Chapter 3. General Virology

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Viruses are the smallest unicellular organisms that are obligate intracellular. They differ from bacteria and other prokaryotes in many ways.

- They possess either DNA (deoxyribonucleic acid) or RNA (ribonucleic acid), but never both
- They cannot be grown on artificial cell-free media
- They do not have a cell wall or cell membrane or cellular organelles

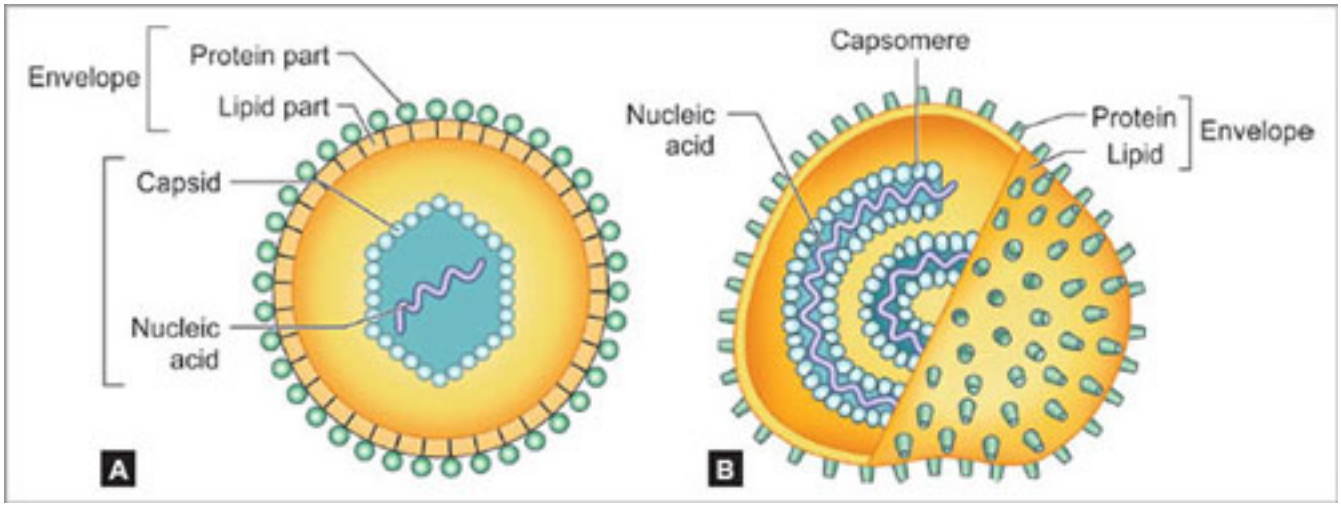
- They lack the enzymes necessary for protein and nucleic acid synthesis
- They are not susceptible to antibacterial antibiotics.

MORPHOLOGY OF VIRUS

The virus particle comprises a *nucleic acid* surrounded by a protein coat called as *capsid*, together known as the nucleocapsid. Some viruses also have an outer *envelope (Figs. 3.1A and B)*.

- **Nucleic acid:** Viruses have only one type of nucleic acid, either DNA or RNA but never both. Accordingly, they are classified as DNA viruses and RNA viruses. The nucleic acid may be single or double-stranded, segmented, or unsegmented
- **Capsid:** It is composed of several protein subunits called capsomeres. It protects the nucleic acid core from the external environment
- **Symmetry:** It is the arrangement of capsomeres with respect to the surrounding nucleic acid, which can be of three types
    1. *Icosahedral symmetry:* The capsomeres are arranged surrounding the nucleic acid in a cubical shape; e.g. all DNA viruses (except poxviruses) and most of the RNA viruses *(Fig. 3.1A)*
    2. *Helical symmetry:* The capsomeres are coiled surrounding the nucleic acid in the form of a helix or spiral. Examples include—myxoviruses, rhabdoviruses, etc. *(Fig. 3.1B)*
    3. *Complex symmetry:* Poxviruses possess complex symmetry.
- **Envelope:** Certain viruses possess an envelope surrounding the nucleocapsid. The envelope is lipoprotein in nature
    - The envelope is lipoprotein in nature. The protein spikes (called peplomeres) are embedded into the lipid layer
    - Peplomers bind to specific receptors on the host cells, thus facilitating the entry of the virus
    - Peplomers are antigenic; therefore, antibodies against them are protective
    - **Examples** of enveloped viruses include—influenza virus, hepatitis B, and coronavirus.

