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METHODS OF CULTIVATION OF VIRUSES

As viruses are intracellular obligatory parasites, they always need living cells for their growth. They cannot be grown on any artificial media. There are three methods employed for the cultivation of animal viruses

1. Animal inoculation
2. Embryonated eggs or chick embryo method.
3. Tissue culture or cell culture.

1. **Animal Inoculation –** Susceptible experimental animals like Mice, Monkey, Rabbits, Guinea Pigs etc. are used for the cultivation of viruses.

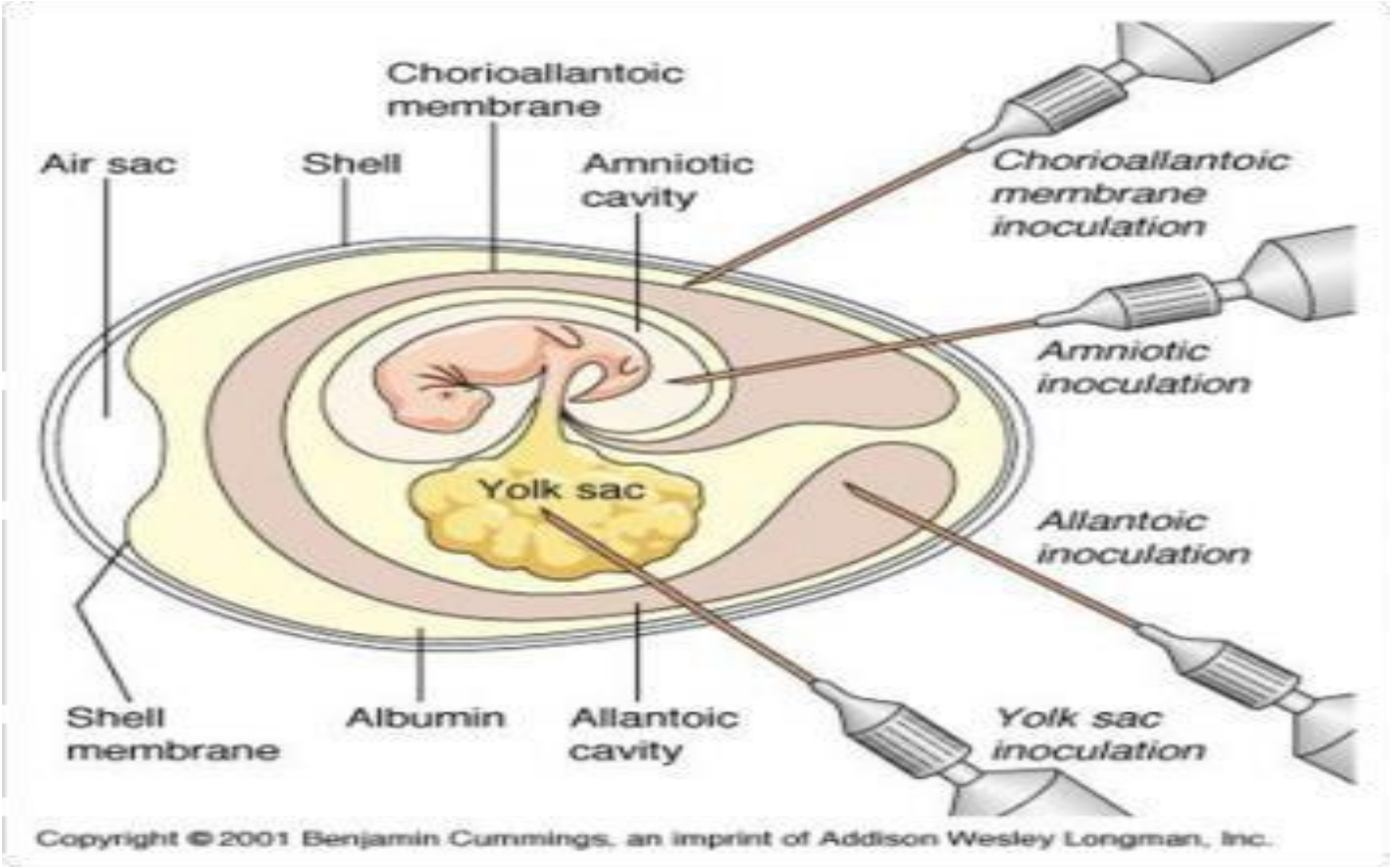
Virus sample to be cultivated should injected into the experimental animal. It is important to select specific host animal for particular viruses. Route of inoculation of viral sample in the host cell also play important role in cultivation of viruses. Other factors such as **age and immunity of host animal also affect the growth of viruses in the host.** Eg. Mice are the most widely employed animals in virology. It can be inoculated by routes like intracerebral, subcutaneous, intraperitoneal or intranasal. The growth of the virus in inoculated animals may be indicated by death, disease or visible lesion. Disadvantages of animal inoculation are that immunity may interfere with viral growth.

