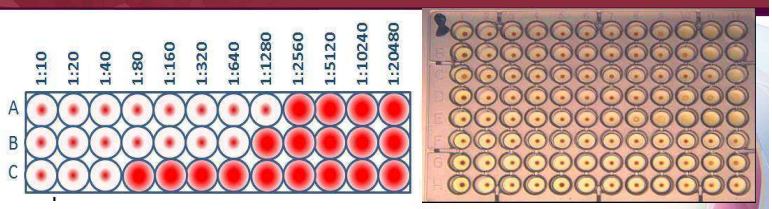




Haemagglutination Inhibition Assay (HIA or HI)

1. Dispense 25 µL of PBS into each well of the 96 microwell plate.
2. Shake each serum sample and dispense 25 µL 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 µL 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 µL 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.





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 ul = reciprocal of highest dilution
e.g. an endpoint of 1:8 = 8 HIU/50 ul
At this dilution, the antibodies were still capable of recognizing and binding to the antigens on the virus.

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.

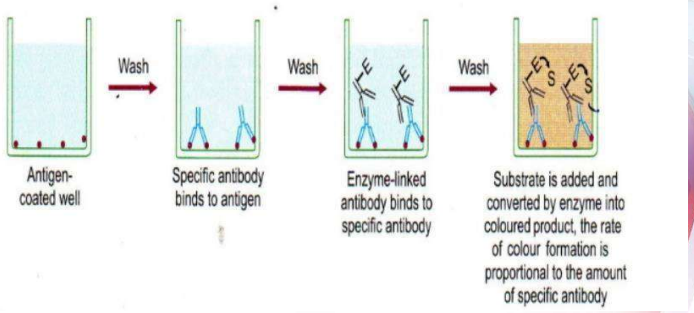
In this technique, antigen is coated on the microtiter well. Serum or some other sample containing primary antibody is added to the microtiter well and allowed to react with the coated antigen.

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.



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)

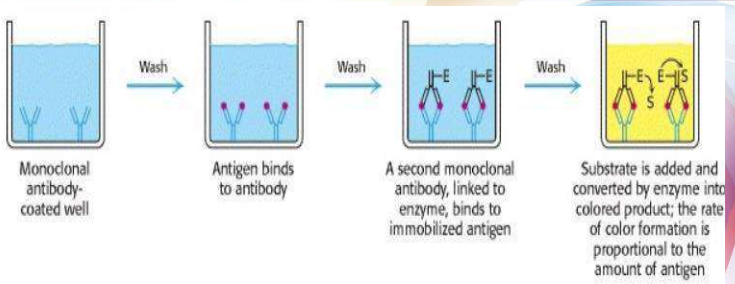
At the end of this lecture

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.



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

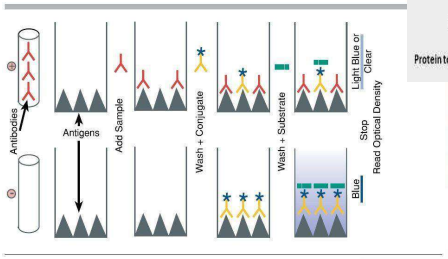
The steps competitive ELISA are:

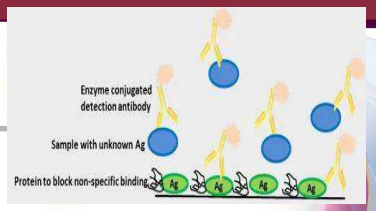
- Unlabeled antibody is incubated in the presence of its antigen (sample).
- These bound antibody/antigen complexes are then added to an antigen-coated well.
- The plate is washed, so unbound antibodies are removed. (The more antigen in the sample, the more Ag-Ab complexes are formed and so there are 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 65ºC 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 42ºC for 50 minutes.
- Heat inactivate the enzyme at 65ºC to 70ºC 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 -20ºC)
- Incubate sample at 37ºC 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 -20ºC 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)

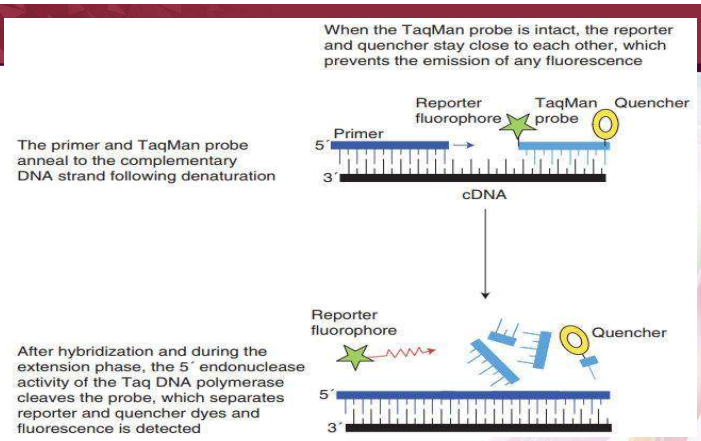
- Quantitative PCR utilizes polymerase chain reaction chemistry to amplify viral DNA or RNA to produce high enough concentrations for detection and quantification by fluorescence.
- Quantitative detection can be achieved using a wide variety of fluorescence detection strategies, including sequence specific 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

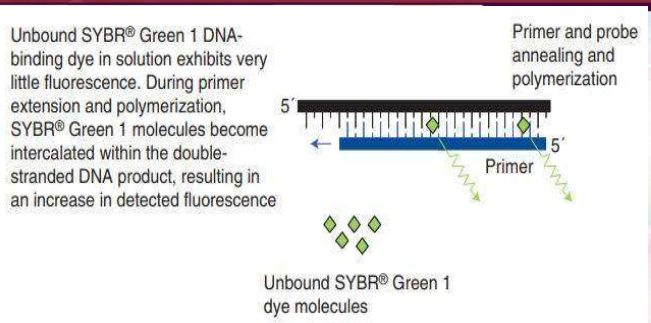
TaqMan Probe

- 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.



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



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 (JPV).

Plaque Assay

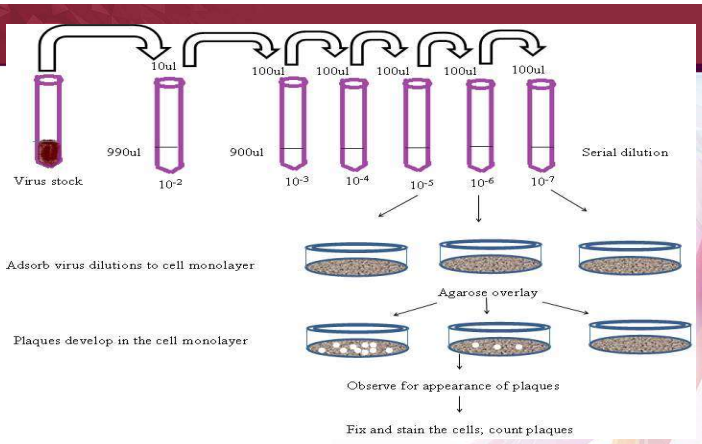
- Plaque: an area of cells in a monolayer which display a cytopathic effect, e.g. appearing round and darker than other cells under the microscope, or as white spots when visualized by eye; the plaque center may lack cells due to virus-induced lysis
- Plaque-forming unit (PFU): a virus or group of viruses 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 x 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°C water bath. Prewarm culture media to 37°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 $TCID_{50}$/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 $TCID_{50}$ 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-Karber
  - 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 Immunodeficience 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 contamined fluids or blood,
- use of contamined injection materials (syringues, catheters), or prosthetic devices,
- and the transplantation of noncontrolled contamined 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 oughing 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:

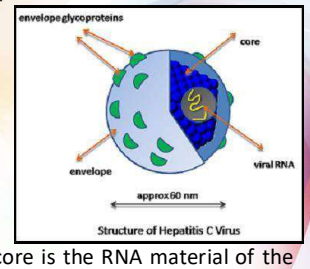
Structure, Mode of transmission, Mechanism, Causes, Symptoms Diagnosis and Treatment of HCV

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

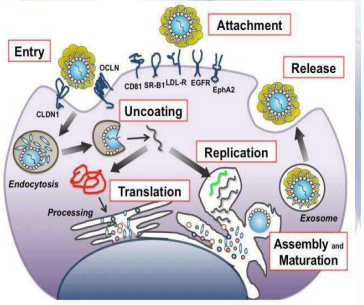
4-Translation: the genomic RNA is directly translated in a polyprotein precursor

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, andsexual transmission
- Transmission through occupational, perinatal, and sexual routes is less efficient as compared to transmission through large or repeated percutaneous exposures.

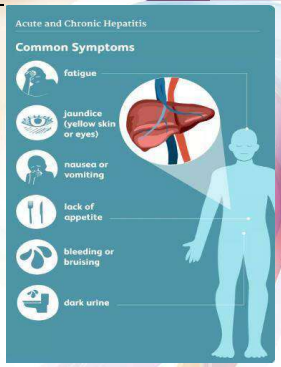
Diagnosis, Prevention and Treatment

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 treatmen. 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 reteroviruses 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

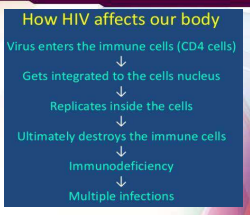
Clinical Manifestations

Four stages of HIV infection:

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

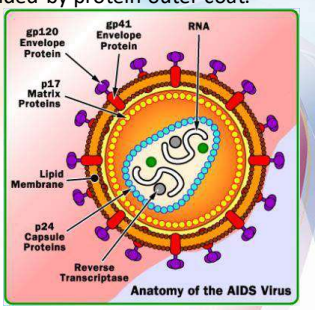
AIDS

- 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

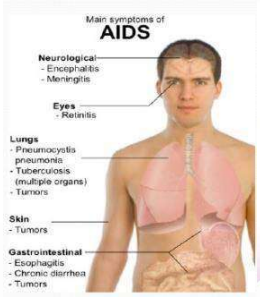
Lab Diagnosis of HIV

Direct Tests

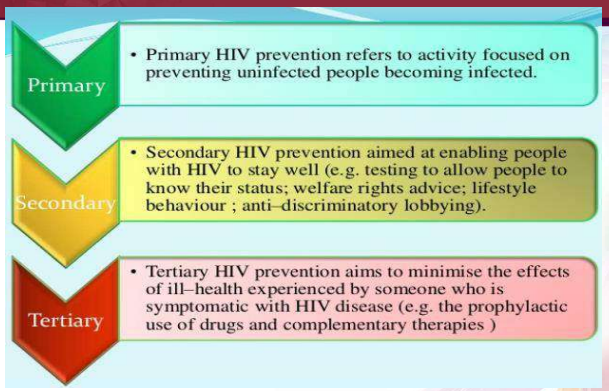
- ELISA (enzyme-linked-immunosorbent-serological assay
- Recombinant DNA Techniques
- Viral Isolation in culture
- PCR

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

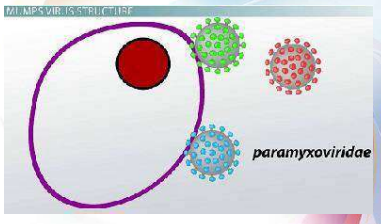
- **Measles** is a highly contagious infectious disease caused by the **measles** virus.
- It is a childhood infection caused by Virus.
- Also called rubeola
- Measles can be serious and even fatal for small children. While death rates have been falling worldwide as more children receive the measles 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.

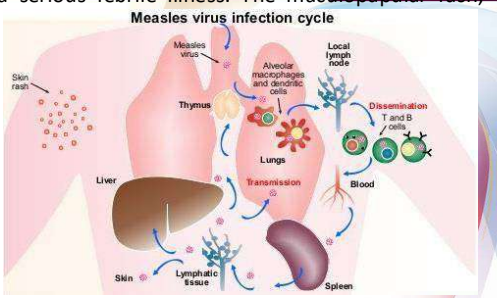


Virulence Factors

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 in 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:
       - he 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 Mesales

- 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.

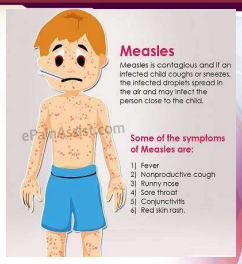
Reservoir

- 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 encephaletis 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), bubar polio (affects the brainstem, & bulbospinal polio (affects both the spine and brainstem). Occurs in 0.1-2% cases

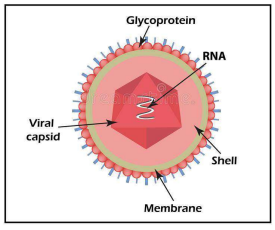
Structure & Transmission of Poliovirus

- **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:-

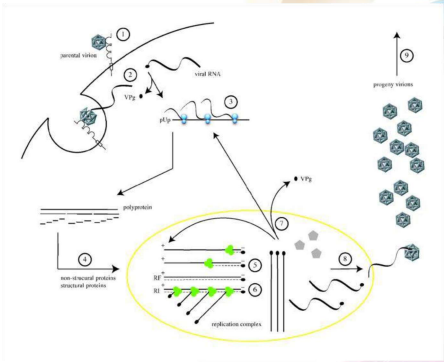
Abnormal reflexes, Back stiffness, Difficulty in lifting the head or legs when lying flat or the back stiff neck, trouble bending the neck.

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

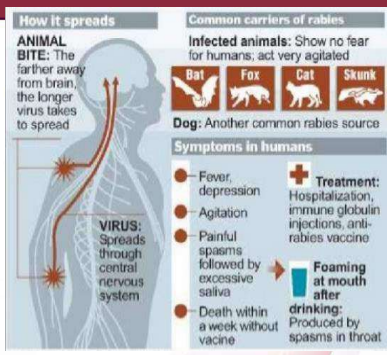
ii-Attacking and biting moving at stationary objects

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

iv-Hydrophobia, Photophobia, Aerophobia

v-Paralysis, Seizures

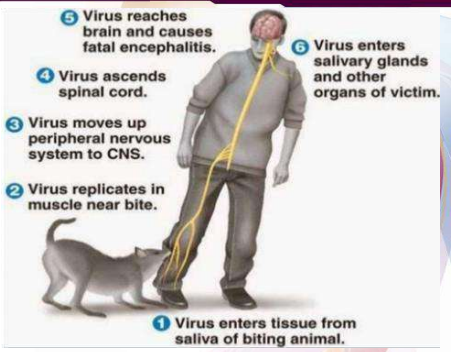
- Death within 2 weeks of showing signs



Pathogenesis

Stages of Rabies Infection

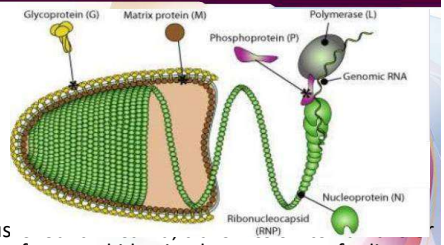
- Non Specific encephalitis
- Acute neurological encephalitis
- Coma
- Death



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 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 immunoflourescence
- ❖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 immunoglobin (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

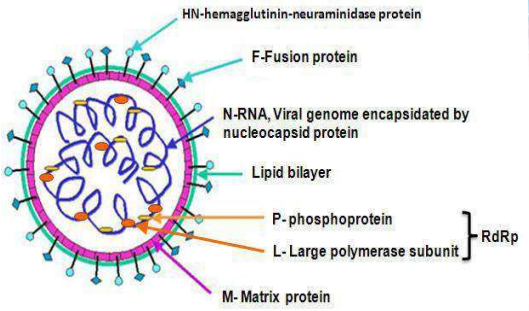
- First identified in Newcastle in 1926, (Java)
- ND is contagious and fatal viral disease affecting most species of birds (chickens, turkeys, pigeons, parrots, ducks, geese, quails) and human.
- Considered the most serious poultry disease worldwide
- Respiratory tract and multi-organ systemic disease 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 to100% & 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 neurologicdisease 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).

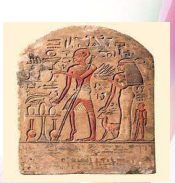
Virology

History Of Virology

History Of Virology

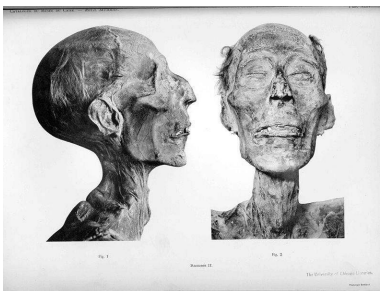
First written record

- Hieroglyph from Memphis (capital of ancient Egypt)
- 3700 BC
- Temple priest showing signs of paralytic poliomylietis



History Of Virology

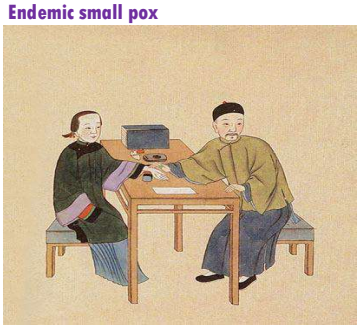
1196 BC



- Pharoah Ramses V
- Dies in 1196 BC
- Well preserved mummified body kept in Cairo museum
- Believed to have small pox
- Pustular lesions on face

History Of Virology

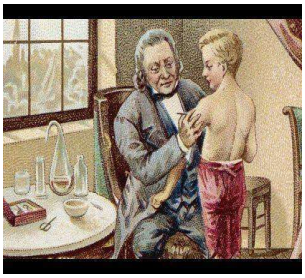
Endemic small pox



- Small pox endemic in China by 1000 BC
- Survivors did not suffer from re-infection
- Variolation was developed

History Of Virology

May 1796



- Edward Jenner introduced the concept of vaccination
- Cow pox against small pox
- Adopted worldwide in 19th century

History Of Virology

Martinus Beijerinick

- Tobacco Mosaic Virus
- First to develop modern idea of virus
- Referred as *contagium vivum fluidium* (soluble living germ)

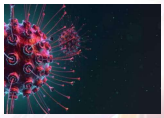
Virology

Importance of Virology

Importance Of Virology

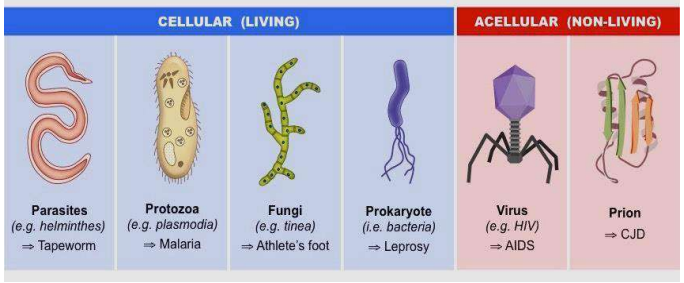
UNIQUE PROPERTIES

- Living as well as non-living characteristics
- Unable to cultivate in laboratory easily
- Simplest structure
- Complex interaction with host
- Replication within host



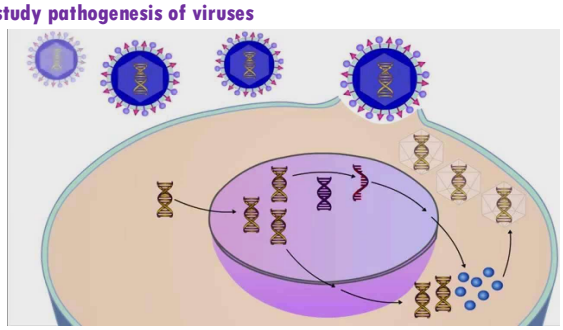
Importance Of Virology

1. To study an important class of pathogens



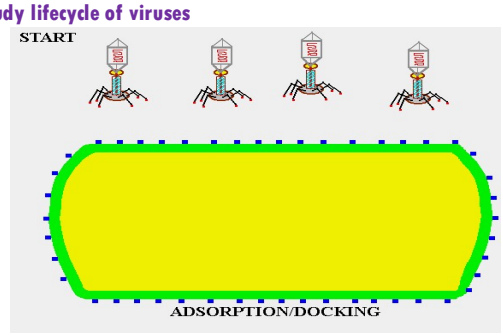
Importance Of Virology

2. To study pathogenesis of viruses



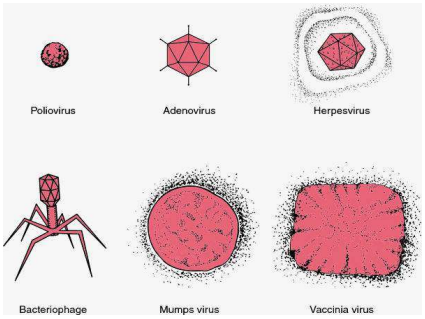
Importance Of Virology

3. To study lifecycle of viruses



Importance Of Virology

4. To study structure of viruses



Importance Of Virology

5. To study viral cultivation in lab

a. Tissue culture
b. Embryonated egg
c. Live animal

Virology

VIRUSES

Living or non-living

Viruses Living or non-living

Are virus living entities?

Ambiguous question

Viruses Living or non-living

What is life?

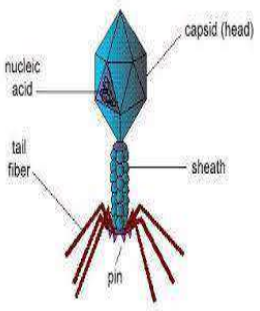
- Definition of life:
  - A complex set of processes resulting from the actions of proteins specified by nucleic acids
- Nucleic acids of living things are in action all the time

Viruses Living or non-living

What is Virus ?

- Viruses are complicated assemblies of molecules, including proteins, nucleic acids, lipids, and carbohydrates
- Viruses can only replicate themselves by infecting a host cell and therefore cannot reproduce on their own.

Viruses Living or non-living



CONCLUSION

- Viruses are:

"exceptionally complex aggregation of non-living chemicals"

OR

" exceptionally simple living organisms"

Virology

ORIGIN OF VIRUSES

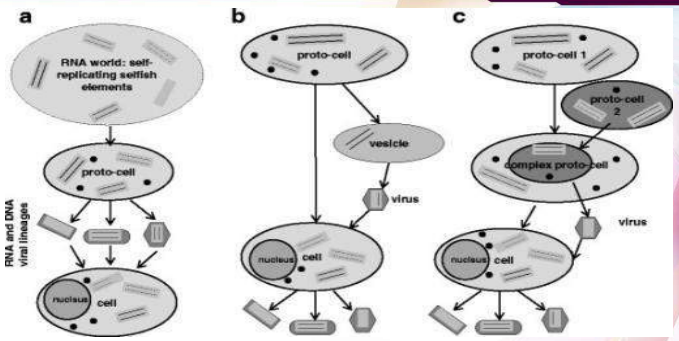
ORIGIN OF VIRUSES

THEORIES

Evolved to be:
Economic form of microbial life

Most efficient
Three theories for evolution

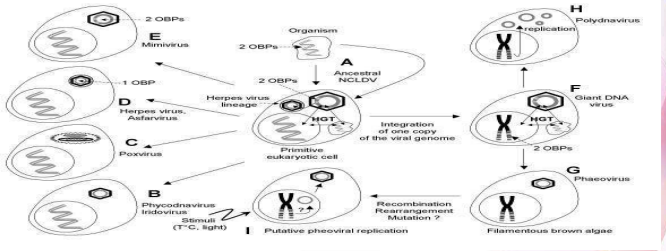
ORIGIN OF VIRUSES



ORIGIN OF VIRUSES

FIRST THEORY

They may have originated and evolved with the other life forms



ORIGIN OF VIRUSES

SECOND THEORY

They may have arisen from segments of the cellular nucleic acids which acquired the ability to replicate at the expense of host cell

ORIGIN OF VIRUSES

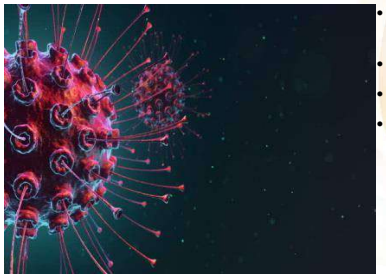
THIRD THEORY

- Regressive theory
- Viruses arose from free living organisms which gradually lost genetic information
- Until they became totally dependent on the biosynthetic pathways of their host cells

Virology

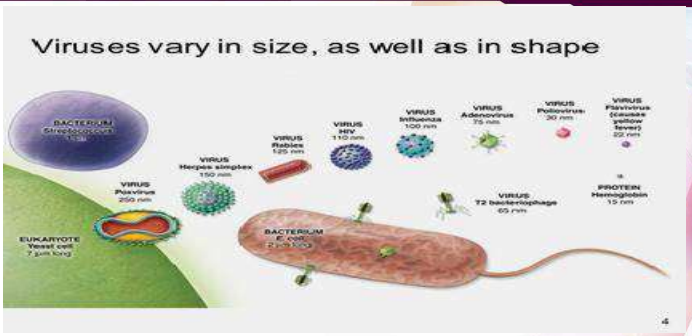
Viral size

Viral size

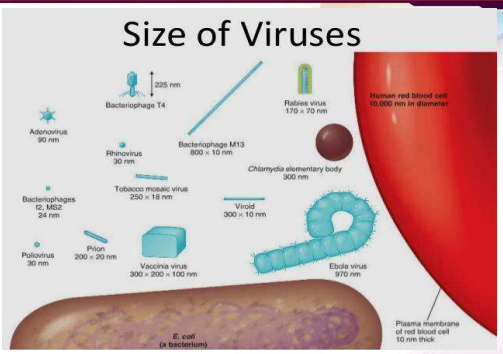


- Determined by electron microscopy
- Vary in size
- Most are smaller than bacteria
- Some are as the same size of bacteria (vaccinia)

Viral size



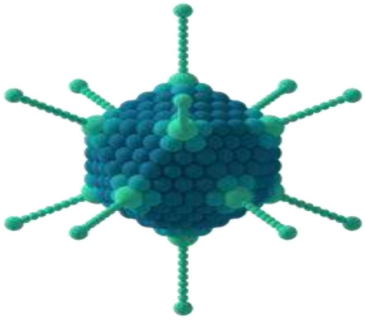
Viral size



Virology

FUNCTIONAL CHEMISTRY OF VIRUSES

Function chemistry of viruses



CAPSID

- Outer protein coat
- Infectious component of virion
- Infectivity depends on break down of protein capsid (Capsomeres)
- Mostly host specific

Function chemistry of viruses

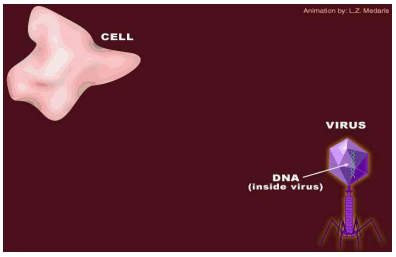
SPECIFICITY

Most viruses infect specific host cells ie are host specific.

Host specificity is due to:

- **specific attachment sites on the host cells called receptors**
  - **Receptor sites for bacteriophage are found in bacterial cell walls or fimbrae or flagella**
  - **Animal cell membranes contain receptors for animal viruses**
- **availability of cellular factors required for viral multiplication in the host cells**

Function chemistry of viruses



INFECTIVITY

- For infectivity in host cells:

1. Viral nucleic acid
2. Viral structural sub components (spikes or glycoproteins)
3. Enzymes induced in host cells (polymerase enzymes)

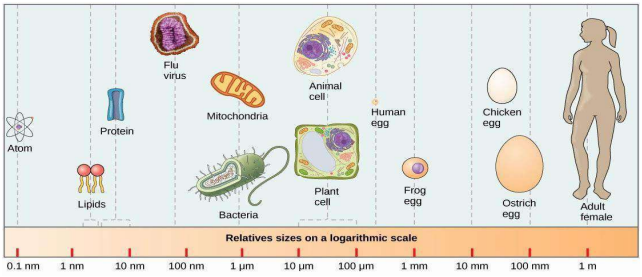
Virology

VISUALIZATION OF VIRAL PARTICLES

Visualization of Viral particles

Size

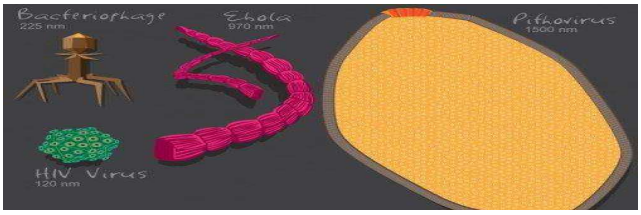
- Small size Not visible through simple microscope



Visualization of Viral particles

Variation

- Shape greatly varies
- Diversity
- Effects pathogenicity



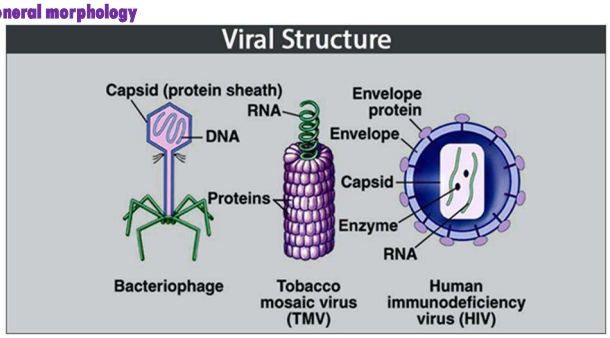
Visualization of Viral particles

General morphology

1. Icosahedral
2. Helical
3. Complex

Visualization of Viral particles

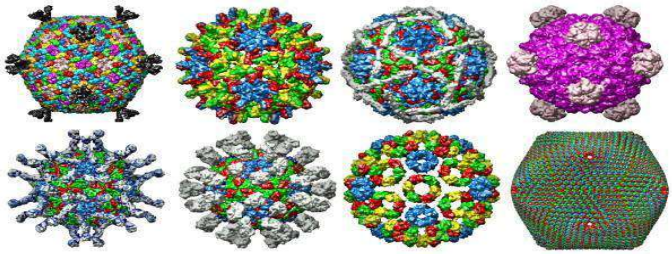
General morphology



Virology

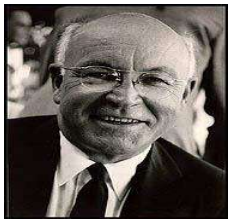
BIOCHEMICAL AND CELLULAR BACKGROUND OF VIRUSES

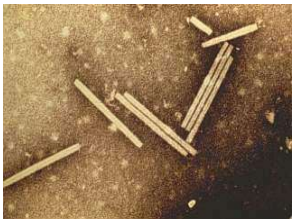
BIOCHEMICAL AND CELLULAR BACKGROUND OF VIRUSES



BIOCHEMICAL AND CELLULAR BACKGROUND OF VIRUSES

Wendell M. Stanley
1946 Nobel Prize in Chemistry
Crystallized Tobacco Mosaic Virus and
Systematically Investigated its Biochemistry

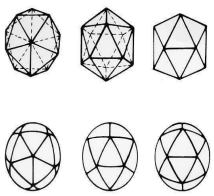




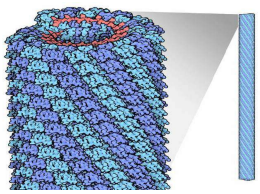
BIOCHEMICAL AND CELLULAR BACKGROUND OF VIRUSES

Symmetry of Viral Capsids

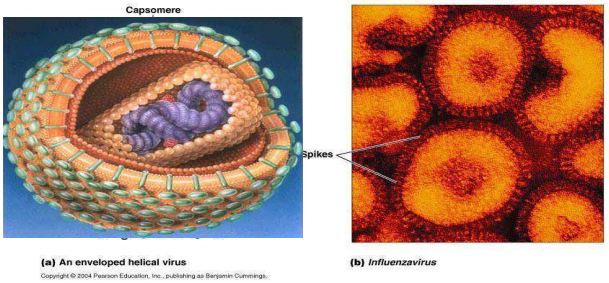
Icosahedral



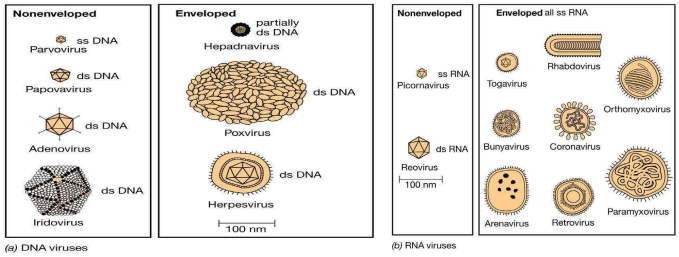
Helical



ENVELOPED VIRUSES



SURVEY OF HUMAN VIRUSES



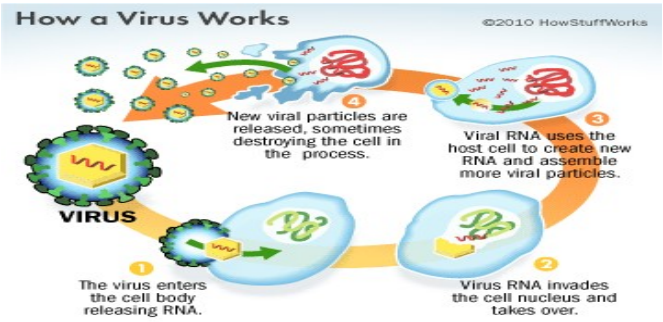
Virology

Living characters in host cells

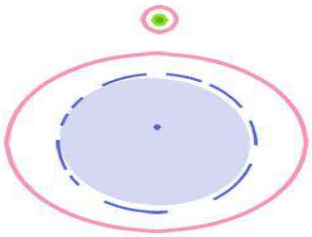
Living characters in host cells

- Molecular hijackers
- Biologically inert
- Dependent on host cells for replication and protein synthesis
- Lack in their own enzymes or organelles etc
- Sometimes alter the host genome

Living characters in host cells



Living characters in host cells



Replication

- Using host machinery
- Force replication in host
- Increase in number
- Leave the cell simply or either by bursting the host cell

Living characters in host cells

Effects on host

- None
- Genetic alterations
- Physical damage
- Oncogenesis

Shelter

- Take shelter in specific host cells
- Take nourishment
- Complete lifecycles
- Continuity of viral life

Virology

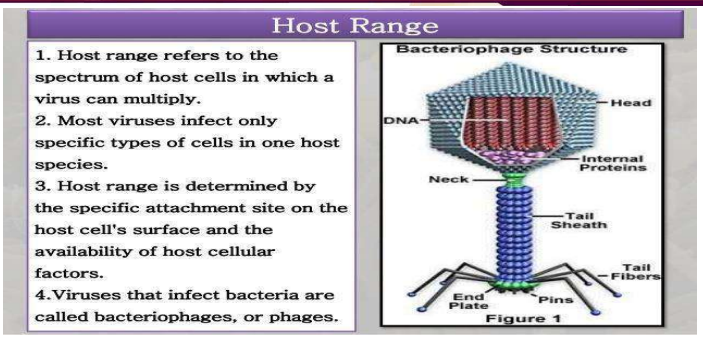
HOST RANGE

Host Range

What is a host range?