Figs. 3.1A and B. Structure and symmetry of virus: *A.* Enveloped virus with icosahedral nucleocapsid; *B.* Enveloped virus with helical nucleocapsid.



- **Size of the viruses:** Viruses are extremely small, and vary from 20–400 nm in size. The smallest virus is parvovirus (20 nm) and the largest is poxvirus (400 nm)
- **Shape of the viruses:** Mostly animal viruses are spherical shaped, with some exceptions:
  - Rabies virus: Bullet-shaped
  - Poxvirus: Brick-shaped
  - Adenovirus: Space vehicle-shaped
  - Rotavirus: Wheel-shaped.

CLASSIFICATION

There are various DNA and RNA virus groups, which further comprise several important viruses infecting humans as enlisted in **Table 3.1**.

VIRAL REPLICATION

Viruses undergo a complex way of cell division. Replication of viruses passes through seven sequential steps:

Attachment → Penetration → Uncoating →

Biosynthesis → Assembly → Maturation →

Release.

1. **Adsorption/attachment**: It is the most specific step of viral replication. It involves receptor interactions between virus and host surface receptors
2. **Penetration:** After attachment, the virus particles penetrate into the host cells
3. **Uncoating:** Lysis of capsid due to host lysozymes and release of the nucleic acid
4. **Biosynthesis** of various viral components: i) nucleic acid, ii) capsid protein, iii) enzymes iv) other regulatory proteins
5. **Assembly:** Viral nucleic acid and proteins are packaged together to form progeny viruses (nucleocapsids)
6. **Maturation:** Following assembly, maturation of daughter virions takes place either in the nucleus or cytoplasm, or membranes
7. **Release** of daughter virions occurs either by:
   - Lysis of the host cells
   - Budding through the host cell membrane.

PATHOGENESIS OF VIRAL INFECTIONS

Most of the viral infections progress through the following steps inside the human body:

- Transmission (entry into the body)

- Primary site of replication
- Spread to a secondary site
- Manifestations of the disease.

Transmission

Viruses enter the human body through various routes *(Table 3.2)*.

Primary Site of Replication

- Some viruses are restricted to the portal of entry where they multiply and produce local diseases

Table 3.1. Important DNA and RNA viruses infecting humans.

DNA Virus Groups	***DNA Viruses***
Herpesviruses	Herpes simplex virus 1 and 2, Varicella-zoster virus, Cytomegalovirus (CMV), Epstein-Barr virus (EBV), Human herpesvirus-6,7 and 8
Poxviruses (largest virus in size)	Variola virus (smallpox), Molluscum contageosum virus
Papovaviruses	Human papillomavirus
Parvoviruses (smallest virus in size)	Parvovirus B19
Hepadnavirus	Hepatitis B virus
Adenoviruses	Human adenovirus
RNA Virus Groups	*RNA Viruses*
Myxoviruses	Influenza viruses–A, B, and C Parainfluenza viruses, mumps virus, measles virus, respiratory syncytial virus, Nipah virus
Coronaviruses	Coronaviruses (SARS-CoV, MERS-CoV and SARS-CoV-2)
Arboviruses	Dengue virus, yellow fever virus, chikungunya virus, Kyasanur forest disease virus, Japanese B encephalitis virus, Zika virus
Retroviruses	HIV (human immunodeficiency virus)
Rabies virus	Rabies virus
Picornaviruses	Poliovirus, Coxsackievirus, enterovirus, rhinovirus
RNA hepatitis viruses	Hepatitis A, C, D, E viruses

DNA Virus Groups	*DNA Viruses*
RNA viruses causing gastroenteritis	Rotavirus, calicivirus, Norwalk virus, astrovirus
Miscellaneous RNA viruses	Filoviruses–Marburg virus and Ebola virus, Rubella virus

- On the other hand, most of the viruses first multiply locally to initiate a silent local infection, which is followed by the spread via lymphatics to regional lymph nodes (most viruses) or via blood (e.g. poliovirus) or via neuronal spread to reach the central nervous system or CNS (e.g. rabies virus).