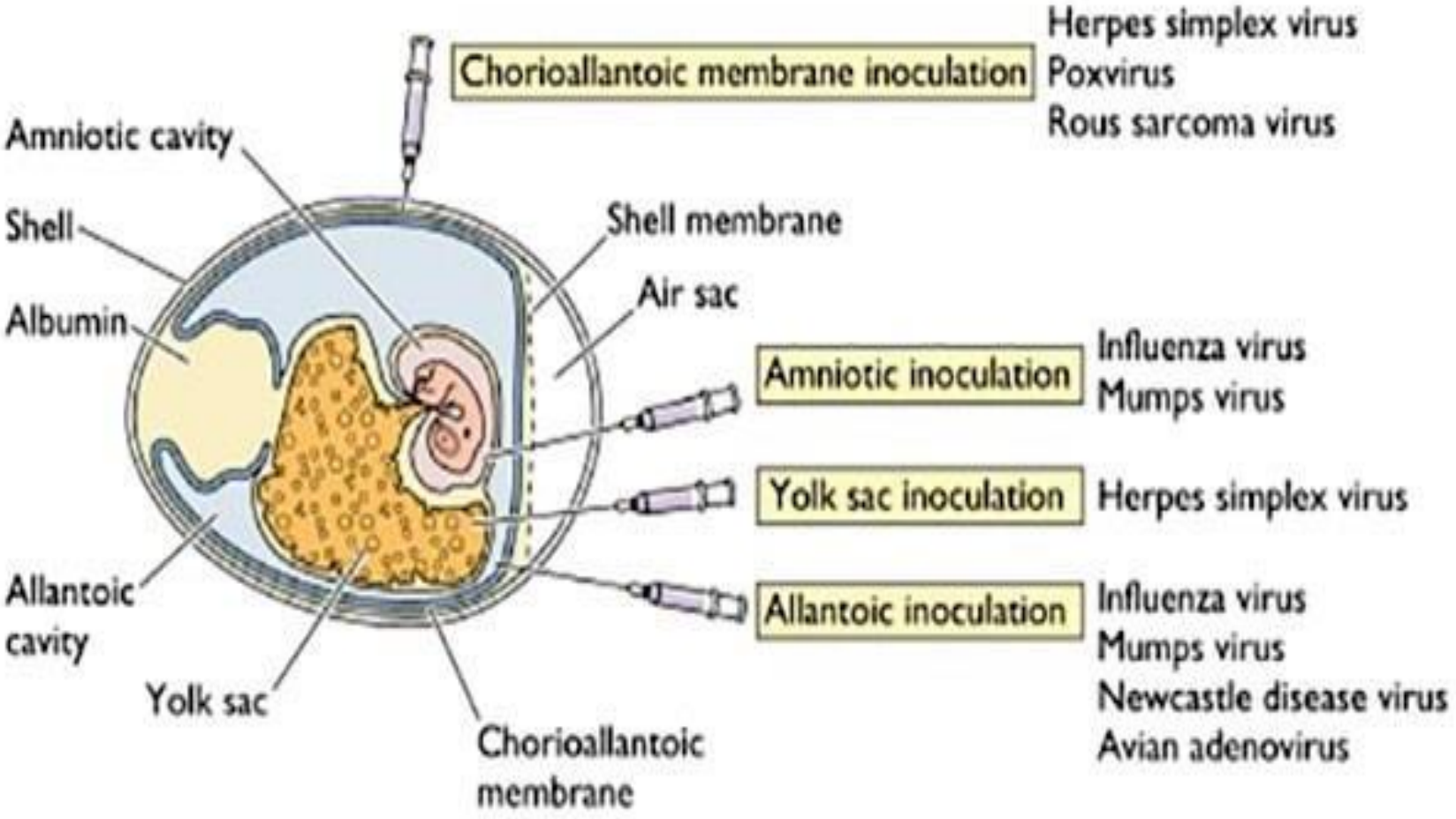
2. Embryonate Eggs or Chick embryo method

