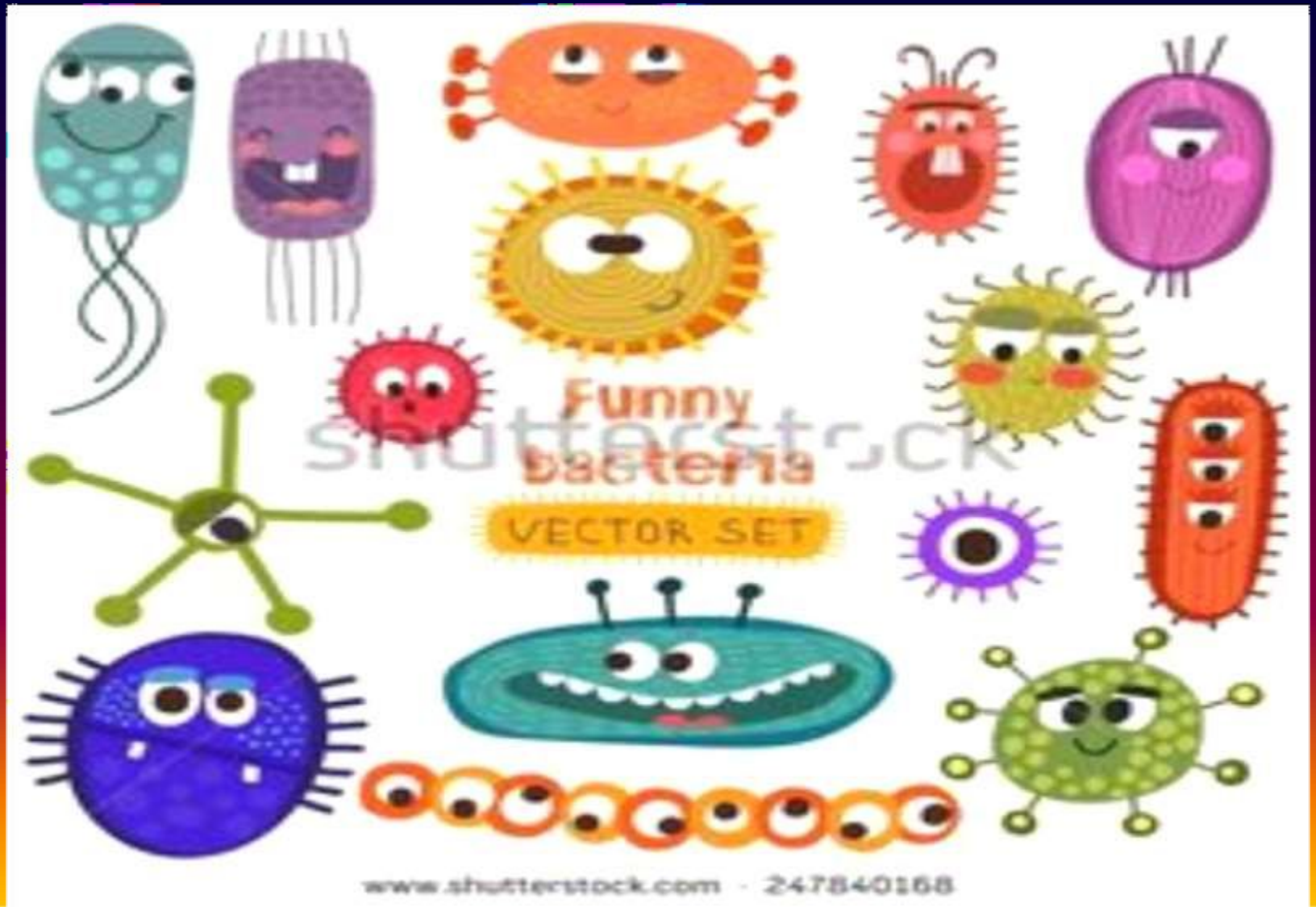
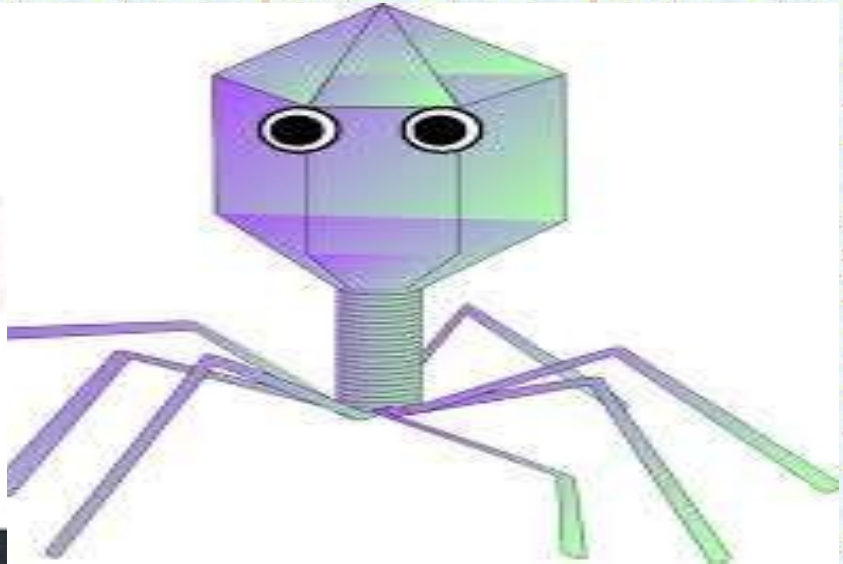
CULTIVATION OF VIRUSES



VIRUSES

- Viruses are obligate intracellular parasites.
- They multiply only inside the living host cells. Animals, plants, humans, bacteria, fungus, protozoa and algae.