- The spectrum of host cells that the virus can infect.

Host Range



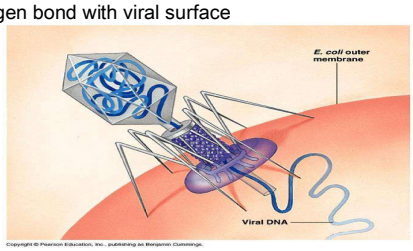
Host Range

Viral host range

- Plants
- Vertebrates
- Invertebrates
- Protists
- Bacteria
- Fungi

Host Range

- Most viruses are host specific
- Bind to specific receptors on cells
- Animal viruses: receptors on plasma membrane
- Form hydrogen bond with viral surface



Virology

Viral specificity

Viral Specificity

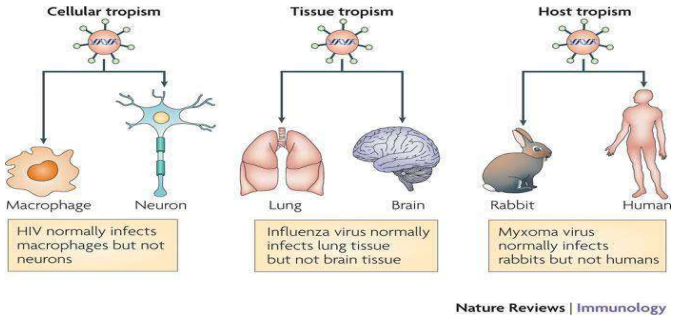
- All kingdoms can be infected by viruses
- Viruses that infect one kingdom are unable to infect another
- Viruses might infect closely related organisms
- Usually species specific

Viral Specificity

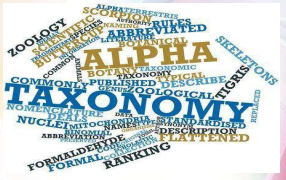
Examples

VIRUS	HOST
Tobacco Mosaic Virus	Tobacco plant
Rabies	Mammals
Swine flu	Pigs and humans
Foot and Mouth disease	Hooved animals
Bird Flu	Birds and human
Myxoma virus	Rabbits

Viral Specificity



Virology



TAXONOMY I

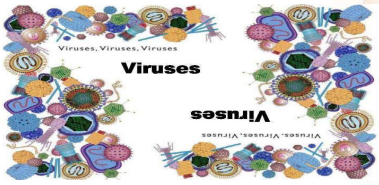
Taxonomy

WHAT IS TAXONOMY?

- Naming of something

  OR
- Scheme of classification and nomenclature of organisms
- Viral taxonomy is the classification of viruses based on viral nature

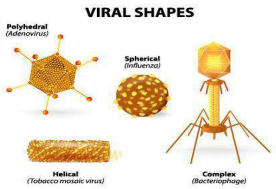
Taxonomy



SCHEMES FOR CLASSIFICATION

- International Committee on Taxonomy of Viruses (ICTV) 1966
- Baltimore system of classification 1971

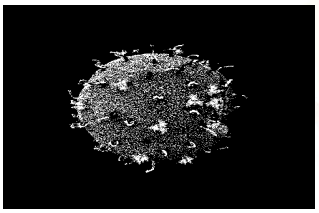
Taxonomy



ED ON PHENOTYPE

- orphology
- ructure of capsid
- esence of envelope
- pe of nucleic acid
- ode of replication
- Host organism
- Diseases caused

Taxonomy



ICTV CLASSIFICATION

•A class where members have several properties in common e.g.

- Host
- Ecological niche
- Pathogenicity
- Mode of transmission
- Habitat

Taxonomy

LEVEL OF CLASSIFICATION

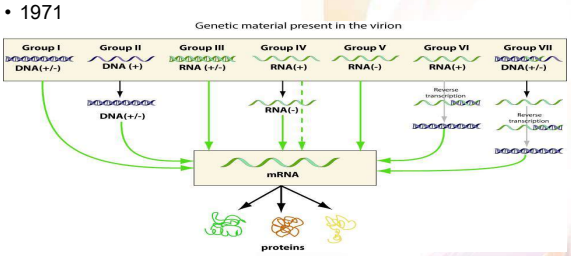
ORDER	FAMILY	SUBFAMILY	GENUS	SPECIES
• -virales	• -viridae	• -virinae	• -virus	

Virology

TAXONOMY-II

Taxonomy

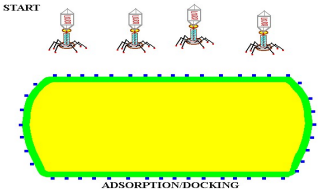
- BALTIMORE CLASSIFICATION
- David Baltimore
- 1971



Taxonomy

DEPENDS ON

1. Expression of nucleic acid
2. Stranded
3. Segmented/ Sense
4. Method of Replication



Taxonomy

7 CLASSES:

GROUP I: double stranded DNA viruses (Adeno, Herpes)

GROUPII: single stranded DNA viruses (Parvo)

GROUP III: double stranded RNA viruses (Reo)

GROUP IV: positive sense single stranded RNA viruses (Polio,Picorna)

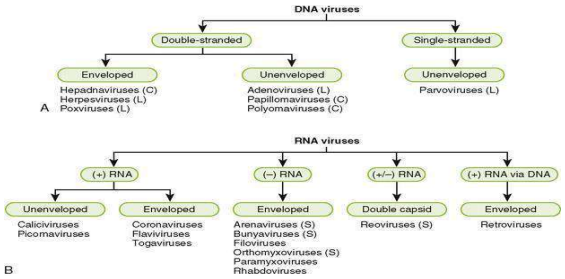
GROUP V: negative sense single stranded RNA viruses (ortho)

GROUP VI: Single stranded RNA viruses (Retero)

GROUP VII: Double stranded DNA viruses (Hepadna)

Taxonomy

VIRAL CLASSIFICATION



Virology

ARTIFICIAL TRANSMISSION OF VIRUSES IN PLANTS

Artificial transmission of viruses in plants

BARRIERS

- External surface
- Structural differentiation of host
- Internal barriers
- Direct transmission only through wounds or poorly protected tissues

Artificial transmission of viruses in plants

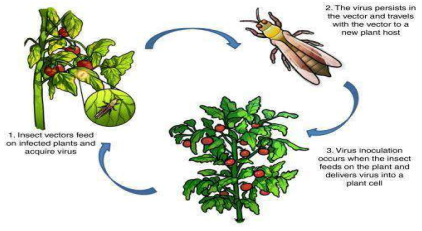
MECHANICAL TRANSMISSION

- Few plant viruses
- TMV and WMV by root inoculation
- Potato viruses by infected tuber plantation

Artificial transmission of viruses in plants

- **SEED TRANSMISSION**
- Rare
- But exists

Artificial transmission of viruses in plants



- **VECTOR TRANSMISSION**
- Most common
- Grass hopper
- Aphids
- Leaf hoppers
- White flies
- Mites
- Beetles

Artificial transmission of viruses in plants

DISEASES

- Rice dwarf virus
- Maize streak
- Tomato spoiled wilt virus
- Pigeon pea sterility virus

Virology

ARTIFICIAL TRANSMISSION OF VIRUSES IN ANIMALS

Artificial transmission of viruses in animals

REASON

- Viruses that cannot be cultivated on cell culture or embryonated eggs
- To experiment on animals rather human beings

Artificial transmission of viruses in animals

ANIMAL INOCULATION

•Route: Subcutaneous, intracerbral, intraperitoneal, Intradermal or intraocular
•Virus multiplies in host and disease develops

Artificial transmission of viruses in animals

ANIMALS USED

- Monkey
- Rabbit
- Guinea pig
- Rat
- Hamsters mice

Artificial transmission of viruses in animals

GROWTH CONFIRMATION

- Pathogenesis
- Lesions
- Immune response
- Oncogenesis
- Death

Artificial transmission of viruses in animals

ADVANTAGES

- Clinical manifestations are determined
- Primary isolation of viruses
- Used for study of immune response

Virology

MICROSCOPY

Microscopy

MICROSCOPE

- An instrument used to see objects that are too small for the naked eye.

Microscopy

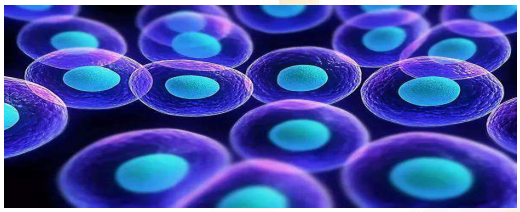
MICROSCOPY

- The science of investigating small objects using such an instrument is called microscopy

Microscopy

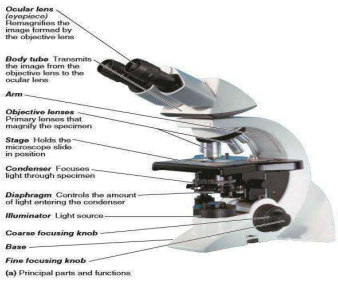
MICROSCOPIC

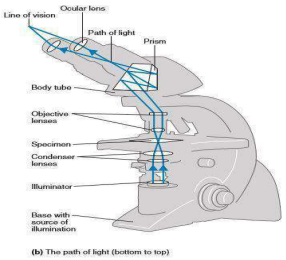
- Microscopic means invisible to the eye unless aided by a microscope



Microscopy

PARTS OF MICROSCOPE





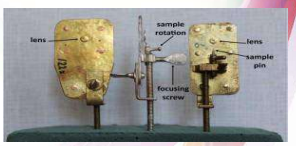
Virology

HISTORY OF MICROSCOPY

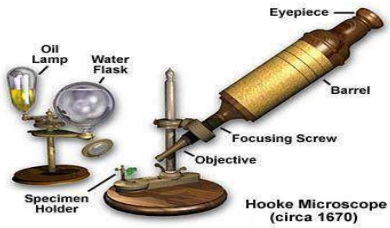
History of Microscopy

FIRST MICROSCOPE

- First simple microscope by Leeuwenhoek
- 17th century



History of Microscopy



ROBERT HOOKE

- Robert Hooke in 1665 observed cork cells

History of Microscopy

COMPOUND MICROSCOPE

- Zaccharias Janssen made the first compound microscope

History of Microscopy

BETTER MICROSCOPE

- In 1830 a better microscope introduced by Joseph Lister

History of Microscopy

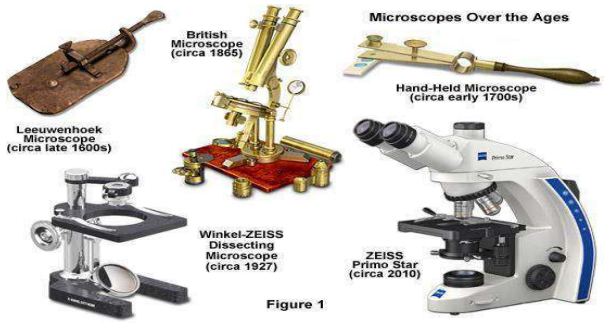


Figure 1

Virology

LIGHT MICROSCOPY

Light Microscopy

LIGHT MICROSCOPY

- Light microscopy refers to the use of any kind of microscope that uses visible light to observe specimens.

Light Microscopy

TYPES:

- Compound light microscopy
- Dark field Microscopy
- Phase-contrast Microscopy
- Differential interference contrast (DIC) Microscopy
- Fluorescence Microscopy
- Confocal Microscopy

Light Microscopy

COMPOUND LIGHT MICROSCOPY

- Light rays from an illuminator (the light source)
- Pass through a condenser, which has lenses that direct the light rays through the specimen
- From here, light rays pass into the objective lenses
- The image of the specimen is magnified again by the ocular lens (eyepiece)

Light Microscopy

MAGNIFICATION

- Objective lens **X** Eyepiece lens
- It is the ratio of the size of an object seen under microscope to the actual size observed with unaided eye.

Light Microscopy

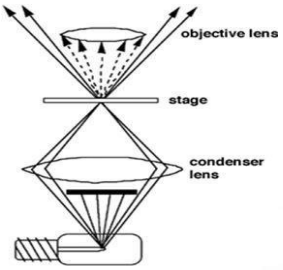
RESOLUTION:

- refers to the ability of the lenses to distinguish two points that are a specified distance apart
- The resolving power of human eye is 0.25 mm
- The light microscope can separate dots that are 0.25µm apart.
- The electron microscope can separate dots that are 0.5nm apart.

Virology

DARK FIELD MICROSCOPY

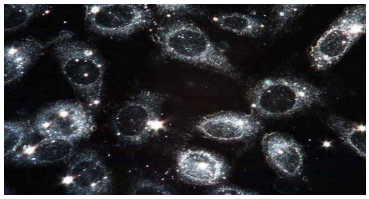
Dark Field Microscopy



DARK FIELD MICROSCOPY

- Refers to the use of microscope that excludes the unscattered beam from the image
- As a result, the field around the specimen (i.e., where there is no specimen to scatter the beam) is generally dark

Dark Field Microscopy



USE:

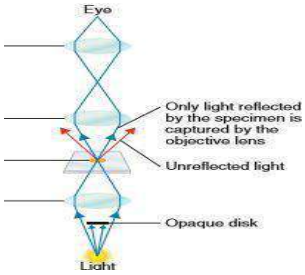
•Used for live microorganisms that either are invisible in the ordinary light microscope, cannot be stained by standard methods.

Dark Field Microscopy

DARK FIELD MICROSCOPY

- Uses a darkfield condenser containing an opaque disk
- The disk blocks light that would enter the objective lens
- Only light that is reflected off (turned away from) the specimen enters the objective lens
- Because there is no direct background light, the specimen appears light against a black background—the dark field.

Dark Field Microscopy



Virology

DIFFERENTIAL INTERFERENCE CONTRAST MICROSCOPY

DIC Microscopy

DIC MICROSCOPY

- Also known as Nomarski interference contrast microscopy
- used to enhance the contrast in unstained, transparent samples
- DIC works on the principle of interferometry to gain information about the optical path length

DIC Microscopy

WORKING:

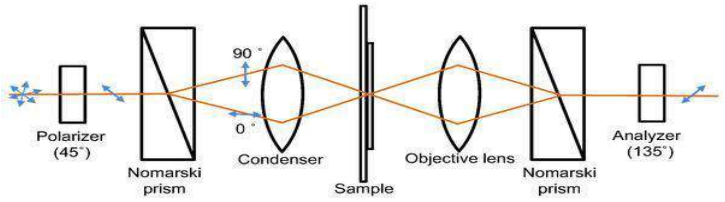
- DIC microscope uses two beams of light instead of one
- prisms split each light beam, adding contrasting colors to the specimen
- image is brightly colored and appears nearly three-dimensional

DIC Microscopy

LIGHT PATH

- Unpolarized light enters and becomes polarized
- Then it enters the prism and gets separated into two beams
- These rays are focused by condenser
- Rays travel through specimen
- Then enter objective lens
- Second prism recombines the rays forming an image

DIC Microscopy



Virology

CONFOCAL MICROSCOPY

Confocal Microscopy

CONFOCAL MICROSCOPY

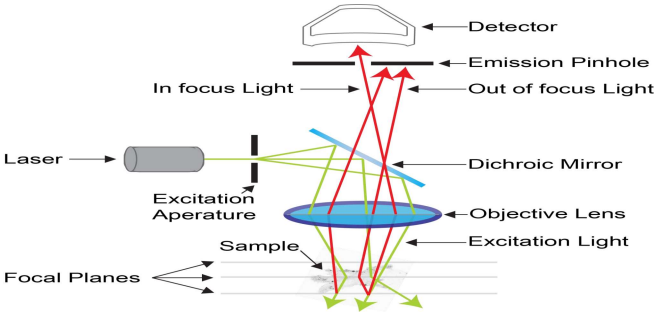
- An optical imaging technique for increasing optical resolution and contrast of a micrograph
- by using a spatial pinhole to block out-of-focus light in image formation

Confocal Microscopy

PRINCIPLE:

- used to reconstruct three-dimensional images
- Like fluorescent microscopy, specimens are stained with fluorochromes so they will emit or return light
- But instead of illuminating the entire field, one plane of a small region of a specimen is illuminated with a short-wavelength (blue) light which passes the returned light through an aperture aligned with the illuminated region

Confocal Microscopy



Confocal Microscopy

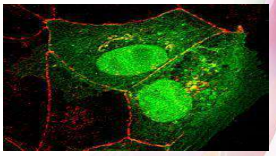
PROPERTIES:

- Each plane corresponds to an image of a fine slice that has been physically cut from a specimen
- Successive planes and regions are illuminated until the entire specimen has been scanned
- Confocal microscopy can image cells in detail only to a depth of less than 100 mm

Confocal Microscopy

IMAGE

- Point laser source
- 3D dimensional fraction pattern
- Better resolution of Wide field illumination



Confocal Microscopy

TYPES:

1. Confocal laser scanning microscope
2. Spinning disk confocal microscope
3. Microlens enhanced or dual spinning disk confocal microscope
4. Programmable array microscope (PAM)

Virology

TWO PHOTON MICROSCOPY

Two Photo Microscopy

TWO PHOTON MICROSCOPY

- It is a fluorescence imaging technique that allows imaging of living tissue up to about one millimeter in depth.

Two Photo Microscopy

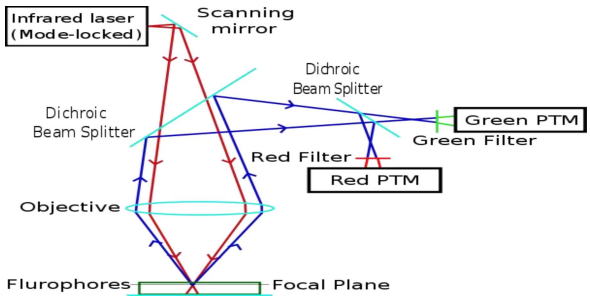
PRINCIPLE:

Specimens are stained with a fluorochrome

Two-photon microscopy uses long-wavelength (red) light, and therefore two photons, instead of one, are needed to excite the fluorochrome to emit light. The longer wavelength allows imaging of living cells in tissues up to 1 mm (1000 mm) deep

Two Photo Microscopy

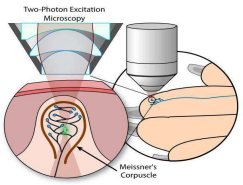
TWO PHOTON MICROSCOPY ILLUSTRATION



Two Photo Microscopy

APPLICATIONS:

- Applicable for very thin transparent cells (skin)
- Non-invasive optical biopsy



Virology

SCANNING ACOUSTIC MICROSCOPY

Scanning Acoustic Microscopy

SCANNING ACOUSTIC MICROSCOPY

- A device that uses focused sound to image an object
- Applicable in biological and medical research
- Developed in 1974

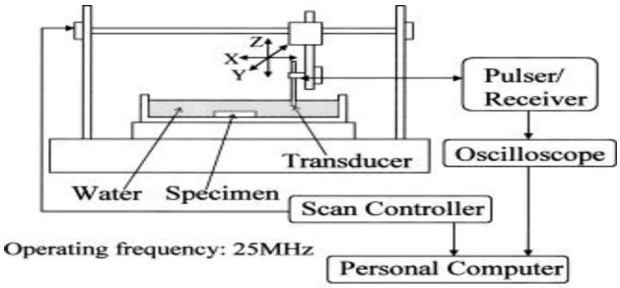
Scanning Acoustic Microscopy

PRINCIPLE:

- Scanning acoustic microscopy (SAM) basically consists of interpreting the action of a sound wave sent through a specimen
- A sound wave of a specific frequency travels through the specimen, and a portion of it is reflected back every time it hits an interface within the material

Scanning Acoustic Microscopy

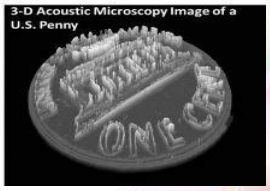
SAM ILLUSTRATION:



Scanning Acoustic Microscopy

IMAGE

- Resolution= 1 µm
- Both 2D and 3D image



Scanning Acoustic Microscopy

USE:

SAM is used to study living cells attached to another surface, such as cancer cells, artery plaque, and bacterial biofilms that foul equipment

Virology

TRANSMISSION ELECTRON MICROSCOPY

Transmission Electron Microscopy

TEM MICROSCOPY

A technique that uses a beam of electrons

- Transmitted through the specimen
- Ultra-thin section of specimen
- Image on imaging device

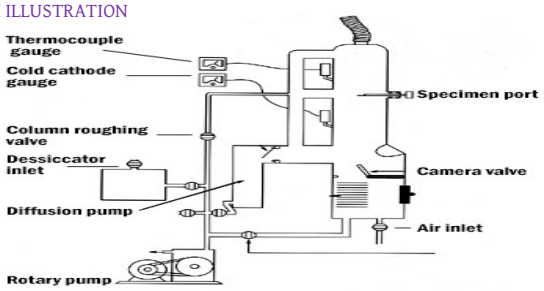
Transmission Electron Microscopy

PRINCIPLE:

- Focused beam of electrons from an electron gun passes through ultrathin section of the specimen
- Beam gets focused on a small area of the specimen by an electromagnetic condenser lens
- Instead of being placed on a glass slide, the specimen is usually placed on a copper mesh grid

Transmission Electron Microscopy

TEM ILLUSTRATION



Transmission Electron Microscopy

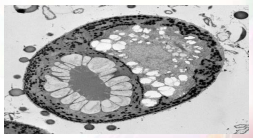
IMAGE FORMATION:

- Electrons passes through the specimen and then through an electromagnetic objective lens, which magnifies the image
- The electrons are focused by an electromagnetic projector lens onto a fluorescent screen or photographic plate
- The final image, called transmission electron micrograph, appears as many light and dark areas, depending on the number of electrons absorbed by different areas of the specimen

Transmission Electron Microscopy

IMAGE

- The transmission electron microscope can resolve objects as close together as 10 pm
- The objects are generally magnified 10,000 to 100,000*



Transmission Electron Microscopy

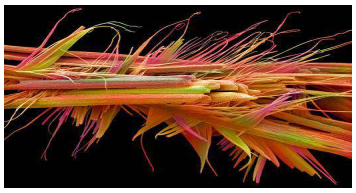
DYE:

- Contrast can be greatly enhanced by using a "dye" that absorbs electrons and produces a darker image in the stained region
- Lead, osmium, tungsten, and uranium (Stains)
- These metals can be fixed onto the specimen (positive staining)
- or used to increase the electron opacity of the surrounding field (negative staining)

Transmission Electron Microscopy

LIMITATIONS

- Only a very thin section of a specimen (about 100 nm) can be studied effectively
- Specimens must be fixed, dehydrated, and viewed under a high vacuum to prevent electron scattering
- These treatments not only kill the specimen, but also cause shrinkage and distortion



Virology

SCANNING ELECTRON MICROSCOPY

Scanning Electron Microscopy

SEM MICROSCOPY

- A microscope that produces images of a sample by scanning the surface with a focused beam of electrons
- Produces various signals that contain information about the surface topography and composition of the sample

Scanning Electron Microscopy

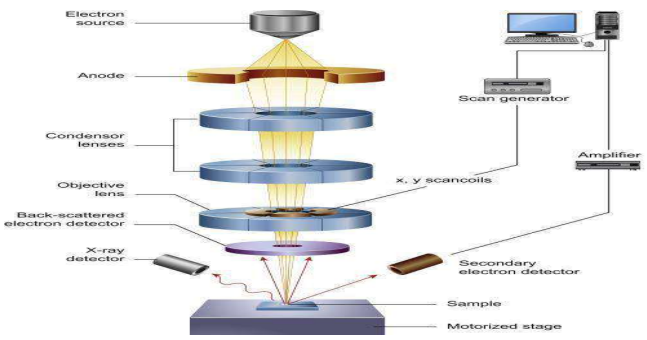
PRINCIPLE:

•An electron gun produces a finely focused beam of electrons called the primary electron beam

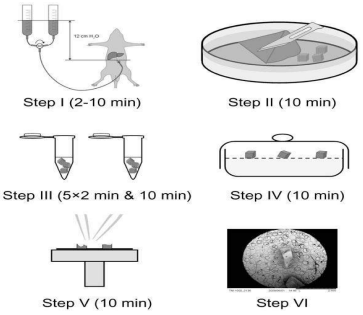
• These electrons pass through electromagnetic lenses and are directed over the surface of the specimen

•The primary electron beam knocks electrons out of the surface of the specimen, and the secondary electrons thus produced are transmitted to an electron collector, amplified, and used to produce an image on a viewing screen or photographic plate. The image is called a scanning electron micrograph

Scanning Electron Microscopy



Scanning Electron Microscopy



SAMPLE PREPARATION:

- Prepared in presence of vacuum and beam of electrons
- Mounted on specimen holder
- Specimens get conductive by beam of electrons

Scanning Electron Microscopy

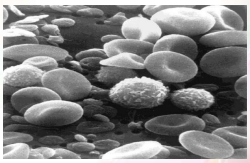
IMAGE

- Provides striking three-dimensional views of specimens
- it can resolve objects as close together as 10 nm, and objects are generally magnified 1000 to 10,000

Scanning Electron Microscopy

USES:

- useful in studying the surface structures of intact cells and viruses
- Also used for fractography



Virology

SCANNED PROBE MICROSCOPY

Scanned Probe Microscopy

SCANNED PROBE MICROSCOPY

- Forms images of surfaces using physical probe that scans the specimen
- Founded in 1981

Scanned Probe Microscopy

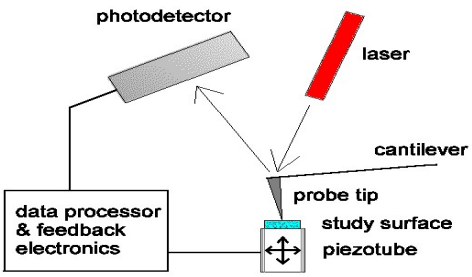
PRINCIPLE:

- The interaction between the sharp probe and surface provides 3D topographic image of surface at the atomic scale
- Two most popular modes:

1. Scanning Tunneling Microscopy (STM)
2. Atomic Force Microscopy (AFM)

Scanned Probe Microscopy

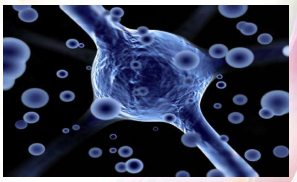
SPM ILLUATRATION:



Scanned Probe Microscopy

IMAGE FORMATION:

- Scans tip over surface
- A value is recorded
- Displayed as heat map



Scanned Probe Microscopy

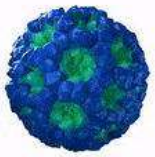
LIMITATIONS:

- Detailed shape of probe is difficult to determine sometimes
- Slower in image acquiring
- Maximum image size is smaller
- Cannot examine buried solid-solid or liquid-liquid interfaces

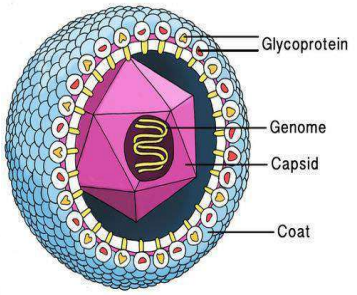
Virology

CAPSID

Symmetry and Architecture



Capsid; Symmetry and Architecture



Capsid; Symmetry and Architecture

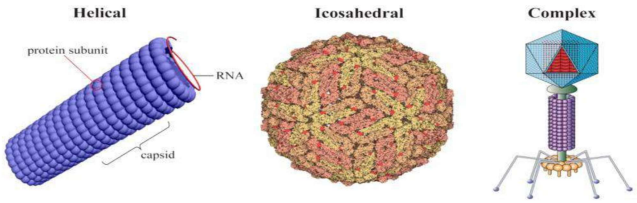
COMPOSITION

- Proteinic nature
- Composed of protomers and/or
- capsomers

Capsid; Symmetry and Architecture

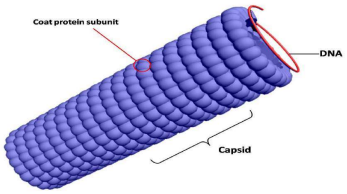
TYPES

Helical Icosahedral Polyhedral



Capsid; Symmetry and Architecture

HELICAL SYMMETRY



- Rod like structure
- Genome in the centre of helix
- Helix made by repeating units in a spiral
- Examples, Tobacco Mosaic Virus

Capsid; Symmetry and Architecture

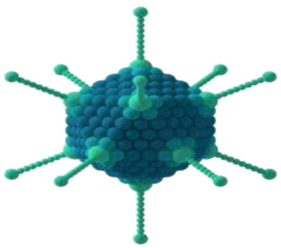
HELICAL SYMMETRY

- Virus particles elongated or pleomorphic (not spherical)
- Nucleic acid also spiral
- Capsomeres arranged around Nucleic acid

Capsid; Symmetry and Architecture

ICOSAHEDERAL SYMMETRY

- Characteristic of many spherical viruses
- In adenovirus (icosahedral) composition consists of : Hexon, Pentone base, and fibre



Capsid; Symmetry and Architecture

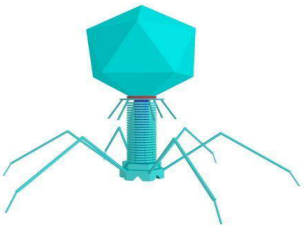
ICOSAHEDERAL SYMMETRY

- Also called cubic capsid
- Mostly spherical
- Particles composed of 20 equilateral triangles, 12 vertices and 2,3,5 rotational symmetry

Capsid; Symmetry and Architecture

POLYHEDERAL SYMMETRY

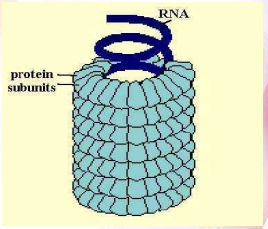
- Also called complex
- Neither helical nor icosahederal symmetry
- E.g Bacteriophage



Virology

CAPSID SYMMETRY

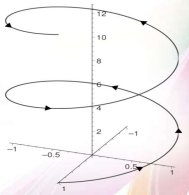
Helical



Helical Symmetry

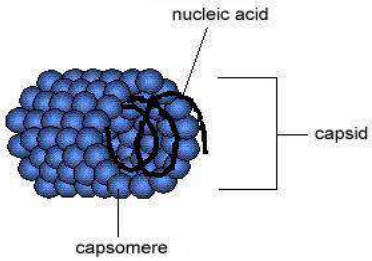
WHAT IS HELICAL SHAPE?

