

Module1: General Concepts

Lecture 1: Virus history

The history of virology goes back to the late 19th century, when German anatomist **Dr Jacob Henle** (discoverer of Henle's loop) hypothesized the existence of infectious agent that were too small to be observed under light microscope. This idea fails to be accepted by the present scientific community in the absence of any direct evidence. At the same time three landmark discoveries came together that formed the founding stone of what we call today as **medical science**. The first discovery came from **Louis Pasteur** (1822-1895) who gave the spontaneous generation theory from his famous swan-neck flask experiment. The second discovery came from **Robert Koch** (1843-1910), a student of Jacob Henle, who showed for first time that the anthrax and tuberculosis is caused by a bacillus, and finally **Joseph Lister** (1827-1912) gave the concept of sterility during the surgery and isolation of new organism.

The history of viruses and the field of virology are broadly divided into three phases, namely discovery, early and modern.

The discovery phase (1886-1913)

In 1879, **Adolf Mayer**, a German scientist first observed the dark and light spot on infected leaves of tobacco plant and named it **tobacco mosaic disease**. Although he failed to describe the disease, he showed the infectious nature of the disease after inoculating the juice extract of diseased plant to a healthy one. The next step was taken by a Russian scientist **Dimitri Ivanovsky** in 1890, who demonstrated that sap of the leaves infected with tobacco mosaic disease retains its infectious property even after its filtration through a Chamberland filter. The third scientist who plays an important role in the development of the concept of viruses was **Martinus Beijerinck** (1851-1931), he extended the study done by Adolf Mayer and Dimitri Ivanofsky and showed that filterable agent form the infectious sap could be diluted and further regains its strength after replicating in the living host; he called it as "*contagium vivum fluidum*". **Loeffler and Frosch** discovered the first animal virus, the foot and mouth disease virus in 1898 and subsequently **Walter Reed** and his team discovered the yellow fever virus, the first human virus from Cuba in 1901. Poliovirus was discovered by **Landsteiner and Popper** in 1909 and two years later **Rous** discovered the solid tumor virus which he called Rous sarcoma virus.

The early phase (1915-1955)

In 1915, **Frederick W. Twort** discovered the phenomenon of transformation while working with the variants of vaccinia viruses, simultaneously **Felix d'Herelle** discovered **bacteriophage** and developed the assay to titrate the viruses by plaques. **Wendell**

Stanley (1935) first crystallized the TMV and the first electron micrograph of the tobacco mosaic virus (TMV) was taken in 1939. In 1933 **Shope** described the first papillomavirus in rabbits. The vaccine against yellow fever was made in 1938 by **Thieler** and after 45 years of its discovery, polio virus vaccine was made by **Salk** in 1954.

The modern phase (1960-present)

During this phase scientists began to use viruses to understand the basic question of biology. The superhelical nature of polyoma virus DNA was first described by **Weil** and **Vinograd** while **Dulbecco** and **Vogt** showed its closed circular nature in 1963. In the same year **Blumberg** discovered the hepatitis B virus. **Temin** and **Baltimore** discovered the retroviral reverse transcriptase in 1970 while the first human immunodeficiency virus (HIV) was reported in 1983 by **Gallo** and **Montagnier**. The phenomenon of RNA splicing was discovered in Adenoviruses by **Roberts, Sharp, Chow** and **Broker**. In the year 2005 the complete genome sequence of 1918 influenza virus was done and in the same year hepatitis C virus was successfully propagated into the tissue culture.

Many discoveries are done using viruses as a model. The transcription factor that binds to the promoter during the transcription was first discovered in SV40. The phenomenon of polyadenylation during the mRNA synthesis was first described in poxviruses while its presence was first reported in SV40. Many of our current understanding regarding the translational regulation has been studied in poliovirus. The oncogenes were first reported in Rous sarcoma virus. The p53, a tumor suppressor gene was first reported in SV40.