- Viruses are host specific and grow only in selective hosts. Virologists use only a suitable host system for cultivation of a virus.
- Viruses cannot grow in artificial media . They must require living cells to support their replication.
- There is no universal cell that will support all viruses.

Main purpose of virus cultivation

- **To isolate and identify viruses in clinical samples.**
- **To prepare viruses for vaccine production.**
- **To do research on viral structure, replication, genetics and effects on host cells.**

Development in cultivation of viruses

- **Reed and colleagues (1900)** used human volunteers for their pioneering work on yellow fever . Due to the serious risk involved, human volunteers are used only when no other method is available and when virus is relatively harmless.

- Monkeys were used for the isolation of the poliovirus by **Landsteiner and Popper (1909).** However, due to their cost and risk to handlers, monkeys find only limited applications in virology.

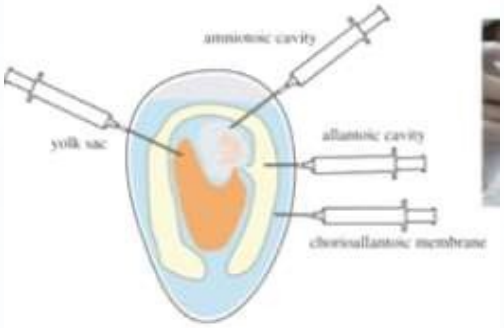
- Theuse of white mice, pioneered by **Theiler (1903)** extended the scope of animal inoculation greatly.

- **Good Pasture** in **1931** first used the embryonated hen's egg for the cultivation of virus and this method is further developed by **Burnet.**
- The first application of tissue culture in virology was by **Steinhardt and colleagues (1913),** who maintained the vaccinia virus in fragments in rabbit cornea.
- **Maitland (1928)** used chopped tissue in nutrient media for cultivation of vaccinia viruses.
- The turning point which made tissue culture the most important method for cultivation of virus.

METHODS FOR CULTIVATION OF VIR

Inoculation of virus into animals.

Inoculation of virus into embryonated eggs.



Tissue culture.

1). Inoculation of Virus in Animals

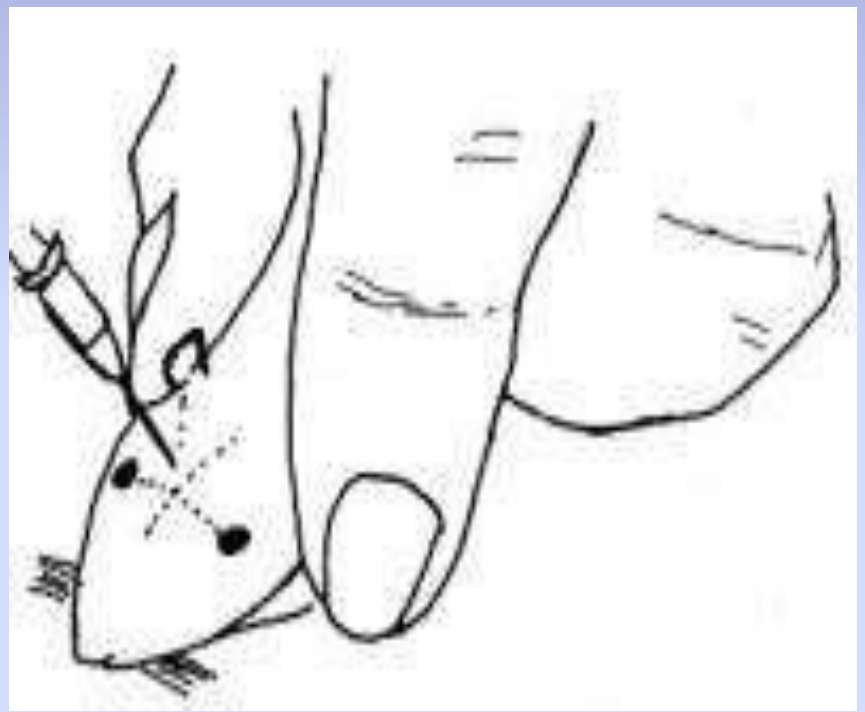
- Viruses which are not cultivated in embryonated egg and tissue culture are cultivated in laboratory animals. e.g: mice, guinea pig are used.
- The selected animals should be healthy and free from any communicable diseases.
- Suckling mice (less than 48 hours old) are most commonly used.

