

Lecture (2) : Cell cultures and Viral Diagnosis

The topics are:

- 1- Preparation of Cell culture
- 2- Type of cell culture
- 3- Diagnosis of Viral Infection
- 4- Inclusion bodies

The purpose of the lecture

- 1- Explain how to prepare the cell cultures and what are the types of cell cultures
- 2- Why do cell cultures use most often in viral diagnosis
- 3- What are the cytopathic effect.
- 4- What are the Inclusion bodies and their importance in viral diagnosis

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Preparation of Cell culture

Viruses are obligate intracellular parasites that require living cells in order to replicate. Cultured cells, eggs and laboratory animals may be used for virus isolation. Although embryonated eggs and laboratory animals are very useful for the isolation of certain viruses, cell cultures are the sole system for virus isolation in most laboratories.

To prepare cell cultures, tissue fragments are first dissociated, usually with the aid of trypsin or collagenase. The cell suspension is then placed in a flat-bottomed glass or plastic container (petri dish, a flask, a bottle, test tube) together with a suitable liquid medium. e.g. Eagle's, and an animal serum. After a variable lag, the cells will attach and spread on the bottom of the container and then start dividing, giving rise to a primary culture. Attachment to a solid support is essential for the growth of normal cells.

Note : Cell cultures vary greatly in their susceptibility to different viruses?

Cell cultures are separated into 3 types:-

1. **Primary cells** - prepared directly from animal or human tissues and can be subcultured only once or twice e.g. primary monkey or baboon kidney
2. **Semi-continuous diploid cells** - which are derived from human fetal tissue and can be subcultured 20 to 50 times e.g. human diploid fibroblasts
3. **Continuous cells** - derived from tumours of human or animal tissue e.g. The advance of cell culture

Advantages of cell culture for virus diagnosis are including :

- 1-Relative ease,
- 2-Broad spectrum and sensitivity.

Disadvantage:

- 1-It is limited by the difficulty in maintaining cell cultures,
- 2- variability of cell cultures.
- 3- Contamination by endogenous viral agents and bacteria may occur.

Diagnosis of Viral Infection

Viruses produce **cytopathic effect (CPE)** which include: change in cell morphology, cell lysis, vacuolation, syncytia formation and inclusion bodies.

Viral cytopathic effect (CPE)	
CPE	Features
Change in cell morphology	Cell rounding Degeneration Aggregation Loss of attachment of substrate
Histologic changes	Inclusion bodies in the nucleus or cytoplasm. Migration of chromatin
Syncytia formation	Multinucleated giant cells caused by virus-induced cell—cell-fusion
Changes in cell surface	Viral antigen expression Haemadsorption (haemagglutinin expression)

What are Inclusion bodies?

Inclusion bodies are virus- specific intracellular masses produced during replication of virus in host cells and visible under the light microscope. They are far larger than a single mature virus particle (elementary body), size ranges from 20-25 μm and can be seen by light microscope.

Inclusion bodies formed by different viruses show distinct shape, size, location and staining properties and their presence in an infected cell is a presumptive histological evidence of viral infection.