

Cultivation of viruses

Systems for the propagation of viruses

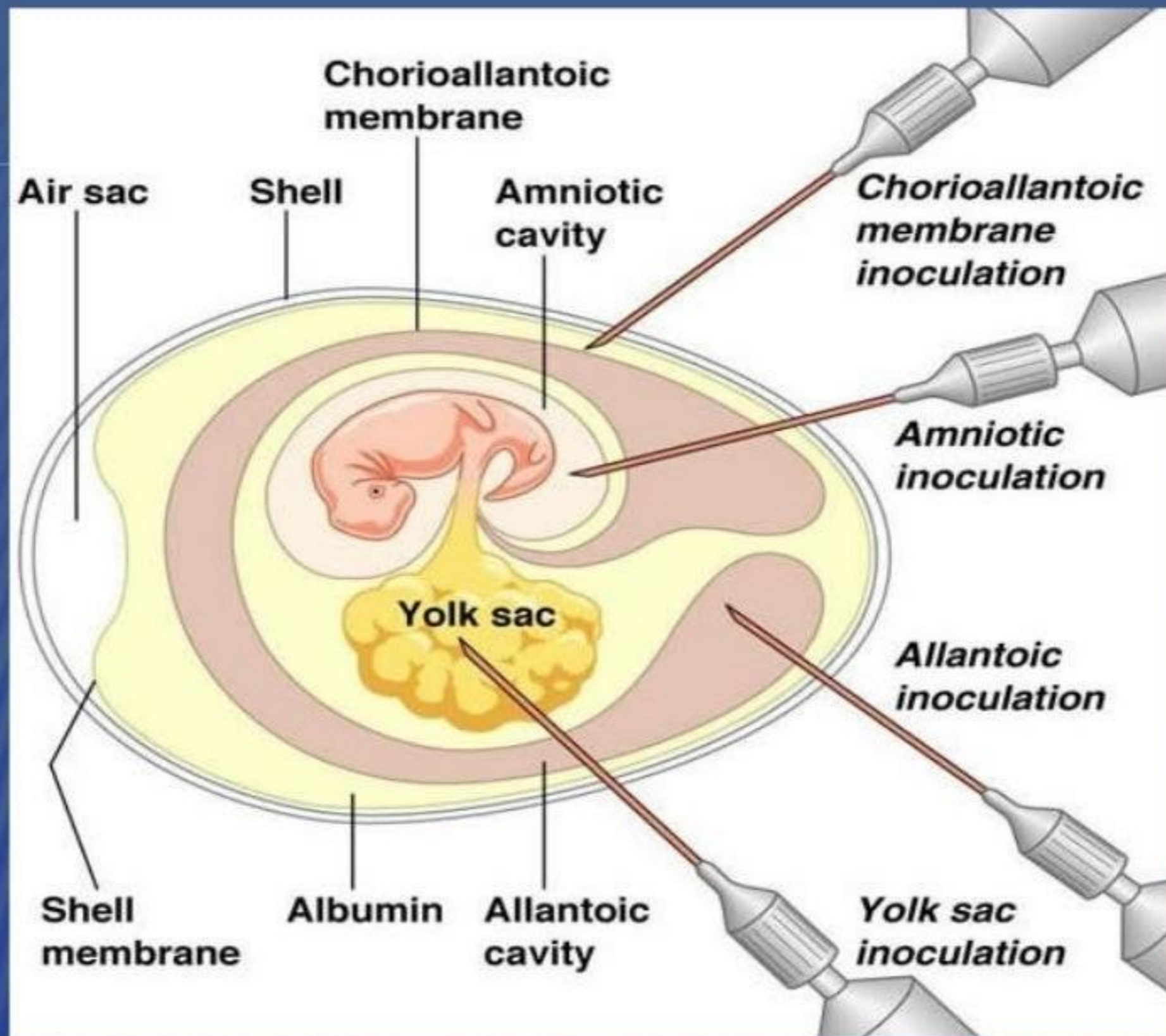
- ❖ Human volunteers
- ❖ Animal inoculation
- ❖ Embryonated eggs
- ❖ Tissue culture
 - a) Organ culture
 - b) Explant culture
 - c) Cell culture