Important discoveries

Date	Discovery
1796	Cowpox virus used to vaccinate against smallpox by Jenner.
1892	Description of filterable infectious agent (TMV) by Ivanovsky.
1898	Concept of the virus as a contagious living form by Beijerinck.
1901	First description of a yellow fever virus by Dr Reed and his team.
1909	Identification of poliovirus by Landsteiner and Popper.
1911	Discovery of Rous sarcoma virus.
1931	Virus propagation in embryonated chicken eggs by Woodruff and Goodpasture.
1933	Identification of rabbit papillomavirus.
1936	Induction of carcinomas in other species by rabbit papillomavirus by Rous and Beard.
1948	Poliovirus replication in cell culture by Enders, Weller, and Robbins.
1952	Transduction by Zinder and Lederberg.
1954	Polio vaccine development by Salk.
1958	Bacteriophage lambda regulation paradigm by Pardee, Jacob, and Monod.
1963	Discovery of hepatitis B virus by Blumberg.
1970	Discovery of reverse transcriptase by Temin and Baltimore.
1976	Retroviral oncogenes discovered by Bishop and Varmus.
1977	RNA splicing discovered in adenovirus.
1983	Description of human immunodeficiency virus (HIV) as causative agent of acquired immunodeficiency syndrome (AIDS) by Montagnier, Gallo)
1997	HAART treatment for AIDS.
2003	Severe acute respiratory syndrome (SARS) is caused by a novel coronavirus.
2005	Hepatitis C virus propagation in tissue culture by Chisari, Rice, and Wakita.
2005	1918 influenza virus genome sequencing.

Lecture 2: Virus diversity

Viruses are minute, non-living entities that copy themselves once inside the living host cells. All living organisms (animals, plants, fungi, and bacteria) have viruses that infect them. Typically viruses are made up of coat (or capsid) that protects its information molecule (RNA or DNA); these information molecules contain the blue prints for making more virus. The viruses are highly diverse in their shape, size, genetic information, and infectivity. Viruses are all around us, on an average a human body encounters billion virus particles every day. Our intestinal, respiratory, and urogenital tract are reservoirs for many different kinds of viruses, it is astonishing that with such constant exposure, there is little or no impact of these organisms in human health. The host defense mechanism is quite strong to remove all these in normal condition, while they cause many nasty diseases only when the person is **immune-compromised**. Although viruses have a limited host range but sometimes they may jump the species barrier and causes fatal disease, recent spread of **swine influenza** is an ideal example of such kind of spread.

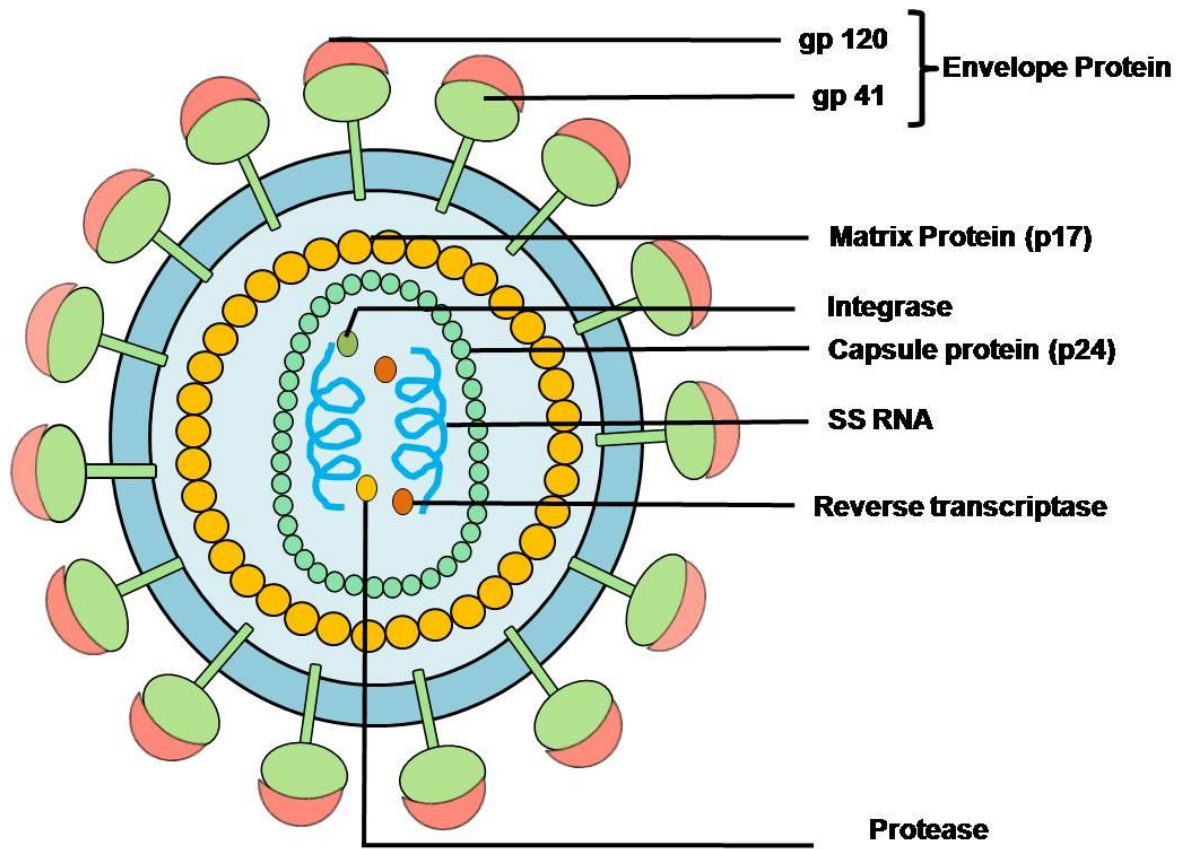
The **epidemic** viruses, such as influenza and severe acute respiratory syndrome (SARS), cause diseases that rapidly spread to a large human population within no time, and seem to attract more scientific and public attention than do **endemic** viruses, which are continually present in a particular population.