- Property that a tangent line at ay point makes a constant angle with a fixed line (axis)



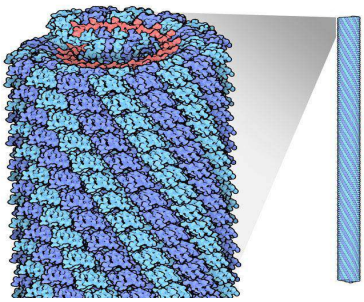
Helical Symmetry

HELICAL VIRUS



- Resembles long rods
- Ribbon-like structure
- Hollow tubes with protein walls

Helical Symmetry

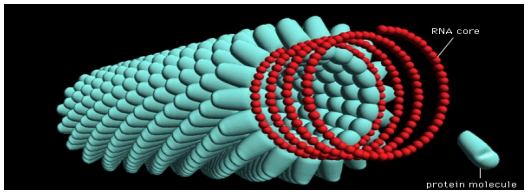


- A single protomer associates together in a helical arrangement to produce a long rigid tube

Helical Symmetry

GENOME

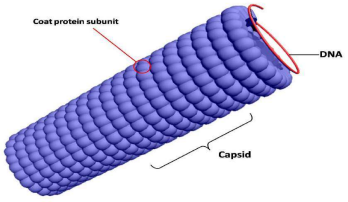
- Genome is wound in a spiral
- Inside the capsid
- Within a groove



Helical Symmetry

LENGTH

- The Nucleic acid determines the length of the helix
- Capsid doesn't extend much beyond the genome end



Helical Symmetry

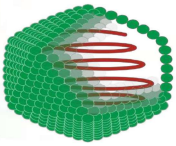
EXAMPLES

- Most are enveloped
- All are RNA viruses
- Tobacco Mosaic Virus (TMV)
- Influenza virus

Virology

CAPSID SYMMETRY

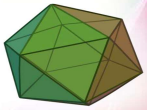
Icosahederal



Icosahederal Symmetry

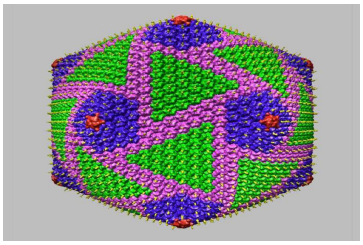
WHAT IS ICOSAHEDERAL SHAPE?

- An icosahedral is a polygon with 12 vertices (corner), 20 facet (sides) and 30 edges



Icosahederal Symmetry

WHY ICOSAHEDERAL?

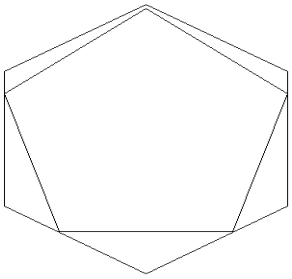


- Most efficient way to enclose a space
- small number of linear genes can specify large structure

Icosahederal Symmetry

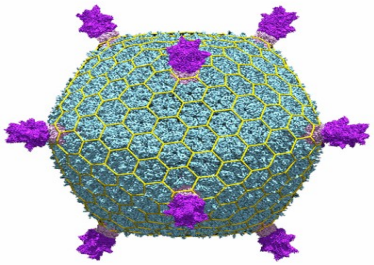
TYPES

- Hexamers (6 units)
- Pentamers (5 units)



Icosahederal Symmetry

CAPSOMERE

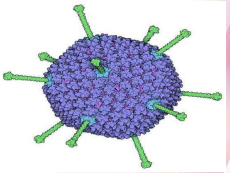


- Composed of knob-shaped structures
- Called capsomeres
- Composed of 5 or 6 protomers

Icosahederal Symmetry

EXAMPLES

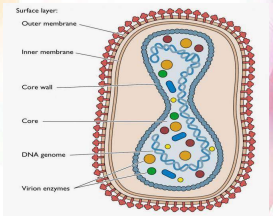
- most stable
- Adenovirus
- Picornavirus



Virology

CAPSID SYMMETRY

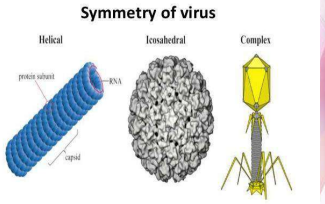
Complex



Complex Symmetry

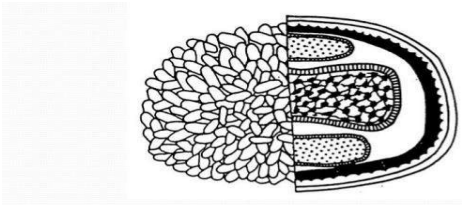
WHAT IS COMPLEX SHAPE?

- Neither helical nor icosahederal



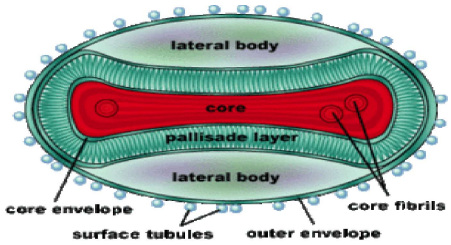
Complex Symmetry

HELICAL VIRUS



- Complex
- Ovoid interior
- Brick-shaped exterior

Complex Symmetry



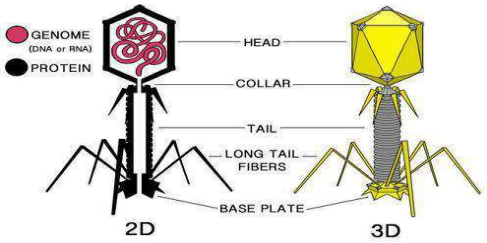
Several

separate capsomeres

Complex Symmetry

GENOME

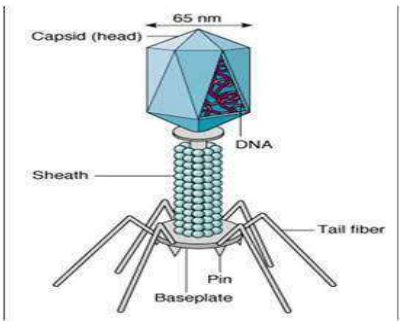
- Genome associated with proteins
- Contained in nucleoid



Complex Symmetry

EXAMPLES

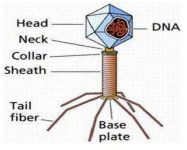
- Bacteriophages
- Poxviruses



Complex Symmetry

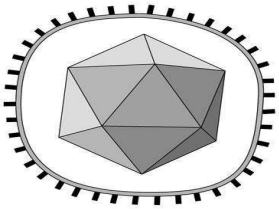
T-PHAGES

- Head and tail
- Called binal symmetry
- Icosahederal head and helical tail symmetry



Virology

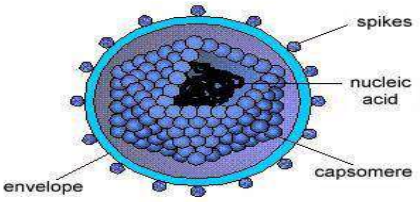
ENVELOPED VIRUSES



Enveloped Viruses

EXAMPLES

- Many animal viruses
- HIV
- Influenza virus
- Herpesvirus
- Adenovirus
- Hepatitis B virus
- Parvovirus



Enveloped Viruses

CONCLUSION

•Surrounds capsid

•Composed of glycoproteins and lipid bilayer

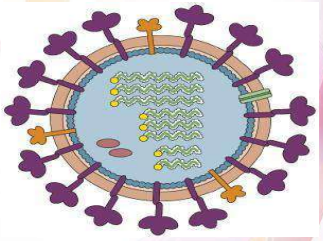
Enveloped Viruses

CONCLUSION

- Helps virus to enter host
- Not all viruses have it (Most animal viruses)

Virology

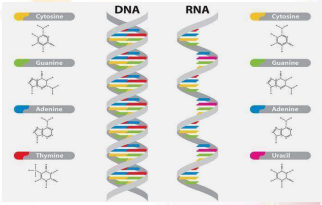
VIRAL GENOME



Viral Genome

NUCLIEC ACID

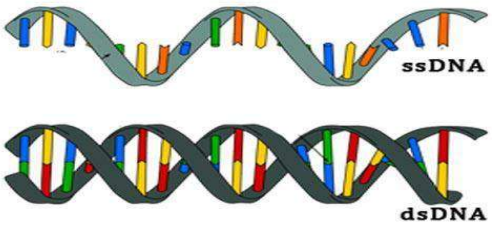
- Either DNA
- Or RNA



Viral Genome

STRANDEDNESS

- Single stranded
- Double stranded



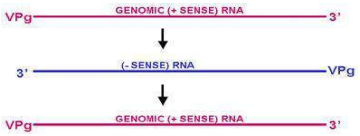
Viral Genome

SHAPE

- Circular
- Linear
- Segmented

Viral Genome

SENSE

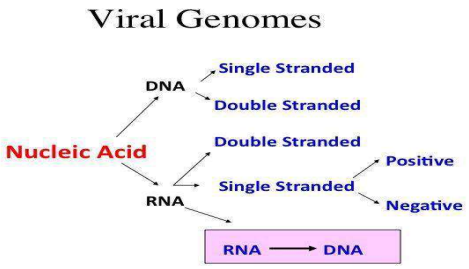


•Positive sense (+)

•Negative sense (-)

•Ambisense (+ /-)

Viral Genome



Viral Genome

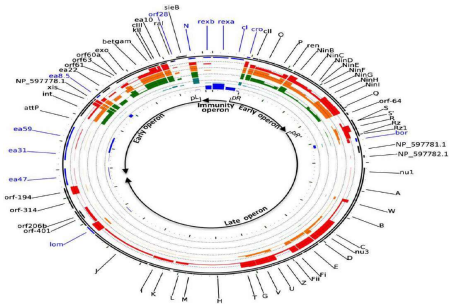
PLANT VIRUSES

- Single stranded
- RNA genome

Viral Genome

BACTERIOPHAGES

- Double stranded
- DNA genome



Viral Genome

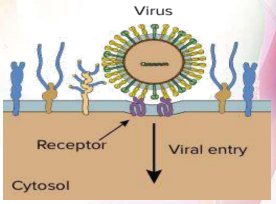
GENOME SIZE

- Varies
- Smallest genome of *Circovirus* (2 kilobases)
- Largest *Pandoravirus* (2 Megabases)

Virology

VIRUS RECEPTORS

Recognition and Binding



Virus Receptors

ENTRANCE

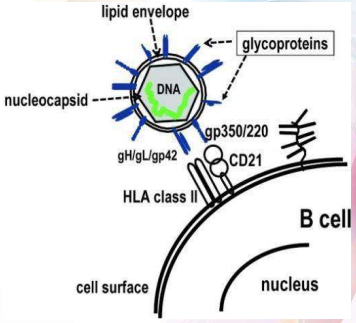
- Virus interacts with host receptors
- Cascade of events
- Leading entry of viruses
- Same for enveloped and nonenveloped

Virus Receptors

RECEPTORS

- Host and virus interaction through receptors
- Receptors can be protein, lipids or carbohydrates

Virus Receptors

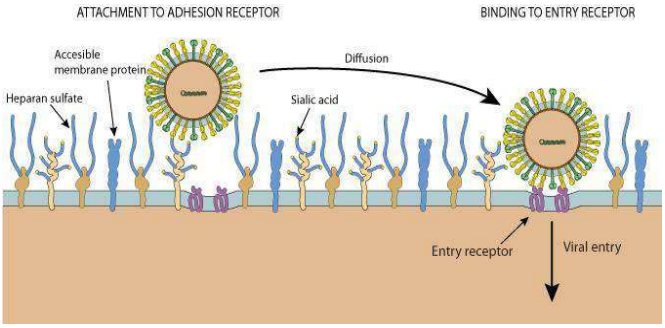


Virus Receptors

RECEPTOR RECOGNITION

- Viruses have various receptor recognition structures on surface
- Frequently have spikes
- Bind on the receptors on host cell surface

Virus Receptors



Virus Receptors

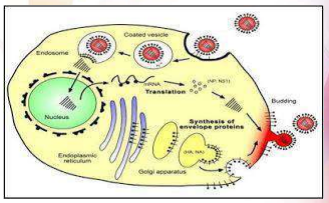
ENTRANCE

Variable

- Pinocytosis/endocytosis
  Membrane fusion
- Viral Penetration

Virology

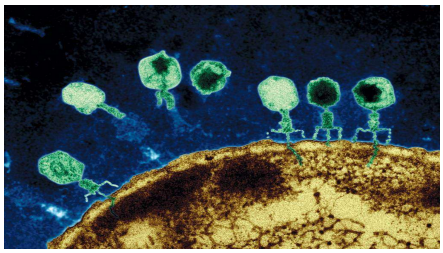
Interaction of Virus and Host Cells



Interaction of Virus and Host Cells

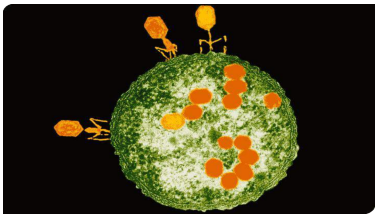
VIRUS-HOST

- Can cause
  - No damage
  - Cell damage
  - Cell death
  - Oncogenesis



Interaction of Virus and Host Cells

NON-IMMUNOGENIC RESPONSE



- Phagocytosis
- Raised body temp
- Hormones
- Malnutrition

Interaction of Virus and Host Cells

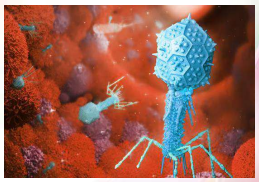
BARRIERS

- Skin
- Lack of membrane receptors
- Mucus
- Epithelium
- Low pH
- Immune system

Interaction of Virus and Host Cells

VIRAL INVASION

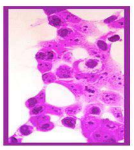
- Virus can escape immune system
- Cause generalized immuno-suppression

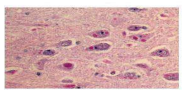


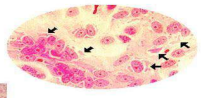
Interaction of Virus and Host Cells

FORMATION OF INCLUSION BODIES

- Nuclear or cytoplasmic aggregates
- Stainable
- Sites of viral multiplication


Adenovirus
nuclear inclusions

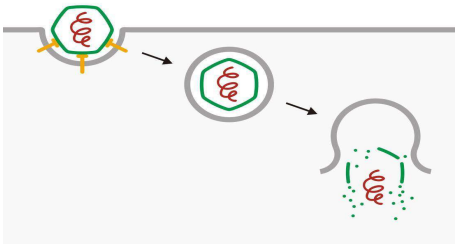

Rabies virus
cytoplasmic inclusions
Negri bodies


RSV
cytoplasmic inclusions

Interaction of Virus and Host Cells

PATHOGENESIS

- Inapparent
- Apparent
- Acute
- Subacute
- Chronic
- Latency



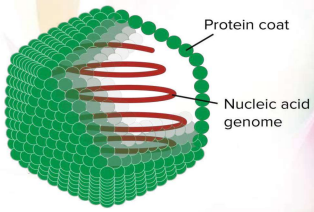
Interaction of Virus and Host Cells

ENTRANCE

- Respiratory tract
- Digestive tract
- Skin
- Conjuctivae
- Sexual contact
- Vectors

Virology

Protein-Nucleic acid Interactions



Protein-Nucleic acid Interactions

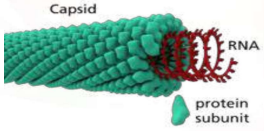
HISTORY

- Late 19th century, Microscopic observation
- Since then researchers have used variety of procedure to understand the relation
- Play a crucial role in biological processes

Protein-Nucleic acid Interactions

PREOTECTION OF GENOME

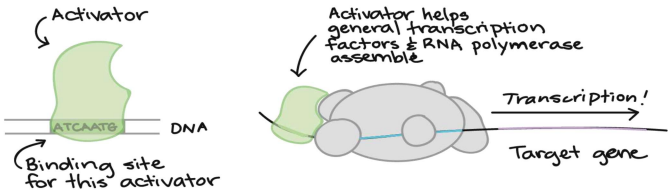
- Primary function of virus
- Capsid proteins protect the genome



Protein-Nucleic acid Interactions

REGULATION OF BIOLOGICAL FUNCTIONS

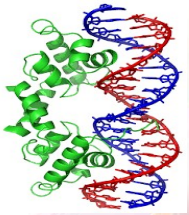
- Usually expression of gene
- Usually transcription factors
- Gene regulation



Protein-Nucleic acid Interactions

STRUCTURE

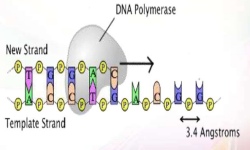
- DNA binding motif
- With the help of different ions



Protein-Nucleic acid Interactions

ENZYMATIC ROLE

- Polymerases and Restriction Endonucleases
- For replication and translation of proteins



Virology

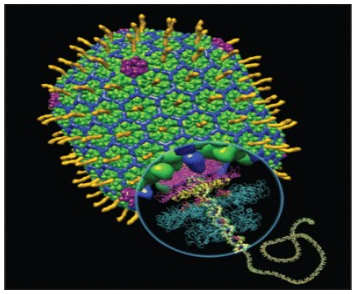
Genome Packaging

Genome Packaging

- Fundamental step in viral lifecycle
- Usually preformed through capsids
- Capsids are reformed sometimes

Genome Packaging

PACKAGING MOTOR PROTEIN



- Driven by hydrolysis of ATP
- Condenses genome into a confined place

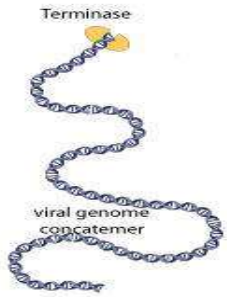
Genome Packaging

STEPS

1. Binding of Terminase
2. Binding to Procapsid portal
3. DNA translocation
4. Contamer Cleavage

Genome Packaging

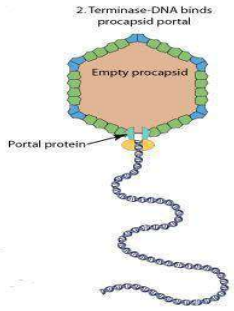
BINDING OF TERMINASE



- First step
- Terminase binds to viral genome

Genome Packaging

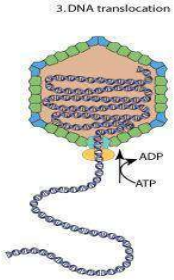
TERMINASE-DNA BINDS TO PROCAPSID PORTAL



- Preassembled capsid or procapsid usually
- Terminase-DNA complex binds to the portal of entry on the capsid

Genome Packaging

DNA TRANSLOCATION

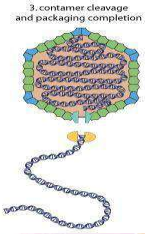


- Portal structure provides entry to DNA
- Packaging motor protein
- Large terminase

Genome Packaging

CONTAMER CLEAVAGE

- DNA cleavage
- Completion of genome packaging



Virology

An Introduction to Viral Genomics

An Introduction to Viral Genomics

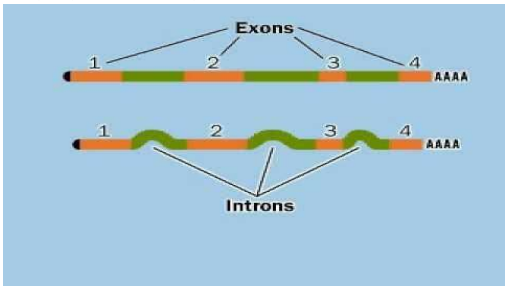
VIRAL GENOME

- More complex than any other species
- DNA or RNA
- Single stranded or double stranded
- Positive sense
- Negative sense

An Introduction to Viral Genomics

INTRONS

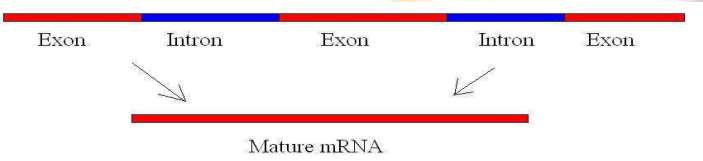
- Non-coding regions on viral genome



An Introduction to Viral Genomics

EXONS

- Coding regions on viral genome



An Introduction to Viral Genomics

MAIN GENES

•All viruses have these:

•Replicase – an enzyme that replicates genome

•Capsid – a protein that protects the genome

An Introduction to Viral Genomics

OTHER PROTEIN GENES

•Protease

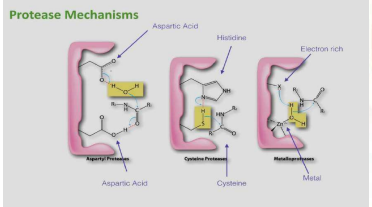
•Glycoprotein

•Host-shut-off proteins

•Anti-host defence proteins

An Introduction to Viral Genomics

PROTEASE



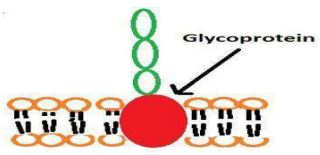
•Processes viral proteins

•Allows assembly

•Maturation to infect other cells

An Introduction to Viral Genomics

GLYCOPROTEIN



•Only in enveloped viruses

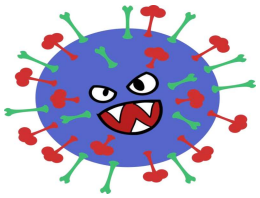
•Allows viral entry into host cell

•Targets specific type of host cells

•Aids in viral assembly

An Introduction to Viral Genomics

HOST-SHUT-OFF PROTEINS



- As name indicates
- Shut off host activities
- Only viral genes are processed

An Introduction to Viral Genomics

ANTI-HOST DEFENCE PROTEINS

- Number and type of these genes vary
- Prevent host defense mechanism from stopping viral replication

Virology

Terminologies used in Viral Genetics

Terminologies used in Viral Genetics

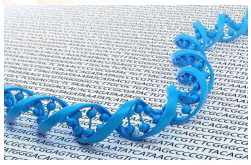
GENE

A segment of DNA molecule that encodes for synthesis of a single polypeptide

Terminologies used in Viral Genetics

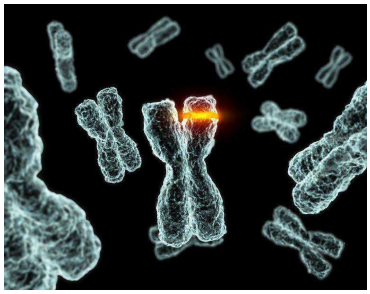
GENOME

- The entire DNA sequence of an individual/species



Terminologies used in Viral Genetics

MUTATION



- A permanent inheritable change in a single gene that results in the existence of two or more alleles occurring at the same locus

Terminologies used in Viral Genetics

PSEUDOTYPES

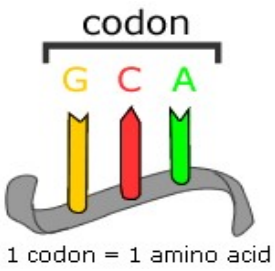
- viruses that have acquired the genotype of one parent and phenotype of another

Terminologies used in Viral Genetics

RECOMBINANTS

- A new combination of genes not found together on the chromosome in either parent
- A recombinant results from crossing over

Terminologies used in Viral Genetics



CODON

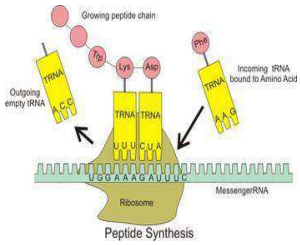
- A sequence of three bases in DNA or RNA that codes for a single amino acid
- Enables specific proteins to be made by specific genes

Terminologies used in Viral Genetics

ANTI-CODON

- A sequence of three bases in tRNA that is complementary to a codon in mRNA
- Enables tRNA to sequence amino acids in the order specified by mRNA

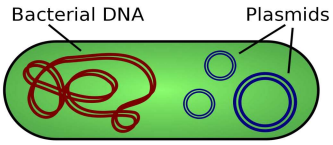
Terminologies used in Viral Genetics



RIBOSOMES

- Complexes of rRNA and protein in cytoplasm that serve as platforms for translation for mRNA into protein

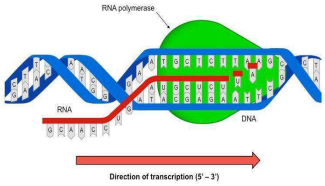
Terminologies used in Viral Genetics



PLASMID

- Extrachromosomal circular DNA in bacteria
- Independently replicate and encode a product

Terminologies used in Viral Genetics



TRANSCRIPTION

- Synthesis of single-stranded RNA by RNA polymerase using DNA as a template
- The process in the nucleus whereby DNA is transcribed into mRNA

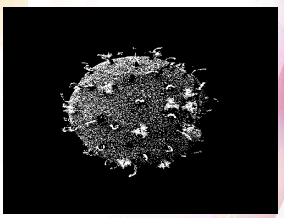
Terminologies used in Viral Genetics

TRANSLATION

- The process of translating the codon sequence in mRNA into polypeptides with the help of tRNA and ribosomes

Virology

Molecular Genetics of Viruses



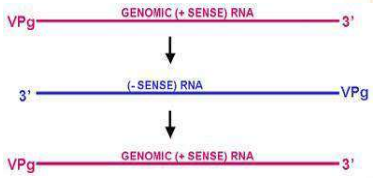
Molecular Genetics of Viruses

VIRAL GENETICS

- Viruses store their genetic info in six different types of genome
- Based on how nucleic acid is transcribed into viral mRNA

Molecular Genetics of Viruses

POSITIVE RNA STRAND



- RNA dependent RNA polymerase produces single stranded negative strand of RNA
- Then it produces positive strand of RNA
- Togavirus, Picornavirus etc.

