Isolation and Techniques of Virus Cultivation

Viruses are obligate intracellular parasites so they depend on host for their survival. They cannot be grown in non-living culture media or on agar plates alone, they must require living cells to support their replication. The primary purpose of virus cultivation is:

1. To isolate and identify viruses in clinical samples.
2. To do research on viral structure, replication, genetics and effects on host cell.
3. To prepare viruses for vaccine production.

Cultivation of viruses can be discussed under following headings:

1. Animal Inoculation
2. Inoculation into embryonated egg
3. Cell Culture

1. Animal Inoculation

- Viruses which are not cultivated in embryonated egg and tissue culture are cultivated in laboratory animals such as mice, guinea pig, hamster, rabbits and primates 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.
- Viruses can also be inoculated by intraperitoneal and subcutaneous route.
- After inoculation, virus multiply in host and develops disease. The animals are observed for symptoms of disease and death.
- Then the virus is isolated and purified from the tissue of these animals.

Advantages of Animal Inoculation

1. Diagnosis, Pathogenesis and clinical symptoms are determined.
2. Production of antibodies can be identified.
3. Primary isolation of certain viruses.
4. Used for the study of immune responses, epidemiology and oncogenesis.

Disadvantages of Animal Inoculation

1. Expensive and Difficulty in choosing of animals for particular virus
2. Some human viruses cannot be grown in animals, or can be grown but do not cause disease.
3. Mice do not provide models for vaccine development.

2. Inoculation into embryonated egg

- Good pasture in 1931 first used the embryonated hen's egg for the cultivation of virus.
- The process of cultivation of viruses in embryonated eggs depends on the type of egg which is used.
- Viruses are inoculated into chick embryo of 7-12 days old.
- For inoculation, eggs are first prepared for cultivation, the shell surface is first disinfected with iodine and penetrated with a small sterile drill.
- After inoculation, the opening is sealed with gelatin or paraffin and incubated at 36°c for 2-3 days.
- After incubation, the egg is broken and virus is isolated from tissue of egg.
- Viral growth and multiplication in the egg embryo is indicated by the death of the embryo, by embryo cell damage,
- **Viruses can be cultivated in various parts of egg like chorioallantoic membrane, allantoic cavity, amniotic sac and yolk sac.**

1. Chorioallantoic Membrane (CAM):

- Inoculation is mainly for growing poxvirus and Herpes simplex virus
- After incubation, visible lesions called pocks are observed, which is grey white area in transparent CAM.

2. Allantoic cavity:

- Inoculation is mainly done for production of vaccine of influenza virus, yellow fever, rabies.
- Most of avian viruses can be isolated using this method.

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.

4. Yolk sac inoculation:

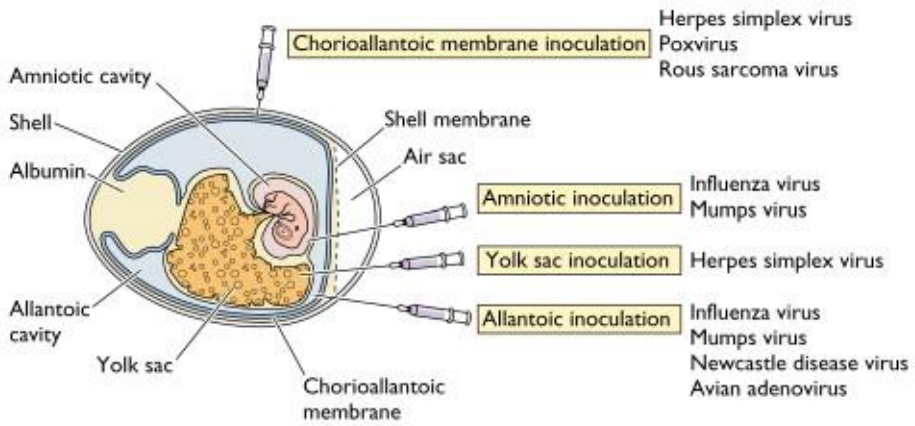
- It is also a simplest method for growth and multiplication of virus.
- It is inoculated for cultivation of some viruses and some bacteria (Chlamydia, Rickettsiae)

Advantages of Inoculation into embryonated egg

1. Widely used method for the isolation of virus and growth and replication.
2. Isolation and cultivation of many avian and few mammalian viruses.
3. Not expensive route.
4. Less labor is needed.
5. They are free from contaminating bacteria and many latent viruses.
6. Widely used method to vaccine production.

Disadvantages of Inoculation into embryonated egg

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



3. Cell Culture (Tissue Culture)

There are three types of tissue culture; organ culture, explant culture and cell culture.

Organ cultures are mainly done for highly specialized parasites of certain organs e.g. tracheal ring culture is done for isolation of coronavirus.

Explant culture is rarely done.

Cell culture is mostly used for identification and cultivation of viruses.

Cell culture is the process by which cells are grown under controlled conditions.

Cells are grown in vitro on glass or a treated plastic surface in a suitable growth medium.

At first growth medium, usually balanced salt solution containing 13 amino acids, sugar, proteins, salts, calf serum, buffer, antibiotics and phenol red are taken and the host tissue or cell is inoculated.

On incubation the cell divide and spread out on the glass surface to form a monolayer.

Types of cell culture

1. Primary cell culture:

2. Diploid cell culture (Semi-continuous cell lines):

3. Heteroploid cultures (Continuous cell lines):

Advantages of cell culture

1. Relative ease, broad spectrum, cheaper and sensitivity

Disadvantage of cell culture

1. The process requires trained technicians with experience in working .
2. State health laboratories and hospital laboratories do not isolate and identify viruses in clinical work.
3. Tissue or serum for analysis is sent to central laboratories to identify virus.