Virology as a discipline is merely 100 years old and the way it expanded in this small period of time is rampant. To group the new emerging viruses in a specific group by specifying certain parameters was initiated in 1966 when international committee on the taxonomy of viruses (ICTV) was formed with the aim to classify the viruses. The ICTV has adopted a norm for the description of the viruses. Name for genera, subfamilies, families, and orders must all be a single word, ending with the suffixes **-virus**, **-virinae**, **-viridae**, and **-virales** respectively. In written usage, the name should be capitalized and italicized.

Viruses are **obligate parasite** which means their absolute dependence on living host system. This property of virus made it a valuable tool to study cell functions and its biology. **Adenovirus** is an example of DNA virus that enters the host nucleus but remains separated from the host genome and at the same time use host cell machinery for

its replication. On the other hand **influenza** is a RNA virus that carries its own enzyme to replicate its genome while the viral proteins are synthesized by using the host cell machinery. Human immunodeficiency virus (HIV) is a **retrovirus**; it contains RNA as a genetic material but it converts into DNA after entering the host cell by an enzyme called **reverse transcriptase**. It also contains enzymes in its virion namely, **integrase** and viral **protease** which helps HIV during maturation process inside the infected cells. Outer surface of HIV virion contains two surface glycoproteins called as **gp120** and **gp41** which helps in the attachment of virus to the cell surface.

Figure 2.1. Schematic diagram of HIV



Lecture 3: Virus Shapes

Early study with tobacco mosaic virus (TMV) strongly suggested that viruses were composed of repeating subunits of protein which was later supported by crystallization of TMV. A major advancement in determining the morphology of virus was the development of negative stain **electron microscopy**. Another modification of classical electron microscopy is **cryo-electron microscopy** where the virus containing samples were rapidly frozen and examined at a very low temperature; this allows us to preserve the native structure of the viruses.

A **virion** is a complete virus particle that is surrounded by the capsid protein and encapsidates the viral genome (DNA or RNA). Sometime structure without nucleic acid can be visible under the electron microscope those structures are called as **empty capsids**. In some of the viruses like paramyxoviruses the nucleic acid is surrounded by the capsid proteins and the composite structures are referred as **nucleocapsid**. Some of the viruses contain the lipid **envelope** which surrounds the nucleocapsids. The envelopes are derived from the **host cell membrane** during the budding process. As the envelopes are derived from host cell membrane they contain many of the surface proteins present in the host cells.

There are two kinds of symmetry found among the viruses: **icosahedral** and **helical**. In theory the icosahedral symmetry may sometime referred as **spherical** based on the external morphology. **Icosahedral** symmetry has **12 vertices**, **30 edges**, and **20 faces**. They also have two, three, or five fold symmetry based on the rotation through axes passing through their edges, faces, and vertices respectively (Figure 3.1). The viruses of this kind look **spherical** in shape. In **helical** symmetry the genomic RNA forms a spiral within the core of the nucleocapsids (Figure 3.2). The viruses of this kind look **rodlike** or **filamentous**. The viruses which contain large DNA genomes are more complex in structure, for example- poxviruses and herpesviruses.