Good pasture (1931) was the first who used hen's embryonated egg for the cultivation of viruses. Embryonated egg provides several sites for the cultivation of viruses. Viz 1. Chorio- allantoic membrane 2. Allantoic cavity 3. Amniotic cavity 4. Yolk sac 5. Embryo

Different site is used for growth of different viruses. Eg. Chorio-allantoic membrance is used for the cultivation of pox virus. Allantoic cavity is employed for the Influenza virus. There are several advantages, chick embryos are packed in their shells and have natural resistant against bacterial contamination. Chick embryo method is cheaper and easy to handle.

Fig :Chick embryo method for cultivation of animal viruses.




Chorioallantoic membrane inoculation
Herpes simplex virus
Poxvirus
Rous sarcoma virus
Amniotic cavity
Shell
Albumin
Shell membrane
Air sac
Amniotic inoculation
Influenza virus
Mumps virus
Yolk sac inoculation
Herpes simplex virus
Allantoic cavity
Allantoic inoculation
Influenza virus
Mumps virus
Newcastle disease virus
Avian adenovirus
Yolk sac
Chorioallantoic membrane

3. Tissue Culture

Steinhardt and colleagues (1913), was the first who used bits of tissue or organ for the cultivation of viruses. Now advance techniques are develop in Tissue culture.

Three types of tissue cultures are available.

1. **Organ culture:-** Small bits of organs are used for the cultivation of virus.
2. **Explants culture:-** Fragments of minced tissue can be grown as 'explants' embedded in plasma clots.
3. **Cell culture:-** This is most common method for viral cultivation and growth of viruses. Different types of cell cultures are used for different viruses. General method is as follows :-

1. Tissue like Monkey Kidney, Rabbit Kidney is taken and treated with proteolytic enzymes such as Trypsin and by mechanical shaking, tissues are dissociated into the component cells. Trypsin, the protecolytic enzyme digest the binding material that binds the cells together in a tissue and results into free cells. **He La cells** (i.e. human cells from cervical cancer region) are also commonly used cell system for the

2. After treatment with trypsin, cells are washed, counted and suspended in the growth medium.
3. Growth medium constitutes all those essential elements required for the growth of cell viz. essential amino acids, vitamins, salts, glucose, bicarbonates (buffer) with atmosphere containing 5% CO_2 and supplemented with 5% calf serum.
4. Antibiotics are added into the growth medium to prevent bacterial contaminants. Some indicators like phenol red, neutral red etc. are added into the growth medium, Change in indicator colour in growth medium, indicates the growth of virus in cell culture.

In such growth medium cells divides and multiply. Then these cells are dispensed in bottle or petri plates. On incubation, cells divides to form a confluent **monolayer sheet** of cells occur within a week. Various types of cell cultures used for virus cultivation are-