Animal inoculation

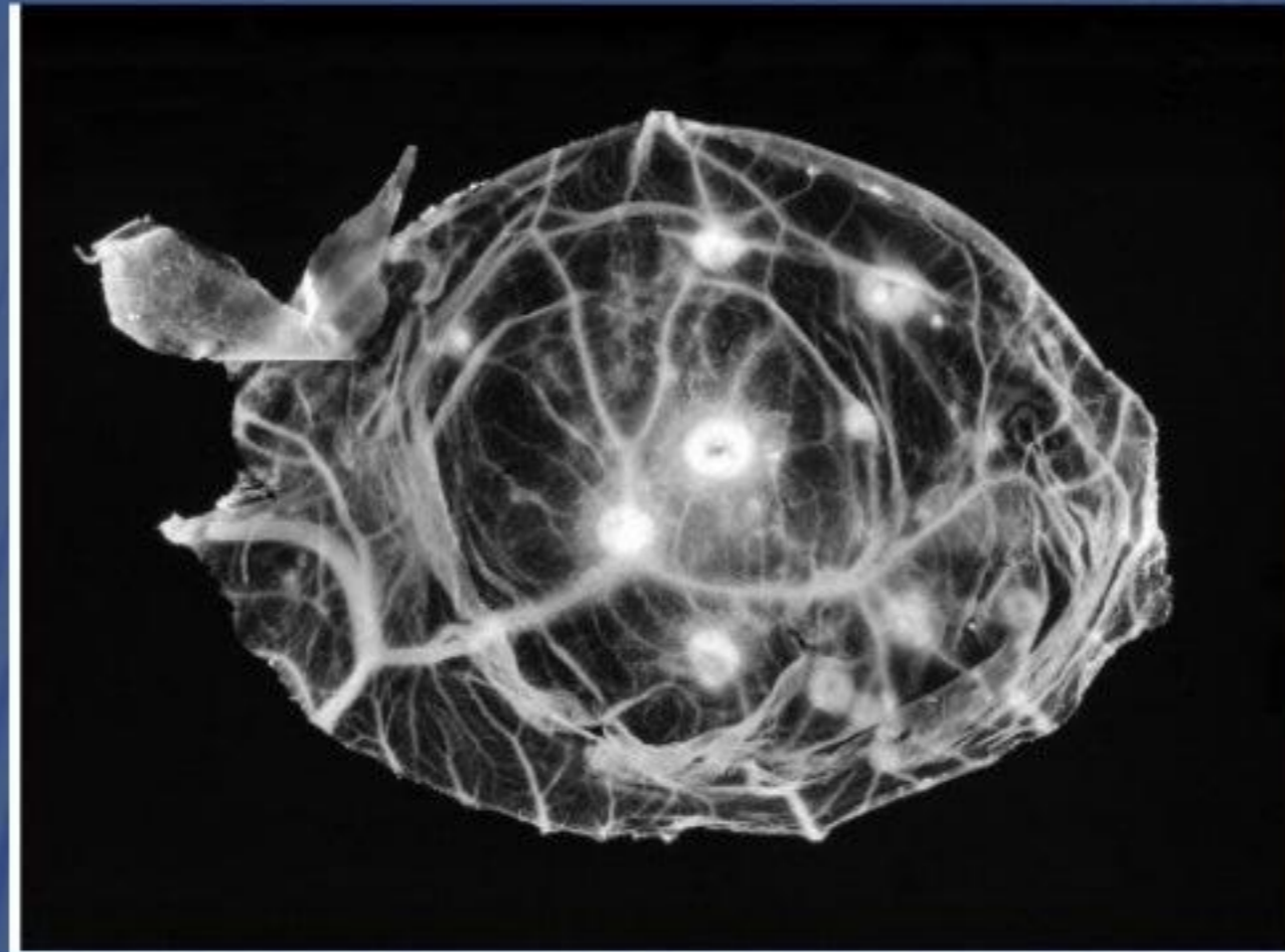
- ✓ Mice, Infant (suckling) mice, rats, monkeys, chickens, guinea pigs, rabbits, ferrets are used for inoculation
- ✓ Growth of the virus in inoculated animals may be indicated by death, disease or visible lesions
- ✓ Animal inoculation is also used for the study of pathogenesis, immune response, epidemiology and oncogenesis
- ✓ Disadvantages of animal inoculation are that immunity may interfere with viral growth and that animals often harbour latent viruses

