

CULTIVATION OF VIRUSES

- ① The cultivation of virus is an essential prerequisite for their isolation
- ① Viruses are obligatory intracellular parasite, virus can only replicate in cells
- ① Three basic laboratory substrate for this purpose these are ;

1. Embryonated Eggs

- Although not all virus can be grown in embryonated egg, many viruses are cultivate in this host
- Eggs are now used for rapid isolation of a few viruses and also used for vaccine or antigen production
- This methods is more economical and convenient than animal inoculation

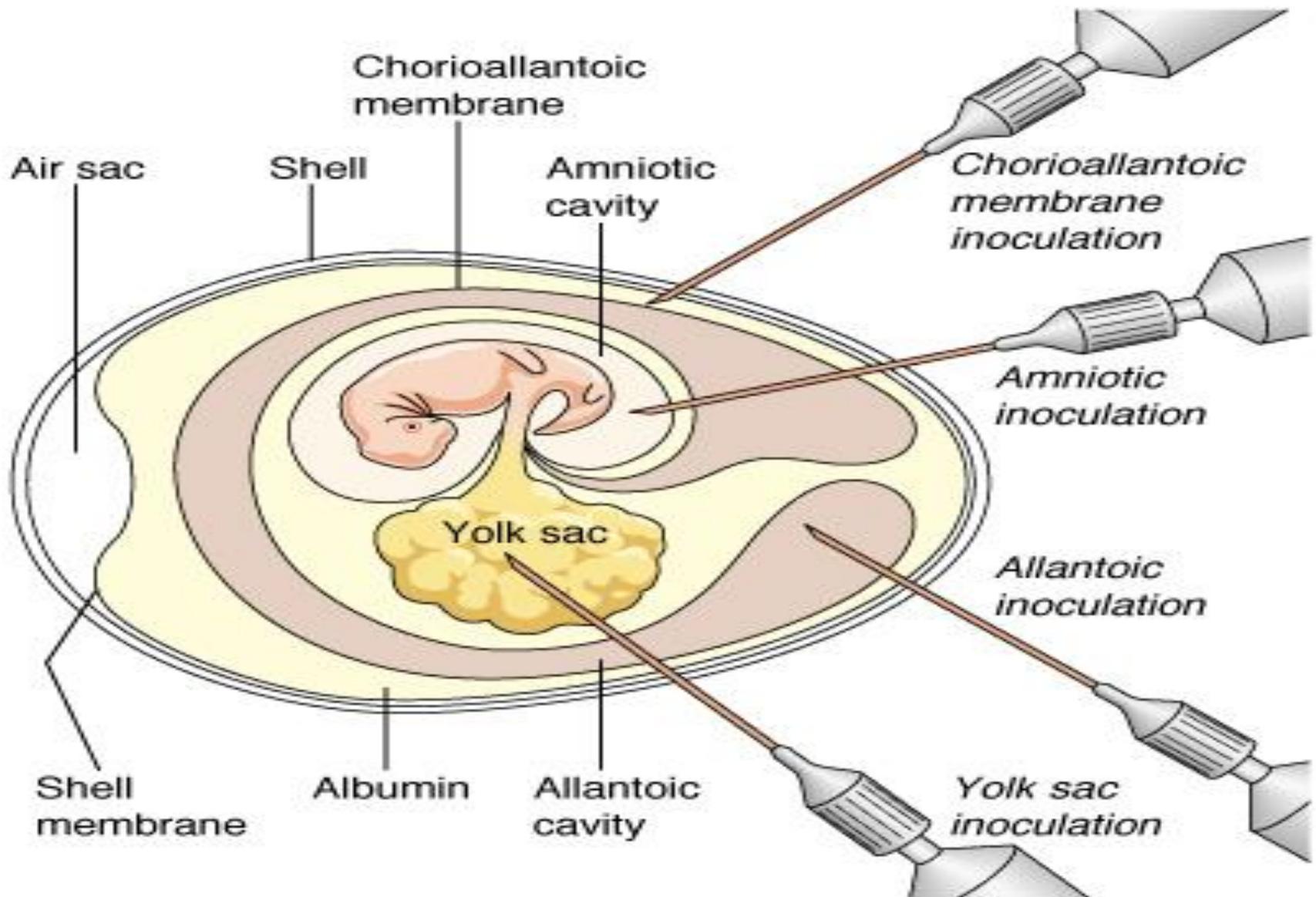
- Embryonated eggs may be infected by various route ; amniotic, allantoic, chorio allantoic, yolk sac, in special cases intravenous and intra embryonal
- The choice of an particular route is again depend upon the ability of tissue to support virus replication
- Before inoculation eggs are candled to check that the embryo is alive
- The presence of virus in inoculated eggs can be detected by mortality, deformities and haemorrhagic of the embryo, lesion in the form