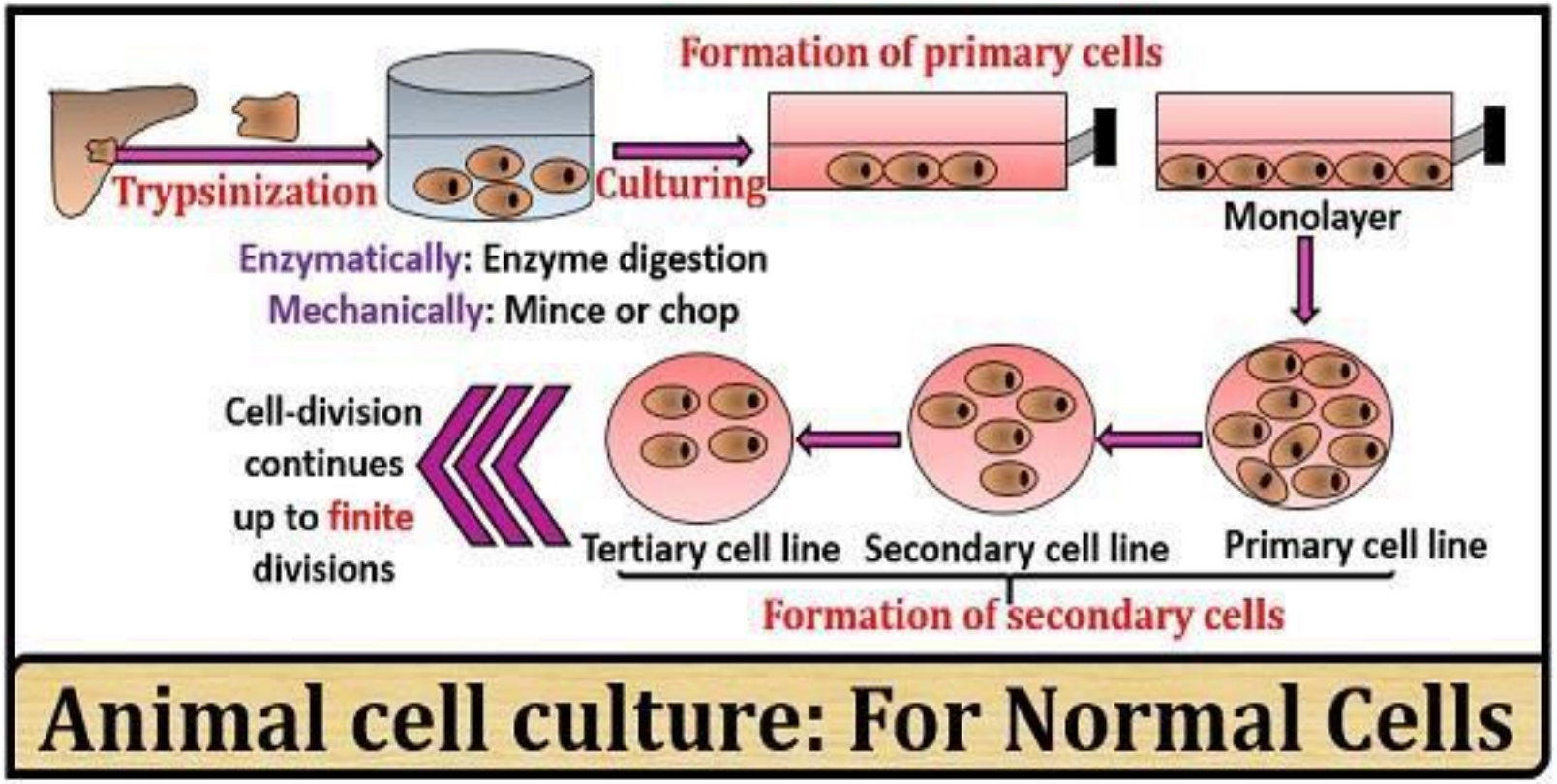
1. Primary cell culture
2. Diploid cell strain
3. Continuous cell lines.

1. Primary cell culture:- Derived form normal cells (eg. Monkey kidney tissue). The fresh monolayer of the cell is referred as Primary culture. They are capable of only limited growth in culture and cannot e maintained in serial culture. Primary cell cultures are used during cultivation of viruses for vaccine production.

2. Diploid cell strains:- These are cells of a single type that retain the original diploid chromosome number and karyotype (appearance of two sets of chromosome) during serial sub cultivation for a limited number of times. They are also employed for the production of viral vaccine.

3. Continuous cell lines:- These are the cells of single type, usually derived from cancer cells, that are capable of continuous serial cultivation indefinitely. Eg. Human cancer cells Viz. **He La, Hep –** 2 and **KB lines** have been used for many years. These cell lines are stored in the cold (-70$_0$c) for use when necessary. The karyotype of these cells is

aneuploid (a variable multiple of the haploid chromosome number.)Continuous cell lines are now widely useful in cultivating many types of viruses.


Formation of primary cells
Trypsinization
Culturing
Monolayer
Enzymatically: Enzyme digestion
Mechanically: Mince or chop
Cell-division continues up to finite divisions
Tertiary cell line
Secondary cell line
Primary cell line
Formation of secondary cells
Animal cell culture: For Normal Cells

