# Isolation and Identification of Viruses

DR. AHMED HASAN MOHAMMED

# Cell Culture

- Living cell cultures that can support their replication are the primary means of isolating pathogenic viruses. The cells are derived from a tissue source by outgrowth of cells from a tissue fragment (explant) or by dispersal with proteolytic agents such as trypsin. They are allowed to grow in nutrient media on a glass or plastic surface until a confluent layer one cell thick (monolayer) is achieved.

# Types of Cell Culture

- The **primary cell culture,** in which all cells have a normal chromosome count (diploid), is derived from the initial growth of cells from a tissue source.
- Redispersal and regrowth produce a **secondary cell culture,** which usually retains characteristics similar to those of the primary culture (diploid chromosome count and virus susceptibility).
- A third type of culture is often termed a **cell strain**. This culture consists of diploid cells, commonly fibroblastic, that can be redispersed and regrown a finite number of times;

# Examples

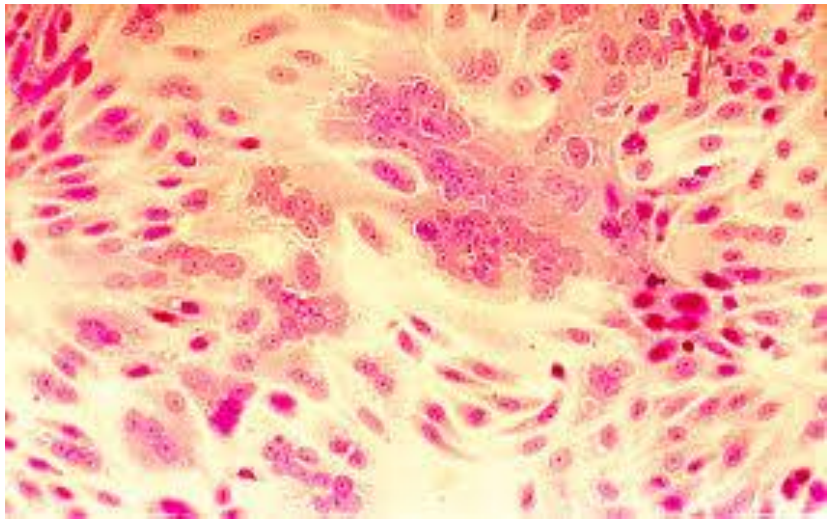
- Monkey and human embryonic kidney cell cultures are examples of commonly used primary and secondary cell cultures.
- Human embryonic tonsil and lung fibroblasts are common cell strains in routine diagnostic use.
- A common cell line in diagnostic use is the *Hep*-2, derived from a human epithelial carcinoma.

# Detection of Viral Growth

- The most common effect is seen with lytic or cytopathic viruses; as they replicate in cells, they produce alterations in cellular morphology (or cell death), which can be observed directly by light microscopy under low magnification (30x or 100x).

# Types of Cytopathic Effects

**Measles and respiratory syncytial viruses cause fusion of cells to produce multinucleated giant cells (syncytia).**



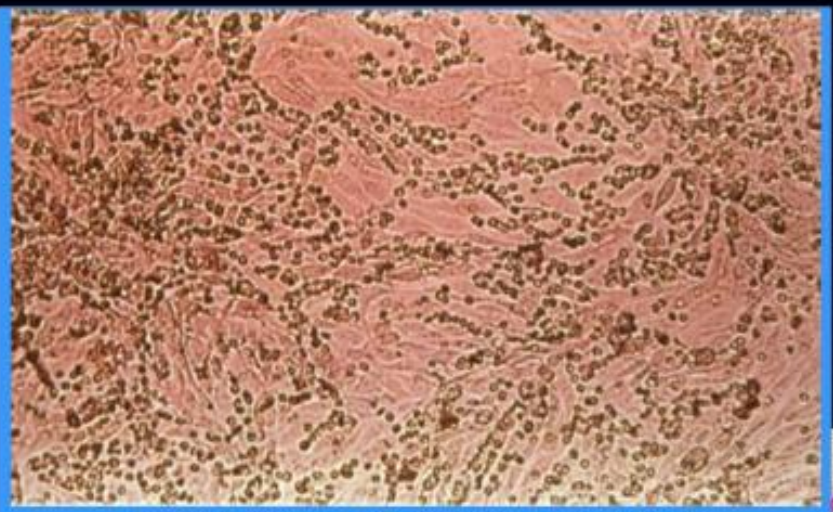
**Enteroviruses often produce cell rounding, pleomorphism, and eventual cell death in various culture systems.**