Embryonated eggs

- ✓ The embryonated egg offers several sites for the cultivation of viruses
- ✓ Inoculation on the chorioallantoic membrane (CAM) produces visible lesions (pocks); pock-forming viruses eg: variola or vaccinia
- ✓ Inoculation into the allantoic cavity provides a rich yield of influenza and some paramyxo viruses; hence employed for vaccine production
- ✓ Inoculation into the amniotic sac is employed for the primary isolation of the influenza virus
- ✓ Yolk sac inoculation is used for the cultivation of some viruses, chlamydiae and rickettsiae



Embryonated egg showing different routes of inoculation



Small pox (variola) virus pocks on CAM of embryonated egg

Tissue culture

Organ culture

- ❖ Small bits of organs can be maintained in vitro for days and weeks, preserving their original architecture and function
- ❖ Useful for the isolation of some viruses which appear to be highly specialised parasites of certain organs (eg: Tracheal ring culture – corona virus, a respiratory pathogen)

Explant culture

- ❖ Fragments of minced tissue can be grown as 'explants' embedded in plasma clots or may be cultivated in suspension (eg: adenoid tissue explant cultures – adenoviruses)

Cell culture

Although embryonated eggs and laboratory animals are very useful for isolation viruses, cell culture is the sole system for virus isolation in most laboratories

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

1. Primary cell cultures
2. Diploid cell cultures (semi-continuous)
3. Continuous cell lines



Primary cell cultures

- ✓ Prepared directly from animal or human tissues and can be subcultured only once or twice (eg: monkey kidney, human embryonic kidney cell cultures)
- ✓ Useful for the isolation of viruses and their cultivation for vaccine production

Diploid cell cultures

- ✓ Derived from human fetal tissue and can be subcultured 20-50 times (human diploid fibroblasts – MRC-5, WI-38 (derived from normal embryonic lung tissue))
- ✓ They are useful for the isolation of some fastidious pathogens
- ✓ They are also useful for the production of viral vaccines (eg: poliomyelitis, rubella, rabies, CMV, VZV)

Continuous cell lines

- ✓ These are cells of a single type, usually derived from cancer cells and capable of continuous serial cultivation indefinitely (eg: HeLa, HEp-2, Vero, KB cell lines)