Different ways of inoculation in mice are:

1) **Intracerebral.**
2) **Subcutaneous.**
3) **Intraperitoneal.**
4) **Intranasal.**

1). Intracerebral

- It occurring or within introduced or administered into the cerebrum. It means when a diseased blood vessel within the brain bursts allowing blood to leak inside the brain.



2). Subcutaneous

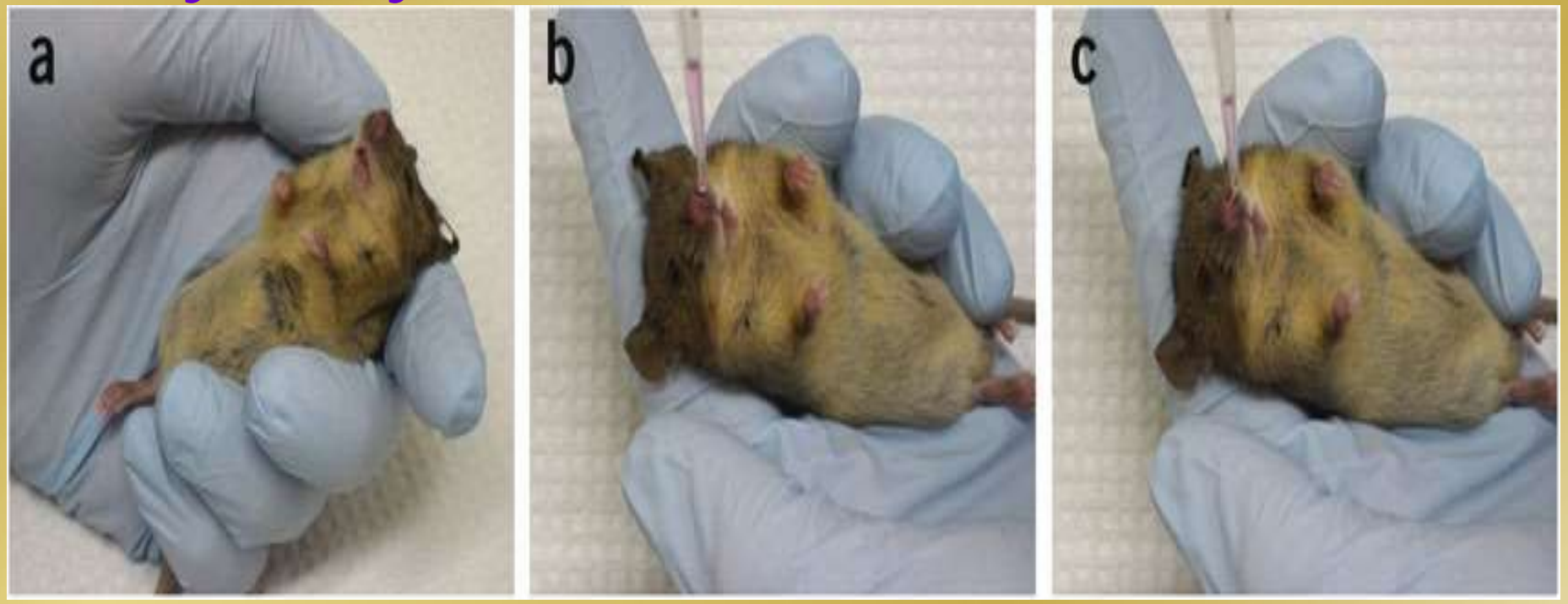
- A subcutaneous injection is an injection in which a needle is inserted just under the skin.

3). Intraperitoneal

- **Intraperitonium injection is the injection of a substance into the peritonium (body cavity).**

4). Intranasal

- ✔ It lying within or administered by way of the nasal structure.



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.

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.

2). Inoculation of virus into embryonated egg

The process of cultivation of viruses in embryonated eggs depend upon the type of egg being used.

Egg provide a suitable means for :

i. The primary isolation and identification of viruses.

ii. The production of vaccines.

