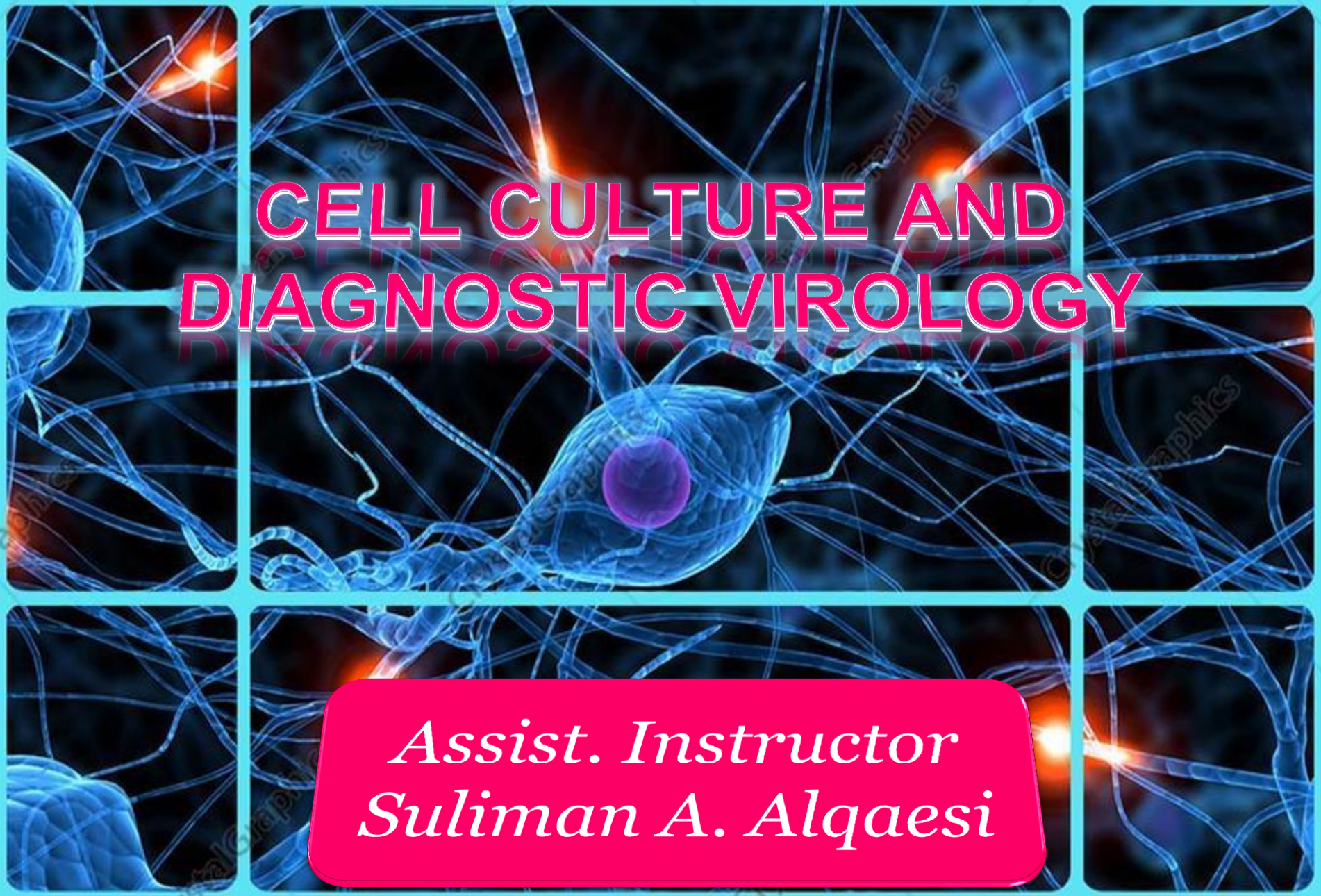


CELL CULTURE AND DIAGNOSTIC VIROLOGY

Assist. Instructor
Suliman A. Alqaesi

CELL CULTURE

- ***Routinely used for growing viruses.***
- ***Is based upon amplification of potentially infectious pathogens.***
- ***Implies intracellular replication of viruses in the cytoplasm or in the nucleus.***