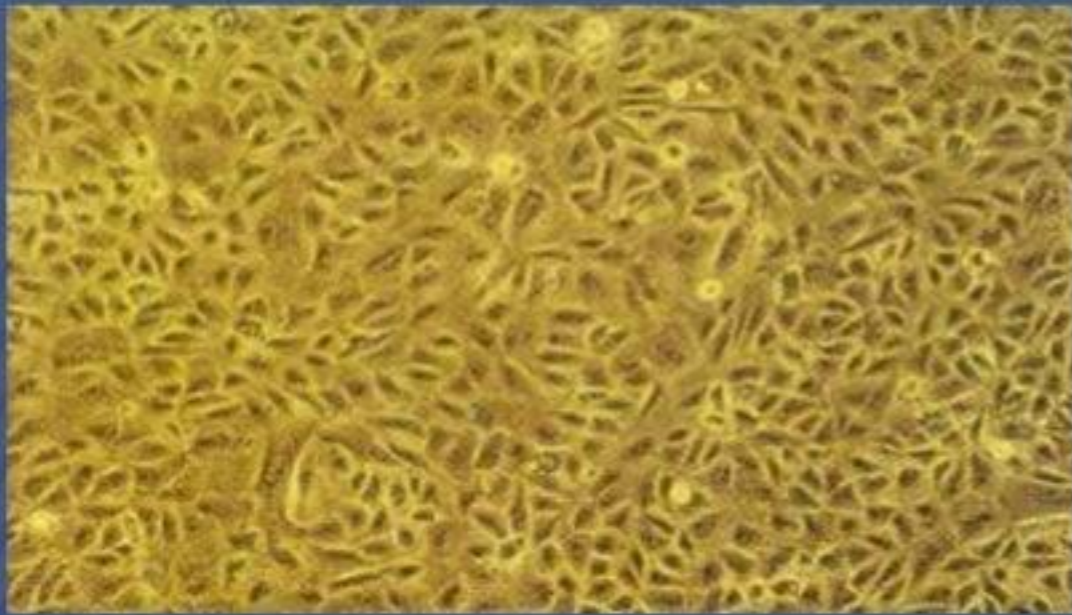
Some continuous cell lines in common use

HeLa	Human carcinoma of cervix cell line
HEp-2	Human epithelioma of larynx cell line
Vero	Vervet monkey kidney cell line
KB	Human carcinoma of nasopharynx cell line
McCoy	Human synovial carcinoma cell line
BHK-21	Baby hamster kidney cell line

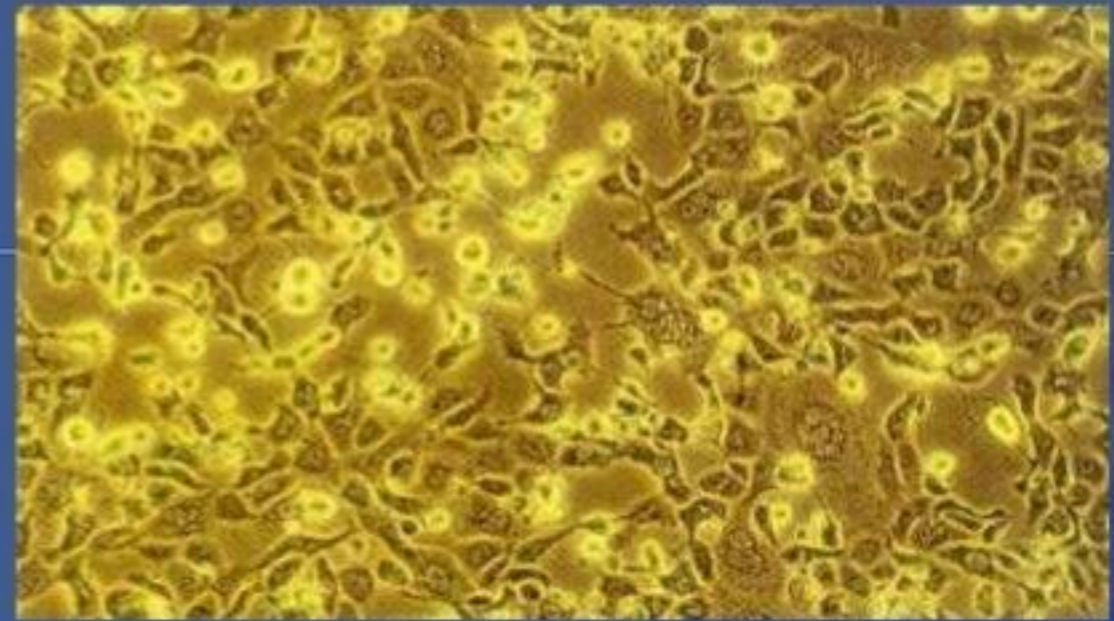
Detection of virus growth in cell cultures