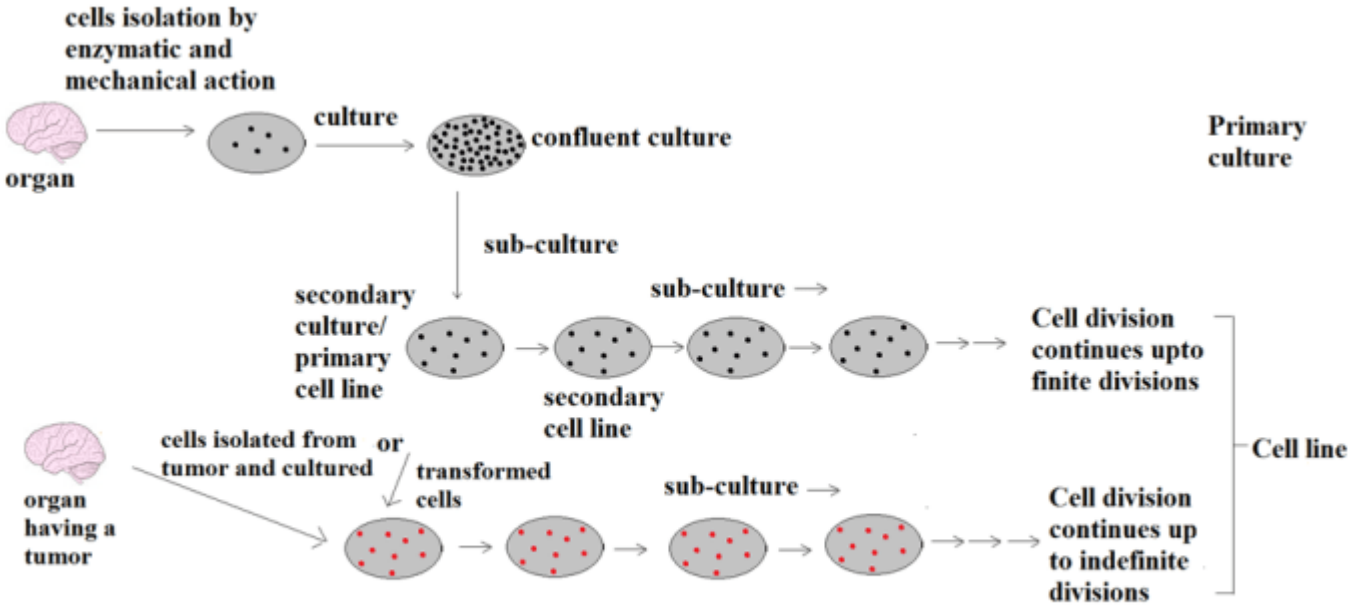
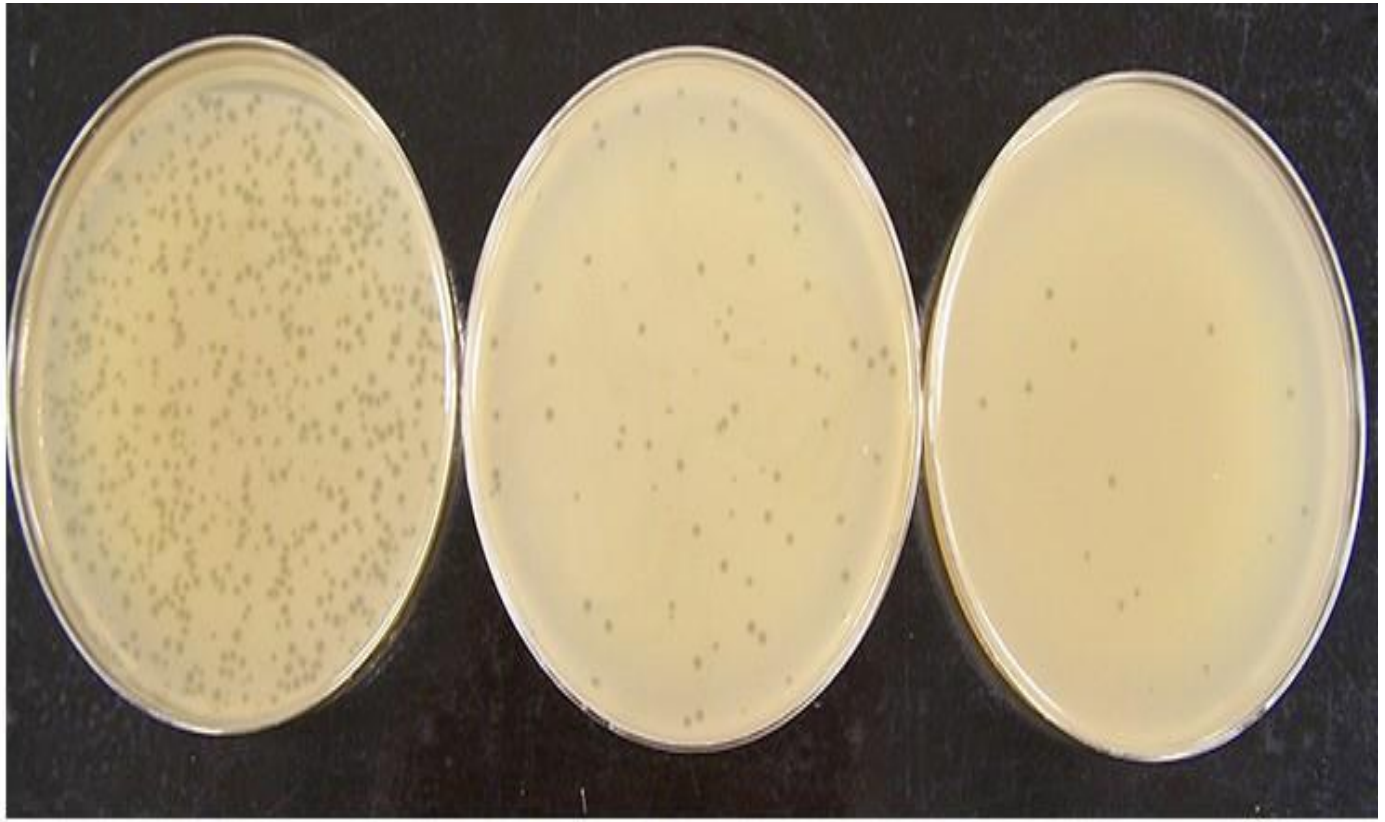