Isolation of Viruses in Cell Culture

- *Viral isolation in cell culture still remains the "gold standard" for many cultivable viruses.*
- *A single cell culture can be used to cultivate a broad spectrum of viral agents.*
- *Viral culture also facilitates the production of high tittered viruses which can be used in:*
  - *Antibody testing*
  - *Viral characterization*
  - *or molecular analysis*

Isolation of Viruses in Cell Culture

- *The ability to culture viruses successfully in the laboratory depends on a number of important factors which include:*
  - ✓ *The sensitivity of the cells used.*
  - ✓ *The viability of the virus.*
  - ✓ *The type of specimens sent to the laboratory.*
  - ✓ *and The culture conditions.*
- *Even when all these considerations are taken into account, not all viruses can be cultured*
- *There are certain viruses that are very difficult to grow or require very specialized culture conditions*

Types of cell cultures

1. ***Primary cells*** *- e.g. Monkey Kidney. These are essentially normal cells obtained from freshly killed adult animals. These cells can only be passaged once or twice.*

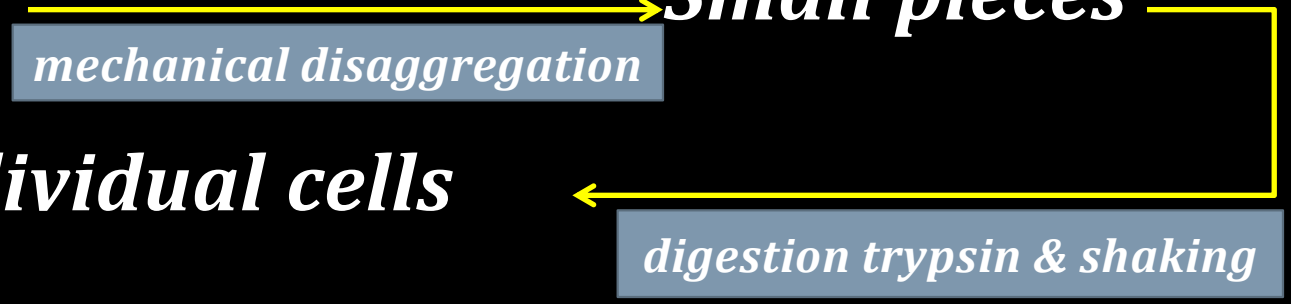
2. ***Semi-continuous cells*** *- e.g. Human embryonic kidney and skin fibroblasts. These are cells taken from embryonic tissue, and may be passaged up to 20 times.*

3. ***Continuous cells*** *- e.g. HeLa (Human cervix cell line), Vero (Vervet monkey kidney), Hep2 (Human epithelioma of larynx). These are immortalized cells i.e. tumour cell lines and may be passaged indefinitely.*

Primary cell culture are widely acknowledged as the best cell culture systems available since they support the widest range of viruses.

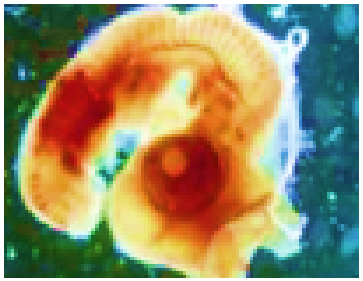
Steps of Cell Culture preparation

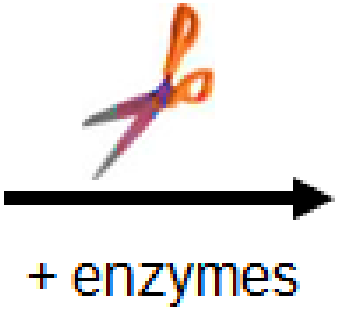
- *Tissues* → *Small pieces* (*mechanical disaggregation*)
- *Small pieces* → *Individual cells* (*digestion trypsin & shaking*)