Figure 3.1. An icosahedral virion structure showing two, three, and fivefold symmetry

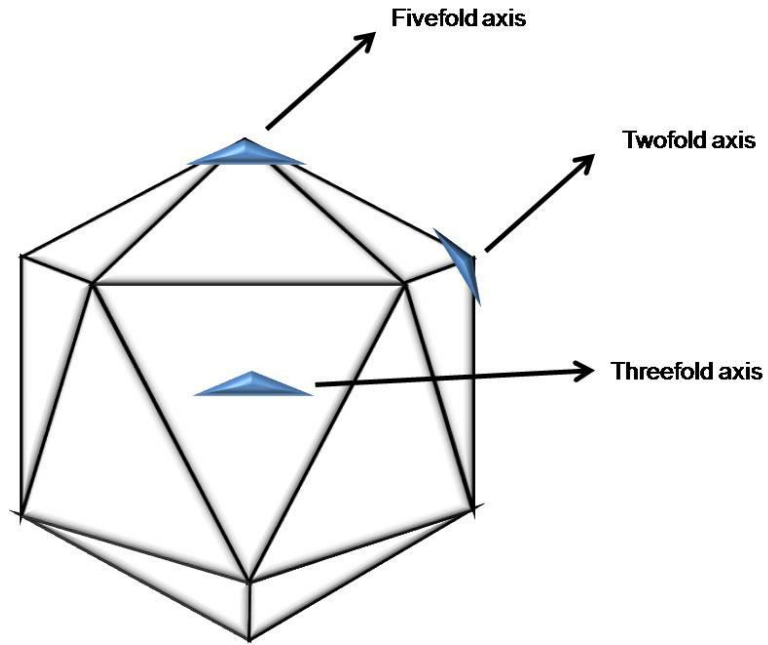


Figure 3.2. Virus structure with helical symmetry.

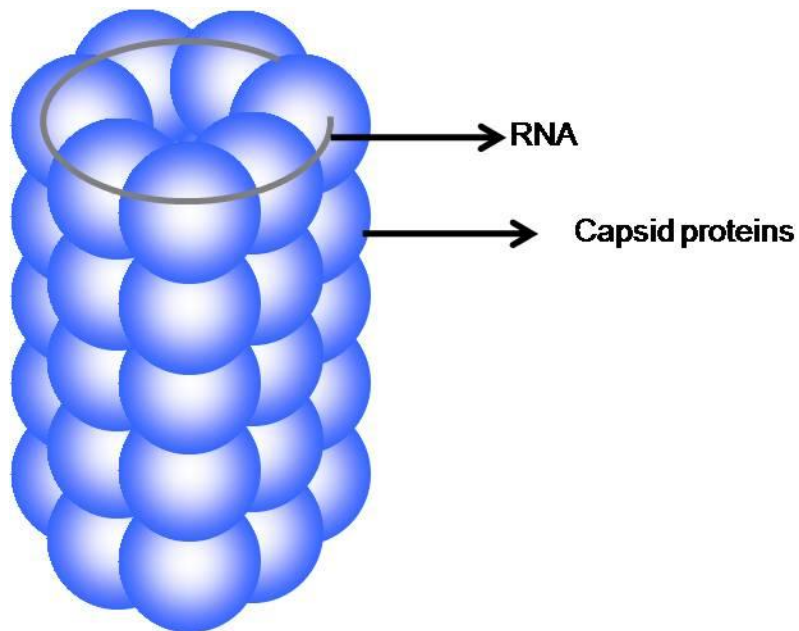


Table 3.1. Shape of viruses belonging to different families

Family	Shape
Poxviridae	Pleomorphic
Iridoviridae	Icosahedral
Asfarviridae	Spherical
Herpesviridae	Icosahedral
Adenoviridae	Icosahedral
Polyomaviridae	Icosahedral
Papillomaviridae	Icosahedral
Hepadnaviridae	Spherical
Circoviridae	Icosahedral
Parvoviridae	Icosahedral
Retroviridae	Spherical
Reoviridae	Icosahedral
Birnaviridae	Icosahedral
Paramyxoviridae	Pleomorphic
Rhabdoviridae	Bullet shaped
Filoviridae	Filamentous
Bornaviridae	Spherical
Orthomyxoviridae	Pleomorphic
Bunyaviridae	Spherical
Arenaviridae	Spherical
Coronaviridae	Spherical
Arteriviridae	Spherical
Picornaviridae	Icosahedral
Caliciviridae	Icosahedral
Astroviridae	Icosahedral
Togaviridae	Spherical
Flaviviridae	Spherical

