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 37^0C 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.

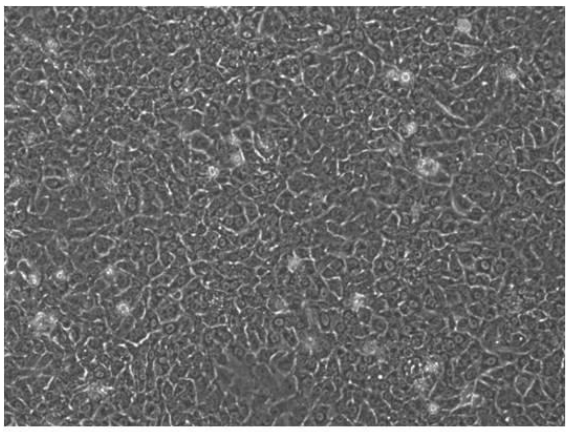
Specimens containing virus should be transported to the laboratory as soon as possible upon being taken. Oral or cloacal swabs should be collected in vials containing virus transport medium. Body fluids and tissues should be collected in a sterile container and sealed properly. If possible all the samples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

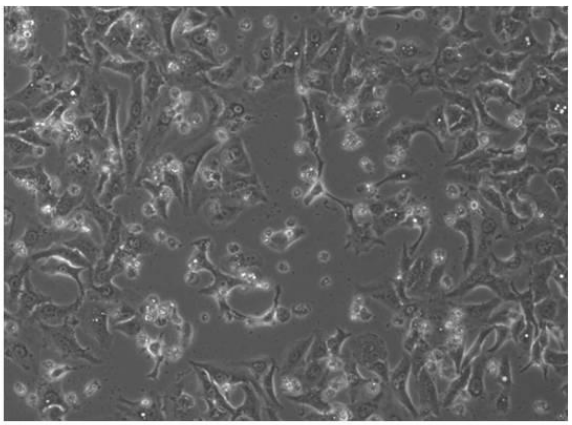
Susceptible cell lines:

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

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 puirity 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