Table 3.2. Mode of transmission of viruses.

Transmission	*Viruses*
Respiratory route (probably the most common route)	• Myxoviruses such as influenza virus • Coronaviruses such as SARS-CoV-2 • Rhinovirus • Varicella-zoster virus • Cytomegalovirus • Rubella virus • Parvovirus • Smallpox virus
Oral route	• Rotavirus and other viral agents causing gastroenteritis • Poliovirus and other enteroviruses • Hepatitis viruses—A and E
Cutaneous route	• Herpes simplex virus-1 • Human papillomavirus • Molluscum contagiosum virus
Vector bite	Arboviruses such as: • Dengue virus *(Aedes)* • Chikungunya virus *(Aedes)* • Japanese encephalitis virus *(Culex)* • Yellow fever virus and Zika virus *(Aedes)* • Kyasanur Forest disease virus (Tick)
Animal bite	Rabies virus
Sexual route	• Herpes simplex virus-2 • Human papillomavirus

Transmission	*Viruses*
	• Hepatitis B, C and rarely D viruses • Human immunodeficiency virus (HIV)
Blood transfusion	• Hepatitis B, C and rarely D viruses • HIV
Needle-stick injury	• Hepatitis B, C and rarely D viruses • HIV
Mother-to-child transmission (including transplacental route)	• Rubella virus • Cytomegalovirus • Herpes simplex virus • Varicella-zoster virus • Parvovirus B19 • Hepatitis B and C viruses • HIV

Spread of Virus

- **Primary viremia:** Viruses spread to the bloodstream either from the primary sites or from the lymph nodes
- **Secondary site of replication:** Viruses are then transported to the reticuloendothelial system (bone marrow, endothelial cells, spleen and liver) where further multiplication takes place
- **Secondary viremia:** From the spleen and liver, viruses spill over into the bloodstream leading to secondary viremia which results in the onset of non-specific symptoms
- **Target organs:** Via the bloodstream, they reach the target organs (lung, brain, skin, etc.). Certain viruses (e.g. rabies) affect the brain, there is no viremia. Instead, the virus reaches the target organ via neuronal spread
- **Tropism** of the viruses for specific organs determines the pattern of systemic illness; e.g. hepatitis viruses have tropism for hepatocytes and thus produce hepatitis
- **Shedding:** Following infection viruses escape the host by shedding either at the portal of entry (e.g. influenza virus) or in the blood (e.g. dengue virus) or near the target organ (e.g. salivary gland for mumps).

Manifestations of Viral Infections

- **Incubation period:** It is the time interval between the entry of the virus into the body and the appearance of the first clinical manifestation
  - The incubation period is shorter if the virus produces lesions near the site of entry, e.g. influenza virus
  - It is longer if the target organ is much far from the site of entry, e.g. poliovirus and rabies virus.
- **Clinical manifestations:** Persons infected with viruses develop either an inapparent (subclinical) infection or apparent (clinical) infection. The symptoms developed depend on the target body sites where the virus multiplies

- Respiratory viruses such as influenza and coronaviruses produce respiratory infections
- Gastroenteritis: Produced by rotavirus
- Neurotropic viruses can produce meningitis (enteroviruses) or encephalitis (rabies)
- Hepatitis viruses produce hepatitis

LABORATORY DIAGNOSIS OF VIRAL DISEASES

Laboratory diagnosis of viral infections is useful for the following purposes:

- **To start antiviral drugs** for infections caused by herpes, CMV, HIV, influenza viruses, etc.
- **Screening of blood donors** for HIV, hepatitis B, and hepatitis C helps in the prevention of transfusion-transmitted infections
- **Surveillance purpose:** To assess the disease burden in the community
- **For outbreak or epidemic investigation**: To initiate appropriate control measures
- **To start post-exposure prophylaxis** of antiretroviral drugs to the health care workers following needle stick injury (Chapter 43)
- **To initiate certain measures:** For example, if the newborn is diagnosed to have hepatitis B infection, then immunoglobulins (HBIG) should be started within 12 hours of birth.