Cytopathic effect

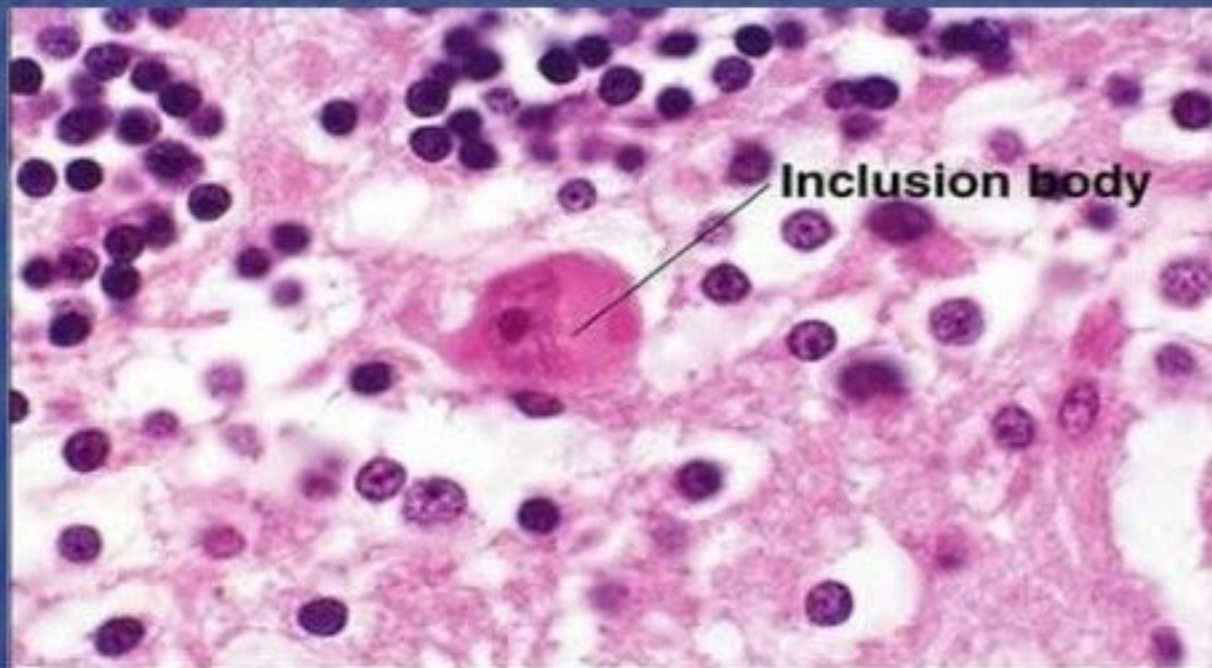
- ❖ Many viruses cause morphological changes in cultured cells, these changes can be readily observed by microscopic examination and these changes are known as 'cytopathic effects' (CPE)
- ❖ Cytopathic effects may be cytocidal (cell death) or non-cytocidal
- ❖ Non-cytocidal effects include acidophilic or basophilic inclusion bodies in the nucleus or cytoplasm, or both; cell fusion, and transformation
- ❖ Cytopathic effects can be so characteristic of individual viruses that they can often be used to identify viruses
(Measles – syncytium formation; adenovirus – large granular clumps)