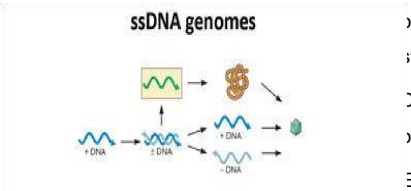
Molecular Genetics of Viruses

POSITIVE/ NEGATIVE DNA STRANDS

- Transcribed into mRNA
- DNA dependent DNA polymerase copies the strand to DNA strands
- RNA dependent RNA polymerase converts RNA into mRNA
- Then translated into viral proteins

Molecular Genetics of Viruses

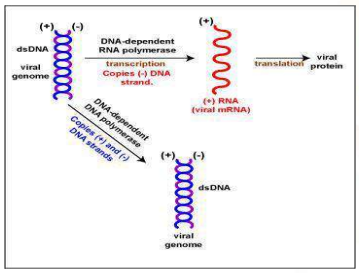
POSITIVE SINGLE STRANDED DNA



- DNA dependent DNA polymerases forms a double stranded DNA strand
- DNA dependent RNA polymerase forms the mRNA
- Example, Parvovirus

Molecular Genetics of Viruses

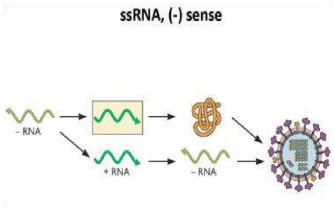
POSITIVE/ NEGATIVE DOUBLE STRANDED DNA



- RNA dependent RNA polymerases forms positive and negative RNA strand
- RNA dependent RNA polymerase forms the mRNA
- Translated into proteins

Molecular Genetics of Viruses

NEGATIVE SINGLE STRANDED DNA



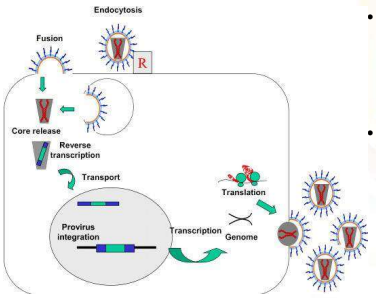
- RNA dependent RNA polymerases forms positive RNA strand
- RNA dependent RNA polymerase forms the mRNA
- Translated into proteins

Molecular Genetics of Viruses

POSITIVE RNA RETEROVIRUSES

- Reverse transcriptase enzyme
- Produces single stranded negative sense DNA
- DNA dependent DNA Polymerase copies to form double stranded DNA

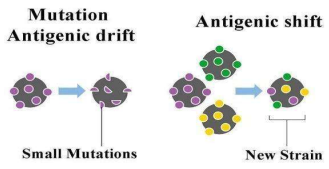
Molecular Genetics of Viruses



- DNA dependent RNA polymerase produces single stranded positive RNA genomes (mRNA)
- Examples, HIV-1, HIV-2, HTLV-1

Molecular Genetics of Viruses

VIRAL GENETICS

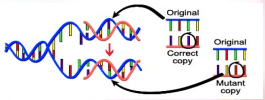


- Viruses usually grow rapidly, producing more chance of mutation
- Genetic changes by various mechanisms:
  - Genetic drift (Bases change)
  - Antigenic Shift (Recombination)

Molecular Genetics of Viruses

MUTATION

- Spontaneous Mutation that arise naturally during replication
- Induced Mutation that is caused by physical or chemical means



Virology

Genetic Techniques in Virology

Genetic Techniques in Virology

TYPES

- In-vitro (Physical analysis)
- In-vivo (viral genotype and phenotpe)

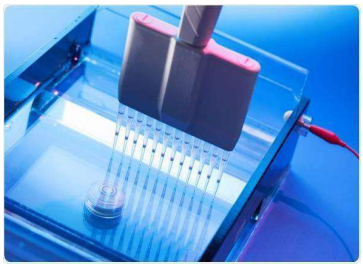
Genetic Techniques in Virology

IN-VITRO

- Physical analysis
- Isolation of nucleic acids from virus preparations
- Direct analysis of DNA using restriction endonucleases enzyme
- Use of vectors in DNA sequencing
- Electron microscopy
- RNA sequence analysis using Reverse transcriptase

Genetic Techniques in Virology

MOLECULAR ANALYSIS



- Molecular cloning
- Gel electrophoresis
- Hybridization of complementary nucleotide sequences

Genetic Techniques in Virology

PHENOTYPIC ANALYSIS

- Standard technique in virology
- Cell-free extracts to translate mRNAs
- Transfection tests
- In-vitro protein formation
- In-vivo protein replication

Virology

LATENT VIRUSES

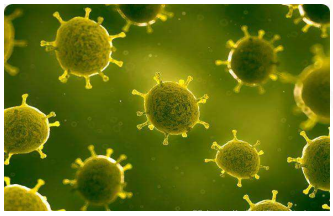
Latent viruses

MEANING

- The pathogenic viruses having the ability to lie dormant (or latent) within host cell
- Called lysogenic part of lifecycle

Latent viruses

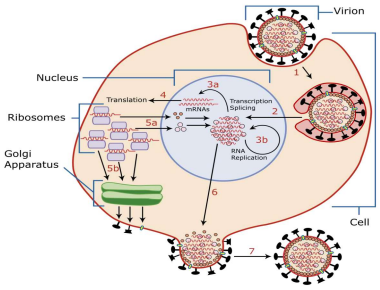
LATENCY



- The phase in which viral proliferation ceases after initial infection
- Viral genome not fully eradicated

Latent viruses

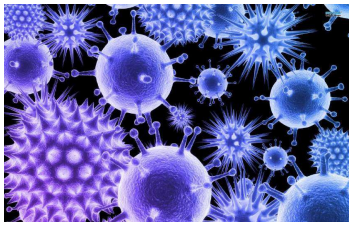
LYTIC PHASE



- The virus activates
- Continues to multiply increases its progeny
- Clinical signs appear

Latent viruses

MECHANISMS



1. Episomal Latency
2. Proviral Latency
3. Maintaining Latency

Latent viruses

EPISOMAL LATENCY

- Use of genetic episomes
- Viral genes float in cytoplasm or nucleus
- More vulnerable to host immune system
- Example, chicken pox and herpes simplex viruses

Latent viruses

PROVIRAL LATENCY

- Virus genome is integrated into the DNA of a host cell
- Automatic host cell division during viral replication
- Viral genome enters nucleus
- Example; HIV

Latent viruses

MAINTAINING LATENCY

- For continuity of infection
- By genes expressed during latency
- To evade host immune systme
- To protect virus genomre to be digested by cellular enzymes

Virology

EXAMPLE OF LATENT VIRUS

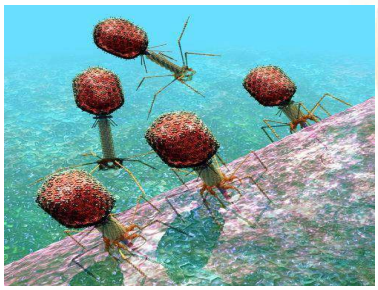
Example of Latent viruses

LATENT VIRAL INFECTIONS

1. Chronic persistent infections
2. Latent Occult Viral infections

Example of Latent viruses

CHRONIC PERSISTENT INFECTIONS



- Enveloped viruses
- e.g *Paramyxoviruses, Reteroviruses, Arenavirus*
- No disruption of celular functions
- Promotes shredding of viral Antigens from the cell surface

Example of Latent viruses

LATENT OCCULT VIRAL INFECTIONS

Some viruses reappear to cause acute disease

Three examples

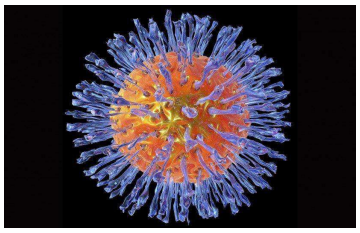
i. HSV

ii. Adenovirus

iii. SSPE

Example of Latent viruses

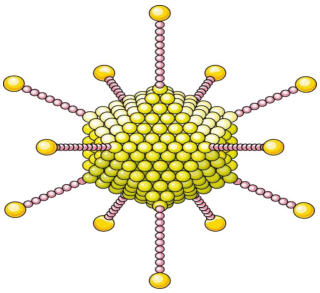
HSV



- Herpes Simplex Virus
- Primary infection in early age
- Found only during recurrent acute episodes
- Virus can be isolated from tissue homogenates

Example of Latent viruses

ADENOVIRUS



- Self-limitng
- Persistent infection of tonsils and adenoids
- Failure in viral isolation
- Absence of mature virion
- DNA in linear episomal form

Example of Latent viruses

MEASLES VIRUS

- Incomplete viral production
- Immature nucleocapsids produced
- Disease depends on immune response
- May cause tumorigenesis

Virology

VIRAL MUTANTS

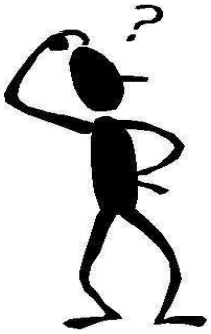
Introduction

Viral Mutants

MUTANT

- A mutant is an organism or a new genetic character arising or resulting from an instance of mutation, which is generally an alteration of the DNA/RNA sequence of the genome or chromosome of an organism

Viral Mutants



RELEVANT TERMS

- Strain
- Type
- Variant
- Mutant

Viral Mutants

DIFFERENCE IN RELEVANT TERMS

- Strain: lines or isolate of same virus
- Type: serotypes of same virus
- Variant: virus, whose phenotype varies from original virus
- Mutant: virus showing genetic alteration

Viral Mutants

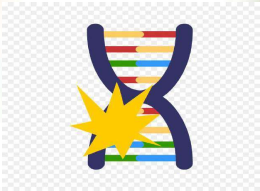
ORIGIN OF MUTATION

- **Spontaneous mutation:**
  - Very high to low rate of mutation
  - Due to difference in type of genome
  - Error rate higher in RNA dependent RNA polymerase
- **Induced Mutation**
  - In-vivo
  - or in-vitro mutagens

Viral Mutants

MAIN REASON

- Replication Error
- Chemicals
- Radiations



Viral Mutants

TYPES OF MUTANT VIRUSES

- Biochemical markers: drug-resistance mutations
- Deletions: similar to non-sense mutations
- Host range: refers to whole host animal
- Non-sense mutation: alteration of coding sequence
- Plaque morphology
- Temperature sensitive
- Cold sensitive
- Reverents

Virology

VIRAL MUTANTS

Spontaneous Mutation

Spontaneous Mutation

RATE

- High to low
- Very high in HIV
- Very low in Herpesvirus
- Resulting mutation in cellular DNA

Spontaneous Mutation

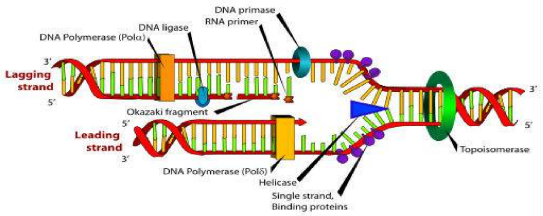
REASON

- Mechanism of genome replication
- RNA dependent RNA polymerase shows higher error rate than DNA dependent DNA polymerase

Spontaneous Mutation

DO RNA VIRUSES HAVE PROOF-READING?

- Yes!
- Yet they show higher rates of mutation than DNA viruses



Spontaneous Mutation

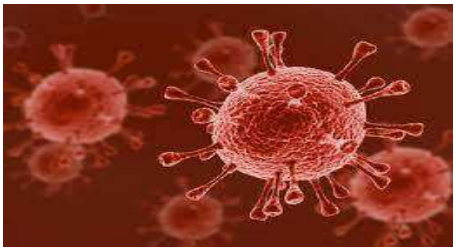
MUTATION: GOOD OR BAD?

- Sometimes good for the virus
- Helps escape the immune system
- But some mutations may be deleterious
- Due to production of defective particles

Spontaneous Mutation

HIV

- Genome is 9.7kb long
- Mutatuion rate can be 0.9 to 9.7 mutaations in every genome copied



Spontaneous Mutation

QUASISPECIES

- Mixture of molecular variants
- Usually the wild-type virus dominates
- Mostly non-infectious
- Important in viral evolution

Spontaneous Mutation

REASON?

- Tautomerism: base change due to repositioning of Hydrogen atom
- De-purination: Loss of purine base
- De-amination and hydrolysis: change of normal base to atypical base
- Slipped strand mis-pairing: Denaturation of new strand
- Replication slipping: error during replication process

Virology

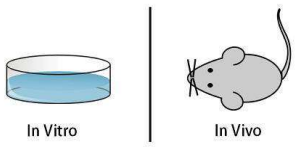
VIRAL MUTANTS

Induced Mutation

Induced Mutation

TYPES:

- In-vitro
- In-vivo



Induced Mutation

IN-VITRO

- Chemically modify nucleic acids
- Do not require replication
- E.g: nitrous acid, hydroxylamine and alkylating agents like nitrosoguanidine

Induced Mutation

IN-VIVO

- Requires Metabolically active nucleic acid that is replicating
- Compounds incorporated intoo newly synthesized nucleic acids
- E.g:5-bromouracil resulting erroneous base pairing, intercalating bases that stack between bases, UV irradiation

Induced Mutation

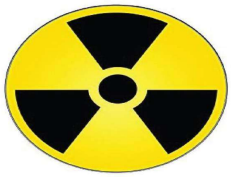
CHEMICAL REASON

- Hydroxylamine
- Base analogs
- Alkylating agents
- DNA intercalating substances
- DNA cross linkers
- Oxidative damage
- Nitrous acid

Induced Mutation

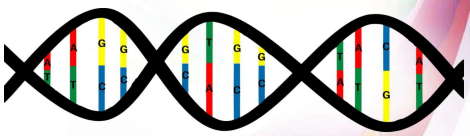
RADIATION REASON

- Ultraviolet rays
- Ionizing radiation



Virology

Types of Mutant Viruses



Types of Mutant Viruses

TYPES

- Biochemical Markers
- Deletions
- Host Range
- Non-Sense
- Plaque morphology
- Temperature sensitive
- Cold Sensitive
- Reverents

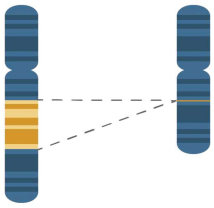
Types of Mutant Viruses

BIOCHEMICAL MARKERS

- Drug resistance mutations
- Altered virulence
- Polymorphism
- Altered proteins or nucleic acids
- Sensitivity to inactivating agents

Types of Mutant Viruses

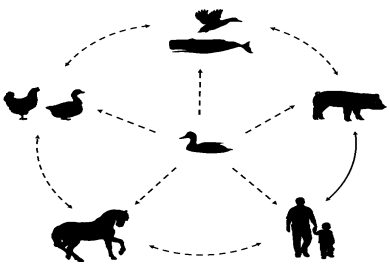
DELETIONS



- Similar to nonsense mutation
- May include one or more viral genes
- Involve non-coding regions of genome
- Defective-interfering particles (DIP)
- Can only revert to wild-type by recombination

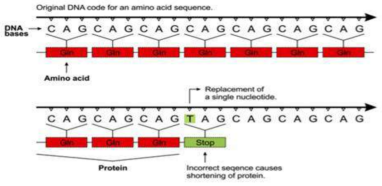
Types of Mutant Viruses

HOST RANGE



- Refers to whole host animal or cell type in vitro
- Conditional mutants

Types of Mutant Viruses



NON-SENSE

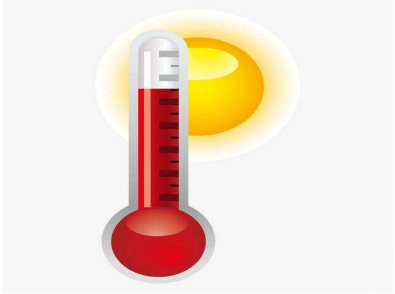
- Alteration of coding sequence of one of the three stop codons (UAG, UAA, UGA)
- Termination of translation
- Low reversion frequency

Types of Mutant Viruses

PLAQUE MORPHOLOGY

- Large plaque mutants or small plaque mutants
- Plaque size referred to temp. sensitivity
- Useful as unselected markers in multi-factorial crosses

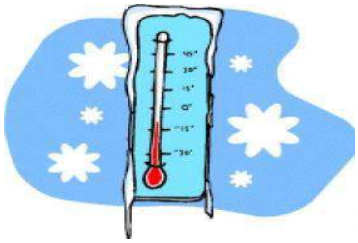
Types of Mutant Viruses



TEMPERATURE SENSITIVE

- Useful for isolation of conditional-lethal mutations
- Usually occur by mis-sense mutation in proteins
- Some activities work on non-permissive temperatures

Types of Mutant Viruses



COLD SENSITIVE

- Opposite to temperature sensitive
- Useful in bacteriophages and plant viruses as their cells can be propagated at low temperatures

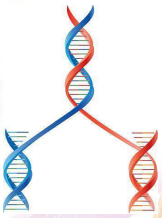
Types of Mutant Viruses

REVERENTS

- Valid type of mutation
- Reverse mutations
- 'back mutation' or 'compensatory mutation'

Virology

VIRAL RECOMBINATION



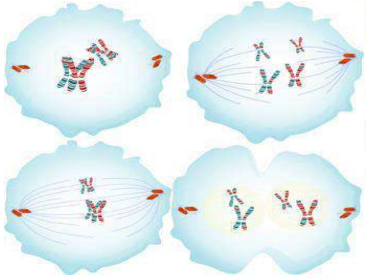
Viral Recombination

DEFINITION

- The exchange or transfer of genetic material between different but closely related viruses infecting the same cell

Viral Recombination

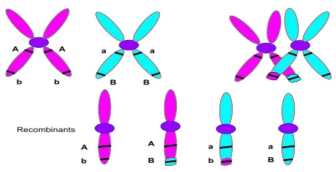
HOW?



- Can also occur between virus and host
- Recombinants contain new genetic information

Viral Recombination

INTRAMOLECULAR RECOMBINATION



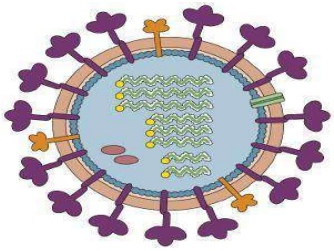
- Usually in DNA viruses
- Dissociation and re-establishment of covalent bonds with nucleic acids
- Template switching mechanism

Viral Recombination

EXAMPLES

- Picornavirus
- Togavirus
- Corona virus
- Influenza A virus

Viral Recombination



RNA VIRUSES

- Randomly with segmented genome
- E.g orthomyxovirus, reovirus, bunyavirus
- Genome segments show recombinations

Viral Recombination

GENETIC REACTIVATION

- Infectious progeny produced from parents, in which one or both are noninfectious
- Mixed infection of cell

Viral Recombination

ADVANTAGE

- Genetic variaility in nature
- Rapid adaptation of viruses
- Virulence changes
- Epidemiology of viruses (e.g. Influenza A)

Virology

SUPRESSION OF VIRAL GENOME

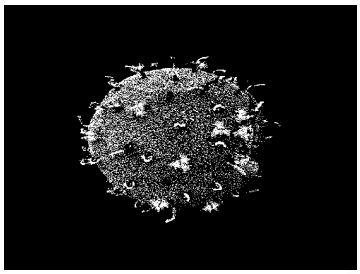
Viral Suppression

DEFINITION

- Inhibition of a mutant phenotype by a second suppressor mutation
- Which may be either in viral genome or in host cell

Viral Suppression

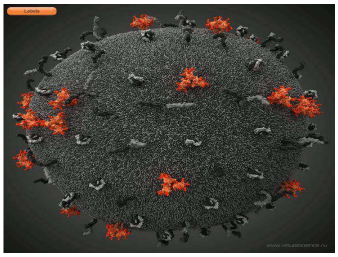
HOW?



- Mechanism different from suppression of chain-terminating amber mutations by host encoded suppressor tRNAs
- That is also referred as informational suppression

Viral Suppression

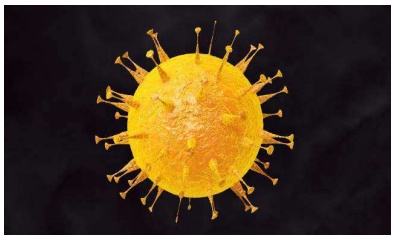
PSEUDOREVERENT



- Usually apparent wild-type strain
- Genetically mutant
- Prokaryotic cells
- Now discovered in reovirus, vaccinia, influenza

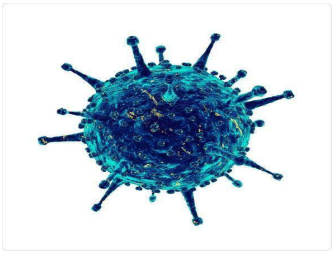
Viral Suppression

REVERSION



- Mutant viruses can revert by three ways:
- True reversion
- Intragenic reversion
- Extragenic Reversion

Viral Suppression



TRUE REVERSION

- Back mutation of original mutation
- Gives wild-type genotype or phenotype

Viral Suppression

INTRAGENIC SUPPRESSION

- Compensatory mutation, which occurs in same gene as originally mutated gene
- Restoring it

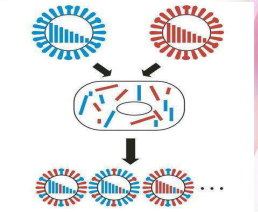
Viral Suppression

EXTRAGENIC SUPPRESSION

- A suppressor mutation in a different gene
- Virus gene or host gene

Virology

GENETIC INTERACTIONS BETWEEN VIRUSES

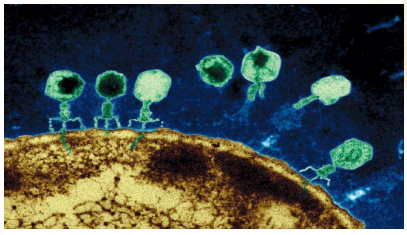


Genetic Interactions between viruses

WHEN?

- Naturally
- More than one infection in host

Genetic Interactions between viruses



Super-infection

- Mixed infection in cell culture
- Two purposes:
- COMPLEMENTATION
- RECOMBINATION

Genetic Interactions between viruses

COMPLEMENTATION

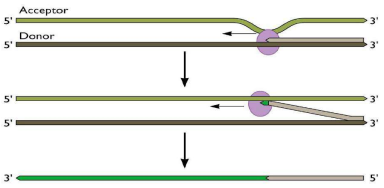
- Assignment of mutants to functional groups
- Resulting production of one or both parent viruses
- One of the viruses provides functional gene product
- Allows functional analysis of unknown mutations

Genetic Interactions between viruses

TYPES OF COMPLEMENTATION

- Allelic (intragenic) complementation where different mutants have defects in same protein
- Non-allelic (intergenic) results from mutants with defects in different genes

Genetic Interactions between viruses



RECOMBINATION

- Physical interaction of viral genomes resulting gene combinations not present in either parents
- Three mechanisms: Intra-molecular recombination by strand breakage and re-ligation, intra-molecular recombination by copy-choice and Reassortment

Genetic Interactions between viruses

INTRA-RECOMBINATION BY STRAND BREAKAGE AND LIGATION

- Occurs in all DNA and RNA viruses that replicate via DNA intermediate
- Mediated by cellular enzymes

Genetic Interactions between viruses

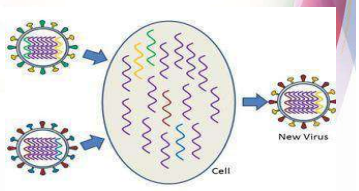
INTRA-MOLECULAR RECOMBINATION BY COPY CHOICE

- In RNA viruses
- During switching of viral polymerase
- Cellular enzymes involved
- Defective Interfering

Genetic Interactions between viruses

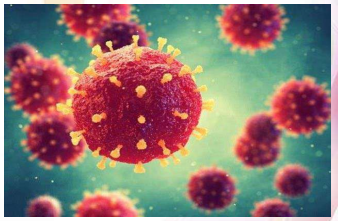
REASSORTMENT

- Random shuffling
- Progeny viruses receive atleast one of the parent gene segment
- Packaging mechanism not understood



Virology

NON-GENETIC INTERACTIONS BETWEEN VIRUSES



Non-Genetic Interactions between viruses

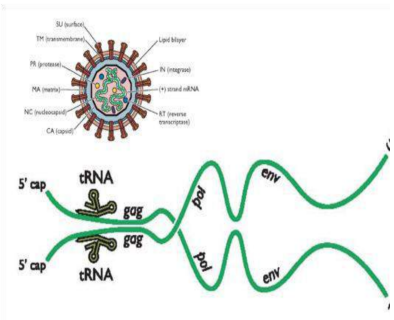
EUKARYOTIC CELLS

- Diploid genome
- Two copies of each chromosome
- Each having own allele of same gene
- Difference in allelic markers at loci

Non-Genetic Interactions between viruses

RETROVIRUS

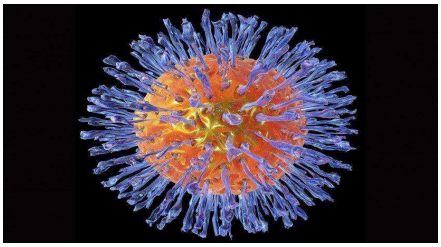
- Truly diploid
- Two copies of entire genome



Non-Genetic Interactions between viruses

HERPESVIRUS

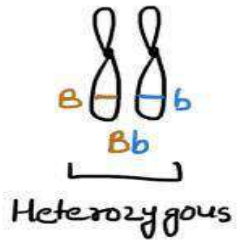
- Partially heterozygous
- Repeated sequences



Non-Genetic Interactions between viruses

HETEROZYGOSIS

- Aberrant packaging of multiple genomes
- May result in multiploid particles
- genetic complexity of population



Non-Genetic Interactions between viruses

SUPERINFECTION

- Interference as a result of superinfection
- Homologous interference against same virus from presence of particles which blocks replication
- Interference by mutation
- Interference by sequestration of virus receptors due to virus-attachment protein

Non-Genetic Interactions between viruses

PHENOTYPIC MIXING

Variable

Genome maybe enclosed in capsid/envelope of another (pseudo-typing)

capsid contains genomic particles of both viruses

Non-specific incorporation of different viral glycoproteins

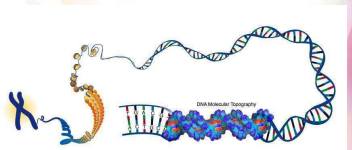
Non-Genetic Interactions between viruses

PHENOTYPIC MIXING AS A TOOL

- Examining biological properties of viruses
- Vesicular stomatitis virus forms pseudotypes containing retrovirus envelope glycoproteins
- Plaque forming properties of VSV, cell tropism properties of retrovirus
- Used for HIV and Retrovirus

Virology

LARGE DNA GENOMES



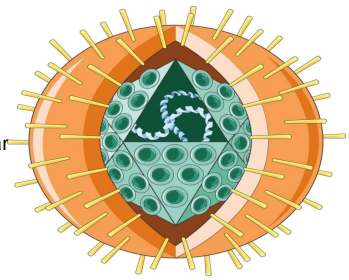
Large DNA genomes

- Viral genome could be considerably big e.g DS DNA genomes
- Generally, Genetically similar to host cells e.g *Adenovirus, herpes ctc.*

Large DNA genomes

HERPESVIRIDAE

- Large family
- More than 100 members
- Eight human viruses, with similar overall structure
- Subdivided into 3 families



Large DNA genomes

SUBFAMILIES

Alphaherpesvirinae

- Latent infections in sensory ganglion, genome size 120-180kbp
- Simplexvirus, Varicellovirus, Human herpes virus-I and II,
- Human herpes virus-III

Betaherpesvirinae

- Restricted host range, genome size 140-253 kbp
- Cytomegalovirus, Muromegalovirus, Roseolovirus, Human herpes virus-V, Mouse cytomegalovirus-I, Human herpes virus-VI and VII

Gammaherpesvrinae

- Infection of lymphoblastic cells, genome size 105-175 kbp
- Lymphocryptovirus, Rhadinovirus, Human herpesvirus-IV, Human herpes virus-VIII

Large DNA genomes

HERPESVIRUS

- large linear double stranded DNA genome containing 35 viral polypeptides encoding variety of enzymes
- Two covalently joined sections, Unique long (U_L)and Unique short regions(U_S) by inverted repeats
- Allows structural rearrangements of unique regions

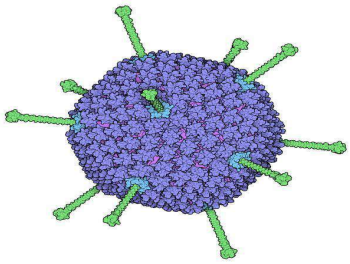
Large DNA genomes

HERPES SIMPLEX VIRUS

- 152 kbps ds DNA, 80 genes
- Densly packed with overlapping reading frames
- Each gene expressed by own promoter
- Extensively mapped by conventional genetic analysis
- Most intensively studied virus

Large DNA genomes

ADENOVIRUS

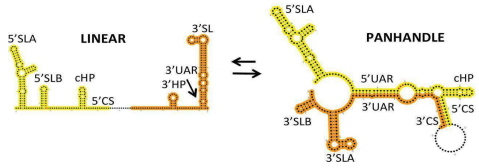


- Linear double stranded DNA genome of 30 to 38 kbp, 30-40 genes
- Terminal sequence is inverted repeat of 100 to 140bp
- Denatured single strands form panhandled structure

Large DNA genomes

PANHANDLED STRUCTURES

- Important in replication
- 55kDA protein known as terminal protein
- Attached to 5' end of each strand
- Acts as primer and initiates synthesis of new strands



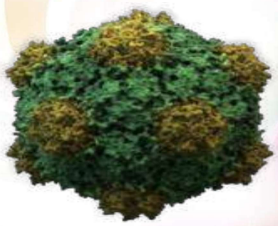
Large DNA genomes

EXPRESSION

- Promoters
- Splicing patterns

Virology

VIRUSES WITH SMALL DNA GENOMES



Small DNA genomes

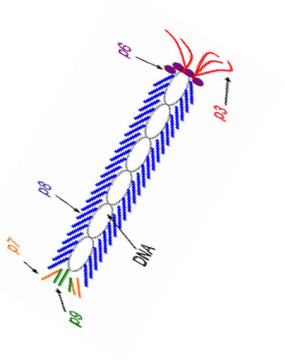
M-13 PHAGE

- Simple genome
- 6.4kb
- Single stranded
- Positive sense
- Circular DNA encoding ten genes

Small DNA genomes

M-13 PHAGE

- Icosahederal symmetry
- Capsid can be expanded by protein subunits
- Hence, genomic size can also be increased



Small DNA genomes

LAMBDA PHAGE

- Rigid packaging restraints
- Only DNA of between 95 and 110% of normal genome size can be packaged into the capsid
- 49kbp
- Linear genome

Small DNA genomes

LAMBDA PHAGE

- Substrate packaged into phage heads during assembly consist of long concatemers of phage DNA
- DNA apparently whirled by phage head
- Complete genome cleaved by phage coded endonuclease
- *Cos-site*
- DNA ligase

Small DNA genomes

T-4 PHAGE

- 160 kbp
- Double stranded DNA
- Linear genome

Small DNA genomes

T-4 PHAGE

- Terminal redundancy
- Long Contactmers of DNA genome
- Longer than complete genome

Small DNA genomes

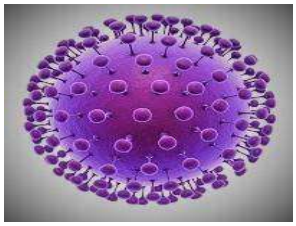
PARVOVIRUS

- Linear, non-segmented, single stranded negative sense genome, 5kb
- Most packaged as negative sense
- A portion of positive sense strands , very small
- Two genes *cap* and *rep*

Small DNA genomes

PARVOVIRUS

- Splicing pattern for each gene
- Pallindromic sequences for hair-pin structures



Small DNA genomes

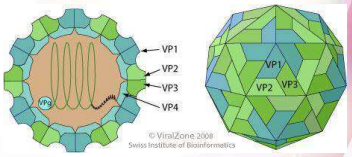
POLYOMAVIRUS

- Double stranded, circular DNA, 5 kb
- DNA in super-coiled form
- Four cellular histones
- Maximum information in minimum space

Virology

PICORNAVIRUS

Properties



PicornaVirus

PROPERTIES

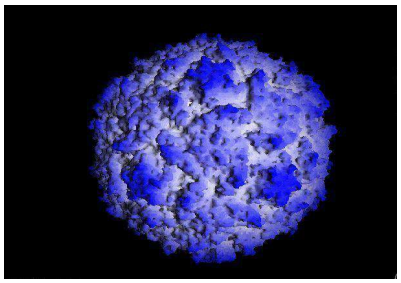
- Non-enveloped
- Icosahedral symmetry

Biochemicaly,

- Ether- resistant
- Acid stable

PicornaVirus

- One of the largest families
- PICO RNA
  - Small sized
  - RNA genome
- 9 genera
- 230 members



PicornaVirus

- A picornavirus is a virus belonging to the family *Picornaviridae*, and order Picornavirales.
- Vertebrates, including humans, serve as natural hosts.
- Birds also serve as host
- Picornaviruses are nonenveloped viruses that represent a large family of small, cytoplasmic, plus-strand RNA (~7.5kb) viruses .
- 30-nm icosahedral capsid.