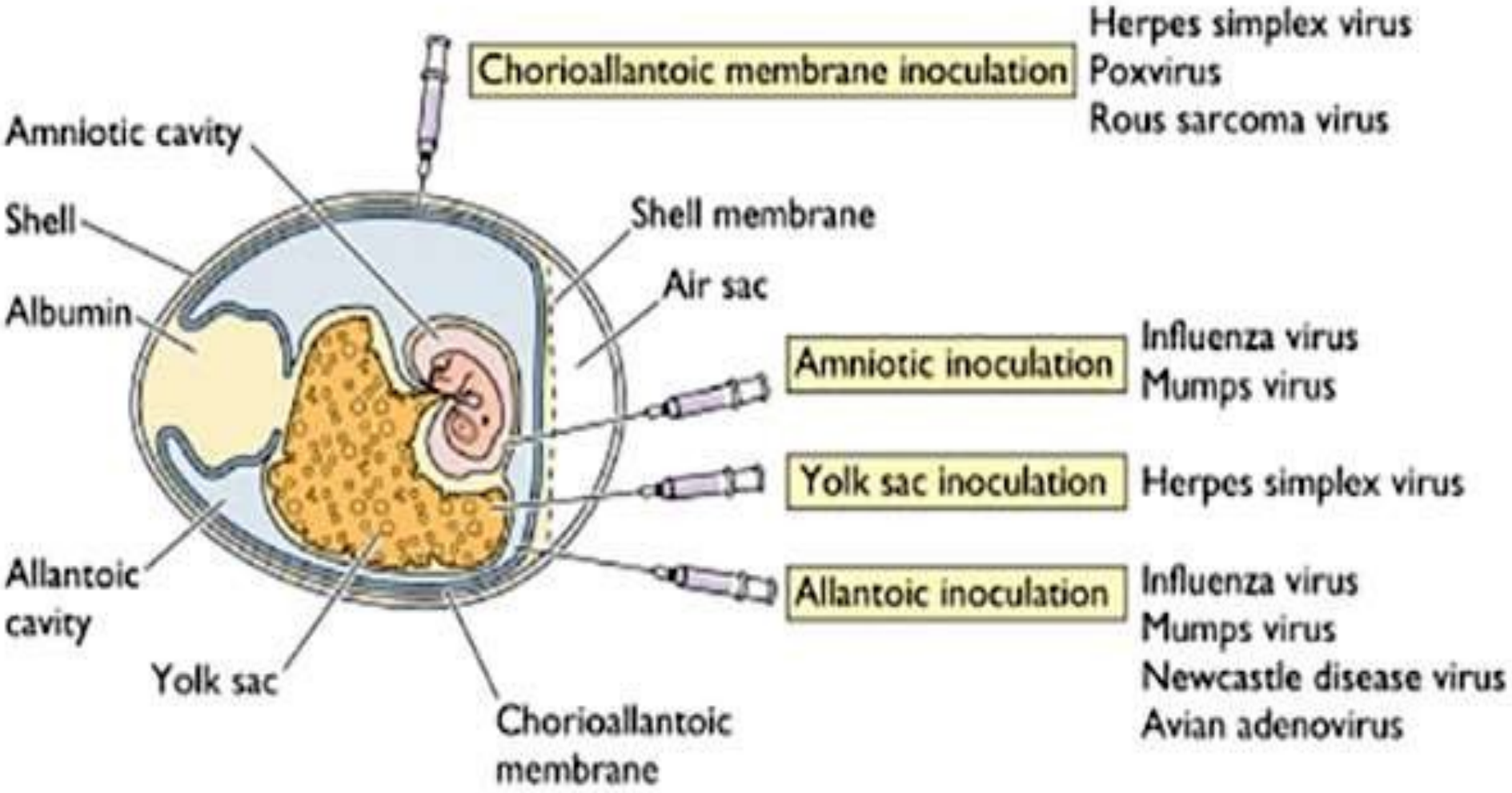
iii. The maintaince of stock culture.

Viruses are inoculated into chick embryo of 7-12 days old.

For inoculation , eggs are first prepared for cultivation , the sheel surface are first prepared for cultivation , the shell surface is first disinfected with iodine and penetrated with a small sterile drill.

After incubation , the egg is broken and virus is isolated from tissue of egg.

Inoculation of virus into embryonated eggs



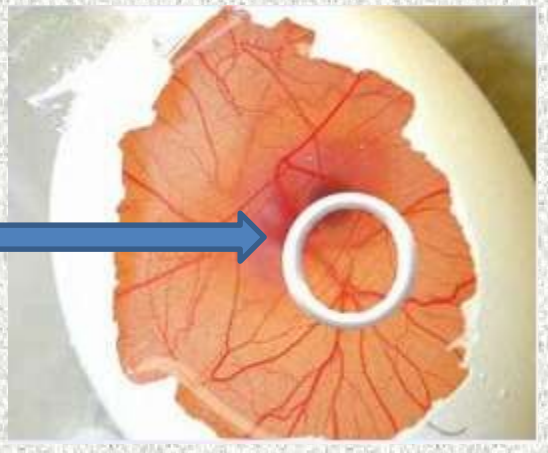
- **Virus growth and multiplication in the egg embryo is indicated by the death of the embryo , by embryo cell damage, or by the formation of typical lesions on the egg membrane.**
- **Viruses can be cultivated in various parts of egg like :**

1). Chorioallantoic membrane (CAM)