Normal cell

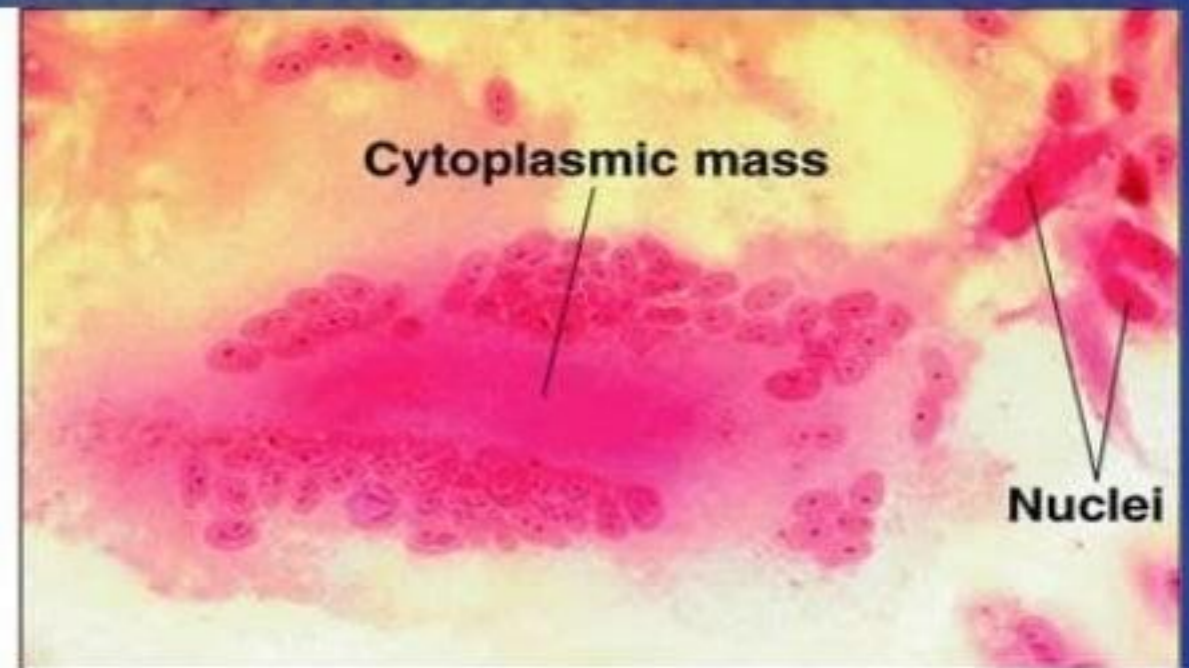


CPE



(a)

LM 25 μ m



(b)

LM 2 μ m

(a) Cytoplasmic inclusion body caused by rabies virus in brain tissue
(b) Syncytium formed by cell fusion due to infection by measles virus

Metabolic inhibition

When viruses grow in cell cultures, cell metabolism is inhibited and there is no acid production

Hemadsorption

When hemagglutinating viruses grow in cell culture, their presence can be indicated by the addition of guinea pig erythrocytes to the cultures

Interference

The growth of a non-cytopathogenic virus in cell culture can be tested by the subsequent challenge with a known cytopathogenic virus

Transformation

Oncogenic viruses induce cell transformation and loss of contact inhibition, so that growth appears in a piled-up fashion producing microtumors

Immunofluorescence

Viral assay

The virus content of a specimen can be assayed in two ways

1. Total virus particles: Electron microscopy and haemagglutination
2. Assay of infectivity (with reference to the infectious virions only)

a) Quantal assays

- ❖ Only indicate the presence or absence of infectious viruses but using serial dilutions of virus suspensions and with the aid of statistical methods, reasonably accurate estimates of infectivity can be obtained
- ❖ End points used for infectivity titration are the death of the animal, production of hemagglutinin in allantoic fluid or the appearance of CPE in cell cultures
- ❖ The titers are expressed as the '50 percent infectious dose' (ID_{50}) or LD_{50}