PicornaVirus

- There are currently 80 species in this family, divided among 35 genera.
- These include the *Enterovirus*, *Aphthovirus*, *Cardiovirus*, *Rhinovirus* and *Hepatovirus* genera.
- The viruses in this family can cause a range of diseases including paralysis, meningitis, hepatitis and poliomyelitis.

PicornaVirus

Enteroviruses	Rhinoviruses	Other picornaviruses
• poliomylietis • Echovirus • Bovine enterovirus • Simian Enterovirus • Avian Enterovirus	• Human cold virus • Equine rhinovirus • Bovine rhinovirus • Foot and mouth disease • Vesicular exanthema	• Avian encephalomylietis • Mouse encephalitis • Feline picornavirus

Virology

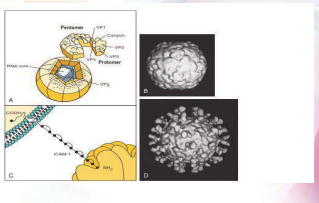
PICORNAVIRUS

Genome

Picornavirus

GENOMIC PROPERTIES

- Resembles mRNA
- Positive sense
- Single stranded RNA
- 7200 to 8450 bases



Picornavirus

GENOME

- The genome is non-segmented and positive-sense.
- The geome RNA is unusual because it has a protein on the 5' end that is used as a primer for transcription by RNA polymerase.
- This primer is called VPg genome range between 2–3 kb.
- VPg contain tyrosine residue at the 3’ end.
- Tyrosine as a –OH source for covalently linked to 5’ end of RNA.

Picornavirus

- Picornaviruses are non-enveloped, with an Icosahedral capsid.
- The capsid consists of 60 protomers.
- Each protomer consists of 4 polypeptides
- These peptides are called VP (viral protein) 1, 2, 3 and 4.
- VP2 and VP4 polypeptides originate from one protomer known as VP0
- VP0 is cleaved to give the different capsid components.

Picornavirus

REPLICATION

- The mRNA encodes RNA dependent RNA polymerase.
- This polymerase makes complementary minus strands of RNA.
- RNA will act as template to make more plus strands.

Picornavirus

REPLICATION

an overview of the steps in picornavirus replication attachment,

- entry
- translation
- transcription/genome replication
- Assemble
- exit

Virology

PICORNAVIRUS

Important diseases

PICORNAVIRUS

POLIOVIRUS

- Causes polio
- Deadly neurological conditions
- Mostly subclinical
- 1% suffer from paralytic legs
- Deadly respiratory failure

PICORNAVIRUS

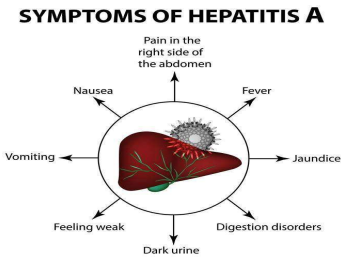
RHINOVIRUS

- Most common virus
- Common flu in humans
- Rhinovirus infection proliferates in temperatures between 33–35 °C (91–95 °F)

PICORNAVIRUS

HEPATITIS A

- Loss of appetite
- Jaundice
- Dark urine
- No long term consequences



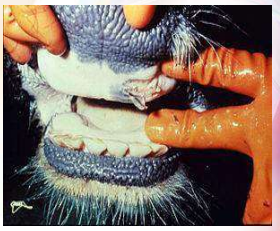
PICORNAVIRUS

FOOT AND MOUTH DISEASE

- In hooved animals
- Lesions around foot and mouth
- Inability to walk
- Fast spread
- Significant economic damage

PICORNAVIRUS

Lesions



Virology

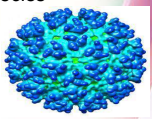
TOGAVIRUS

General properties

TOGAVIRUS

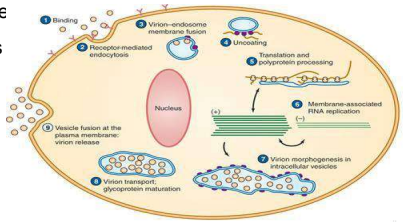
PROPERTIES

- Enveloped
- Positive single stranded RNA
- Icosahedral
- 2 genera having 32 species
  - Arbovirus
  - Rubula virus



TOGAVIRUS

- Early nonstructural and late structural protein synthesis
- Cytoplasmic replication
- Budding at plasma membrane



TOGAVIRUS

GENOME

- Linear, non-segmented, single-stranded, positive sense RNA
- 10,000–12,000 Nucleotides
- The virus is enveloped and forms spherical particles (65–70 nm diameter)
- The capsid is icosahedral, constructed of 240 monomers, having a triangulation number of 4.

TOGAVIRUS

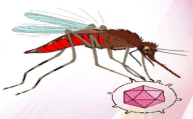
REPLICATION

- Entry into the host cell is achieved by attachment of the viral E glycoprotein to host receptors
- which mediates clathrin-mediated endocytosis.
- The receptors for binding are unknown
- Glycoprotein petal-like spikes act as attachment proteins.
- After virus attachment and entry into the cell, gene expression and replication takes place within the cytoplasm.

TOGAVIRUS

ARBOVIRUS

- Arthropod born virus (mosquitoes)
- Broad host range
- (Humans and plants)
- Zoonotic



Virology

TOGAVIRUS
Genome

TOGAVIRUS

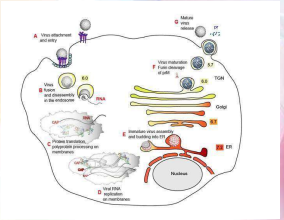
GENETIC CHARACTERS

- Positive sense
- Single stranded RNA
- Around 11.7kb
- The 5'-terminus has a methylated nucleotide cap.

TOGAVIRUS

REPLICATION

- Viral RNA acts as both genome as well as mRNA
- After fusion of viral capsid with cell receptor
- RNA released in cytoplasm



TOGAVIRUS

VIRION STRUCTURE

- Togavirus virions consist of an envelope and a nucleocapsid.
- The virions are spheical to pleomorphic in shape, and about 70 nm in diameter.
- Glycoprotein spikes evenly cover the surface of the virion.
- The genome is contained in the T=4 icosahedral capsid, which measures 40 nm in diameter.

TOGAVIRUS

REPLICATION

- Nonstructural proteins formed first
- Replication within cytoplasm

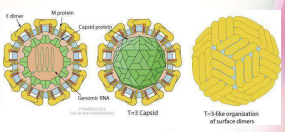
Virology

FLAVIVIRUS
Properties

FLAVIVIRUS

CHARACTERISTICS

- RNA virus
- Enveloped



FLAVIVIRUS

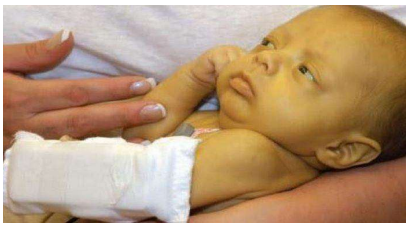
DENGUE FEVER VIRUS

- Mosquito borne
  - *Aedes Egyptei*
- Infects bone marrow (break bone fever)
- Low platelet level
- Hemorrhegic fever
- Can lead to renal failure
- Septicemia and death

FLAVIVIRUS

YELLOW FEVER

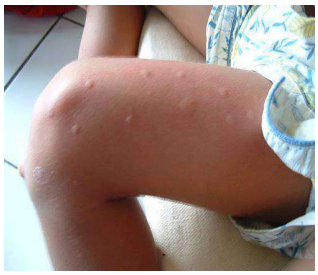
- *Aedes* mosquito
- Jaundice
- Backache
- Bloody diarrhea
- Bloody vomiting
- Vaccine available



FLAVIVIRUS

WEST NILE VIRUS

- Birds are reservoir
- Mosquitoes Vector
  - Culex
- Encephalitis
- Meningitis
- Flaccid paralysis
- Seizures and coma



FLAVIVIRUS

HEPATITIS C

- Transmission: Exposure to infected blood
- Highly antigenic variable
- Jaundice
- 60-80% become chronic cases
- Cirrhosis
- Primary cause of hepatocellular carcinoma
- Interferon treatment

Virology

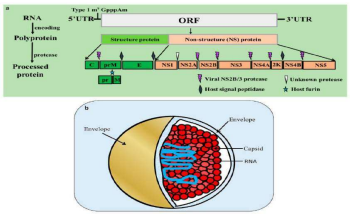
FLAVIVIRUS

Genome

FLAVIVIRUS

GENOME

- RNA genome
- Positive sense
- Non-segmented



FLAVIVIRUS

TWO GROUP HYPOTHESIS

On the basis of morphological information

Obtained using electron microscopy

supported the hypothesis of the existence of at least two virus groups

FLAVIVIRUS

- One group includes non-enveloped virus
- Family Reoviridae (genera Orbivirus, Coltivirus and Seadornavirus)
- Viruses with an overall diameter of 60–80 nm
- Icosahedral symmetry
- Several concentric capsid layers that surround a segmented double-stranded RNA (dsRNA) genome

FLAVIVIRUS

- Second group includes enveloped viruses (inactivated by ether and deoxycholate)
- 50–60 nm in diameter
- Infectious ssRNA genome of positive polarity.
- Two antigenically distinct subgroups i.e.
- Group A and Group B.

FLAVIVIRUS

MEDICAL IMPORTANCE

- Mostly serologically related to each other
- Antibodies of one useful against others
- Antigenic variability of Hepatitis C proteins
- The 17D vaccine is one of the most efficient vaccines ever developed and was derived from a strain of Flavivirus isolated from a man who recovered from infection by the virus.

Virology

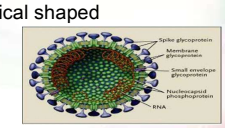
CORONAVIRUS

Structure

Coronavirus

STRUCTURE

- Enveloped/ encapsulated
- Club shaped projections on envelope
- 80-160nm diameter
- Helical shaped



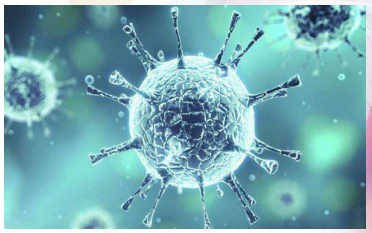
Coronavirus

- *Coronaviruses* are species belonging to the subfamily Coronavirinae in the family Coronaviridae, in the order Nidovirales.
- Coronaviruses are enveloped viruses with a positive sense single-stranded RNA genome and with a nucleocapsid of helical symmetry.
- The Genomic size of coronaviruses ranges from approximately 26 to 32 Kb, the largest for an RNA virus

Coronavirus

COMMON COLD

- Second most common cause of common cold
- SARS (Severe Acute Respiratory syndrome)
- GI tract infections in kids



Coronavirus

SARS

- Zoonotic origin
- Acute respiratory form
- Outbreaks leads to enormous number of deaths
- Flu and Fever
- Lethargy
- Cough
- Pnemonia

Coronavirus

MERS

- Middle east respiratory syndrome
- Fever
- Cough
- Shortness of breath
- Sometimes diarrhea
- Mortality rate: 35%
- Camels are major reservoir hosts

Virology

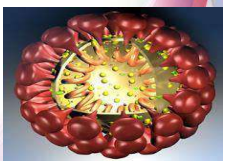
CORONAVIRUS

Genome

Coronavirus

GENOME

- Positive sense
- Single stranded
- RNA
- 27,000 -30,000 kb genome



Coronavirus

- Enveloped
- Spherical
- About 120 nm in diameter
- The RNA genome is associated with the N protein to form the nucleocapsid (helical for the genus coronavirus, and tubular for the genus torovirus)
- Linear ssRNA

Coronavirus

- 27-32kb in size (the largest of all RNA virus genomes)
- Capped
- Polyadenylated
- The leader RNA (65-89 bp) at the 5' end of the genome is also present at the end of each subgenomic RNAs.

Coronavirus

REPLICATION

CYTOPLASMIC

- Attachment of the viral S protein receptors
- Endocytosis of the virus into the host cell
- Fusion of virus membrane with endosomal membrane
- Genome is released into the cytoplasm.
- Synthesis and proteolytic cleavage of the replicase polyprotein.

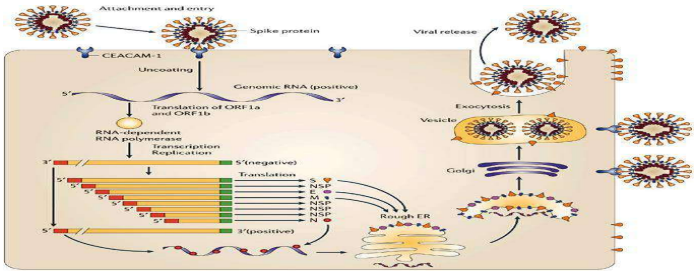
Coronavirus

REPLICATION

- A dsRNA genome is synthesized from the genomic ssRNA(+).
- The dsRNA genome is transcribed by providing viral mRNAs/new ssRNA(+) genomes.
- Synthesis of structural proteins encoded by subgenomic mRNAs.
- Assembly and budding at membranes of the endoplasmic reticulum (ER), the intermediate compartments, and/or the Golgi complex.
- Release of new virions by exocytosis.

Coronavirus

REPLICATION IN CYTOPLASM



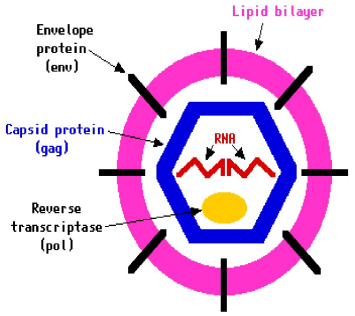
Virology

RETROVIRUS

RETROVIRUS

BASICS

- An envelope
- Envelope protein
- A capsid
- Two molecules of RNA
- Reverse transcriptase



RETROVIRUS

REPLICATION PATTERN

Once inside the host cell cytoplasm, the virus uses its own RT enzyme to produce DNA from its RNA genome

The reverse of the usual pattern, thus *retro* (backwards)

The new DNA is then incorporated into the host cell genome by an integrase enzyme referred to as a provirus

RETROVIRUS

REPLICATION PATTERN

The host cell then treats the viral DNA as part of its own genome, transcribing and translating the viral genes along with the cell's own genes

The proteins are produced which are required to assemble new copies of the virus.

It is difficult to detect the virus until it has infected the host.

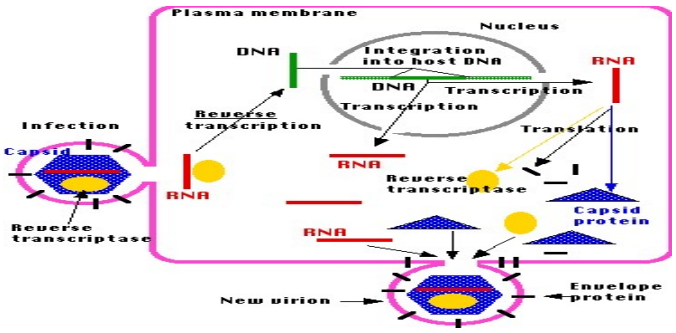
Infection will persist indefinitely

RETROVIRUS

REPLICATION PATTERN

- The gag gene --- capsid protein
- The pol gene --- molecules of reverse transcriptase
- The env gene --- molecules of the envelope protein

RETROVIRUS



RETROVIRUS

HUMAN IMMUNODEFICIENCY VIRUS

- Most important immunosuppressing virus
- Causes immuno-supression
- HIV-1 and HIV-2
- AIDS

RETROVIRUS

DISCOVERY

- In February 1997
- Parkinson's disease
- Transplant of pig tissue to human beings
- Recipients immune system need to suppress to avoid rejection
- HIV-1 and HIV-2, retroviruses of primates

RETROVIRUS

TRANSMISSION

- blood
- Semen
- pre-seminal fluid
- rectal fluids
- vaginal fluids
- breast milk
- **Blood transfusion**
- **Syringes**

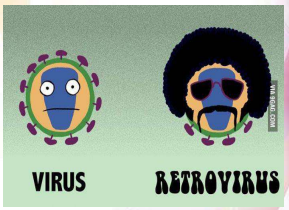
Virology

RETROVIRUS

Characteristics

RETROVIRUS

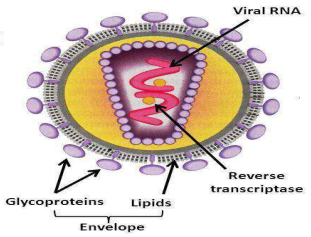
Most challenging virus for human beings so far.....



RETROVIRUS

Retrovirus Structure

- All retroviruses:
  - Are surrounded by a viral envelope
  - Are icosahedral in shape
  - Contain 2 identical molecules of RNA
  - Contain the enzyme *reverse transcriptase*



RETROVIRUS

- Virions of retroviruses consist of enveloped particles about 100 nm in diameter.
- The virions also contain two identical single-stranded RNA molecules 7–10kb in length.
- Although virions of different retroviruses do not have the same morphology or biology
- All the virion components are very similar.

RETROVIRUS

Virion components

- Envelope
- Nucleic acid (RNA)
- Protiens

RETROVIRUS

ENVELOPE

- composed of lipids
- obtained from the host plamsa membrane
- glycoprotein encoded by the env gene.

Functions

- protection from the extracellular environment via the lipid bilayer
- enabling the retrovirus to enter/exit host cells through endosomal membrane trafficking
- the ability to directly enter cells by fusing with their membranes.

RETROVIRUS

NUCLEIC ACID

- dimer RNA
- cap at the 5' end and a poly(A) tail at the 3' end
- terminal noncoding regions for replication
- internal regions that encode virion proteins
- The 5' end includes four regions, which are R, U5, PBS, and L.

PROTEINS

- consisting of gag proteins, protease, pol proteins, and env proteins

RETROVIRUS

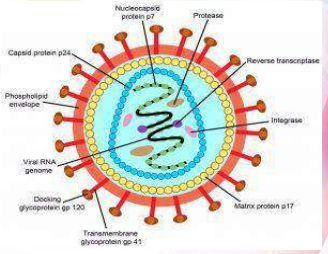
SALIENT CHARACTERISTICS

- Retroviruses are the only animal viruses that integrate into the host cell's genome
- The retroviral genomes can accommodate changes to its configuration.
- The retroviral life cycle begins in the nucleus of an infected cell.
- Retroviruses offer gene therapy researchers aid for delivering genes to target cells at high efficiency that allows for long-term, stable expression of introduced genetic elements

Virology

RETROVIRUS

Genome



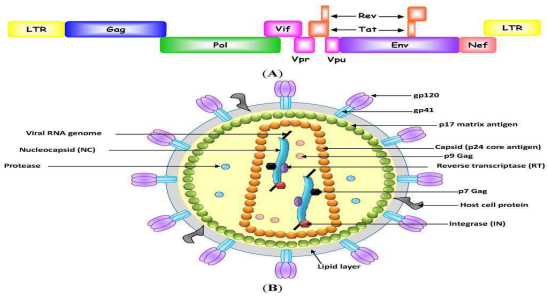
RETROVIRUS

GENETIC CHARACTERS

- Single stranded RNA viruses
- Positive sense
- enveloped
- Changes to DNA by RT enzyme

RETROVIRUS

Genomic Structure



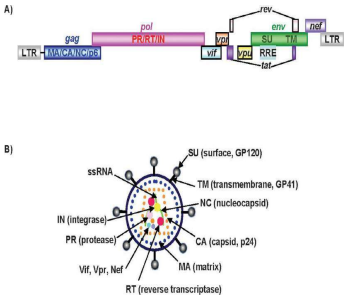
RETROVIRUS

Genetic makeup

Diploid nature of virus

3 very imp. genes:

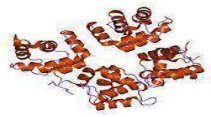
- GAG
- ENV
- POL



RETROVIRUS

GAG

- Gag gene makes protein p24
- Which serves as capsule around RNA
- Cone shaped



RETROVIRUS

ENV

- Envelope glcoproteins
- gp41 and gp120
- Gp41: transmembrane protein
- Gp120: outer glycoprotein
- Host receptor

RETROVIRUS

POL

- Reverse transcriptase Polymerase
- Integrase activity
- DNA from RNA

Virology

RETROVIRUS
Lifecycle

Virology

RETROVIRAL REPLICATION CYCLE

1- early steps

from the cell surface to the nucleus

- Retroviral particles must bind specifically to their target cells
- cross the plasma membrane
- reverse-transcribe their RNA genome
- Uncoat the cores
- find their way to the nuclear membrane
- penetrate into the nucleus

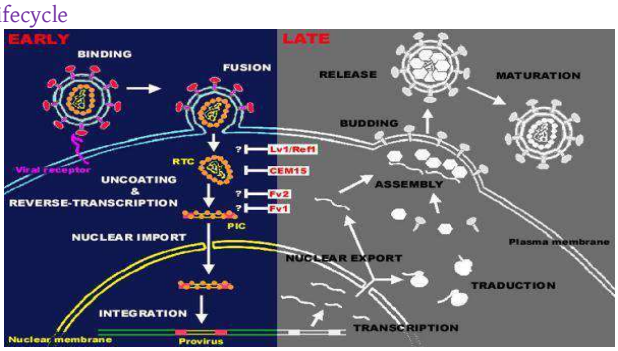
Hijack the cellular machinery

Virology

2- LATER STAGE

- Transcription
- Transduction
- Assembly
- Maturation
- Budding
- Release

- Lifecycle



Life Cycle of a Retrovirus - YouTube.MP4

Virology

POSITIVE SENSE RNA PLANT VIRUS

Positive Sense RNA Plant Virus

LUTEOVIRIDAE

- Sobemovirus

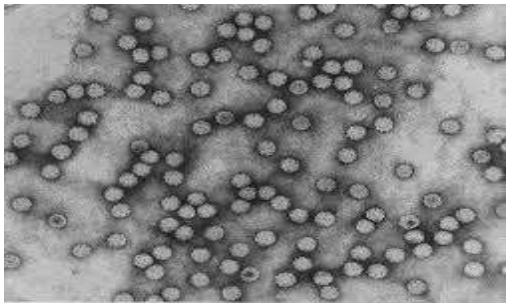
Positive Sense RNA Plant Virus

SEQUIVIRIDAE

- Sadwavirus
- Chevavirus

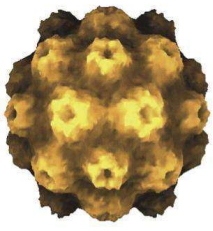
Positive Sense RNA Plant Virus

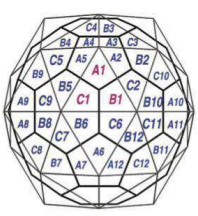
TOMBUSVIRIDAE

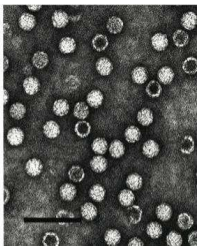


Positive Sense RNA Plant Virus

TYMOVIRIDAE







Positive Sense RNA Plant Virus

COMOVIRIDAE

- Idaeovirus

Positive Sense RNA Plant Virus

BROMOVIRIDAE

- Cucumovirus
- Bromovirus
- Ilavirus
- Alfamovirus
- Ourmiavirus
- Tobamovirus
- Hordievirus
- Pecluvirus
- Furovirus
- Pomovirus
- Bemivirus

Positive Sense RNA Plant Virus

OTHERS…

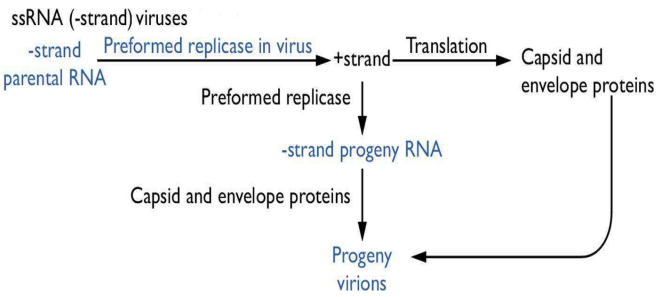
- Flexiviridae
- Potyviridae
- Closteroviridae

Virology

NEGATIVE SENSE RNA VIRUSES

Negative Sense RNA viruses

REPLICATION



Negative Sense RNA viruses

Family	Subfamily	Genus	Species
Bornaviridae		*Bornavirus*	Borna disease virus
Bunyaviridae		*Bunyavirus* *Hantavirus* *Phlebovirus*	Bunyamweravirus Hantaanvirus Rift valley fever
Orthoviridae		*Influenza virus A,B,C* *Thogotovirus*	Influenza A,B,C Thogotovirus
Paraviridae	*Paramyxovirinae* *Pneumovirinae*	*Respirovirus* *Morbillivirus* *Rubulavirus* *Pneumovirsu* *Metapneumovirus*	Sendai virus Measles Virus Mumps virus Human respiratory synctial virus
Rhabdoviridae		*Lyssavirus* *Vessiculovirus* *Ephemerovirus* *Novirhabdovirus*	Rabies virus Vessicular Stomatitis Virus Bovine Ephemeral fever virus

Virology

BUNYAVIRUS

Characteristics

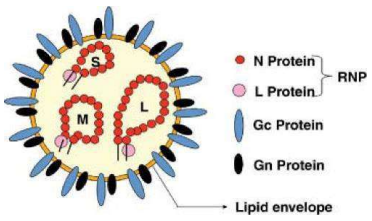
Bunyavirus

GENERAL PROPERTIES

- Enveloped RNA viruses
- Found in Arthopods and rodents ; occasionally infecting humans
- Some of them also infect plants
- Vector-borne except Hantaviruses
- Vectors (mosquitos, tick, or sandfly)
- Hantaviruses are transmitted through contact with deer mice feces
- Vector activity, for example, mosquito-borne viruses are more common in the summer

Bunyavirus

MORPHOLOGY



- Spherical
- 90-100nm
- Lipid envelope
- Spikes
- Lack matrix proteins

Bunyavirus

CLASSIFICATION

- Largest family
- 5 genera:

1. Orthobunyavirus
2. Hantavirus
3. Nairovirus
4. Phleovirus
5. Tospovirus

Bunyavirus

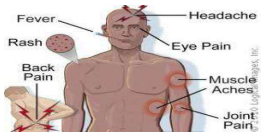
DISEASES

Family: Peribunyaviridae

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

Family: Nairoviridae

Crimmean Hemmorhegic Congo fever
Vector: ticks



Bunyavirus

DISEASES

Family:Phenuiviridae

- Rift velley fever
- Vector: mosquitoes

Family:Hantaviridae

- Hantavirus
- Vector: aerosolized excreta from these mammals

Virology

BUNYAVIRUS

Genome

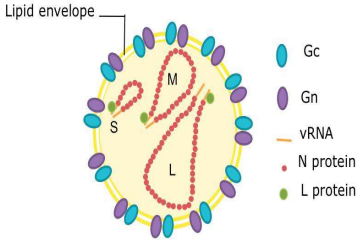
Bunyavirus

GENOMIC PROPERTIES

- Negative sense RNA virus
- Three segments (segmented)
  - Large (L)
  - Medium (M)
  - Small (S)
- 11-19kbs

Bunyavirus

MORPHOLOGY



- Three segments (segmented)
  - Large (L)
  - Medium (M)
  - Small (S)

Bunyavirus

SEGMENT SIZE

- Vary

Segment	Size
L	6.3-12
M	3.5-6
S	1-2.2

Bunyavirus

FUNCTIONS

- The L segment encodes the RNA Dependent RNA-polymerase, necessary for viral RNA replication and mRNA synthesis.
- The M segment encodes the viral glycopro teins, which project from the viral surface and aid the virus in attaching to and entering the host cell.
- The S segment encodes the nucleocapsid protein (N).

Bunyavirus

- The L and M segment are negative sense.
- the S segment is ambisense (Phlebovirus and Tospovirus)
- The S segment codes for the viral nucleoprotein (N) in the negative sense and a non structural (NSs) protein in ambisense.

Virology

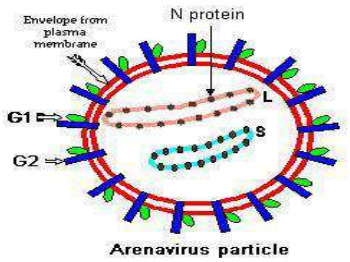
ARENAVIRUS

Characteristics

Arenavirus

GENERAL PROPERTIES

- Name from Latin "arenosus" meaning sand
- Sandy appearance of ribosomes
- two single-stranded RNA segments
- Virion Pleomorphic or spherical
- 60 to 300nm (variable)



Arenavirus particle

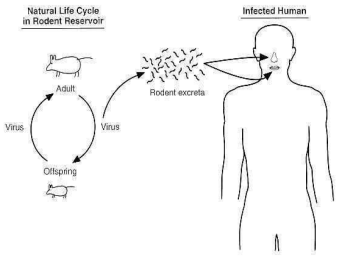
Arenavirus

- family *Arenaviridae*
- Hosts; rodents and occasionally humans
- Snakes
- At least eight arenaviruses are known to cause human disease
- The diseases derived from arenaviruses range in severity

Arenavirus

DISEASES

- Transferred from rodents to humans
- Aseptic meningitis
- Viral hemorrhagic fever



Arenavirus

DISEASE

- Lymohocytic Choriomeningitis Virus infection
- Hemmorhegic fever from a number of virus belonging to this family

Virology

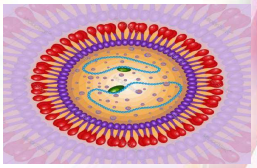
ARENAVIRUS

Genome

Arenavirus

GENOMIC CHARACTERS

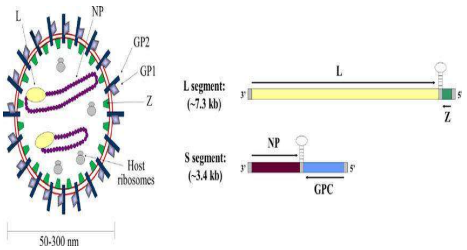
- Two single stranded RNA strands
- Ambisense RNAs
- Negative sense



Arenavirus

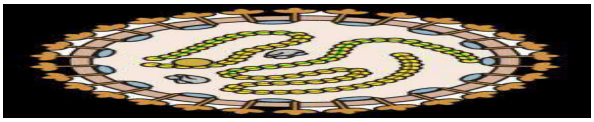
SEGMENTED GENOME

- Segmented genome
  - Small S
  - Large L



Arenavirus

- The S-segment RNA is approximately 3.5 kb
- Encodes the viral nucleocapsid protein (NP) and glycoprotein (GPC).
- The L-segment RNA is approximately 7.2 kb
- Encodes the viral RNA-dependent RNA-polymerase (L) and a small RING-domain containing protein (Z).