Direct Demonstration of Virus

- **Electron microscopy:** Detection of viruses by electron microscopy (EM) is increasingly used nowadays. Viruses can be identified based on their distinct appearances; for example:
  - Rabies virus—bullet-shaped
  - Rotavirus—wheel-shaped
  - Coronavirus—petal-shaped peplomers
  - Adenovirus—space vehicle-shaped
  - Astrovirus—star-shaped peplomers.
- **Fluorescent microscopy:** Direct immunofluorescence (Direct-IF) technique is useful to detect viral particles in clinical samples. Its clinical applications are:
  - Diagnosis of rabies virus antigen in skin biopsies, corneal smear of infected patients
  - Rapid diagnosis of respiratory infections caused by influenza virus, rhinoviruses, and respiratory syncytial virus
- **Light microscopy:** It is useful for demonstration of inclusion bodies by histopathological staining of tissue sections, which helps in the diagnosis of certain viral infections (see highlight box below).

Inclusion Body

They are the aggregates of viral proteins and other products of viral replication that confer altered staining property to the host cell.

Role in Laboratory Diagnosis

Inclusion bodies are characteristic of specific viral infections. They have distinct size, shape, location and staining properties by which they can be demonstrated in virus infected cells under the light microscope.

Location

They may be present either in the host cell cytoplasm or nucleus or both

- **Intracytoplasmic inclusion bodies:** They are seen as pink structures (acidophilic)

  # Paschen bodies-variola virus

  # Molluscum bodies-molluscum contagiosum virus

  # Negri bodies-rabies

- **Intranuclear inclusion bodies:** They are basophilic.

  # Cowdry type A inclusions-e.g. Torres body in yellow fever

  # Cowdry type B inclusions-in poliovirus and adenovirus

- **Both intracytoplasmic and intranuclear inclusions-** in cytomegalovirus (owl's eye appearance) and measles.

Detection of Viral Antigens

Detection of viral antigens in serum and other samples can be done by techniques such as enzyme-linked immunosorbent assay (ELISA), immunochromatographic test (ICT), enzyme-linked fluorescence assay (ELFA), etc. Some important antigen detection tests include:

- HBsAg antigen detection for hepatitis B virus infection from serum
- SARS-CoV-2 antigen (nucleocapsid protein) detection in nasopharyngeal swabs.

Detection of Viral Antibodies

Antibody detection from serum is one of the most commonly used methods in diagnostic virology. Techniques such as ELISA, ELFA, ICT are widely used; for example:

- Anti-hepatitis C antibodies in serum
- Antibodies against HIV antigens from serum
- Anti-dengue IgM/IgG antibodies from serum.

Molecular Methods

Molecular techniques have eased the diagnosis of viral infections. They are more sensitive, specific and yield quicker results.

- **Polymerase chain reaction (PCR)** is useful to detect viral DNA in clinical specimens

- **Reverse transcriptase-PCR (RT-PCR)** is used for the detection of RNA viruses in clinical specimens
- **Multiplex PCR** can simultaneously detect genes of common organisms responsible for a clinical syndrome; for example, multiplex PCR for respiratory infection simultaneously detects genes of many respiratory viruses in clinical specimens
- **Real time-PCR (rt-PCR):** It is considered as the gold standard method for the diagnosis of several viral infections such as influenza, COVID-19, etc. It has several advantages such as—
  - Quantifying viral nucleic acid in the samples, hence used to monitor the treatment response
  - Takes lesser time
  - More sensitive and specific than PCR.

Isolation of Virus

Viruses cannot be grown on artificial culture media. They are cultivated by animal inoculation, embryonated egg inoculation, or tissue cultures.