Lecture 4: Virus Size

Viruses are generally much smaller than the bacteria and its average size varies from 25-300 nm in diameter. They are visible under electron microscope and only the largest and complex viruses are seen under light microscope with high resolution. Among all, the smallest viruses belong to the families *Circoviridae*, *Parvoviridae* and *Picornaviridae* which measure about 20 - 30 nm in diameter while the largest one belongs to *Poxviridae* that measures around 250-300 nm in diameter. Recently, scientists isolated a new form of virus that infects amoeba and grouped it under a separate family *Mimiviridae*. The members of the family *Mimiviridae* range from 400-800 nm in diameter.

On an average a bacterial cell is about 1400 nm in diameter while an average epithelial cell is about 20,000 nm. Considering both viruses and bacteria to be nearly spherical a bacterial cell has a volume about 30,000 times greater than a virus while an epithelial cell is about 60 million times larger.

Table 4.1. Size of viruses belonging to different families

Family	Size (nm)
Poxviridae	300
Iridoviridae	135-300
Asfarviridae	170-220
Herpesviridae	150
Adenoviridae	80-100
Polyomaviridae	40-50
Papillomaviridae	55
Hepadnaviridae	50
Circoviridae	12-27
Parvoviridae	15-25
Retroviridae	80-100
Reoviridae	60-80
Birnaviridae	60
Paramyxoviridae	150-250
Rhabdoviridae	100
Filoviridae	80
Bornaviridae	80-100
Orthomyxoviridae	80-120
Bunyaviridae	80-120
Arenaviridae	50-280
Coronaviridae	120-150
Arteriviridae	60-70
Picornaviridae	30
Caliciviridae	30-40
Astroviridae	30
Togaviridae	70
Flaviviridae	40-60

Lecture 5: Components of genomes

In general the viruses are made up of nucleic acids (genome), proteins (capsid), and lipids (envelope). Viral genomes can be **either DNA or RNA**, when once inside a host cell it directs synthesis of new viral proteins, and replication of new viral genomes. Capsid is a protein covering that surrounds and protects the viral genome. It is made up of many small subunits called as **capsomeres** which determine the shape of the virus. The arrangement and composition of the capsomeres varies among the virus families. Envelopes are the lipid bilayer membranes that are derived from the host cell membrane when virus “buds” out from the plasma membrane or passes through a membrane-bound organelle (such as the Golgi body or endoplasmic reticulum). The envelope contains sometimes glycoprotein (protein with carbohydrate) in the form of spikes which helps them in the attachment during the time of infection to the host cell surface (gp120 in HIV). In non-enveloped viruses, grooves present in the capsid and specific capsid proteins may bind to the cell surface receptor.

The most important and characteristic feature of a living organism is replication of its genetic information. The mechanism of genome replication is done with greater economy and simplicity among different viruses. Different families of viruses have their genome made of either double stranded (ds) DNA or single stranded (ss) DNA or RNA. The viruses that contain RNA genome may have either positive, negative, or mixed (ambisense) polarity. In addition, they either have single or multiple segments in their genome with linear or circular topology. Each of the above parameters have their consequences for the pathways of viral genome replication, viral gene expression, and virion assembly.

Among the families of viruses that infect animals and human, those containing RNA genome outnumber those containing DNA genome. This disparity is even more in case of plant viruses (no double stranded DNA virus that infect plant is known).

5.1. Viruses encode enzymes and follow unique pathways:

Almost all viruses encode unique proteins and enzymes, moreover they follow unique pathways to transfer their genetic information. This phenomenon is more pronounced in case of RNA viruses, they either use RNA dependent RNA polymerase or in case of retrovirus (HIV) RNA dependent DNA polymerase to complete their replication cycle. Both of these processes require unique enzymes that are encoded by the virus following infection to the host cells and are generally absent elsewhere.

The RNA dependent RNA polymerase and reverse transcriptase have minimal proofreading ability, as a result their error rate is very high (1 in 10,000) as compared to the DNA replication. This means that an RNA virus particle will contain 1 or more mutation from its parental wild type virus. Presence of many different subspecies of virus particle in a population is also called as **quasispecies** nature of RNA viruses. The error prone activity of RNA virus polymerase restricts the upper size limit of the genome

above which they cannot survive. As a result of this phenomenon most of the RNA virus have their genome size in the range of 5-15kb (coronavirus 30kb). The opposite is true in case of DNA viruses where proofreading and error repair activity ensures accurate replication of the viral DNA as big as 800 kb. The fact that DNA is more stable chemically than RNA likely explains us why all thermophilic hosts contain viruses that have dsDNA as their genetic material.