2). Allantoic cavity

3) Amniotic sac

4) Yolk sac Pock

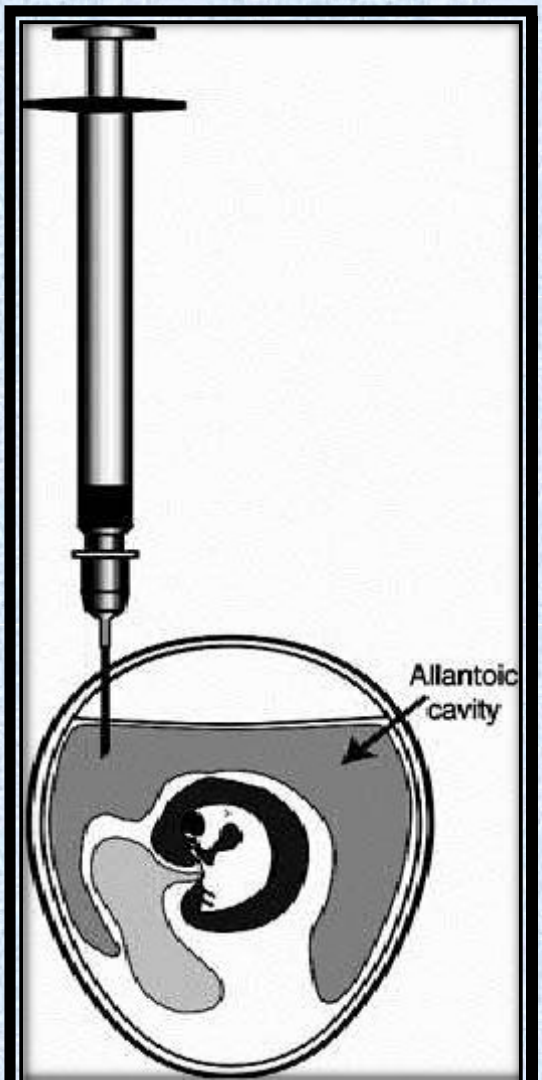


1). Chorioallantoic membrane (CAM)

- Inoculation is mainly for growing poxvirus.
- After incubation and visible lesions called pocks are observed , which is grey white area in transparent CAM.
- Herpes simplex virus is also grown.
- Single virus gives single pocks.
- This method is suitable for plaque studies.

2). Allantoic cavity:

- Inoculation is mainly done for production of vaccine of influenza virus , yellow fever , rabies.
- Most of viruses can be isolated using this method.
- Allantoic inoculation is a quick and easy method that yields large amounts (8-15ml) of virus-infected egg fluids.

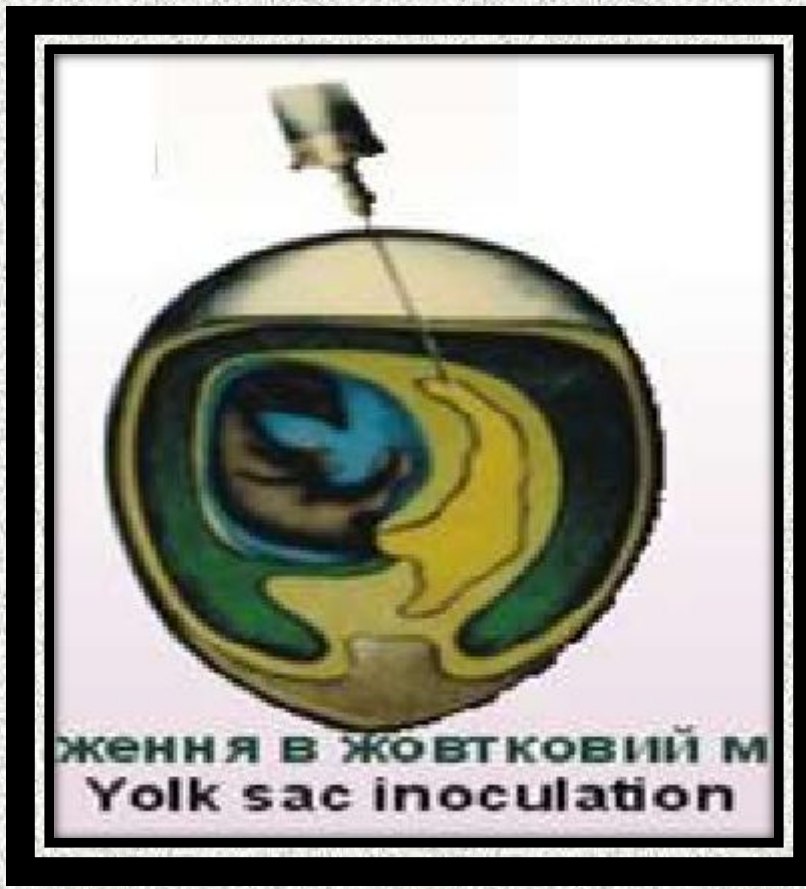


3). Amniotic sac :

- Inoculation is mainly done for primary isolation of influenza virus and the mumps virus.
- Growth and replication of virus in egg embryo can be detected by haemagglutination assay.
- The virus is introduced directly into the amniotic fluid that bathes the developing embryo.

4). Yo l k sac l nocu lation :

- It is also a simplest method for growth and multiplication of virus.
- It is inoculated for cultivatio of som n viruses and e bacteria (Chlamydia , som Rickettsiae).
- Immune interferenc mechanis e can be detected in most of avian m viruses (Influenza)



Advantages of inoculation into embryonated egg

- Widely used method for the isolation of virus and growth.
- Cost effective and maintenance is much easier.
- The embryonated eggs are readily available.
- They are free from contaminating bacteria and many latent viruses.
- Ideal substrate for the viral growth and replication.
- less labour is needed.
- Widely used method to grow virus forsome vaccine production.
- Defense mechanisms are not involved in embryonated eggs.

Disadvantage of inoculation into embryonated egg

- The site of inoculation for varies with different virus . That is , each virus have different sites for growth and replication.