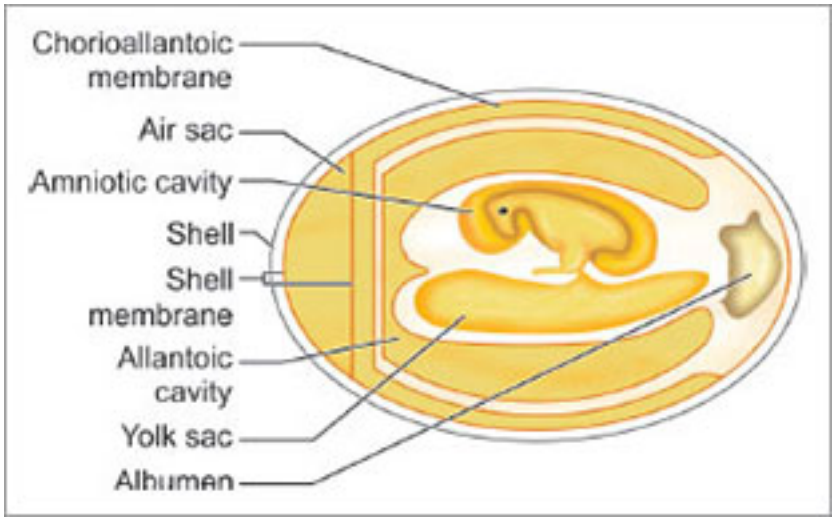
Animal Inoculation

Animal inoculation is largely restricted only for research purposes and for limited diagnostic purposes such as—primary isolation of arboviruses and coxsackieviruses.

Egg Inoculation

The use of egg inoculation in viral diagnostics is greatly limited now. An embryonated hen's egg has four sites that are specific for the growth of certain viruses *(Fig. 3.2)*.

Fig. 3.2. Schematic diagram of an embryonated egg.



- **Yolk sac inoculation:** Used for arboviruses (e.g. JE virus) and some bacteria such as *Rickettsia* and *Chlamydia*
- **Amniotic sac:** Used for the isolation of influenza virus
- **Allantoic sac:** It is used for the production of viral vaccines such as—influenza vaccine, yellow fever (17D) vaccine

- **Chorioallantoic membrane:** Used for the isolation of poxviruses. They produce visible lesions over the chorioallantoic membrane called pocks.

Tissue Culture

The tissue culture technique was widely used in the past in diagnostic virology. It is of three types—(i) organ culture, (ii) explant culture, and (iii) cell line culture.

Cell line culture is the only isolation method that is in use now.

Types of Cell Lines

There are three types of cell lines —(i) primary cell lines, (ii) secondary cell lines, and (iii) continuous cell lines.

1. **Primary cell lines:** They are capable of very limited growth in culture, maximum up to 5–10 divisions. Examples include—human amnion cell line and chick embryo cell line
2. **Secondary cell lines:** They can divide a maximum of up to 10–50 divisions. Examples include—human fibroblast cell line and human embryonic lung cell starin
3. **Continuous cell lines:** They are derived from cancerous cell lines, and hence are capable of indefinite growth. Examples include—
    - HeLa cell line (human carcinoma of cervix cell line)
    - Vero cell line (vervet monkey kidney cell line).

Detection of Viral Growth in Cell Cultures

The following methods are used to detect the growth of the virus in cell cultures.

- **Cytopathic effect (CPE):** It is defined as the morphological change produced by the virus in the cell line detected by a light microscope. Examples of CPE effect produced by viruses include—
    - Syncytium formation by the measles virus
    - Granular clumps (like bunches of grapes) by adenovirus
    - Rapid crenation and degeneration of entire cell sheet by enteroviruses.
- **Other methods** to detect viral growth include—
    - Detection of viral antigens by direct immunofluorescence assay
    - Viral genes detection by using PCR.

TREATMENT OF VIRAL DISEASES

Only for limited viral diseases, effective antiviral drugs are available. Commonly used antiviral drugs for viral diseases are as follows:

- Acyclovir for herpes simplex virus and VZV infections
- Ganciclovir for CMV infections
- Oseltamivir for H1N1 flu
- Telbivudine, tenofovir, lamivudine for hepatitis B.

Interferons (IFNs)

IFNs-α, β have antiviral action; produced by many cell types such as macrophages (IFN-α) and fibroblasts (IFN-β). INF-γ does not have antiviral action.