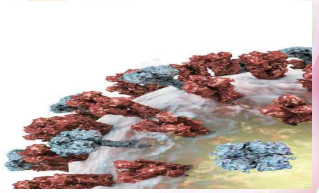
Arenavirus

- The Z protein forms homo oligomers and a structural component of the virions.
- The formation of these oligomers is an essential step for particle assembly and budding. Binding between Z and the viral envelope glycoprotein complex is required for virion infectivity.
- Z also interacts with the L and NP proteins.
- Polymerase activity appears to be modulated by the association between the L and Z proteins.
- Interaction between the Z and NP proteins is critical for genome packaging.

Virology

ORTHOMYXOVIRIDAE

Characteristics



Orthomyxoviridae

NOMENCLATURE

- Greek word
- Orthos meaning proper
- Myx meaning mucus

Orthomyxoviridae

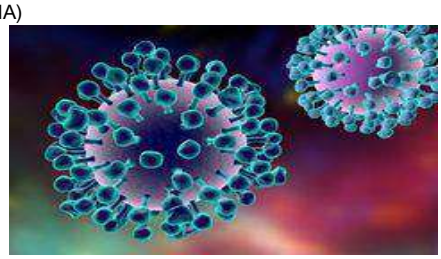
MORPHOLOGY

- Spherical/ pleomorphic
- Enveloped
- 80-120nm
- Spikes on Influenza A and B
- Labile to heat, pH variations, detergents

Orthomyxoviridae

PROTEINS

- The hemagglutinin (HA)
- The neuraminidase (NA)



Orthomyxoviridae

GENERA

- Influenza virus A
- Influenza virus B
- Influenza virus C
- Influenza virus D
- Isavirus
- Thogotovirus
- Quaranjavirus

Orthomyxoviridae

DIEASES

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

Virology

ORTHOMYXOVIRIDAE

Genome

Orthomyxoviridae

GENOMIC PROPERTIES

- Helical nucleopcapsid
- Segmented genome
- Negative sense
- Single stranded RNA
- 10 – 13.6kb
- 12000-15000kb

Orthomyxoviridae

RNPs

- The segmented genome is encapsidated with each segment in a separate nucleocapsid called as *Ribonucleoprotein complexes (RNPs)*

Orthomyxoviridae

- terminal sequences of RNA bound to trimeric RdRp.
- All these RNPs are surrounded by one envelope

Orthomyxoviridae

segment	Protein
PB1	Polymerase
PB2	Polymerase
PA	Polymerase
HA	Hemagglutinin
NP	Nucleoprotein
NA	Neuraminidase
M	Membrane protein(s)
NS	non-structural protein(s)

Orthomyxoviridae

REPLICATION

- Entrance through body fluids
- Virus binds through hemagglutinin-glycoprotein and sialic acid receptors
- Virus enters by endocytosis
- Proteins and polymerase forms complex
- New protein synthesis
- Replication in the nucleus
- Eight segments of genome formed

Virology

PARAMYXOVIRIDAE
Characteristics

Paramyxoviridae

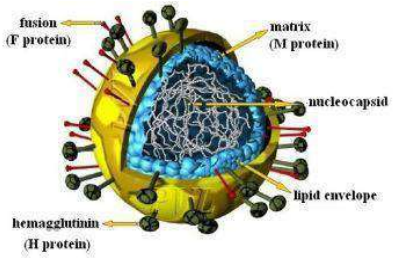
NOMENCLATURE

- Para means beyond
- Myxo means mucus
- Paramyxo and orthomyxo were formerly known as myxoviruses

Paramyxoviridae

PROPERTIES

- Pleomorphic
- 150-300nm diameter
- Enveloped
- Single stranded RNA



Paramyxoviridae

- Spikes on envelope
- Sensitive to heat, chemicals and radiations
- Host: Vertebrates
- Genera: 7
- Species: 49

Paramyxoviridae

PROTEINS

- N – the nucleocapsid protein associates with genomic RNA and protects the RNA from nuclease digestion
- P – the phosphoprotein binds to the N and L proteins and forms part of the RNA polymerase complex
- M – the matrix protein assembles between the envelope and the nucleocapsid core, it organizes and maintains virion structure

Paramyxoviridae

PROTEINS

- F – the fusion protein projects from the envelope surface as a trimer, and mediates entry
- H/HN/G – the cell attachment proteins span the viral envelope and project from the surface as spikes.
- L – the large protein is the catalytic subunit of RNA dependant RNA polymerase (RDRP)
- Accessory proteins – a mechanism known as RNA editing allows multiple proteins to be produced from the P gene.

Virology

PARAMYXOVIRUS
Genome

Paramyxoviridae

GENOMIC CHARACTERS

- Nucleocapsid: helical
- 13-18nm
- Non Segmented linear
- Negative sense
- Single stranded RNA
- 15-16kb
- Contain 6-10 genes

Paramyxoviridae

Extracistronic regions

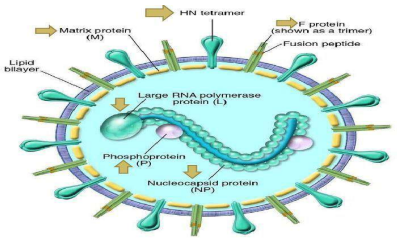
- A 3' leader sequence, , which acts as a transcriptional promoter.
- A 5' trailer sequence, 50–161 nucleotides long

Intergenomic regions

- between each gene
- which are three nucleotides long for morbilliviruses, respiroviruses and henipaviruses
- variable length (1-56 nucleotides) for rubulaviruses

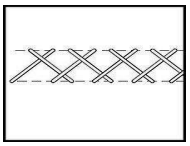
Paramyxoviridae

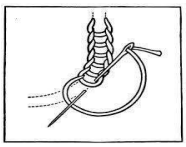
- Fusion Protein
- Haemagglutinin-Neuraminidase
- Matrix protein
- Phosphoprotein
- Nucleoprotein
- Polymerase protein



Paramyxoviridae

- Characteristic herring bone appearance
- Nucleocapsid core contains:
  - Genomic RNA
  - Nucleocapsid protein
  - Polymerase protein





Virology

RHABDOVIRIDAE

General Properties

Rhabdoviridae

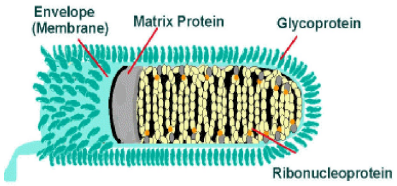
MEANING

- Greek word
- Rhabdos meaning rod
- Referring to shape of virus
- Mononegavirales

Rhabdoviridae

MORPHOLOGY

- Rod or bullet shaped
- Enveloped
- Bacilliform
- Helical neucleocapsid
- 75-180nm long
- RNA genome
- 18 genera

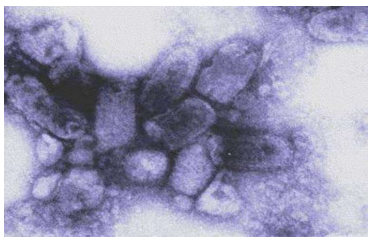


Rhabdoviridae

PROTEINS

- large protein (L)
- glycoprotein (G)
- nucleoprotein (N)
- phosphoprotein (P)
- matrix protein (M)

Rhabdoviridae



MEMBERS

- Vessicular stomatitis Indiana Virus
- Lyssavirus
- Ephemerovirus
- Cytorhabdovirus
- Nucleorhabdovirus

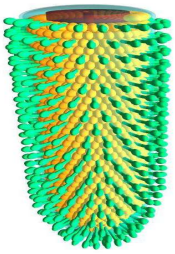
Rhabdoviridae

VESICULOVIRUS

- Infects human, cattle, horses
- Flu-like illness
- Fever
- Siezures
- Diarrhea
- Vomiting
- Coma
- Death

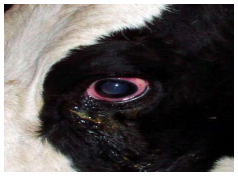
Rhabdoviridae

LYSSAVIRUS



- Infects human
- Bat and rodent carrier
- Throughout world
- Causes rabies
- Anxiety, confusion, agitation, paranoia, hallucinations
- Death

Rhabdoviridae



EPHEMEROVIRUS

- Infects cattle primarily
- Causes Bovine Ephemeral Fever
- Fever, stiffness, lameness
- Nasal and ocular discharges
- High morbidity and low mortality

Rhabdoviridae

CYTORHABDOVIRUS

- Plant viruses
- Bacilliform
- Mature in cell cytoplasm
- lettuce necrotic yellow virus
- barley yellow striate mosaic virus

Rhabdoviridae

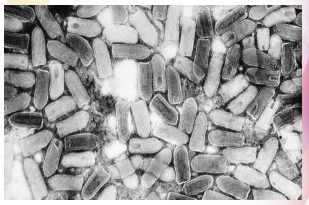
NUCLEORHABDOVIRUS

- Plant viruses
- Bacilliform
- Linear genomes encoding 5 to 6 proteins
- Nuclear viral replication transmitted by insect bite

Virology

RHABDOVIRIDAE

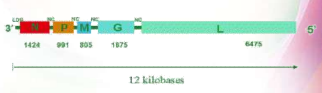
GENOME



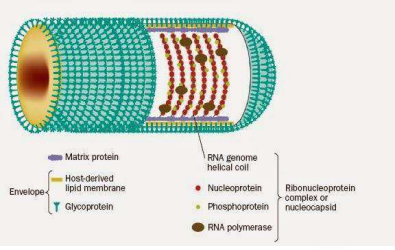
Rhabdoviridae

Genomic Properties

- Single stranded
- RNA



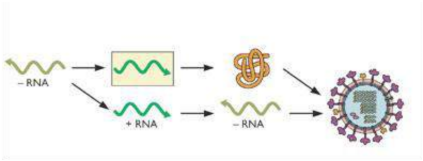
Rhabdoviridae



GENES

- Five proteins:
- Large protein (L)
- Glycoprotein (G)
- Nucleoprotein (N)
- Phosphoprotein (P)
- Matrix protein (M)

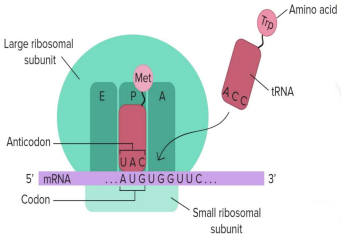
Rhabdoviridae



TRANSCRIPTION

- 1 L and 3 P proteins
- Right after entry
- From 3' to 5'
- Produces mRNA

Rhabdoviridae



TRANSLATION

- Translated on free ribosomes
- G protein translated on RER
- Single peptides of G protein

Rhabdoviridae

REPLICATION

- Cytoplasmic
- Attachment of G protein
- Endocytosis
- Negative strand of RNA transcribed
- Virus leaves by budding

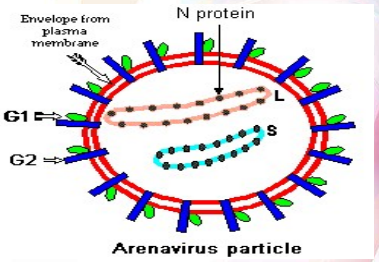
Virology

SEGMENTED AND MULTIPARTITE GENOME

Multipartite and Segmented Genome

ARENAVIRUS

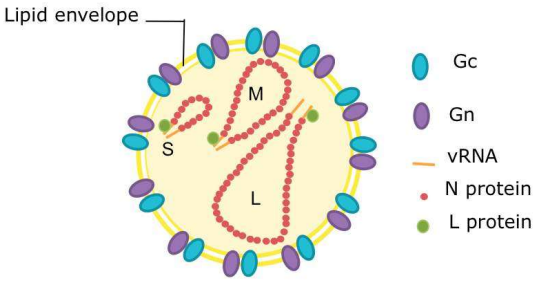
2 segments



Multipartite and Segmented Genome

BUNYAVIRUS

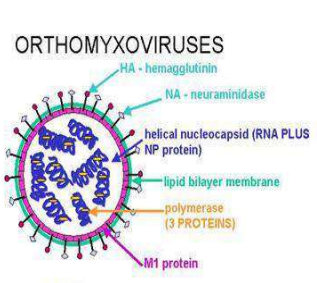
3 segments



Multipartite and Segmented Genome

ORTHOMYXOVIRUS

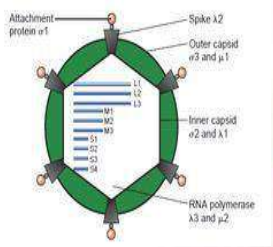
8 segments



Multipartite and Segmented Genome

REOVIRUS

10 segments



Virology

REVERSE TRANSCRIPTION

Reverse Transcription

Reverse transcription is a process in which a double standard DNA molecules ae made from a single stranded RNA

The name of this method originated by tis opposite direction to transcription process

Reverse Transcription

It involves the presence of

- Reverse transcriptase,

an enzyme

- A primer

some bases
of nucleotides segments

- Reverse transcriptase inhibitor

leucine rich repeat protein

Reverse Transcription

Reverse Transcription

Virology

TRANSPOSONS

Importance

Transposons and its Important

TRANSPOSONS

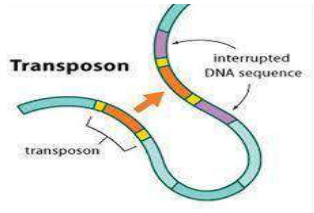
A sequence of DNA that can change its position, from place to place within a genome

Transposons and its Important

DISCOVERY

- 1940s
- Barbera McClintock

Transposons and its Important



TYPES

Two groups:

- Simple transposons
- Reterotransposons

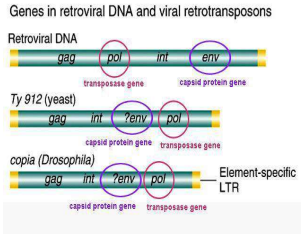
Transposons and its Important

SIMPLE TRANSPOSONS

- In prokaryotes
- Which do not undergo reverse transcription
- e.g. genome of enterobacteria phage Mu

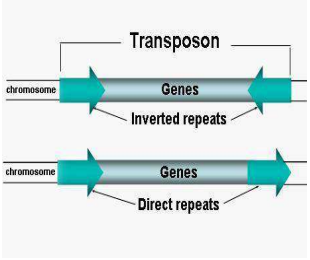
Transposons and its Important

RETROTRANSPOSONS



- Closely resemble retroviral genome
- Bound by long repeat units (LTRs)
- Move by means of transcription/ reverse transcription/ integration mechanism
- Found in eukaryotes
- In *Metaviridae* and *Pseudoviridae*

Transposons and its Important



PROPERTIES

- Responsible for high mutation
- Promote genetic rearrangements like deletion, inversion, duplication etc
- Often accompanied by replication
- Control their own transposition functions

Virology

Isolation, Cultivation and Identification of Viruses

Isolation, Cultivation and Identification

ISOLATION

➢Centrifugation

- Differential centrifugation- high vs low speed to separate cells from viruses
- Gradient centrifugation - separate by size or density

Isolation, Cultivation and Identification

CULTIVATION

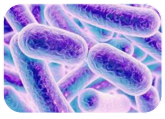
- To cultivate the virus in specific host

Isolation, Cultivation and Identification

CULTIVATION


Animal


Plant


Bacteria

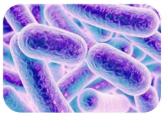

Insect

Isolation, Cultivation and Identification

CULTIVATION


Animal


Plant


Bacteria


Insect

Isolation, Cultivation and Identification

METHOD OF CULTIVATION

- Cell culture
- Organized tissue
- Live animal
- Embryonated egg

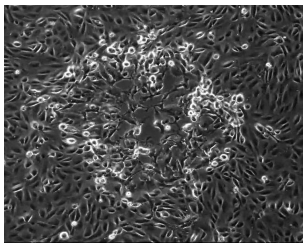
Isolation, Cultivation and Identification

IDENTIFICATION

- Plaque formation
- Cytopathic Effects
- Fluorescent Antibody technique
- Nucleic Acid hybridization
- Electron Microscopy

Isolation, Cultivation and Identification

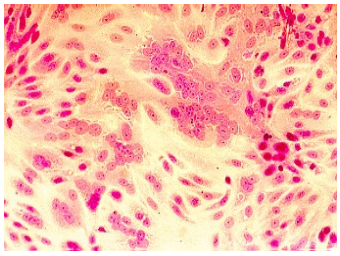
PLAQUE FORMATION



- a visible structure formed within a cell culture
- The bacteriophage viruses replicate and spread, thus generating regions of cell destructions known as plaques

Isolation, Cultivation and Identification

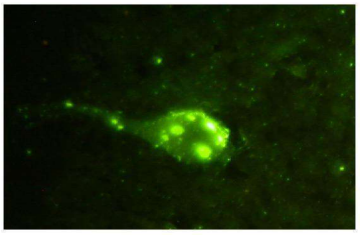
CYTOPATHIC EFFECTS



- Syncytia
- Cell rounding
- Membrane proliferations
- Vacuolization
- Inclusion bodies
- Focus (foci)
- Hemadsorption

Isolation, Cultivation and Identification

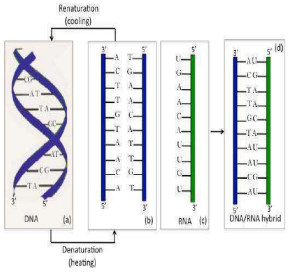
FLUORESCENT ANTIBODY TECHNIQUE



- based on the observation that virus proteins are (antigen) present in the tissues
- e.g rabies virus present in nervous tissues

Isolation, Cultivation and Identification

NUCLIEC ACID HYBRIDIZATION

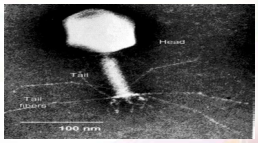


A technique in which single-stranded nucleic acids (DNA or RNA) are allowed to interact so that complexes called hybrids are formed by molecules with similar, complementary sequences

Isolation, Cultivation and Identification

ELECTRON MICROSCOPY

- uses a beam of **electrons** to create an image of the specimen
- Inclusion bodies formed



END

Virology

Growing Bacteriophages in Laboratory

Growing Bacteriophages

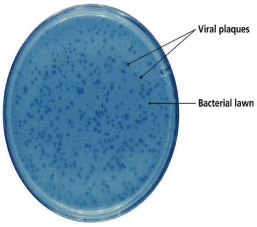
METHODS

Two methods

- In liquid media
- On solid media

Growing Bacteriophages

ON SOLID MEDIA



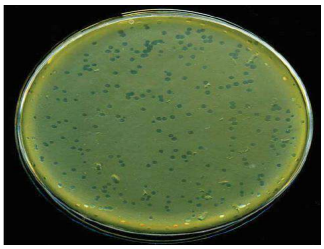
- Like bacteria
- Helps in plaque test to detect and count viruses
- Bacteriophage sample mixed with host bacteria and melted agar

Growing Bacteriophages

INNOCULATION

- Mixed agar poured into petri plate
- Petri plate already containing hardened layer of agar media
- Mixture solidifies into top thin layer

Growing Bacteriophages

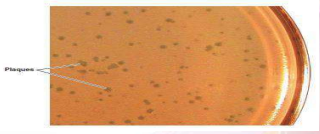


- ❖Each virus infects bacteria
- ❖Multiplies
- ❖Releases new viruses
- ❖New viruses infect other bacteria
- ❖All bacteria get destroyed

Growing Bacteriophages

PLAQUE FORMATION

- Produces a number of clearings or plaques
- Visible by naked eye
- Uninfected bacteria form turbid



END

Virology

Growing Animal Viruses in Embryonated Eggs

Growing Animal viruses

METHODS

1. Animals
2. Cell culture
3. **Embryonated eggs**

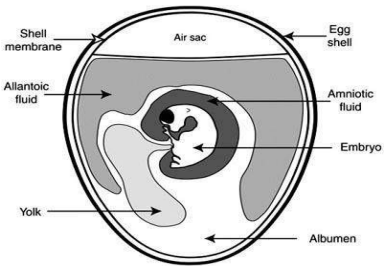
Growing Animal viruses

ADVANTAGE

- Convenient
- Inexpensive
- Host for many viruses

Growing Animal viruses

PROCEDURE



- Hole is drilled in the shell
- Viral suspension inoculated
- Many routes

Growing Animal viruses

VIRAL GROWTH

- Death of embryo
- Damage to embryo
- Pocks or lesions on egg membrane

Growing Animal viruses

PURPOSE

- Isolation and growth of virus
- Growth for vaccination

Growing Animal viruses

ROUTES

1. Chorioallantoic membrane inoculation
2. Amniotic inoculation
3. Allantoic inoculation
4. Yolk sac inoculation

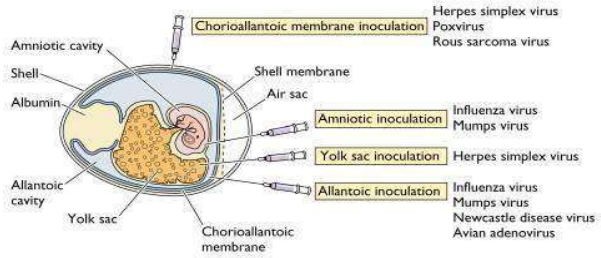
END

Virology

Chorioallantoic Membrane Inoculation

Chorioallantoic Membrane Inoculation

ROUTES OF INOCULATION

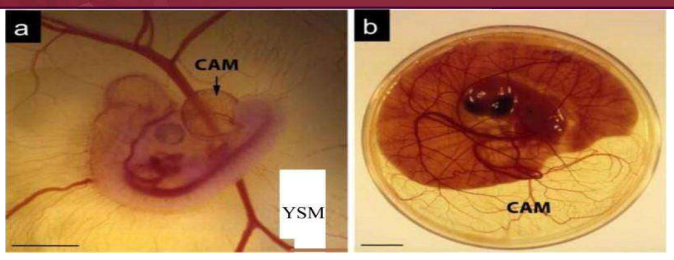


The different routes of inoculation into the egg are shown, as well as the different compartments in which viruses replicate.

Chorioallantoic Membrane Inoculation

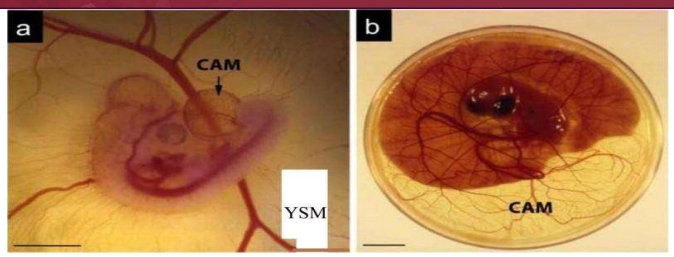
- The chicken chorioallantoic membrane (CAM) is a simple, highly vascularized extraembryonic membrane, which performs multiple functions during embryonic development. Over the last two decades, interest in the CAM as a robust experimental platform to study blood vessels has been shared by specialists working in bioengineering, development, morphology, biochemistry, transplant biology, cancer research and drug development

Chorioallantoic Membrane Inoculation



Chicken embryo and associated extra-embryonic structures. a, Chicken embryo at day 4 post-fertilization the highly vascularized chorioallantoic membrane (CAM) that expands from the hind-gut. The yolk sac membrane (YSM), also highly vascularized, is seen in the background; b Embryo at day 12 post-fertilization into a 10 cm petri dish.

Chorioallantoic Membrane Inoculation



Chicken embryo and associated extra-embryonic structures. a Chicken embryo at day 4 post-fertilization. Note the highly vascularized chorioallantoic membrane (CAM) that expands from the hind-gut. The yolk sac membrane (YSM), also highly vascularized, is seen in the background; b Embryo at day 12 post-fertilization into a 10 cm petri dish.

Chorioallantoic Membrane Inoculation

Virology

Allantoic Cavity Inoculation

Allantoic Cavity inoculation

- Taking Flu virus as an example
- Virus inoculated in allantoic cavity

Chorioallantoic Membrane Inoculation

Virology

Intraembryonic, Intracranial and Intravenous Inoculation

I/E, I/C and I/V inoculation

- Inoculated
- Incubated for few days
- Lesions or death observed

I/E, I/C and I/V inoculation

- These inoculations of embryos are not widely used.
- Embryonic inoculation:
- Embryos are inoculated and incubated for a period of one to six days during which they are observed twice a day for the evidence of death of embryo. Some viruses, such as equine encephalomylietis virus kill the embryo within 24 hours while others kill the embryos in 2 to 3 days. Some viruses readily multiply within the embryonic tissue and are seldom lethal.

I/E, I/C and I/V inoculation

- Embryo death is accompanied by collapse of the large blood vessels of the chorioallantois so that they are difficult to see upon candling the egg, whereas they are quite distinct while the embryos are alive. Normal movements of the embryo are no longer observed if the embryo is moribund or dead.
- If the embryo is not dead 5 or 6 days of incubation, it is killed or examined for lesions.
-

I/E, I/C and I/V inoculation

jove

II. Preparation and Setup for Stereotaxic Delivery of Viral Vectors

Virology

SEROLOGICAL METHODS

Agglutination and Precipitation

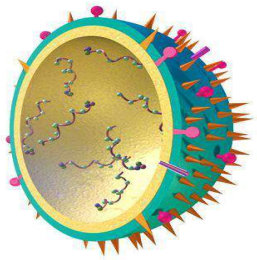
Agglutination and Precipitation

PRINICPILE

- Viruses maybe sensitized by specific antibody
- In the presence of electrolyte the virus particles cling to each other forming aggregates
- Observed through examinations
  - microscopic
  - macroscopic

Agglutination and Precipitation

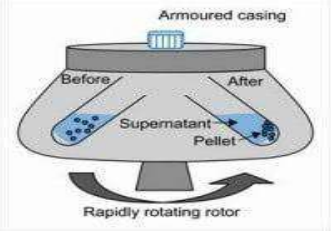
FLOCCULUATION



- Size of virus is intermediate between bacteria and protein molecule
- Hence the term, Flocculation is more appropriate

Agglutination and Precipitation

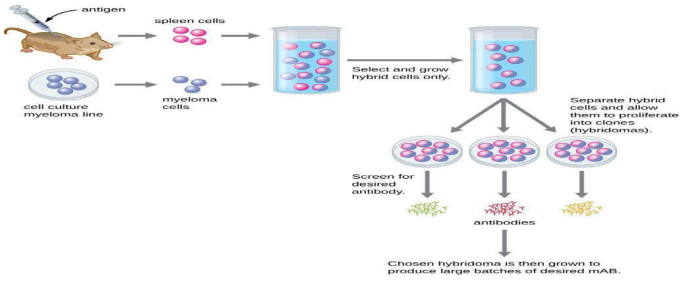
ANTIGEN PREPARATION



- Centrifugation
- Washing in buffered solution
- Recentrifugation

Agglutination and Precipitation

LINE OF ACTION



Agglutination and Precipitation

APPLICATIONS

- Two ways:

1. If virus is known, antibodies can be detected
2. If known serum is available, an unknown virus can be detected

Agglutination and Precipitation

CHALLENGES

- Preparation of antigen is difficult
- Viruses must be grown in in the animal tissues

END

Virology

SEROLOGICAL METHODS

Complement Fixation

Complement Fixation

- The complement fixation test is an immunological test used to detect the presence of either specific antibody or specific antigen on the basis of complement fixation
- The complement fixation is a system of serum proteins that react with antigen-antibody complexes.
- If this reaction occurs on a cell surface, it will result in the formation of trans-membrane pores and therefore destruction of the cell.

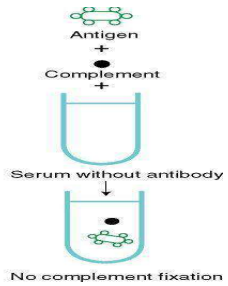
Complement Fixation

HOW IT WORKS

- Viruses as antigen
- Virus antigen for this test obtained from infected animal tissues
- Tissues such as, lungs or brain

Complement Fixation

APPLICATIONS



- Detection of presence of antibodies in serum
- Identification of unknown virus by using known antibody

Complement Fixation

SHORT PROCEDURE

- Serum is separated from the patient.
- Patients naturally have different levels of complement proteins in their serum. To negate any effects this might have on the test, the complement proteins in the patient's serum must be destroyed and replaced by a known amount of standardized complement proteins.
  - The serum is heated in such a way that all of the complement proteins—but none of the antibodies—within it are destroyed.
  - A known amount of standard complement proteins are added to the serum. (These proteins are frequently obtained from guinea pig serum.)
- The antigen of interest is added to the serum.
- Sheep red blood cells (sRBCs) which have been pre-bound to anti-sRBC antibodies are added to the serum.