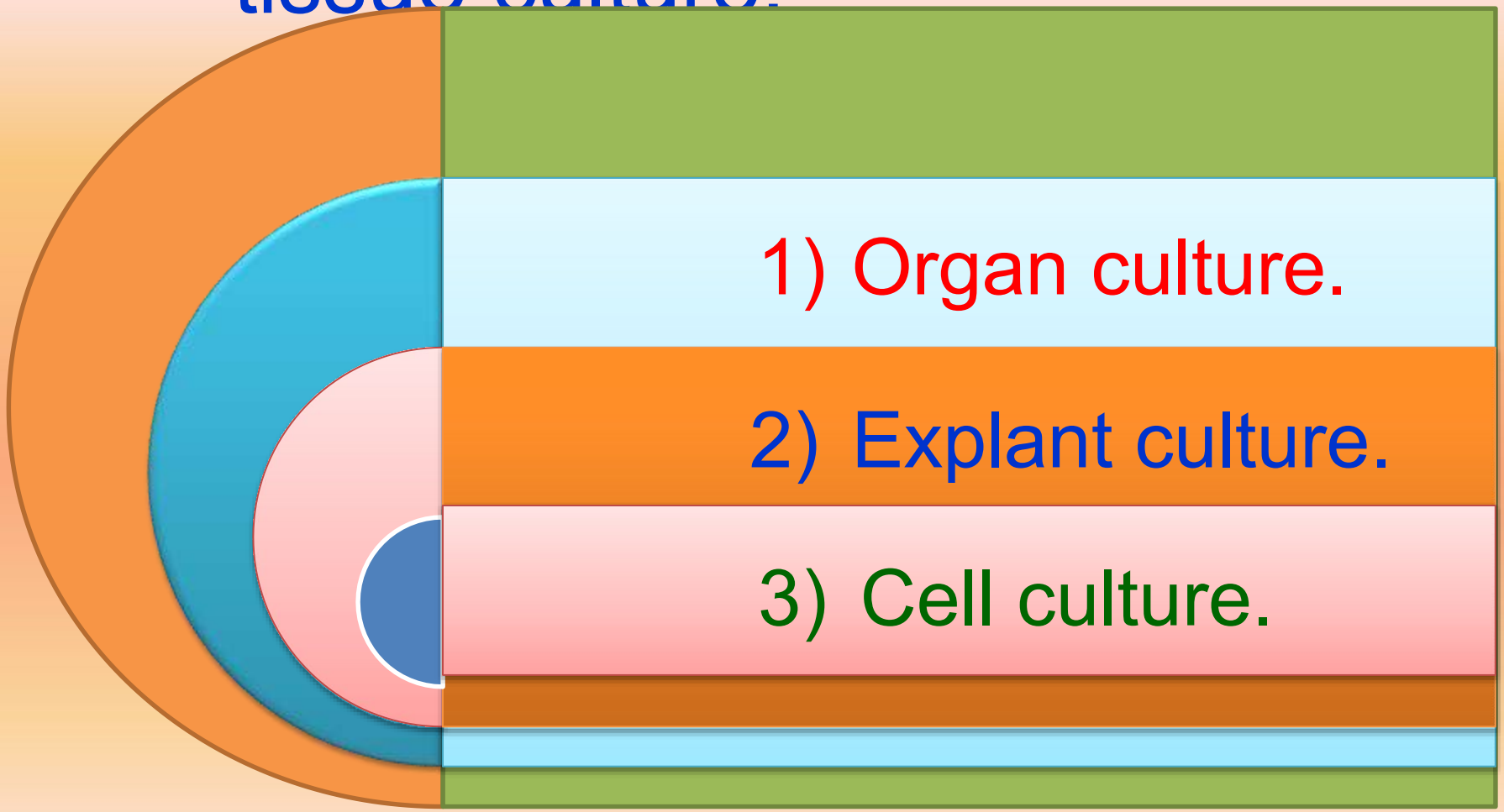
3). Tissue culture

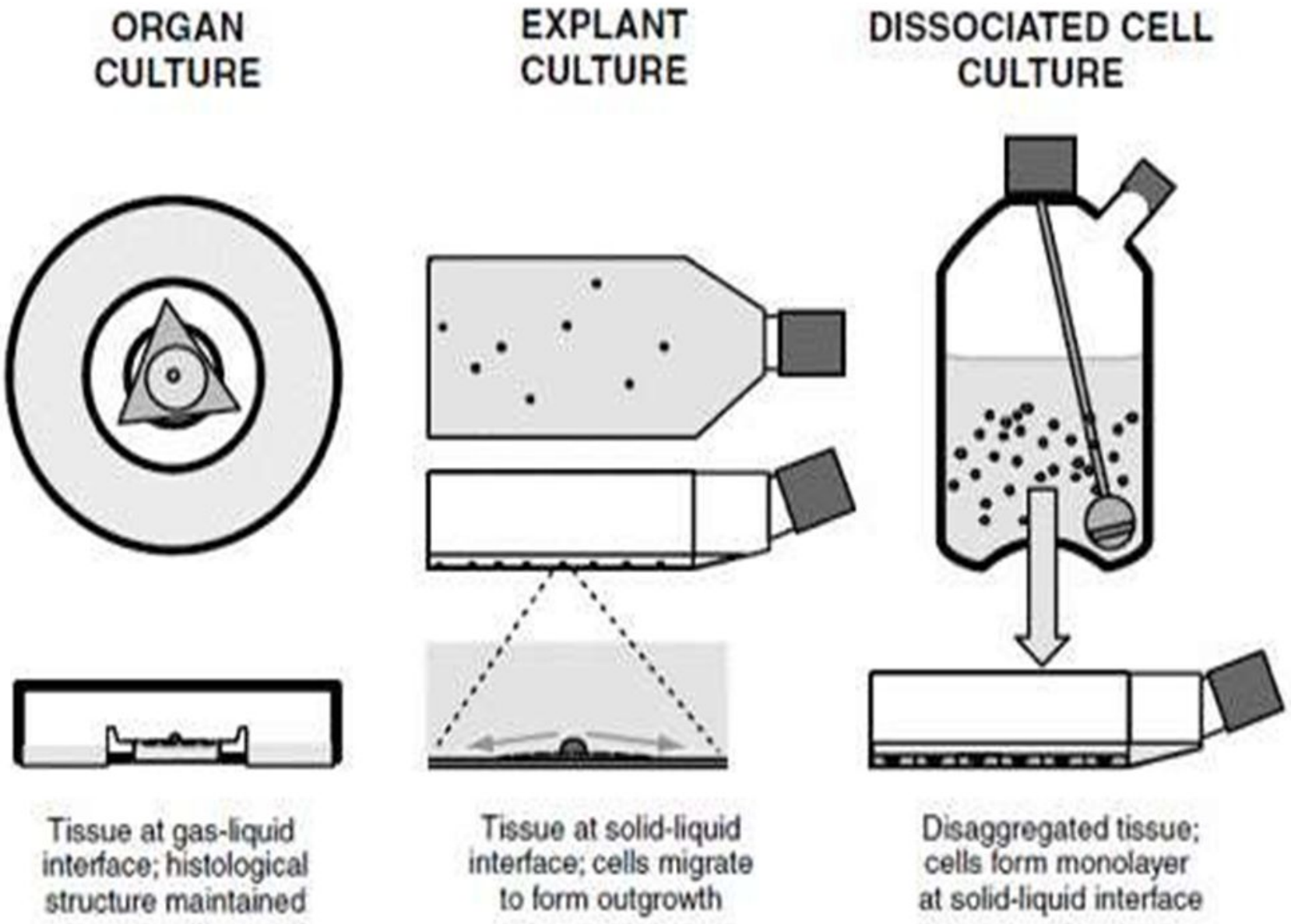
- Cultivation of bits of tissues and organs in vitro had been used by physiologists and surgeons for the study of morphogenesis and wound healing.

- Before 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 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:




ORGAN CULTURE
EXPLANT CULTURE
DISSOCIATED CELL CULTURE
Tissue at gas-liquid interface; histological structure maintained
Tissue at solid-liquid interface; cells migrate to form outgrowth
Disaggregated tissue; cells form monolayer at solid-liquid interface

1). Organ culture

Small bits of organs can be maintained in vitro for days and weeks, preserving their original architecture and function. Formalin is used for the preservation.

Organ culture is useful for the isolation of some viruses which appear to be highly specialized parasites of certain organs.

Example: Tracheal ring organ culture is employed for the isolation of coronavirus, a respiratory pathogen.

2). Explant culture

A small portion of tissue excised from animal's body is explant.

The explants taken from laboratory animals such as mice , rabbit , guinea pigs , hamester and man can be grown in petri dishes.

An explant is aseptically transferred into a sterile petri dish by using a fine tipped forceps and then a coverslip is placed over that explant.

Enough volume of medium is poured into the petri dish , which is then incubated at 37 ^{0}C untill cell growth.

Example : Adenoid tissue explant culture were used for the isolation

3). Cell culture

This is the type of culture routinely employed for growing viruses.

Tissues are dissociated into the components of cells by the action of proteolytic enzymes such as trypsin and mechanical shaking.

The essiential constitutents of the growth medium are physiologic amounts of essiential amino acids and vitamins , salts , glucose and a buffering system generally consisting of bicarbonate in equilibrium with atmosphere containing about 5% carbon dioxide.

This is supplemented with up to 5% calf serum.

Antibiotics are added to prevent the bacterial contaminants and phenol red as indicator.

Such media will enable most cell types to multiply with a division time of 24-48 hours.

The cell suspension is dispensed in bottles, tubes or petri dishes.

The cell adhere to the glass surface and on incubation , divide to form a confluent monolayer sheet of cells covering the surface within about a week.

Cell culture tubes may be incubated in a sloped horizontal position , either as ‘stationary culture’ or may be in special ‘roller drums’ to provide better aeration.

Some fastidious virus grow only in such roller cultures.