- **Mechanism of action**: IFNs are part of innate immunity; the body's first line of antiviral defense. They are nonspecific in action; produced quickly following viral infection
- **Inducers:** Certain RNA viruses can induce IFN synthesis
- **Application:** IFN-α is used in the following clinical conditions:
  - Topically—used in rhinovirus infection, genital warts, and herpetic keratitis
  - Systemically—used in chronic hepatitis B, C, and D infections.

IMMUNOPROPHYLAXIS FOR VIRAL DISEASES

Viral Vaccines (Active Immunization)

Viral vaccines confer prolonged and effective immunity. Vaccines for viral infections may be available either in live, killed, or subunit forms.

Killed Viral Vaccines

Killed vaccines are available for various viral agents.

- **Preparation:** They are prepared by inactivating viruses with heat, phenol, formalin, or beta-propiolactone
- **Advantages:** They are more stable and are considered safe when given in immunodeficiency or pregnancy
- **Disadvantages:** Killed vaccines are associated with more adverse side effects due to reactogenicity
- **Examples:** Common killed viral vaccines for human use are:
  - Rabies non-neural vaccine—e.g. HDC (human diploid cell) vaccine
  - Killed injectable polio vaccine (IPV).

Subunit Vaccines

In subunit vaccines, only a particular antigen of the virus is used; prepared by DNA recombinant technology, e.g. hepatitis B vaccine.

Live Vaccines

Live vaccines are available for various viral agents.

- **Preparation:** They are prepared by attenuation by serial passages
- **Advantages:** Live vaccines provide a stronger and long-lasting immunity and are administered as a single dose (except OPV)
- **Disadvantages:** Live vaccines are risky in immunodeficiency or pregnancy. They are less stable than killed vaccines
- **Examples:** Common killed viral vaccines for human use are:

- Live oral polio vaccine (OPV)
- MMR vaccine for measles, mumps, and rubella.

Passive Immunization (Immunoglobulin)

Passive immunization is indicated when an individual is immunodeficient or when early protection is needed (i.e. for post-exposure prophylaxis). Currently, human immunoglobulins are available for many viral infections such as mumps, measles, hepatitis B, rabies, and varicella-zoster.

Combined Immunization

Simultaneous administration of vaccine and immunoglobulin in post-exposure prophylaxis is extremely useful. It is recommended for:

- Hepatitis B (neonates born to HBsAg positive mothers or for unvaccinated people following exposure)
- Rabies (for exposures to severe class III bites).

EXPECTED QUESTIONS

1. **I. Write short notes on:**
    1. Laboratory diagnosis of viral infections.
    2. Interferons.
    3. Inclusion bodies.
    4. Viral vaccines.
2. **II. Multiple Choice Questions (MCQs):**
    1. **All of the following are RNA viruses,** *except:*
        a. Enterovirus
        b. Human adenoviruses
        c. Coxsackievirus
        d. Hepatitis A virus
    2. **All of the following viruses are transmitted by the respiratory route,** *except:*
        a. Influenza virus
        b. Rotavirus
        c. Respiratory syncytial virus
        d. Rhinovirus
    3. **All of the following are intracytoplasmic inclusion bodies,** *except:*
        a. Negri bodies

b. Molluscum bodies

c. Cowdry type A inclusions

d. Guarnieri bodies

4. **Which of the following vaccine is a killed vaccine?**

a. Mumps vaccine

b. Measles vaccine

c. Rubella vaccine

d. IPV

5. **The largest virus in size is:**

a. Herpes simplex virus

b. Hepatitis B virus

c. Poxvirus

d. Adenovirus

6. **The smallest virus in size is:**

a. Picornaviruses

b. Parvovirus

c. Hepatitis D virus

d. Adenovirus

7. **Which of the following is continuous cell line?**

a. HeLa cell line

b. Amnion cell line

c. Chick embryo cell line.

d. Human fibroblast cell line

8. **Amniotic sac of embryonated hen's egg is used for isolation of:**

a. HIV

b. Influenza virus

c. Hepatitis B virus

d. Poliovirus

1. b	**2.** b	**3.** c	**4.** d	**5.** c	**6.** b	**7.** a	**8.** b