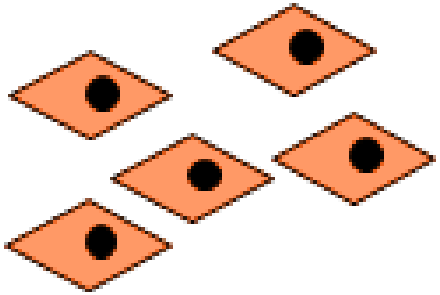


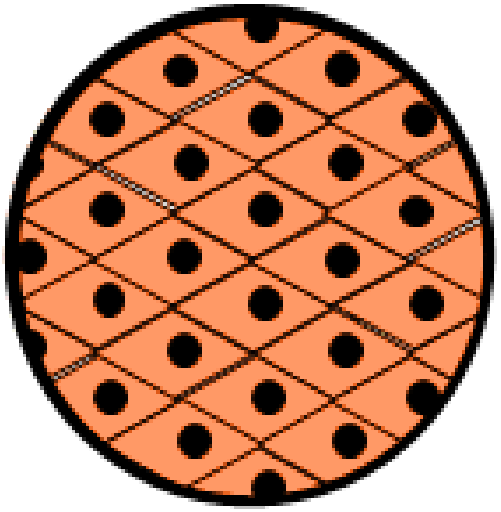
- *Cells are washed & suspended in a growth medium.*
- *Growth medium – Minimum Essential Medium (MEM): essential amino acids, vitamins, salts, glucose & bicarbonate in 5% CO2 with 5% fetal calf or calf serum, antibiotics & phenol red indicator*