- of pock, oedema of the developing membrane, the presence of specific antigen in the fluid as shown by HI test, SN test, FAT and other serological test

2 Tissue culture

- Tissue or organ enplanted from animal and maintained or grown invitro in a suitable media

Embryonic eggs



Several methods for growing cells invitro

1) cell culture

This terms used to denote the growing of cells invitro including the culture of single cell;

a) Suspension culture

- Some cell lines can be adapted to grow in suspension in vessels where the medium is continuously stirred and aerated

b) Monolayer cell culture

Sheets of cells , only one cell thick grown on the surface of glass or plastic vessels

2.) Organ culture; the growth of tissue or part of organ invitro

Types of culture

1) Primary cell culture

- A culture started from cells, tissues or organs taken directly from an animal
- A primary culture may be regarded as such until it is subcultured for the first time

2) Continuous cell lines

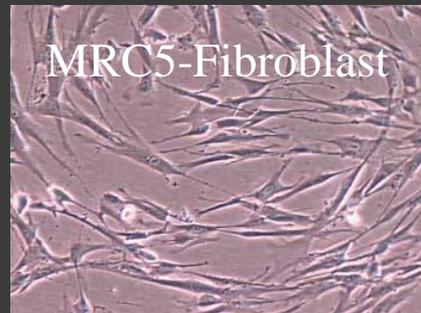
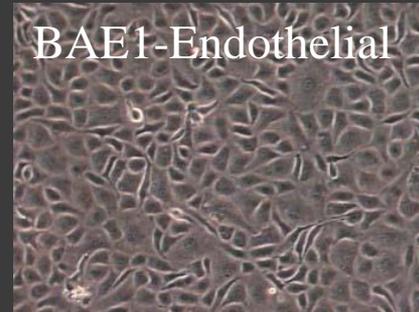
- Cells of a single type that are capable of propagation invitro indefinately
- The cultivation of cells or tissue culture invitro require nutrien media which is usually consist of balanced salt solution, glucose, vitamin, coenzyme,aminoacids, serum, sodium bicarbonate, phenol red



Recognition of viral growth in culture

1) Cytopathic Effect (CPE)

- Many viruses kill the cells, developed morphological evidence of cell damage are known as CPE, which that are visible with the inverted microscope
- They can be seen as ; formation of giant cell, complete destruction of cells, presence of inclusion bodies, syncytium



MORPHOLOGY CELL

2) Metabolism effect

Metabolic effect of the cells as shown by the change of colour of the medium, change in pH medium from red to yellow through orange