b) Quantitative assays

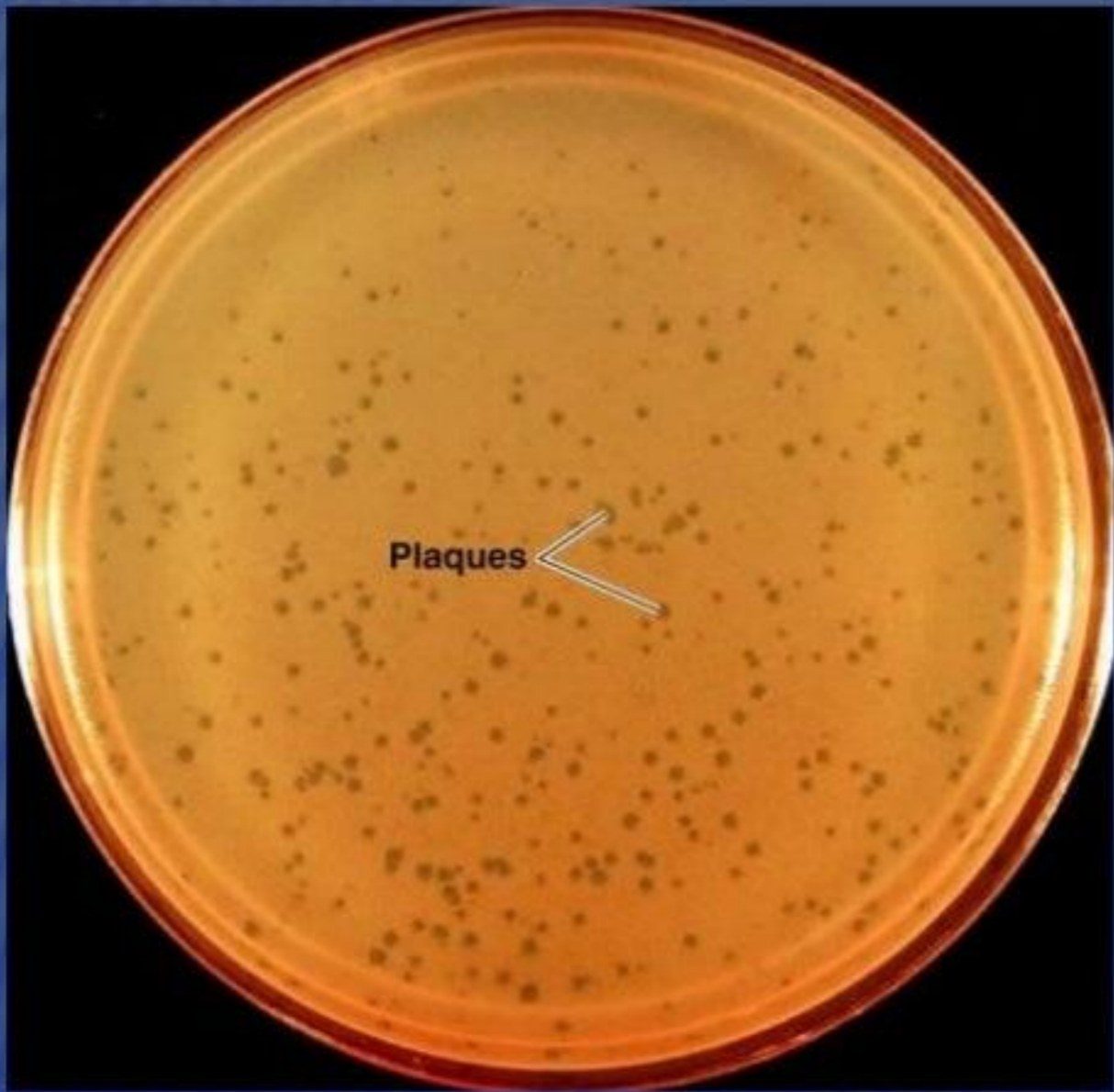
- ❖ Similar to the estimation of bacterial viable counts by colony counting

Two methods are available;