Primary cell culture

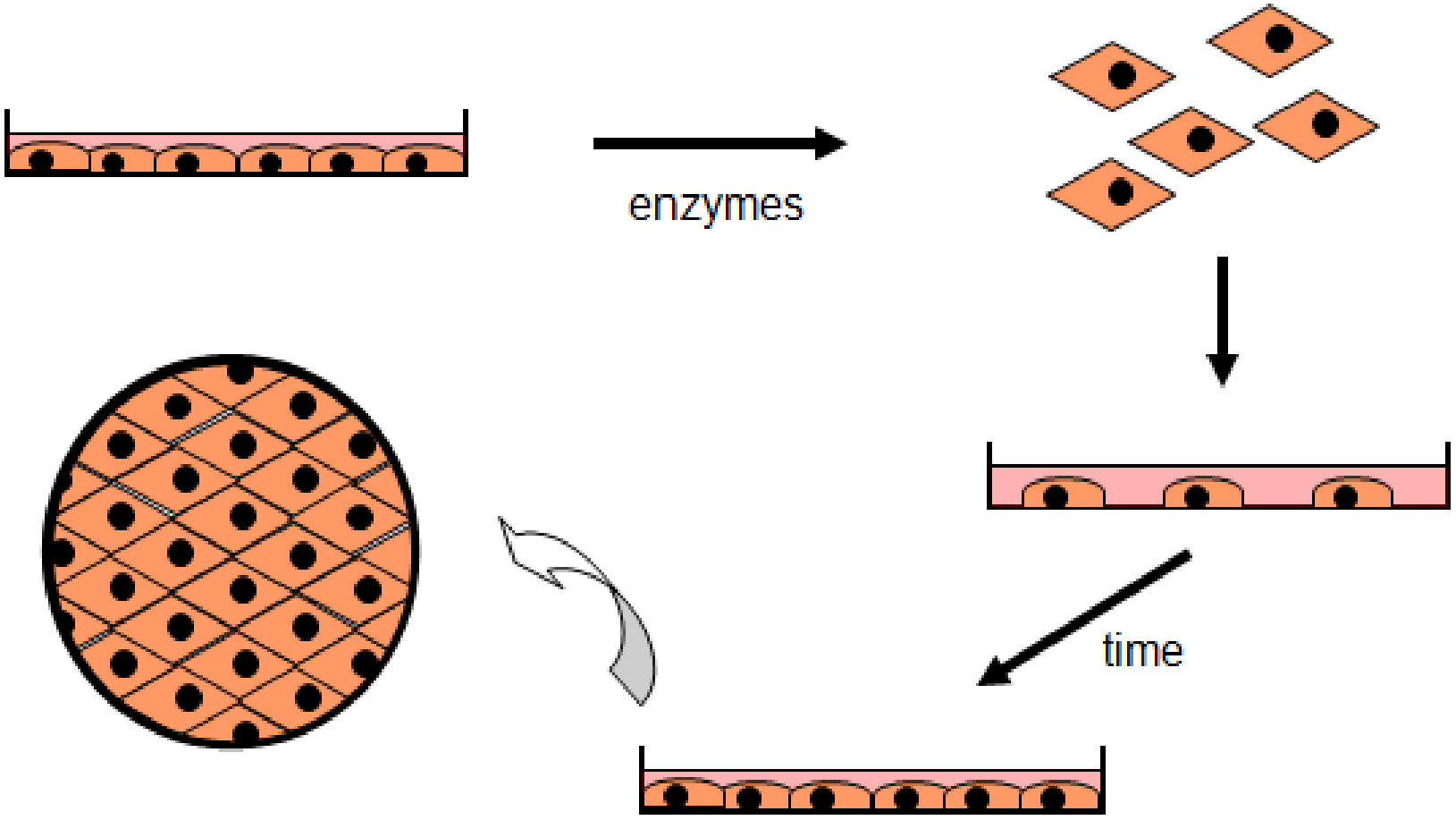








Subculture



Growth media

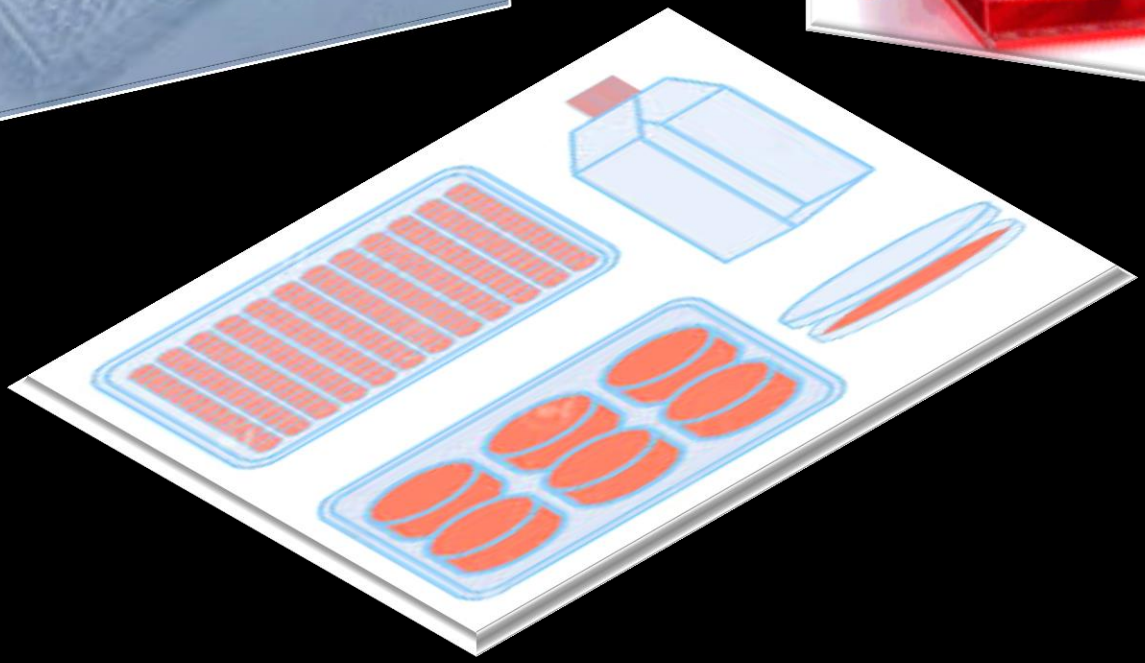
Steps of Growth medium preparation

Dissolving

Filtration

Incubation

Types of vessels use in cell culture



Specimens used to culture viruses

- Blood specimens
  - EDTA
  - Heparin
  - Serum
- Stool , rectal swabs.
- Throat swabs.
- Naso-pharyngeal aspirates.
- Urine
- Saliva
- Cerebro-spinal fluid
- Biopsy
  - Skin
