Methods for Cultivation of Virus

Since the viruses are obligate intracellular parasites, they cannot be grown on any inanimate culture medium. Viruses can be cultivated within suitable hosts, such as a living cell. Generally three methods are employed for the virus cultivation.

1. Inoculation of virus into animals.
2. Inoculation of virus into embryonated eggs.
3. Tissue culture

Inoculation of Virus in Animals

Laboratory animals are widely used for routine cultivation of virus; they play an essential role in studies of viral pathogenesis. Live animals such as monkeys, mice, rabbits, guinea pigs, ferrets are widely used for cultivating virus. Monkeys were used for the isolation of Poliovirus. But due to their risk to handlers, monkeys find only limited applications in Virology. Mice are the most widely employed animals in virology. The different routes of inoculation in mice are intracerebral, subcutaneous, intraperitoneal or intranasal. After the animal is inoculated with the virus suspension, the animal is observed for signs of disease, visible lesions or is killed so that infected tissues can be examined for virus.

Advantages:

1. Animal inoculation may be used as diagnostic procedure for identifying and isolating a virus from a clinical specimen.

2. Mice provide a reliable model for studying viral replication.
3. Gives unique insight into viral pathogenesis and host virus relation.
4. Used for the study of immune responses, epidemiology and oncogenesis.

Disadvantages:

- Expensive and difficulties in maintenance 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 disease.
- Mice do not provide models for vaccine development.
- It will lead to generation of escape mutants.
- Issues related to animal welfare systems.

Inoculation of Virus into Embryonated eggs

Prior to the advent of cell culture, animal viruses could be propagated only on whole animals or embryonated chicken eggs. Good pasture in 1931first 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. The egg used for cultivation must be sterile and the shell should be intact and healthy. A hole is drilled in the shell of the embryonated egg, and a viral suspension or suspected virus- containing tissue is injected into the fluid of the egg. Viral 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 pocks or lesions on the egg membranes. An embryonated egg offers various sites for the cultivation of viruses (Fig 3). The different sites of viral inoculation in embryonated eggs are:

- Chorioallantoic membrane(CAM).
- Amniotic Cavity.
- Allantoic Cavity.
- Yolk sac.
- Embryo.
- Air sac.

Chorioallantoic Membrane(CAM)

Is mainly employed in the growth of poxvirus. Virus growth and replication in the CAM is indicated by visible lesions (pocks); grey white area in transparent CAM. Herpes simplex virus is also grown. Each pock is derived from a single virion. The morphology of the pocks may vary depending on the nature of the virus. Under optimal conditions, each infectious virus particle can form one pock. Hence this method is suitable for plaque studies. Herpes simplex virus can also be inoculated via CAM.

Allantoic Cavity

Is the most popular and simple method for viral inoculation. Allantoic inoculation is employed for the growth and replication of the influenza virus for vaccine production. This will provide a rich yield of influenza and some paramyxoviruses. Other allantoic vaccines include Yellow fever and rabies vaccines. Duck eggs provide a better yield of rabies virus and were used for the preparation of the inactivated non-neural rabies vaccines. But they need a longer incubation period than embryonated hen's egg. Most of avian viruses can be isolated using this method.

Amniotic Cavity

The amniotic sac is employed inoculated for primary isolation of influenza a virus and the mumps virus. Growth and replication of virus in egg embryo can be detected by haemagglutination assay.

Yolk Sac

It is also a simplest method for growth and multiplication of virus. Mostly mammalian viruses are isolated using this method. Immune interference mechanism can be detected in most of avian viruses. This method is also used for the cultivation of some bacteria like Chlamydiae and Rickettsiae.

Advantages:

- Widely used method for the isolation of virus and growth.
- Ideal substrate for the viral growth and replication.
- Isolation and cultivation of many avian and few mammalian viruses.
- Cost effective and maintenance is much easier.
- Less labor is needed.
- The embryonated eggs are readily available.
- Sterile and wide range of tissues and fluids
- They are free from contaminating bacteria and many latent viruses.
- Specific and non specific factors of defense are not involved in embryonated eggs.
- Widely used method to grow virus for some vaccine production.

Disadvantages:

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

procedure

For propagation of influenza virus, pathogen-free eggs are used 11-12 days after fertilization. The egg is placed in front of a light source to locate a non-veined area of the allantoic cavity just below the air sac. This is marked with a pencil. After all the eggs have been 'candled' in this way, a small nick is made in the shell at this position using a jeweler's scribe. Next, a hole is drilled at the top of the egg with a Dremel motorized tool. If this is not done, when virus is injected, the pressure in the air sac will simply force out the inoculum.

After all the eggs have been nicked and drilled, they are inoculated with virus using a tuberculin syringe – a 1 ml syringe fitted with a 1/2 inch, 27 gauge needle. The needle passes through the hole in the shell, through the chorioallantoic membrane, and the virus is placed in the allantoic cavity, which is filled with allantoic fluid. The two holes in the shell are sealed with melted paraffin, and the eggs are placed at 37 degrees C for 48 hours.