Based on their origin, chromosomal characters and the number of generations through which they can be maintained, cell culture are classified into three types :

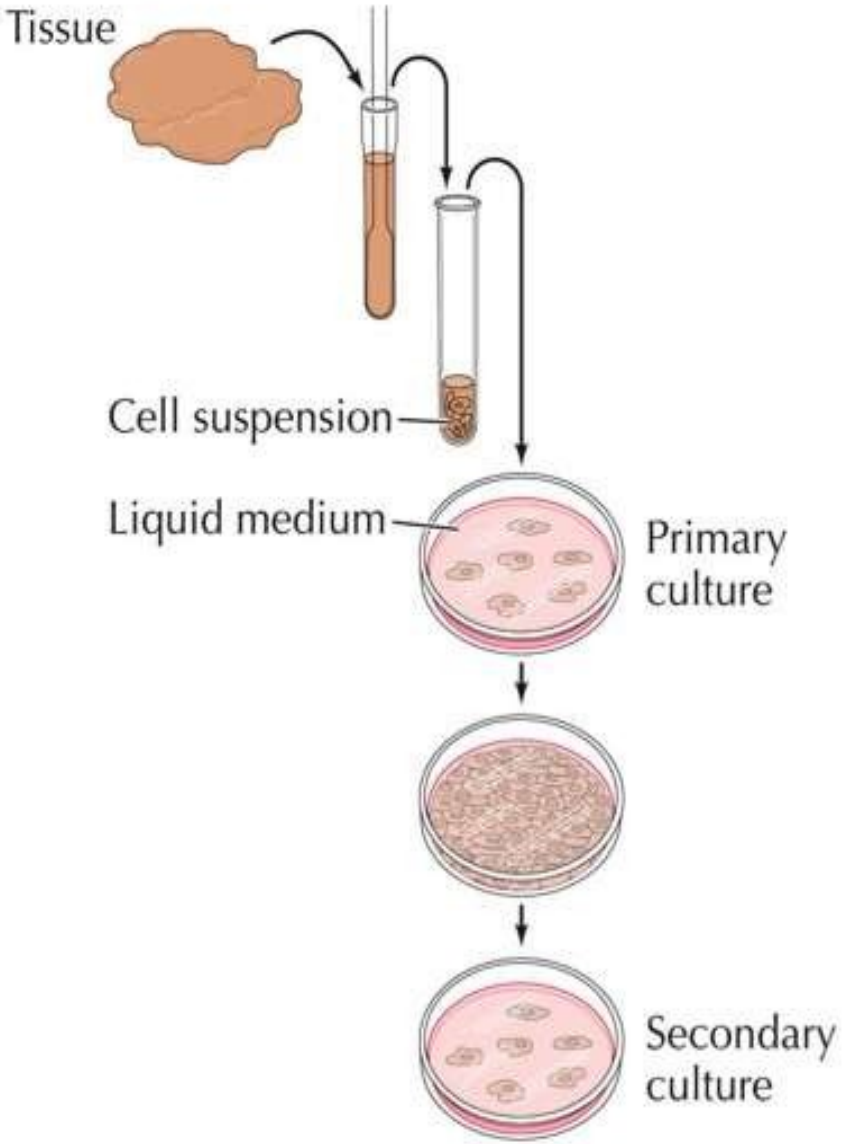
Types of cell culture

1). Primary cell culture.

2). Secondary cell culture.

3). Continuous cell lines.

1.41 Culture of animal cells



THE CELL, Fourth Edition, Figure 1.41

1). Primary cell culture

The cell culture established directly from cells taken from animal's tissue is called primary culture.

It is capable of only limited growth and hence it can be subcultured once or twice.

Examples : Kidney cells of monkey and man , embryo cells , alveolar cells are usually grown in primary cell cultures.

A small volume of cell suspension is aseptically transferred to a culture flask or petri dish containing nutrient medium , with the help of pipette.

The culture vessel is incubated at $37^{0}C$ for a few days to get a primary culture.

Primary cell cultures are widely used for the isolation of animal viruses and cultivation of viruses for vaccine production.

2). Secondary cell culture

The cell culture established from primary cell culture are called secondary culture or sub-culture.

As secondary cell cultures can be maintained and subcultured for 20-50 times , they are called semi-continuous cells.

Examples : Human embryonic kidney cells and skin fibroblast cells.

Secondary cell cultures are used for the isolation of wide group of animal viruses. Some secondary cultures are used for vaccine production.

Monolayer produced as a result of primary cell culture is detached from the bottom of the culture flask by adding trypsin or EDTA.

It is then cut into small fragments. 2 or 3 fragments are inoculated into a roller drum containing nutrient medium and the roller drum is incubated at 37^{0}C for few days.

The fragments of monoculture grow into large monolayers. These are called secondary culture.

3). Continuous cell lines

- Animal cells capable of indefinite growth are called continuous cell lines or cell lines.
- These are the cells of a single type , usually derived from cancer cells, that are capable of continuous serial cultivation indefinitely.
- Standard cell lines derived from human cancers, such as HeLa, KB cell lines have been used in laboratories throughout the world for many years.

- These cell lines may bemaintained by serial subcultivation or stored in the cold (-70^{0}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 donot isolate and viruses in clinical work. identify
- Tissue or serum for analysis is sent to central laboratories to identify virus.