Table 5.1. Nature of genome of viruses belonging to different families

Family	Nature of Genome
Poxviridae	dsDNA
Iridoviridae	dsDNA
Asfarviridae	dsDNA
Herpesviridae	dsDNA
Adenoviridae	dsDNA
Polyomaviridae	dsDNA
Papillomaviridae	dsDNA
Hepadnaviridae	dsDNA-RT
Circoviridae	ssDNA
Parvoviridae	ssDNA
Retroviridae	ssRNA-RT
Reoviridae	dsRNA
Birnaviridae	dsRNA
Paramyxoviridae	NssRNA
Rhabdoviridae	NssRNA
Filoviridae	NssRNA
Bornaviridae	NssRNA
Orthomyxoviridae	NssRNA
Bunyaviridae	NssRNA
Arenaviridae	NssRNA
Coronaviridae	ssRNA
Arteriviridae	ssRNA
Picornaviridae	ssRNA
Caliciviridae	ssRNA
Astroviridae	ssRNA
Togaviridae	ssRNA
Flaviviridae	ssRNA

dsDNA= double stranded DNA

ssDNA= single stranded DNA

dsRNA= double stranded RNA

ssRNA= single stranded RNA

NssRNA= single stranded RNA with negative polarity

Figure 5.1. Diversity among the viruses belonging to different groups



Lecture 6: Isolation and purification of viruses and components

6.1. Virus Isolation

Viruses are obligate intracellular parasites that require living cells in order to replicate. Generally cell culture, embryonated eggs and small laboratory animals are used for the isolation of viruses. Embryonated eggs are very useful for the isolation of influenza and paramyxoviruses. Although laboratory animals are useful in isolating different kind of viruses, cell culture is still a preferred way for virus isolation in many of the laboratories.

For primary cell cultures, tissue fragments are first dissociated into small pieces with the help of scissors and addition of trypsin. The cell suspension is then washed couple of times with minimal essential media and seeded into a flat-bottomed glass or plastic container bottle after resuspending it with a suitable liquid medium and fetal calf serum. The cells are kept in incubator at 37⁰C for 24 to 48hrs depending on the cell type. This allows the cells to attach the surface of the container and its division following the normal cell cycle.

Cell cultures are generally of 3 types:-

1. **Primary culture** – These are prepared directly from animal or human tissues and can be subcultured only once or twice e.g. chicken embryo fibroblast.
2. **Diploid cell culture** – They are derived from neonatal tissues and can be subcultured 5-10 times. e.g. human diploid fibroblasts cells.
3. **Continuous cells** – They are derived from tumor tissues and can be subcultured more than 10 times. e.g. Vero, Hep2, Hela.

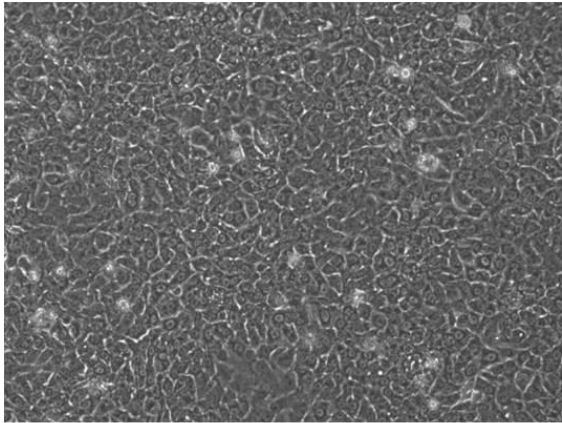
Specimens containing virus should be transported to the laboratory as soon as possible upon being taken. Oral or cloacal swabs should be collected in vials containing virus transport medium. Body fluids and tissues should be collected in a sterile container and sealed properly. If possible all the samples should be maintained and transported in a cold condition for higher recovery rates.