fig: animal cell culture

Plaques formation indicates cultivation of viruses on cell culture

(a)



(b)

DETECTION OF VIRAL GROWTH

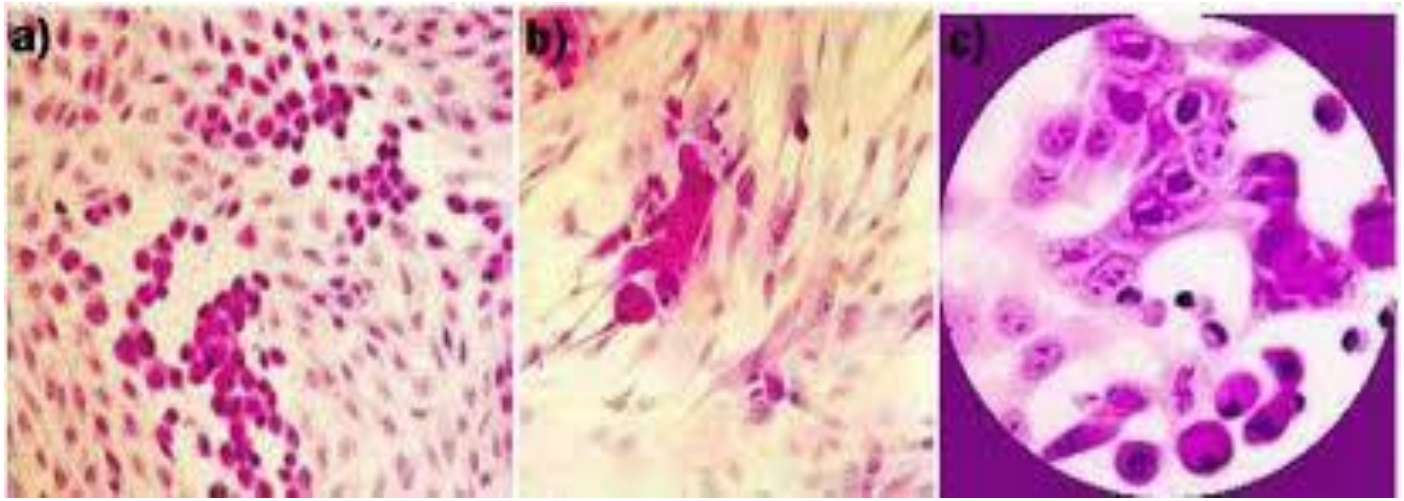
Viral growth in cell culture can be detected by the following methods

a. Cytopathic effect (CPE)