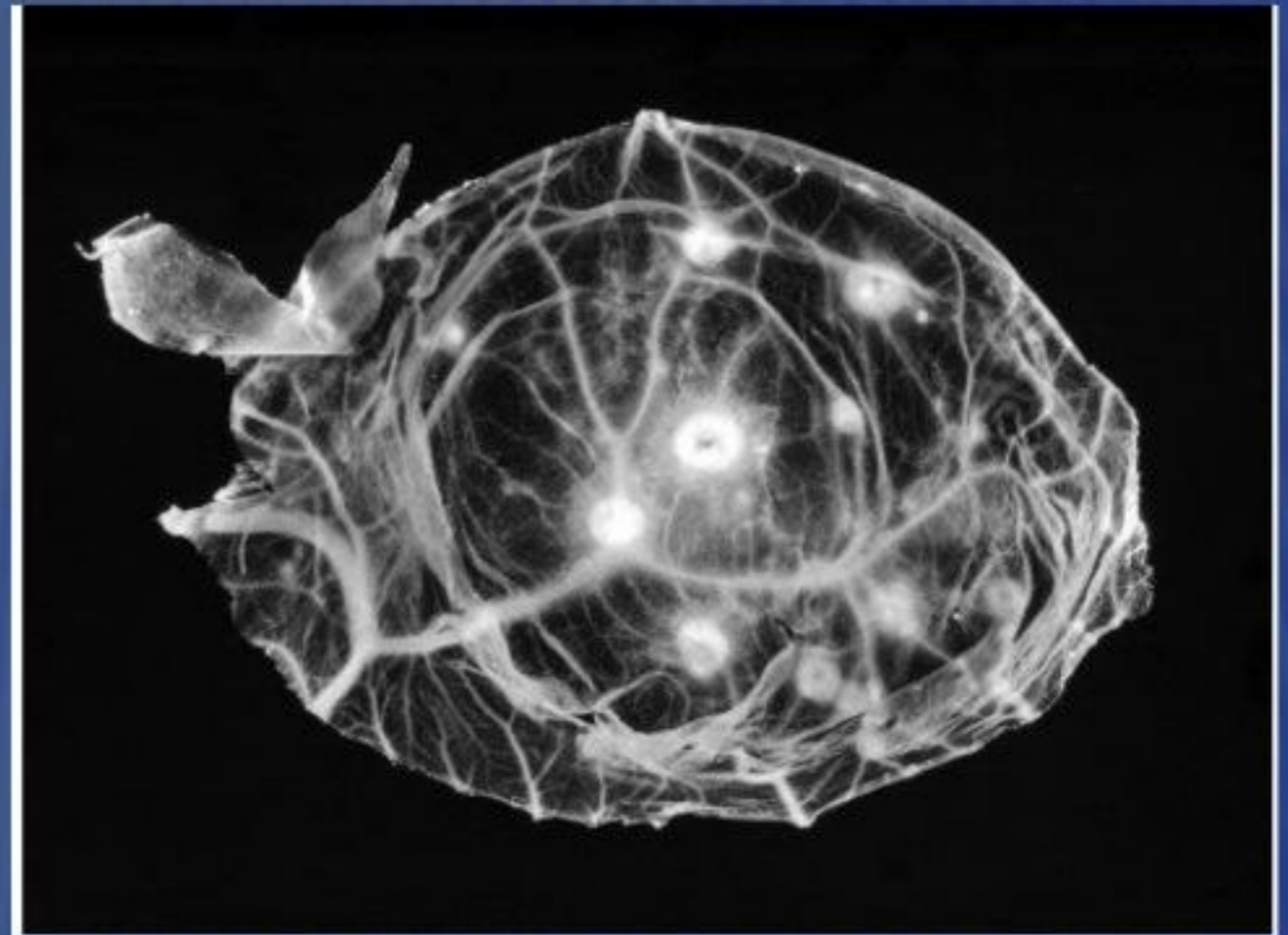
1. Plaque assay

- ✓ A viral suspension is added to a monolayer of cultured cells in a bottle or petri dish, and after adsorption, the medium is removed and replaced with a solid agar gel
- ✓ Each infectious viral particle gives rise to a localised focus of infected cells that can be seen with the naked eye. Such foci are known as 'plaques'

2. Pock assay



Plaque assay



Pock assay