Upon receipt, the samples should be inoculated into cell culture depending on the history and symptoms of the disease. The infected cell culture flask should be observed every day for any presence of cytopathic effect (CPE). Certain kind of samples, such as faeces and urine are toxic to the cell cultures and may produce a CPE-like effect. When virus specific CPE is evident, it is advised to passage the infected culture fluid into a fresh cell culture. For cell-associated viruses such as cytomegaloviruses, it is required to trypsinize and passage the intact infected cells. Viruses such as adenovirus can be subcultured after couple of time freezing and thawing of the infected cells.

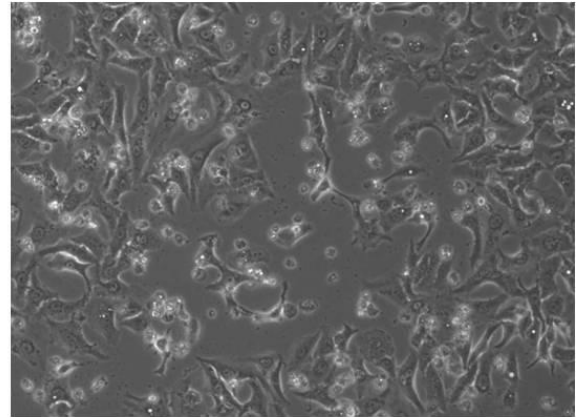
Susceptible cell lines:

Influenza virus- MDCK cells, Vero cells.
Paramyxoviruses- DF-1 cells, Vero cells.
Adenoviruses- HEK cells, HuH7 cells.
Herpesviruses- LMH cells.
Respiratory syncytia virus- Hep2 cells, Vero cells.

Figure 6.1. Virus induced CPE in cell culture



Normal human epithelial cells



Adenovirus infected human epithelial cells

6.2. Purification of virus and components:

6.2.1. Ultracentrifugation:

The viruses are usually purified with the help of ultracentrifugation. The machine is capable of rotating the samples at 20,000-100,000 rpm under the density gradient of CsCl₂ or sucrose. Density at which viruses neither sink nor float when suspended in a density gradient is called as **buoyant density**. The rate at which viral particles sediment under a defined gravitational force is called as **sedimentation coefficient**. The basic unit is the Svedberg (S) which is 10⁻¹³ sec. The S value of a virus is used to estimate its molecular weight.

Types of sedimentation medium:

A. Sucrose cushions or gradient - A fixed concentration or a linear gradient of sucrose is used. Increasing the density and viscosity of the medium decreases the rate at which virus sediments through them. In general a "cushion" of sucrose is prepared at the bottom of the centrifuge tube and the sample containing virus is overlaid over the cushion. Since most viruses have greater densities than sucrose, separation is based on S values. This

method can be used to separate molecules with relatively close S values. Sometime glycerol is also used in place of sucrose.

B. CsCl₂ gradient centrifugation - A linear gradient of CsCl₂ in buffer is prepared in the ultracentrifuge tube. As the concentration of the CsCl₂ is increased the density of the medium increases in the tube so that density is low at the top and high at the bottom. Viral particle centrifuged through this medium will form a band at a position equal to their buoyant density. These are useful to separate viruses of different densities. Limitation of this method is that CsCl₂ can permanently inactivate some viruses.

6.2.2. Other techniques for separation:

Viruses can also be separated by electrophoresis and column chromatography but these are not the preferred way to separate virus while sometimes they are used to separate viral nucleic acids or proteins. Both the methods separate the virus on the basis of charge and/or size. Virus contains a variety of charged macromolecule on its surface which contributes to its electrophoretic mobility or ion-exchange characteristics. Viruses are sometimes ligated with the charged group to be separated by ion exchange chromatography. Molecular sieve chromatography can also be used to purify the viruses where large pores are formed with the help of special agarose through which virus particles can enter.

6.3. Purity of viruses:

Many methods are used to assess the purity of virus. The ratio of UV absorption at 260 and 280 nm during a spectrophotometric analysis (260/280) is a characteristic feature to measure the purity of a virus sample and is dependent on the amount of nucleic acid and protein present in the virion. Serological methods such as enzyme-linked immunosorbent assay (ELISA), radioimmuno precipitation assay (RIPA), western blot, virus neutralization test (VNT), and complement fixation are also used to check the purity of a virus sample. These methods require antibodies specific to viral proteins that may be monoclonal (single type of antibody specific to a single viral protein) or polyclonal (several different antibodies that may recognize several viral proteins or epitopes). Plaque assay is also performed in order to isolate the single colony from a pool of quasispecies viruses.

Figure 6.2. A general approach for purifying a virus from tissue culture cells