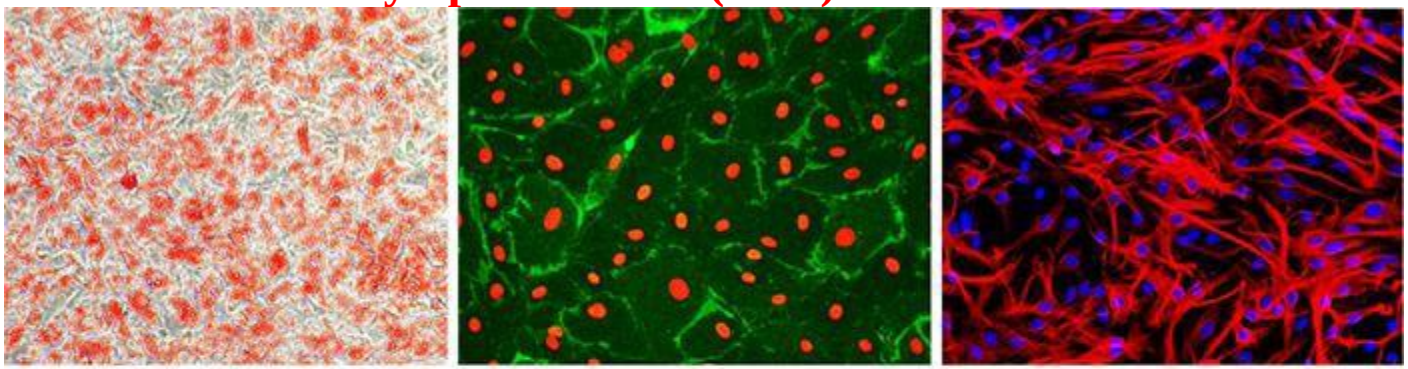
Due to the viral growth, morphology of cultured cell change, these changes can be readily observed under microscope. These morphological changers in cell culture is called **Cytopathic effect (CPE)** and viruses causing CPE are called **cytopathogenic virus.'** Eg. Adeno virus cause large granular clumps in cell culture.

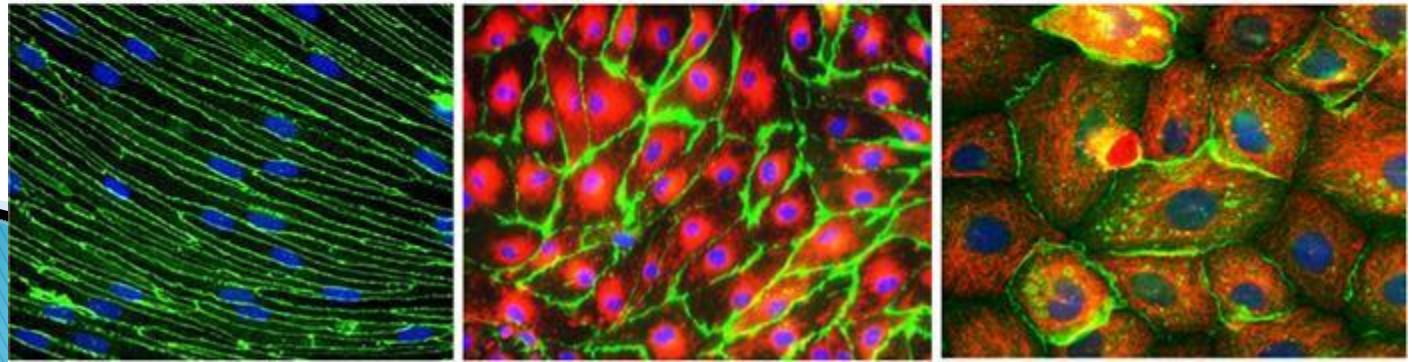
b. Fluorescence Antibody Technique (FAT):- In this technique, cell from virus infected cultures can be stained by fluorescent conjugated antigen. Fluorescent dye such as fluorescein isothioacynate and rhodamine are generally used to tag with antibodies. FAT is very useful in testing for rabies virus in clinical specimen within few hours with 100% accuracy.

c. Haemagglutination :- Haemagglutination is the phenomenon of clumping of RBCs. Viruses such as mumps, measals and influenza can able to agglutinate the RBCs. their presence can be indicated by addition of guinea pig erythrocytes to the culture. If the viruses are cultivated in the cell culture, the erythrocytes will adsorb onto the cell surface also called 'haemadsorption.'



Cytopathic effect (CPE) and FAT





Heamagglutination method for virus detection