Complement Fixation

RESULT INTERPRETATION

- If the patient's serum contains antibodies against the antigen of interest, they will bind to the antigen in step 3 to form antigen-antibody complexes.
- The complement proteins will react with these complexes and be depleted.
- Thus when the sRBC-antibody complexes are added in step 4, there will be no complement left in the serum.
- However, if no antibodies against the antigen of interest are present, the complement will not be depleted and it will react with the sRBC-antibody complexes added in step 4, lysing the sRBCs and spilling their contents into the solution, thereby turning the solution pink

Complement Fixation

Virology

SEROLOGICAL METHODS

Serum Neutralization Test

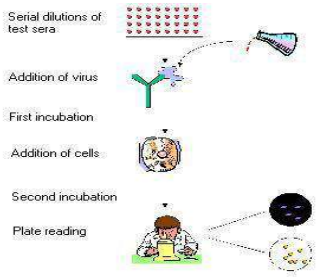
Serum Neutralization Test

HOW IT WORKS

- Used to confirm identity of an unknown viral isolate
- Use of known dilution of specific antiserum mixed with multiple dilution
- Observation of virus to produce Cytopathic effects

Serum Neutralization Test

NECESSITIES



- Amount of virus
- Amount of serum
- Test animal
- Route of inoculation

Serum Neutralization Test

BASIC PRINCIPLES

- Wide range of dilutions used
- Equal volumes of viral dilution
- Equal volume of virus dilution and immune serum used
- Inoculation of 5 to 6 animals
- Equal number of controls
- Observation of animals for signs, lesions or deaths

Serum Neutralization Test

For comparisons

- L.D50 (Lethal dose 50)
- Viral dilutions checked for causing deaths and infectivity
- Titer noted

Serum Neutralization Test

Virology

SEROLOGICAL METHODS

Virus Neutralization Test

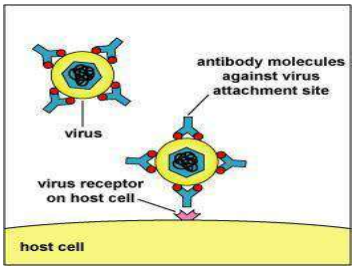
Virus Neutralization Test

INTRODUCTION

- Serological method that detects the presence of viral neutralizing antibodies
- The antibodies bind to viral particles
  - Monoclonal and polyclonal antibodies
- Block viral infectivity – no cell infection

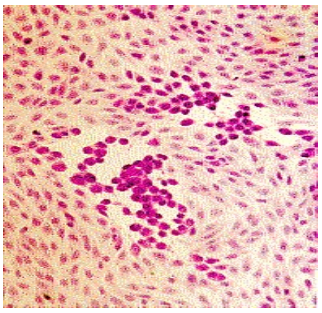
Virus Neutralization Test

- Conducted by:



- Mixing dilutions of antibodies with standardized amount of virus , incubating them and cultured into cells, eggs or animals to have clear cytopathic observation

Virus Neutralization Test



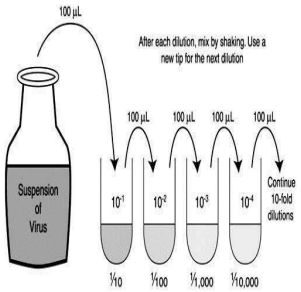
CYTOPATHIC EFFECTS

- Rounding of cells
- Change in texture
  - Granular
  - Hyaline
  - Glassy
- Formation of synctium

Virus Neutralization Test

STEPS

- First step:
  - Serial dilution of serum
  - Known virus
  - Incubated for 1-2 hours at 37°C
- Second step
  - Cell culture inoculated with mixture
  - Incubated at 37°C
  - Direct microscope



Virus Neutralization Test

PROS

- Can be specific
- Sensitive
- Vaccine production/ immunological status

Virus Neutralization Test

CONS

- Very slow
- Intensive
- Required skilled people
- Depends on cell lines

END

Virology

SEROLOGICAL METHODS

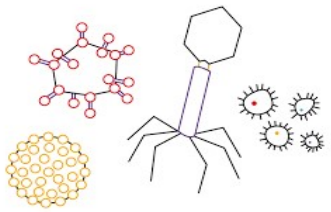
Protection Tests

Protection Test

INTRODUCTION

- Not strictly serological
- For identification of those viruses
- Used to produce successful vaccines
- Procedure also employed for testing commercial serum and vaccine products

Protection Test



- Production of either active or passive immunity in an animal followed by a challenge dose of virulent virus
- It is also called in-vivo neutralization test

Protection Test

- Vaccine prepared for all types of immunity to different lab animals
- When satisfactory immunity is produced, all are challenged with a known virus

Protection Test

EXAMPLES

- Used commercially for Rabies and hog cholera vaccines
- Used by injecting in graded doses

END

Virology

SEROLOGICAL METHODS

Result interpretation of Protection Tests

Protection Test

HYPOTHETICAL TEST

- Suppose we have three types of vaccines
- We inject them in three different guinea pigs
- Observe for immunity

Protection Test

Immunized guinea pigs	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
Eastern type vaccine	5	5	2	1	0
Western type vaccine	1	0	0	0	0
Venezuelan type vaccine	5	5	3	0	0
Controls	5	5	3	1	0

Protection Test

- Disease: equine encephalomylietis
- Control also set

Protection Test

- Vaccine that provides best immunity and no death proves to be the best

END

Virology

SEROLOGICAL METHODS

Haemagglutination /Haemagglutination inhibition Test

HA/HI Test

INTRODUCTION

- Few viruses possess haemagglutinin protein

  that binds to Sialic Acid

  receptors on RBCs

HA/HI Test

SAMPLE

- Blood
- Using anticoagulant like EDTA or Heparin

HA/HI Test

1% RBC'S PREPARATION

- Preparation of 1% RBCs
- Equal amount of blood and Normal Saline (NS) centrifuged in Falcon tube
- Remove supernatant and add the same volume of NS again and centrifuge
- Repeat the procedure until clear supernatant
- Remove the supernatant and add ten times of its volume of NS

HA/HI Test

HAEMAGGLUTINATION TEST

- A ninety six well plate marked according to sample
- Each row assigned to one sample
- Add 50uL NS to all wells
- Add antigen to first well and make two fold dilution till second last well
- Leave last well as control
- Add 25uL RBCs solution to all wells

HA/HI Test

HAEMAGGLUTINATION HAEMINHIBITION TEST

- 96 well plate labelled according to samples
- 25uL antiserum added to first well and make two fold dilution till end
- 0.5uL RBCs added to all wells
- Incubation for 30 minutes

Virology

SEROLOGICAL METHODS

Result interpretation of HA/HI test

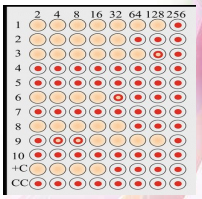
HA/HI Test

INTERPRETATION OF HA TEST

- Button formation showed hemagglutination
- Hazy wells showed no agglutination

HA/HI Test

- One HA calculates on the basis of last well showing button formation
- Button formation at controls



HA/HI Test

- Dilution value shows us 4HA unit value
- To determine one HA unit the value is divided by four

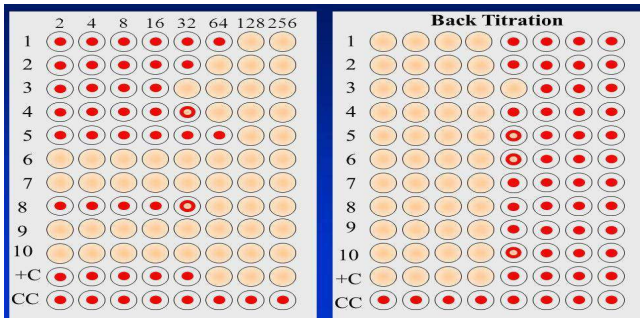
Well	1	2	3	4	5	6	7	8	9	10	11	12
Dilution	1/10	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/10240	Control
Pattern												

HA/HI Test

INTERPRETATION OF HI TEST

- Shows button formation at the start
- Hazy wells at the end
- 8HA units were calculated per 25uL
- By dividing endpoint by 16

HA/HI Test



Virology

CYTOPATHOGENECITY IN TISSUE CULTURE

Tissue Culture

Basic Ingredients

1. Tissue cells
2. Lactalbumin hyrolysate
3. Serum
4. Balanced salt solution

Tissue Culture

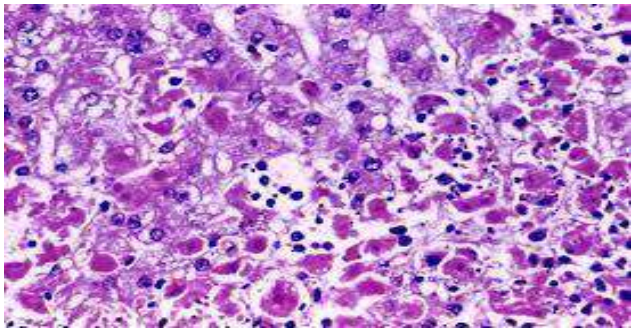
Other Ingredients

1. Ascitic fluid
2. Amniotic fluid
3. Serum ultrafilterate
4. Amino acids
5. Vitamins
6. Glucose
7. Inorganic salts
8. Penicillin and streptomycin

Tissue Culture

- Glassware used must be absolutely clean and free film and toxic substances
- Chemicals and distil water must be free from toxic ions

Tissue Culture



Tissue Culture

Cytopathogenecity

- Abnormalities observed in cells
- Inclusion bodies observed
- Synctia formation

Virology

SEROLOGICAL METHODS

Haemadsorption Test

Haemadsorption

Introduction

- Technique similar to haemagglutination test
- Used for detection of certain viruses in tissue culture

Haemadsorption

Haemagglutinin

- Viruses producing haemagglutinin will adsorb erythrocytes to the surface of cells in which the virus has grown

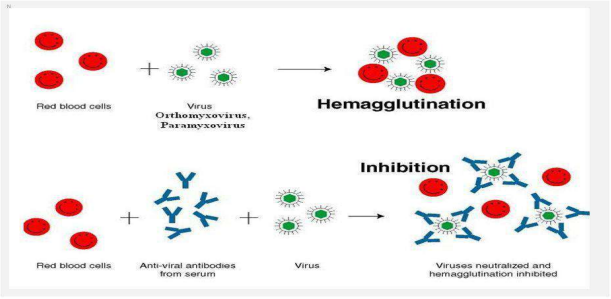
Haemadsorption

PROCEDURE

- A suspension of washed RBCs is added to tissue culture tubes
- Presences of haeadsorbing virus, results in clustering of RBCs
- Rosettes around the infected cells of the culture
- Can be observed microscopically

Haemadsorption

Haemadsorption and agglutination inhibition:



Haemadsorption

USE

- Useful for detection of viruses producing haemagglutinins

Haemadsorption

Inhibition

- Can be inhibited by specific antibody
- Hence haemadsorption-inhibition test is devised for antibody titer

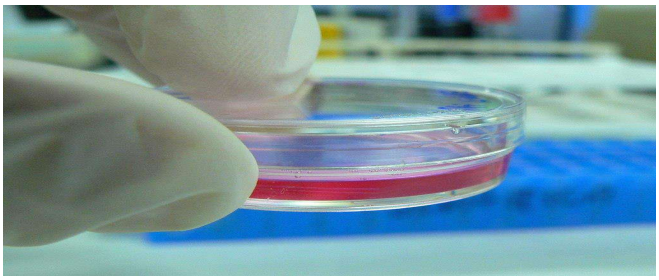
END

Virology

Growing Animal Viruses in Cell Culture

Cell Culture

THE PROCESS BY WHICH CELLS ARE GROWN UNDER CONTROLLED CONDITIONS



Cell Culture

CONTROLLED CONDITIONS

Vary for each cell type

- Essential nutrients (amino acids, carbohydrates etc.
- Growth factors
- Hormones
- Gases (CO_2 or O_2)
- Physio-chemical environment regulators (pH buffer, osmotic pressure, temperature etc.

Cell Culture

History

Cell culture was first successfully undertaken by Ross Harrison in 1907

- Roux in 1885 for the first time maintained embryonic chick cells in a cell culture
- 1878: Claude Bernard proposed that physiological systems of an organism can be maintained in a living system after the death.
- 1885: Roux maintained embryonic chick cells in a saline culture.
- 1897: Loeb demonstrated the survival of cells isolated from blood and connective tissue in serum and plasma.
- 1903: Jolly observed cell division of salamander leucocytes *in vitro.*

1907

Harrison cultivated frog nerve cells in a lymph clot held by the hanging drop' method and observed the growth of nerve fibers *in vitro* for several weeks. He was considered by some as the father of cell culture.

Cell Culture

APPLICATIONS

Areas where cell culture technology is currently playing a major role

- Model systems for

  Studying basic cell biology, interactions between disease causing agents and cells, effects of drugs on cells, process and triggering of aging & nutritional studies
- Toxicity testing

  Study the effects of new drugs
- Cancer research

  Study the function of various chemicals, virus & radiation to convert normal cultured cells to cancerous cells

Cell Culture

APPLICATIONS

Areas where cell culture technology is currently playing a major role

- Virology

  Cultivation of virus for vaccine production, also used to study there infectious cycle.
- Genetic Engineering

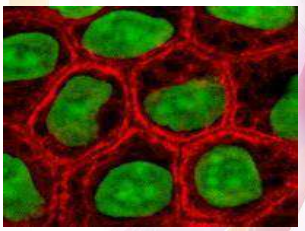
  Production of commercial proteins, large scale production of viruses for use in vaccine production e.g. polio, rabies, chicken pox, hepatitis B & measles
- Gene therapy

  Cells having a functional gene can be replaced to cells which are having non-functional gene

Cell Culture

Culture of Mammalian Cells

Culture of Non-mammalian Cells



Virology

Viral Identification After Growing

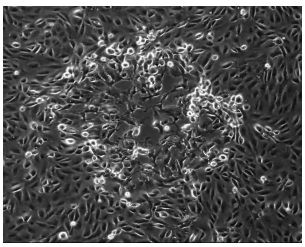
Viral Identification After Growing

IDENTIFICATION

- Plaque formation
- Cytopathic Effects
- Fluorescent Antibody technique
- Nucleic Acid hybridization
- Electron Microscopy

Viral Identification After Growing

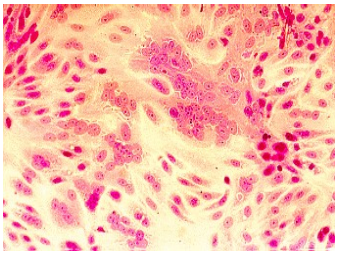
PLAQUE FORMATION



- a visible structure formed within a cell culture
- The bacteriophage viruses replicate and spread, thus generating regions of cell destructions known as plaques

Viral Identification After Growing

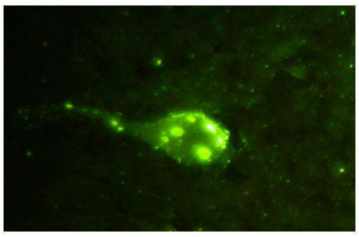
CYTOPATHIC EFFECTS



- Syncytia
- Cell rounding
- Membrane proliferations
- Vacuolization
- Inclusion bodies
- Focus (foci)
- Hemadsorption

Viral Identification After Growing

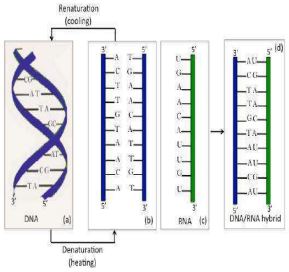
FLUORESCENT ANTIBODY TECHNIQUE



- based on the observation that virus proteins are (antigen) present in the tissues
- e.g rabies virus present in nervous tissues

Viral Identification After Growing

NUCLIEC ACID HYBRIDIZATION

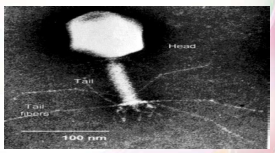


A technique in which single-stranded nucleic acids (DNA or RNA) are allowed to interact so that complexes called hybrids are formed by molecules with similar, complementary sequences

Viral Identification After Growing

ELECTRON MICROSCOPY

- uses a beam of **electrons** to create an image of the specimen
- Inclusion bodies formed



Virology

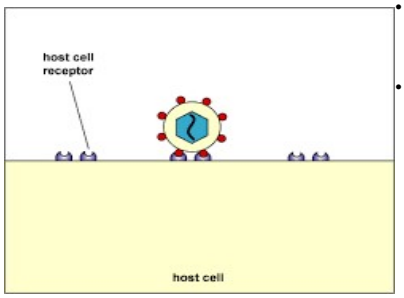
MULTIPLICATION OF VIRUSES

Attachment

Multiplication of Viruses

- Electrostatic phenomenon
- Divalent cations involved
- Viral particles and cell surface have negative charges
- Cations reduce the repulsion

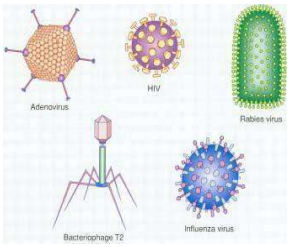
Multiplication of Viruses



- Viral particles and cell surface have negative charges
- Cations reduce the repulsion

Multiplication of Viruses

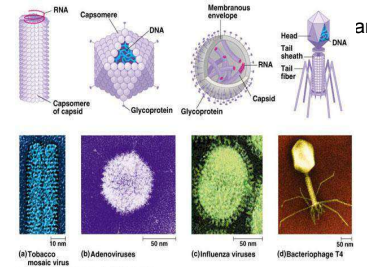
SPECIFICITY



- Specific receptors of cell surface
- Host tropism- Tissue tropism- Cell tropism

Multiplication of Viruses

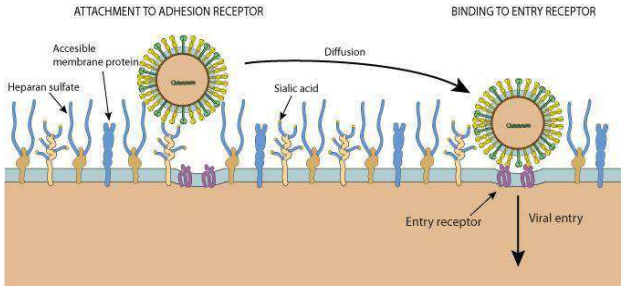
SPECIFICITY



➢Receptor has electrostatic charge arrangement complementary to virus

- Weakening of capsid
- Release of nucleic acid
- Initiation of infection

Multiplication of Viruses



Virology

MULTIPLICATION OF VIRUSES

Entry

Multiplication of Viruses

- After attachment
- Nucleic acid components releases in cytoplasm inside a vacuole
- Active, unspecific, temperature dependent

Multiplication of Viruses

VIRAL ENTRY

- ***Membrane Fusion*** or ***Hemifusion State***: The cell membrane is punctured and made to further connect with the unfolding viral envelope.
- ***Endocytosis***: The host cell takes in the viral particle through the process of endocytosis, essentially engulfing the virus like it would a food particle.
- ***Viral Penetration***: The viral capsid or genome is injected into the host cell's cytoplasm.

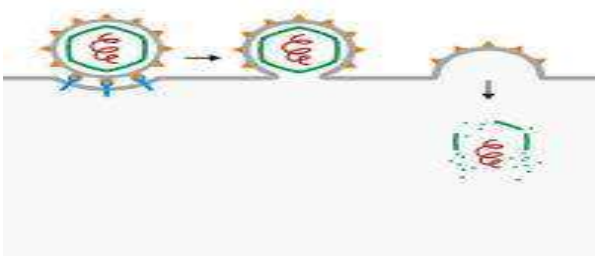
Multiplication of Viruses

VIRAL ENTRY VIA MEMBRANE FUSION

- In viruses with a envelope
- Viral receptors attach to the receptors on the surface of the cell
- The viral envelope fuses with the host cell membrane
- Now-bare virus enters into the cell
- In essence, the virus's envelope "blends" with the host cell membrane, releasing its contents into the cell. Examples: HIV and herpes simplex virus

Multiplication of Viruses

VIRAL ENTRY VIA MEMBRANE FUSION



Multiplication of Viruses

Viral entry via endocytosis

- Viruses with no viral envelope
- Ingested by the host cell through the cell membrane
- Knocking at the door
- Cell will engulf the virus
- Once inside the cell, the virus must now break out of the vesicle by which it was taken up in order to gain access to the cytoplasm.
- Examples include the poliovirus, Hepatitis C virus, FMDV

Multiplication of Viruses

- Many enveloped viruses also enter the cell through endocytosis.

- Entry via the endosome guarantees low pH and exposure to proteases which are needed to open the viral capsid and release the genetic material inside.
- Further, endosomes transport the virus through the cell and ensure that no trace of the virus is left on the surface, which could be a substrate for immune recognition

Multiplication of Viruses

ENTRY VIA GENETIC INJECTION

- Attaching to the surface of the cell via receptors
- Injecting only its genome into the cell
- Leaving the rest of the virus on the surface
- This is restricted to viruses in which only the gene is required for infection of a cell

Example includes the Bacteriophages; for example, when the tail fibers of the T2 phage land on a cell, its central sheath pierces the cell membrane and the phage injects DNA from the head capsid directly into the cell.

Virology

MULTIPLICATION of VIRUSES

Uncoating

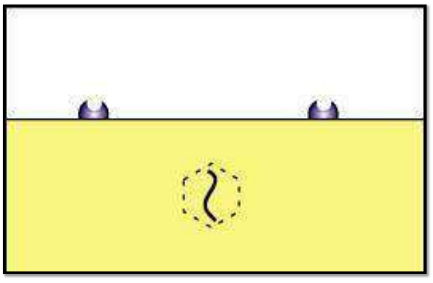
Multiplication of Viruses

Uncoating

- Uncoating means removal of capsid
- Exposing the viral genome in the host cell

Multiplication of Viruses

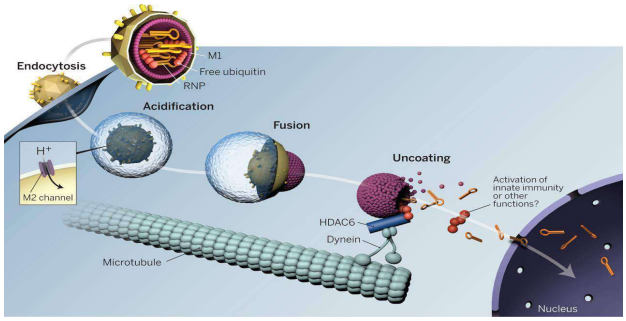
- The Protein coat around genome gets disintegrated
- Cytoplasm
- Cellular enzymes



Multiplication of Viruses

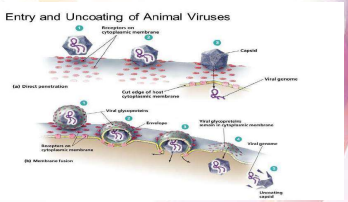
- Vital important step
- Makes genome ready to takeover the host machinery
- Activates cascade of the steps required for viral replication

Multiplication of Viruses



Multiplication of Viruses

- Genome becomes ready to invade the host machinery



Virology

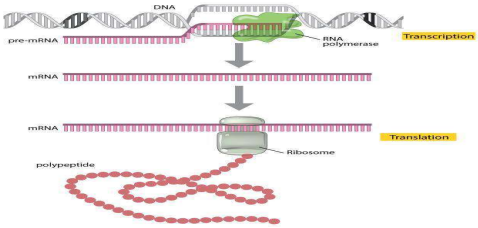
MULTIPLICATION oF VIRUSES

Biosynthesis in DNA viruses

Multiplication of Viruses

TRANSCRIPTION

- All viruses make mRNA and then proteins



Multiplication of Viruses

Differences in mRNA

- Single stranded
- Thymine replaced by Uracil
- mRNA is exactly similar to positive strand

Multiplication of Viruses

Translation

- Information on mRNA changed to proteins
- Viruses that replicate in nucleus
  Use host polymerase
- Viruses that replicate in cytoplasm
  Use own polymerase

Multiplication of Viruses

POLYMERASES

1. Cellular DNA dependent RNA polymerases
2. Viral DNA dependent RNA polymerases

Multiplication of Viruses

SYNTHESIS OF VIRAL PROTEINS

Viral protein synthesis: virus mRNA translated on cell ribosomes into two types of virus protein

a. **Structural:** proteins which make up the virus particle are manufactured and assembled

b. **Non – structural:** not found in particle, mainly enzymes for virus genome replication

Multiplication of Viruses

Synthesis of Viral Genome

Viral nucleic acid synthesis new virus genome synthesized, templates are either the parental genome or with single stranded nucleic acid genomes, newly formed complementary strands

Multiplication of Viruses

https://www.youtube.com/watch?v=nR9e7eXm7gk&t=55s

Virology

MULTIPLICATION of VIRUSES

Biosynthesis of RNA viruses

Multiplication of Viruses

Replication

- RNA viruses usually replicate in cytoplasm

Multiplication of Viruses

Positive sense RNA virus

- Itself acts as mRNA
- They need their own viral RNA dependent RNA polymerase

Multiplication of Viruses

Negative sense RNA virus

- They are a copy of mRNA
- They first make a positive strand with RNA dependent RNA polymerase
- It acts as mRNA
- It will further make copies using RNA dependent RNA polymerase

Multiplication of Viruses

- https://www.youtube.com/watch?v=ZGE4BLuAkuU

Virology

VIRAL DIAGNOSTIC TESTS

Immunofluorescence Assay

Immunofluorescence Assay

APPLICATIONS ON

tissue sections

Cultured cell lines

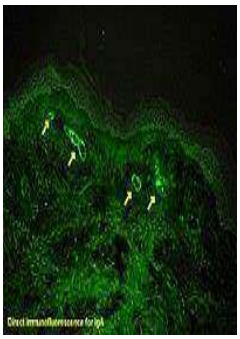
individual cells

Analyze the distribution of

proteins, glycans and small biological and non-biological molecules.

This technique can even be used to visualize structures such as intermediate-sized filaments.

Immunofluorescence Assay



Primary (direct)

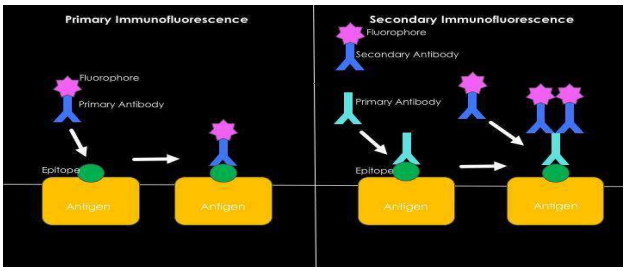
- A single, primary antibody, chemically linked to a Fluorophone
- The primary antibody recognizes the target molecule (antigen)
- Binds to a specific region called the epitope.

Immunofluorescence Assay

Secondary (indirect)

- Two antibodies
- The unlabeled first (primary) antibody specifically binds the target molecule
- The secondary antibody, which carries the fluorophore, recognizes the primary antibody and binds to it

Immunofluorescence Assay



Virology

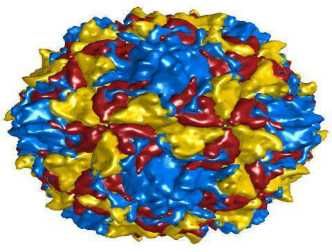
Biochemical Changes Induced in Virus-Infected Cell

Biochemical Changes

BASIC QUESTION?

- Virus induced changes leading to cell death or accelerated growth
- Answer hidden in different proteins coded by viral genome

Biochemical Changes



- e.g the smallest virus i.e. Picornavirus
- Contains 4000-6000 nucleotides
- Genetic information codes 1500 to 2000 amino acids or 10 to 15 proteins

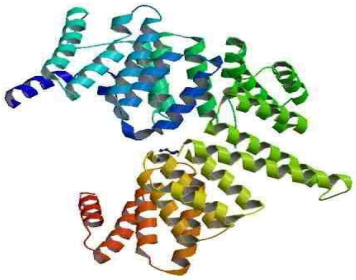
Biochemical Changes

- DNA of vaccinia virus contains 250,000 nucleotide pairs which provide information for 400 to 600 proteins

Biochemical Changes

- Two types of proteins:

1. Early proteins
2. Late proteins



Biochemical Changes

- Early protein synthesis usually due to genetic functioning of parental viral genomes
- Include those which inhibit host cellular DNA replication, host cell RNA formation, host cell protein synthesis

Biochemical Changes

- Late proteins include viral structural proteins and proteins that function in repression or switching off the formation of virus induced enzymes

Biochemical Changes

- Actinomysin prevents transcription of RNA from DNA template
- Puromycin or parafluoroalanine inhibts translation of genetic message from mRNA into protein

END

Virology

Inhibition of Host Cell RNA Synthesis

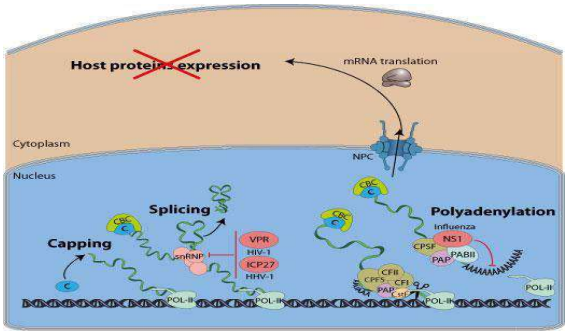
Inhibition of Host Cell RNA Synthesis

- Poliovirus inhibits protein synthesis approximately two hours post infection

Inhibition of Host Cell RNA Synthesis

- e.g the smallest virus i.e. Picornavirus
- Contains 4000-6000 nucleotides
- Genetic information codes 1500 to 2000 amino acids or 10 to 15 proteins

Inhibition of Host Cell RNA Synthesis



Inhibition of Host Cell RNA Synthesis

- DNA of vaccinia virus contains 250,000 nucleotide pairs which provide information for 400 to 600 proteins

Inhibition of Host Cell RNA Synthesis

- When menangioma virus infects mouse origin L cells:
- Marked decrease in cellular enzyme which polymerizes RNA from DNA template

END

Virology

Inhibition of Host Cell Protein Synthesis

Inhibition of Host Cell Protein Synthesis

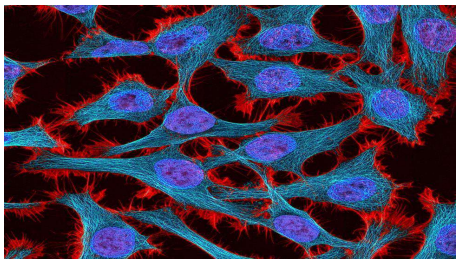
- Most cellular protein is assembled and synthesized on aggregates of ribosomes called polyribosomes

Inhibition of Host Cell Protein Synthesis

- Cellular protein synthesis blocked by interference with synthesis and function of mRNA
- If cellular mRNA synthesis is blocked, the rate of decrease of protein synthesis will increase

Inhibition of Host Cell Protein Synthesis

- Half life of mRNA in actinomycin treated HeLa and L cells is approximately three hours



Inhibition of Host Cell Protein Synthesis

- Cellular protein inhibitory effect can be blocked by a few substances
- E.g the protein inhibitory effect of Newcastle disease virus and Hela cells is blocked by puromycin or ppara-fluorophenyl alanine

Virology

Enzyme Metabolism of Virus Infected Cells

Enzyme Metabolism

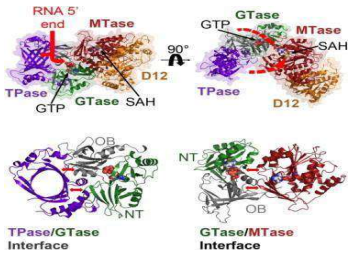
- Enzymes normally speed up different biochemical reactions within host cell
- They help to break down protein, fat or carbohydrate molecules

Enzyme Metabolism

- Induction of new enzymes is followed by cellular infection by virus
- These enzymes not found in normal uninfected cells
- Or have an additive effect with the already present enzymes

Enzyme Metabolism

- Some enzymes are induced *de novo* from information encoded by viral genome



Enzyme Metabolism

- Observed enzyme changes may be secondary from overall disorganization of cell brought by viral development

Enzyme Metabolism

- Particular enzyme activities thought to be regulated by information encoded in the viral genome:

1. Changes in RNA-synthetase
2. Changes in structural protein

END

Virology

Enzymatic Changes in Virus-infected Cells

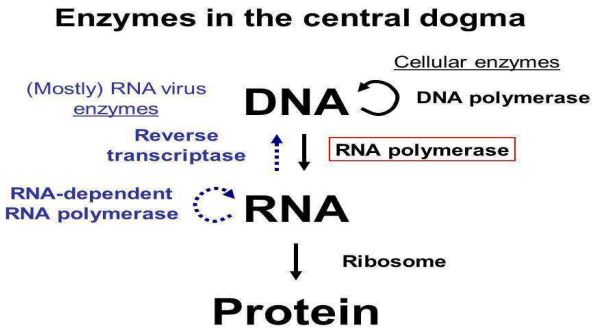
RNA-dependent RNA Polymerase

Enzymatic Changes

RNA dependent RNA polymerase

- RNA-dependent RNA polymerase (RdRP), (RDR), or RNA replicase, is an enzyme that catalyzes the replication of RNA from an RNA template
- This is in contrast to a typical DNA-dependent RNA polymerase, which catalyzes the transcription of RNA from a DNA template

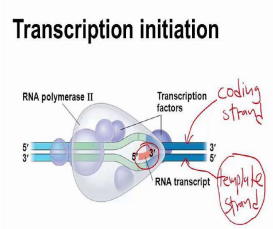
Enzymatic Changes



Enzymatic Changes

NORMAL CELL

- All RNA transcribed from cellular DNA
- Direct transcription
- Instead of formation of mRNA