Fig. 1, Cytopathic effects of enterovirus 71 in rhesus monkey kidney cells

# Detection of Viral Growth

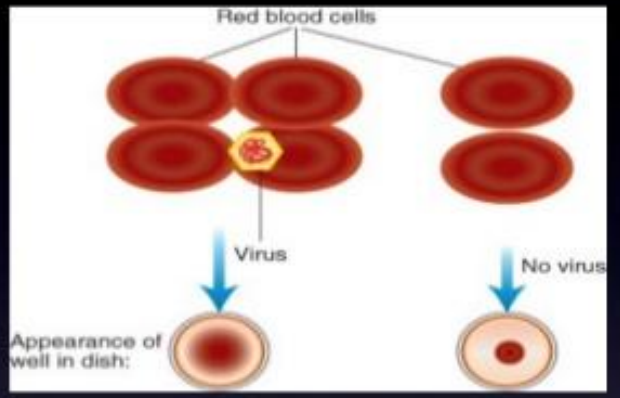
- **Hemagglutinins**. These hemagglutinins may be present on the infected cell membranes, as well as in the culture media, as a result of release of free, hemagglutinating virions from the cells. Addition of erythrocytes to the infected cell culture results in their adherence to the cell surfaces, a phenomenon known as **hemadsorption**.

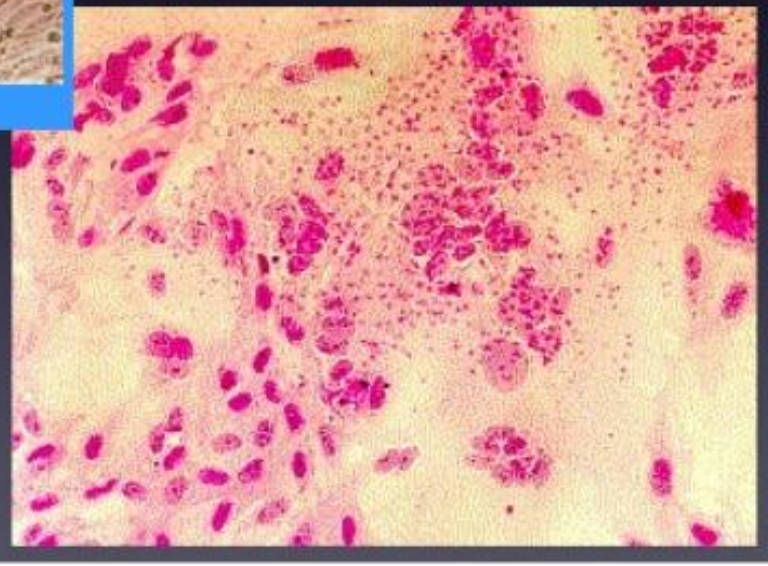
# HEMADSORPTION TEST



Hemadsorption - influenza virus infected cells

MURRAY, 2nd Ed., Fig 53.5



*Hemadsorption of red blood cells onto the surface of a cell sheet infected by mumps virus (Courtesy of Linda Stannard, University of Cape Town).*

# Detection of Viral Growth

- **Interference**. In this situation, the virus that infects the susceptible cell culture produces no CPE or hemagglutinin, but can be detected by "challenging" the cell culture with a different virus that normally produces a characteristic CPE. The second, or challenge, virus fails to infect the cell culture because of interference by the first virus, which is thus detected. This method is obviously cumbersome, but has been applied to the detection of rubella virus in certain cell cultures.