3) Plaque formation

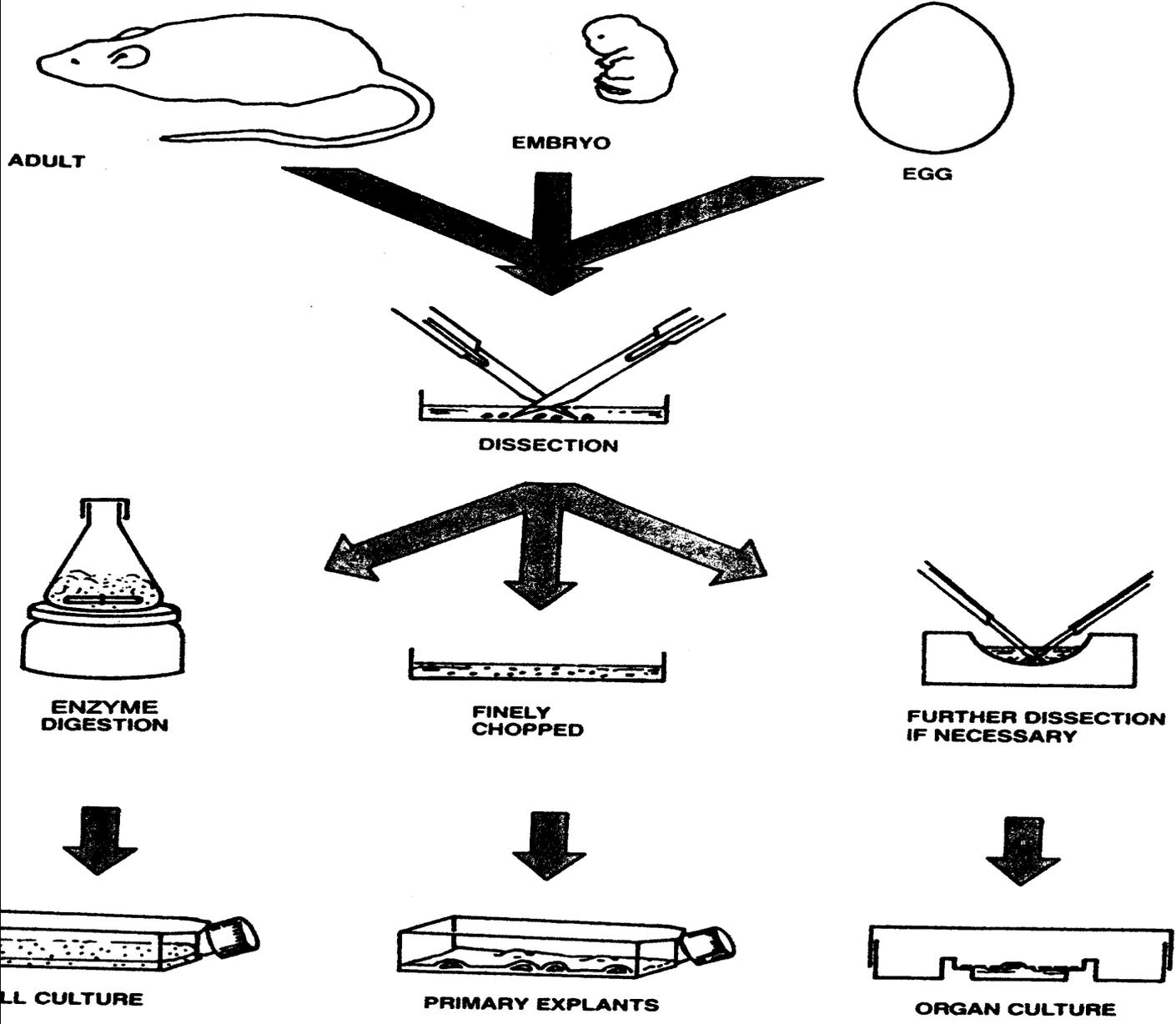
Plaque produce by inoculation virus in monolayer culture with the late addition of semi solid overlay agar which localized viral spread, produce a plaque

4) Interference

The multiplication of virus in the cells often inhibits the multiplication of another virus entering subsequently

3 Animal inoculation

- The ideal animal host for study of virus is of course the natural host; not always possible nor practical
- The animal should be Specific Pathogen Free, because inter current infection can contaminate the propagated virus; animal must also have no prior immunity to the particular virus
- Laboratory animal : mice, rats, hamster, rabbits, guinea pigs, etc. more practical , easier to handle



ISOLATION AND IDENTIFICATION PROCEDURE IN DIAGNOSTIC VIROLOGY

The procedures that are available for the diagnosis of viral disease :

1. Isolation of virus ; embryonated egg, tissue culture, laboratory animal
 - a. Directly by electron microscope
 - b. Indirectly ; detection of antigen or antibody by serological test ; FAT, ELISA etc.
2. Serological test; can be used to detect specific antibody that developed during the course of illness

Effective diagnostic virology depends upon correct selection of clinical specimen and their collection transport to the laboratory under appropriate condition

1. Collection of specimens

Specimen for virus isolation should be collected as early as possible during the course of illness.

Specimen commonly chosen include excretion, secretion various tissue taken at biopsy or autopsy its depend on the nature of illness and the possible identify of the causative virus.

The time of collection specimen

Phase / stage of disease	Virus in specimen	Detection specific ab
Incubation period	Rarely	None
Early stage	Often	Seldom
Acute phase	Often	Often
Recovery	Rarely	Often
Convalescence	Rarely	Usually
Death	Often	None

2. Storage of specimen

- All specimens should be kept cold (4°C), preservation for months or year must be at -70° C or lower in deep freezer or liquid nitrogen
- PBS – glycerol 50% can be used to preserve specimen for virus isolation

3. Inoculation in host system (avian egg, tissue culture, laboratory animal)

- Successful isolation is followed by identification and serological test ; HI, VN, ELISA, RIA etc