Enzymatic Changes

Cell Infected With Rna Virus

- Which replicate in cytoplasm
- Polymerization of viral RNA from cellular RNA template
- This enzyme not found normally

Virology

Enzymatic Changes In Virus-Infected Cells

DNA Polymerase

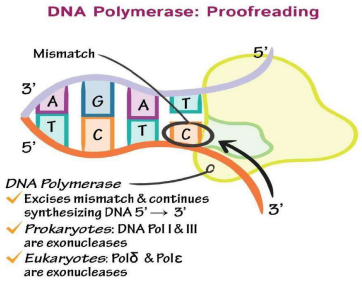
Enzymatic Changes

DNA Polymerase

- DNA polymerase is an enzyme that synthesizes DNA molecules from deoxyribonucleotides, the building blocks of DNA
- These enzymes are essential for DNA replication and usually work in pairs to create two identical DNA strands from a single original DNA molecule

Enzymatic Changes

STRUCTURE



Enzymatic Changes

POXVIRUS

- Poxvirus replicates in cytoplasm
- DNA polymerase does not exist in cytoplasm
- Survival advantage in viral genome is obvious

Virology

Enzymatic Changes in Virus-infected Cells

Thymidine Kinase

Enzymatic Changes

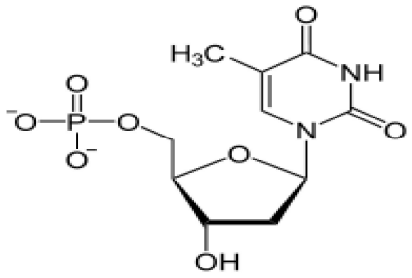
Thymidine Kinase

- Thymidine kinase is an enzyme, a phosphotransferase (a kinase): 2'-deoxythymidine kinase, ATP-thymidine 5'-phosphotransferase
- It can be found in most living cells
- Certain viruses also have genetic information for expression of viral thymidine kinases
- Thymidine kinase catalyzes the reaction:

Thd + ATP → TMP + ADP

Enzymatic Changes

STRUCTURE

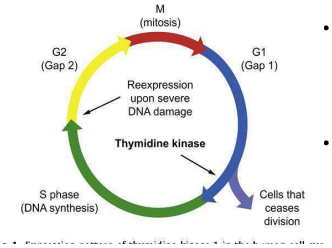


Enzymatic Changes

- Infection with herpesvirus and poxvirus
- Causes increase in thymidine kinase

Enzymatic Changes

Normal function in cell



- Not known until now
- It may phosphhorylate the free deoxyribonucleoside to a usable DNA precursor
- End result may be free thymidine which may be involved in DNA synthesis

Enzymatic Changes

In infected cell

- In vaccinia virus infection
- Enzyme production switched off after ten hours
- Can be inhibited by actinomycin or puromycin
- 40 fold increase in enzyme
- Mechanism yet unknown

Virology

Enzymatic Changes in Virus-Infected Cells

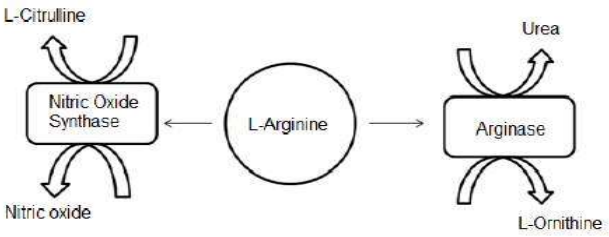
Arginase

Enzymatic Changes

Arginase

- Arginase (EC 3.5.3.1, arginine amidinase, canavanase, L-arginase, arginine transamidinase) is a manganese-containing enzyme
- The reaction catalyzed by this enzyme is: arginine + H_2O → ornithine + urea
- It is the final enzyme of the urea cycle

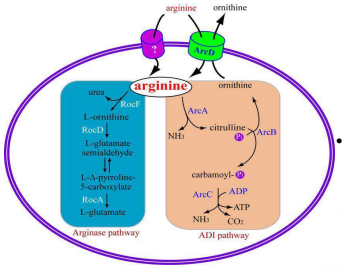
Enzymatic Changes



Enzymatic Changes

- Rabbit papilloma virus induces high levels of arginine in squamous epithelium
- Squamous epithelium normally contains arginine

Enzymatic Changes



- The induced enzyme differs immunologically and physiochemically from normal enzyme
- Released from normal cellular control mechanism

Enzymatic Changes

Speculation: net effect is the depletion of cellular arginine which results in reducing the amount of arginine rich histones synthesized

Virology

Changes in Structural Proteins

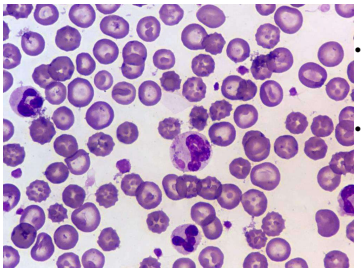
Structural Proteins

INCLUSIONS

- Examination of virus infected cells by light or electron microscope shows viral multiplication and maturation referred as 'inclusions' or 'factories'

Structural Proteins

EXAMPLES

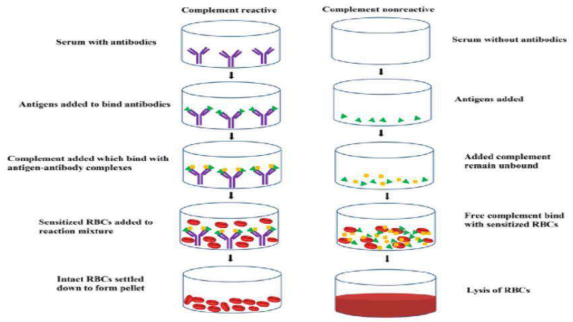


- Inclusion may be large, in case of poxvirus infection
- Or small incase of picornavirus infection
- Or maybe limited by a membrane

Structural Proteins

- New proteins are usually associated with tumors
- Property of oncogenic viruses

Structural Proteins



Structural Proteins

DETECTION

- Complement fixation
- Fluorescent antibody detection
- Graft detection

CONTROL

- Most probably under control of viral genome
- Possibility of cellular response products to virus

Virology

Factors Influencing Viral Multiplication

Factors Affecting Viral Replication

FACTORS

1. Viral concentration
2. Virus-cell systems
3. Elevated temperatures
4. Incomplete viral genomes

Factors Affecting Viral Replication

Viral Concentration

- One infectious unit equals to 10^3 to 10^5 noninfectious particles
- Low ratio associated with slow uncoating of viral particles
- Exception of vaccinia virus

Factors Affecting Viral Replication

Virus-cell Systems

- Blocks in viral development after viral genome starts to function
- E.g herpes simplex virus in dog kidney cells after development of cytopathic effects, inhibition of cell division, synthesis of viral

Factors Affecting Viral Replication

VIRUS-CELL SYSTEMS

- Failure of virus to replicate in dog kidney cells
- Possible that viral DNA acted upon cellular nuclease or that sufficient DNA polymerase not present

Factors Affecting Viral Replication

Elevated Temperatures

- At 40°C, poliovirus inhibits cellular RNA synthesis
- But viral RNA fails o replicate and no viral progeny produced
- Polymerization fails at this temp.

Factors Affecting Viral Replication

Incomplete Genomes

- Incomplete viral genomes
- Like influenza virus
- Produce nucleoproteins, hemagglutinin
- But no mature virus

Virology

Viral Interference

Viral Interference

Principle

- Demonstrated *invitro* and *invivo*
- By infecting cell with a virus followed by a second virus
- Demonstrated when the socnd virus fails to replicate or is inhibited

Viral Interference

Groups

1. Interference mediated by virus induced cellular protein called interferon
2. Interference that is non interforn mediated

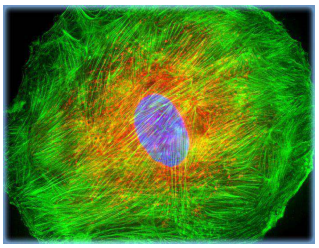
Viral Interference

Interferon

- Interferons are 20,000 molecular weight proteins
- Produced by cells in response to infection
- Interferon producing information resides in host cell genome

Viral Interference

INTERFERON



- Not virus-specific
- E.g chicken fibroblast cell interferon is inactive in mouse cells but inhibitory for viruses grown in chicken fibroblast cell cultures

Viral Interference

ROLE

- Interferons block translation of viral genome in early stages
- Basic mechanism however remains speculative

Viral Interference

- Interferon does not bind to naked viral RNA to reduce infectivity
- Suggests inhibitory action takes place at cellular site
- Role of interferons in recovering is being studied

Virology

Collection of Samples for Viruses

Viral Sample Collection

CONSIDERATIONS

1. What to collect?
   - ✓ Type of specimen
     - e.g septicemic infection → blood
     - Urinary tract infection → urine
     - Respiratory infection → nasal swabs
   - ✓ Quantity of sample
2. When to collect?
   - ✓ Before treatment with antibiotics
   - ✓ Site may vary with time

Viral Sample Collection

TRANSPORT MEDIA

- Allows organisms (pathogens and contaminants) to survive

✓UTM (Universal Transport Medium)

✓PBS/Glycerol transport Medium

(NaCL, KCl, Na2HPO4 in distilled water)

Viral Sample Collection

Specimen	Transport media	Storage condition		Purpose/ Lab investigation
		Transport	Pending test	
Throat swab	VTM	2-8 ºC	-20 ºC	Isolation
NPA/ swab	VTM	2-8 ºC	-20 ºC	Isolation
CSF	No	2-8 ºC	-20 ºC	Isolation, serology
Stool	No	2-8 ºC	-20 ºC	Isolation
Urine	No	2-8 ºC	-20 ºC	Isolation
Serum/ Clotted blood	No	2-8 ºC	-20 ºC 2-8 ºC	Isolation, serology
Whole blood	No	2-8 ºC	2-8 ºC	Isolation, serology

Virology

Preservation of Samples of Virus

Preservation of Viral Samples

Types of Samples

- Tissue samples
- Extracted DNA
- Extracted RNA
- cDNA
- PCR products

Preservation of Viral Samples

Tissue Samples

- can be kept at -80ºC for years
- Temperature should be maintained
- Thawing repeatedly may contaminate the sample

Preservation of Viral Samples

Extracted DNA

- can be kept at -80ºC
- Aliquots of sample
- Properly labeled and sealed

Preservation of Viral Samples

Extracted RNA

- Very sensitive due to presence of RNA everywhere
- Stores at -80ºC for a long period of time
- Can also be kept at -20ºC for short time periods
- Refreezing not recommended

Preservation of Viral Samples

cDNA

- Comparatively more stable than RNA
- Can be kept at -20ºC for several weeks
- Can be kept at -80ºC for many years

Preservation of Viral Samples

Specimen	Storage condition
	Pending test
Throat swab	-20 ^{0}C
NPA/ swab	-20 ^{0}C
CSF	-20 ^{0}C
Stool	-20 ^{0}C
Urine	-20 ^{0}C
Serum/	-20 ^{0}C
Clotted blood	2-8 ^{0}C
Whole blood	2-8 ^{0}C

Virology

Transport of Samples for Viruses

Viral Sample Transport

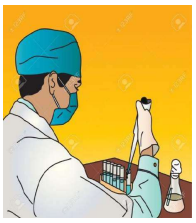
Purpose Of Sop

- Safety from bio-hazardous material
- Results depend on sample collection and handling

Viral Sample Transport

Biosafety consideration

- Zoonotic or transmissible
- Latex gloves, Lab coats, Masks
- New syringes and containers
- Sealed container
- Wash hands after removing gloves
- In event of injury, wash area with soap and water
- Always have a first aid safety kit



Viral Sample Transport

Handling and transport of blood sample

✓Collect in EDTA or Heparin containing containers

✓Store at 4 ^{o}C

✓Transport upright with cushion (to prevent hemolysis)

✓Never freeze blood

Viral Sample Transport

Nasopharyngeal swabs transport

✓All respiratory specimens should be transported in proper medium

✓Transport as quickly as possible to the laboratory to reduce overgrowth by oral flora

✓For transit periods up to 24 hours

- ambient temperature for bacteria
- 4-8°C for viruses

Viral Sample Transport

POSTMORTEM SAMPLES

- Collection
  - ✓Biopsy relevant tissues
    - in formalin for histopathology
    - in transport medium for microbiological testing
    - in sterile saline for isolation of viral pathogens
- Handling and Transport
  - in transport media within 24h at ambient temperature
  - in sterile saline at 4-8^{o}C within 48h

Virology

FILTERATION METHOD

Berkefeld, Mandler and Pasteurchamberland filter

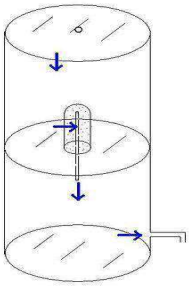
Filtration Method

The Berke Filter

- Manufactured of diatomaceous Earth
- Graded into three porosities
- The V (viel) grade is coarse
- Used for preliminary filtration to remove bacteria or large particles from a solution
- The N (normal) grade is most useful since it retains most bacteria, while all viruses will pass through
- The W (Weing) grade has very small pores that will retain all bacteria and large viruses

Filtration Method

The Berke Filter



Schematic diagram of a Berkefeld filter

Filtration Method

The Mandler Filter

- Made of diatomaceous Earth
- Similar to berkefeld filters
- Three grades
  - Preliminary
  - Regular
  - Fine

Filtration Method

The Mandler Filter

Filtration Method

Pasteur chamberland Filter

- Made of porcelain
- Molded in candle form
- Graded in porosities
- L1 to L13
- L13 is the finest grade

Filtration Method

Pasteur Chamberland Filter

Virology

FILTERATION METHOD

Selas, Seitz and Boerner filter

Filtration Method

The Selas Filter

- Made up of porcelain
- Seven grades of porosity
- Graded as XF, XFF, No. 10, and No. 01 (coarse grade)
- Graded as No. 015, No. 02, No. 03 which retain bacteria

Filtration Method

The Selas Filter

Filtration Method

Seitz Filter

- Asbestos pads
- Manufactured in two grades
- K and EK (coarse)
- Pads in metal holder
- Discarded after one use

Filtration Method

The Seitz Filter

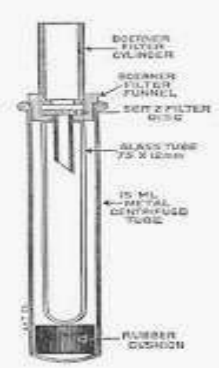
Filtration Method

Boerner Filter

- Same as Seitz
- Except they are small and fitted in small metal receptacle
- Adapts into small test tube
- Centrifuged to force liquid through them

Filtration Method

Boerner Filter



Virology

FILTERATION METHOD

Collodian filter and cellulose filter

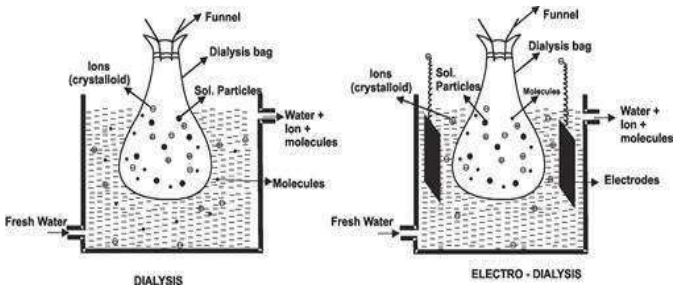
Filtration Method

Collodion Filter

- Made up of nitro-cellulose
- Pore diameter varies
- Variation by addition of various amounts of alcohol, ether, acetone and acetic acid
- Pore diameter: 3um- 10um
- Most useful in determining viral size

Filtration Method

Colloidan Filter



Filtration Method

Cellulose Filter

- Pure cellulose or cellulose derivatives
- Coarse with pore diameter 3um to 0.75 um
- Medium with pore diameter: 0.75um to 0.7um
- Dense with 0.2um
- Very dense below 0.2um

Filtration Method

The Cellulose Filter

Virology

Filteration Technique

Filteration Technique

Filtration

A technique used either to remove solid impurities from an organic solution or to isolate an organic solid

The two types of filtration commonly used

Gravity filtration

Vacuum or suction filtration

Filteration Technique

Gravity Filtration

- Gravity filtration is the method of choice to remove solid impurities from an organic liquid

Filteration Technique

Vacuum Filtration

Vacuum filtration is used primarily to collect a desired solid, for instance, the collection of crystals in a recrystallization procedure

Filteration Technique

- https://www.youtube.com/watch?v=r4GYLuqJV7Y

Virology

Determination of Viral Size by Filteration

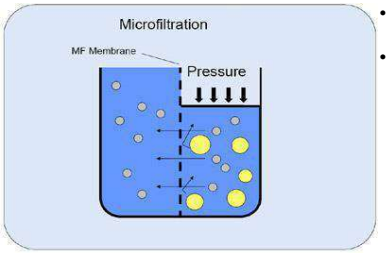
Viral Size

Membrane Technology

- Principle is physical separation
- The extent of removal depends on the size of the particles
- Microfilteration and ultracentrifugation

Viral Size

MICROFILTERATION



- Pore size 0.1 to 1um
- It removes all bacteria
- Only a part of viral contamination is removed

Viral Size

APPLICATIONS

Cold sterilization of beverages and pharmaceuticals

Clearing of fruit juice, wines and beer

Separation of bacteria from water

Separation of oil/ water emulsions

Reverse Osmosis

Solid-liquid separation for pharmacies or food industries

Viral Size

ULTRACENTRIFUGATION

Pore size: 0.001- 0.1 um from liquids

Examples

The dairy industry (milk, cheese)

The food industry (proteins)

The metal industry (oil/ water emulsions separation, paint treatment)

The textile industry

Virology

Centrifugation Techniques

Centrifugation Techniques

Centrifugation

A technique which involves the application of centrifugal force to separate particles from a solution according to their size, shape, density, viscosity of the medium and rotor speed

Centrifugation Techniques

Principle

More-dense components of the mixture migrate away from the axis of the centrifuge (move to the outside), while less-dense components of the mixture migrate towards the axis,

i. e., move to the center.

Centrifugation Techniques

Microcentrifuges

High-speed centrifuges

Ultrcentrifugations

Density

Gradient Centrifugation

Centrifugation Techniques

https://www.youtube.com/watch?v=KEXWd3_fM94

Virology

Determination of Essential Lipids

Determination of Essential Lipids

Why Essential Lipids

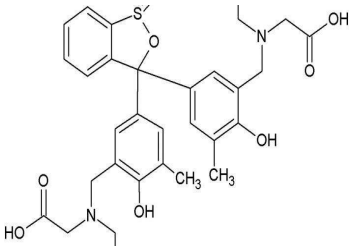
- Used for identification and classification of animal viruses
- On the basis of presence or absence of essential lipids

Determination of Essential Lipids

Principle

Presence of essential lipids determined by mixing virus preparations with either ether

or chloroform



Determination of Essential Lipids

Procedure

- Sufficient ethyl ether is added to virus preparation to make a 20% solution.
- Mixture is incubated at 18-24hrs at 4 °C

Determination of Essential Lipids

- The resultant is poured into a petri dish.
- Ether is allowed to evaporate.

Determination of Essential Lipids

- To expose a virus to chloroform, 0.05ml chloroform is added to 1ml of virus preparation.
- Mix for ten minutes at 4°C.
- Chloroform is removed by centrifugation.

Determination of Essential Lipids

- Essential lipids can be determined by various methods used in labs.

Virology

Determination of Nucleic Acid Types

Determination of Nucleic Acid

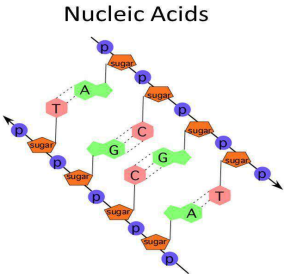
Nucleic acid types

- Double stranded DNA.
- Positive Sense single stranded RNA
- Negative sense single stranded RNA
- Double stranded RNA

Determination of Nucleic Acid

Principle

Use of DNA inhibitors determines type of Nucleic Acid a virus possesses



Determination of Nucleic Acid

DNA inhibitors

- e.g 5-floro-2'
  -deoxyuridine (FUDR)
- 5-bromo-2'
  -deoxyuridine (BUDR)
- 5-iodo-2'
  -deoxyuridine (IUDR)

Determination of Nucleic Acid

Action of DNA inhibitors

- Specific inhibition of viral DNA synthesis when added to cell culture medium

Determination of Nucleic Acid

Interpretation

- If growth of virus is inhibited, it is presumed to contain DNA
- If not, it possesses RNA

END

Virology

Concentration and Purification of Viruses

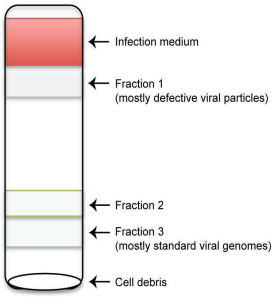
Concentration and Purification

Purpose

- To study many properties of viruses, a purified preparation must be used

Concentration and Purification

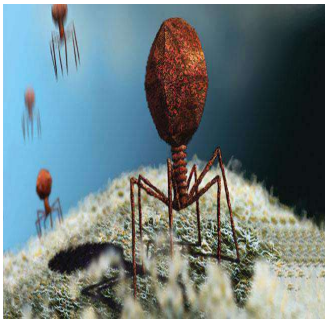
Challenges



- The separation of virus elementary bodies from the components of the cell in which the virus has been grown
- Centrifugation of crude virus containing material at various speed will help overcome this problem

Concentration and Purification

Removal of unwanted substances



- The speed at which majority of elementary bodies are sedimented can be determined
- Resuspension of virus in fresh diluents and resedimentation through several cycles

Concentration and Purification

Density gradient

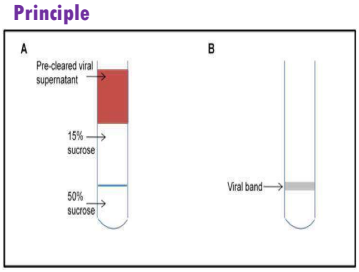
Composed of sucrose or glycerol enables separation of virus from tissue as well as different components

Made by layering fluids of different density in a centrifuge tube

Last layer has greatest density

Concentration and Purification

Principle



- Depending on the relative density, virus in tissue placed on top of the tube will be stratified
- Each settles in the zone of fluid of equal density

Concentration and Purification

Isolation4

Since plastic tubes are used for this technique, it is possible to use a syringe and a needle to draw out the desired layer

Concentration and Purification

Alternate method

Hemagglutination-elution can be applied to virus that attract erythrocytes

Concentration and Purification

Purification of viruses

1. Precipitation with cold ethanol
2. Extraction of tissues/fluids with florocarbons (Genetrons & Freons)

END

Virology

VIRAL DIAGNOSTIC TESTS

Enzyme Linked ImmunoSorbent Assay (ELISA)

Viral Diagnostic Procedures

1. Direct Examination of Specimen

- **Electron Microscopy morphology / immune electron microscopy**
- **Light microscopy histological appearance - e.g. inclusion bodies**
- **Antigen detection immunofluorescence, ELISA etc.**
- **Molecular techniques for the direct detection of viral genomes**

Viral Diagnostic Procedures

2. Indirect Examination

- **Cell Culture - cytopathic effect, haemadsorption, confirmation by neutralization, interference, immunofluorescence etc.**
- **Eggs pocks on CAM - haemagglutination, inclusion bodies**
- **Animals disease or death confirmation by neutralization**

3. Serology

- **Detection of rising titres of antibody between acute and convalescent stages of infection, or the detection of IgM in primary infection.**

Viral Diagnostic Procedures

Other Techniques

Classical Techniques	Newer Techniques
1. Complement fixation tests (CFT)	1. Radioimmunoassay (RIA)
2. Haemagglutination inhibition tests	2. Enzyme linked immunosorbent assay (EIA)
3. Immunofluorescence techniques (IF)	3. Particle agglutination
4. Neutralization tests	4. Western Blot (WB)
5. Single Radial Haemolysis	5. Recombinant immunoblot assay (RIBA), line immunoassay (Liatek) etc.

Viral Diagnostic Procedures

Enzyme Linked ImmunoSorbent Assay (ELISA)

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

- In an ELISA, an antigen must be immobilized on 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.

.

Viral Diagnostic Procedures

The key step, immobilization of the antigen of interest, can be accomplished by direct adsorption to the assay plate or indirectly via a capture antibody that has been attached to the plate.

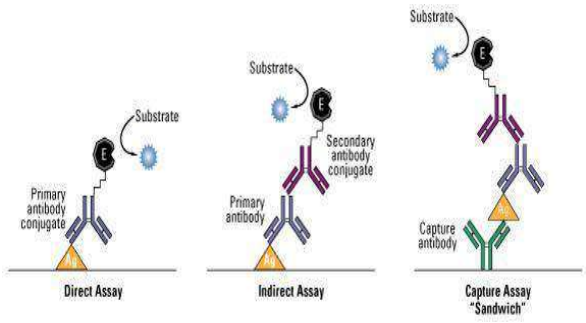
The antigen is then detected either directly (labeled primary antibody) or indirectly (labeled secondary antibody).

The most powerful ELISA assay format is the sandwich assay.

Viral Diagnostic Procedures

This type of capture assay is called a “sandwich” assay because the analyte to be measured is bound between two primary antibodies – the capture antibody and the detection antibody. The sandwich format is used because it is sensitive

Viral Diagnostic Procedures



Viral Diagnostic Procedures

ELISA kits contain pre-coated antibody-plates, detection antibodies, buffers, diluents, standards, and substrates

Virology

VIRAL DIAGNOSTIC PROCEDURES

Histological Examination of Virus infected cells

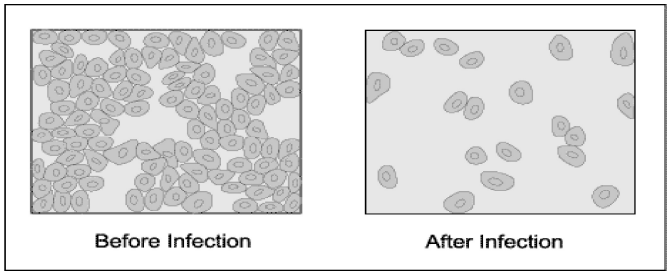
Viral Diagnostic Methods

CULTURE

Grown in :

1. Primary cells
2. Semi-continuous cells
3. Continuous Cells

Viral Diagnostic Methods



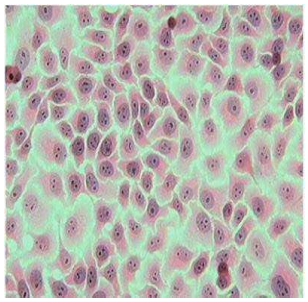
Viral Diagnostic Methods

Identification of growing viruses

- Cytopathic effects (CPE)
- Haemadsorption

Viral Diagnostic Methods

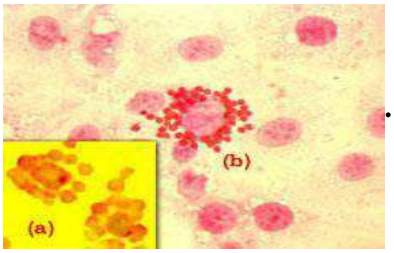
Cytopathic Effects



- Specific or non-specific signs
- Herpes Simplex Virus produces specific CPE
- Enterovirus don't produce CPE

Viral Diagnostic Methods

Haemadsorption



- Cells acquire ability to stick to mammalian RBCs
- Mainly used for influenza and parainfluenza

Viral Diagnostic Methods

Confirmation of virus

Neutralization

Haemadsorption-inhibition

Immunofluoresence

Molecular tests

PCR

ELISA

END

Virology

VIRAL DIAGNOSTIC PROCEDURES

Polymerase Chain Reaction

Viral Diagnostic Procedures

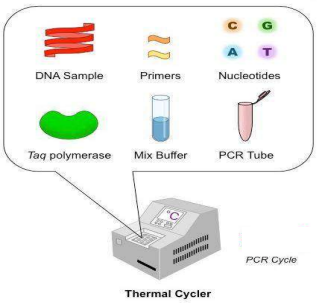
PCR

- Polymerase chain reaction is a laboratory technique used to amplify segments of DNA or RNA
- RNA → (Reverse transcriptase) → DNA
- Cycles: 25-30

Viral Diagnostic Procedures

ESSENTIALS

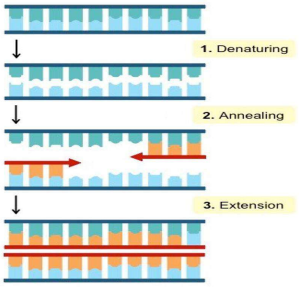
1. DNA sample
2. Primers (Reverse and forward)
3. Nucleotides (dNTP's)
4. Buffer
5. MgCl2
6. Heat stable Taq Polymerase
7. Distilled water
8. PCR tubes and thermal cycler



Viral Diagnostic Procedures

PROCEDURE

1. Step 1:
   - Pre-denaturation
2. Step 2:
   - Denaturation
   - Annealing
   - Extension
3. Step 3:
   - Final polymerization



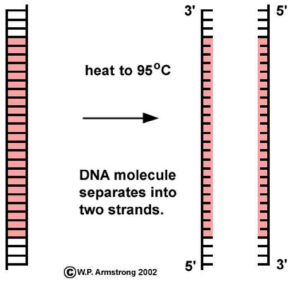
Viral Diagnostic Procedures

DENATURATION

Predenaturation: DNA boiled at 95°C for 5 minutes

Denaturation: 95°C for 15-30 seconds

Hydrogen bonds break & the complementary strands of the double-stranded DNA separate

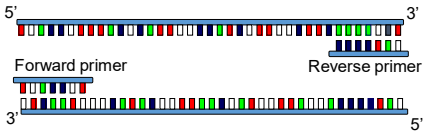


Viral Diagnostic Procedures

ANNEALING

Both primers (i.e. Reverse and forward) bind to their complementary sequences.

Temperature: 40-60°C



Viral Diagnostic Procedures

DENATURATION

- Temperature: 72°C
- MgCl2 facilitates Taq in adding up the nucleotides to the strand.
- Taq polymerase binds to the annealed primers and extends DNA at the 3' end of the chain.
- Final polymerization: Fills gaps

Viral Diagnostic Procedures

Types of PCR

1. Real-Time PCR
2. Nested PCR
3. Multiplex PCR
4. Arbitrary Primed PCR
5. Hot start PCR
6. Inverse PCR

END

Virology

VIRAL DIAGNOSTIC PROCEDURES

Nucleic acid sequence based amplification

Viral Diagnostic Procedures

1. Direct Examination of Specimen

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Viral Diagnostic Procedures

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4. Neutralization tests	4. Western Blot (WB)
5. Single Radial Haemolysis	5. Recombinant immunoblot assay (RIBA), line immunoassay (Liatek) etc.

Viral Diagnostic Procedures

- Strand displacement Amplification
- Polymerase Chain Reaction (PCR)
- Ligase Chain Reaction (LCR)
- **Transcription based amplification**