Techniques of Virus Cultivation–Pros and Cons

Dr. Daljeet Chhabra

Department of Veterinary Microbiology

College of Veterinary Science and A.H., Mhow

NDVSU, Jabalpur

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 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. Vaccine production.

Cultivation of viruses can be discussed under following headings:

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

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.
- The selected animals should be healthy and free from any communicable diseases.
- Viruses can also be inoculated in lab animals by intraperitoneal, subcutaneous, intracerebral and intranasal routes.
- After inoculation, virus multiplies 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. Mice provide a reliable model for studying viral replication.
5. Used for the study of immune responses, epidemiology and oncogenesis.

Disadvantages of Animal Inoculation

1. Expensive and difficulties in maintenance and handling of animals.
2. Difficulty in choosing of animals for particular virus.
3. Some viruses cannot be grown in animals or can be grown but do not cause disease.
4. Mice do not provide models for vaccine development.
5. Issues related to animal welfare systems/ethical issues.

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 mostly into chick embryo of 7-12 days old.
- For inoculation, eggs are first prepared for cultivation; the shell surface is first disinfected with iodine/alcohal and penetrated with a small sterile drill.
- After inoculation, the opening is sealed with gelatin or paraffin and incubated at 36°C-38°C for 2-3 days.
- After incubation, the egg is broken, harvesting is done 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, changes in fluids or by the formation of typical pocks or lesions on the egg membranes.
- Viruses can be cultivated in various parts of egg like chorioallantoic membrane, allantoic cavity, amniotic sac, yolk sac, intravascular, etc.

Advantages of inoculation into embryonated egg

1. Avian embryo culture/inoculation offers an economical and convenient method.
2. Widely used method for the isolation of virus.
3. Ideal substrate for the viral growth and replication.
4. Cost effective and maintenance is much easier.
5. Less labor is needed.
6. The embryonated eggs are readily available.
7. Sterile and wide range of tissues and fluids which can be processed and used further.
8. They are free from contaminating bacteria and many latent viruses.
9. Specific and non specific factors of defense are not involved in embryonated eggs.
10. Widely used method to grow virus for some vaccine production.

Disadvantages of using embryonated eggs

1. Eggs from vaccinated flock may carry antibodies in yolk, which may interfere in growth of specific microorganisms.
2. Some of microbes like Salmonella, Mycoplasma, etc. can pass from infected hen to eggs.

CELL CULTURE

- Cell culture is mostly used for identification and cultivation of viruses.
- Cell culture is the process by which cells are grown *in vitro* under controlled conditions.
- Cells are grown on glass or a treated plastic surface in a suitable tissue culture medium for Monolayer (anchorage dependent) or in flasks as suspension cultures (anchorage independent).
- For Monolayer, first growth medium, usually balanced salt solution containing 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/plastic surface to form a confluent monolayer. After growth of cells maintenance medium is added.
- Virus is then inoculated to observe the cytopathetic effects (CPE) and other purposes.

Advantages of cell culture

1. Relative ease, broad spectrum, cheaper and sensitivity.
2. Use of animals reduced.
3. Used for virus isolation and identification.
4. *In vitro* models allow for control of the extracellular environment.
5. Widely used method to grow virus for some vaccine production.

Disadvantage of cell culture

1. The process requires sophisticated laboratory and trained technicians with experience in working on a full time basis.
2. It is nearly impossible to recreate the *in vivo* environment.
3. The process is time consuming.