  - Organs (fixation with formaldehyde 10%)

Propagation of viruses

Seeding

- In 96 wells over night
- Cell attachment

Exposure

- Inoculation of virus
- Incubation for 24-72 hrs.

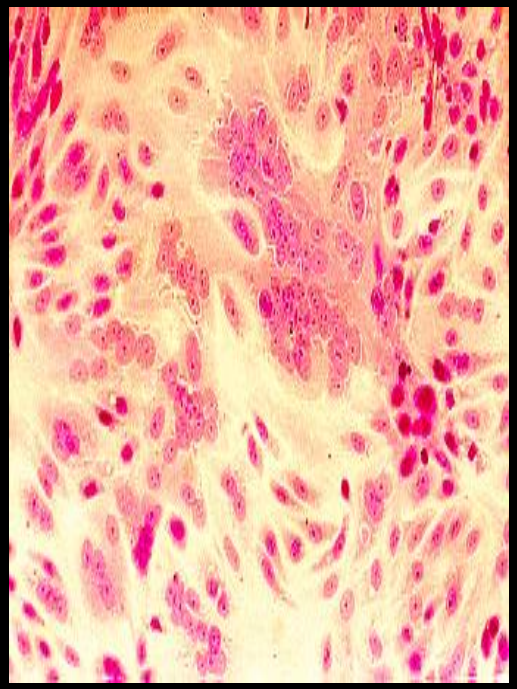
Endpoint

- Viability or Death
- Using specific dye (MTT)

Identification of growing virus

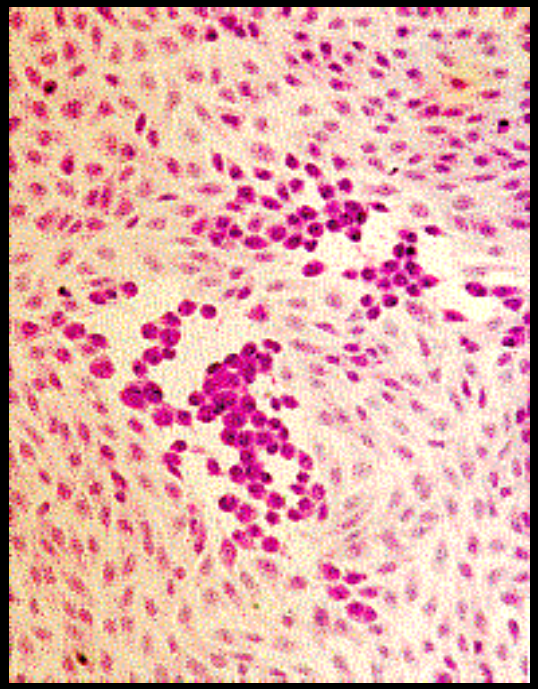
The presence of growing virus is usually detected by:

1. Cytopathic effects (CPE) –



Cell lyses

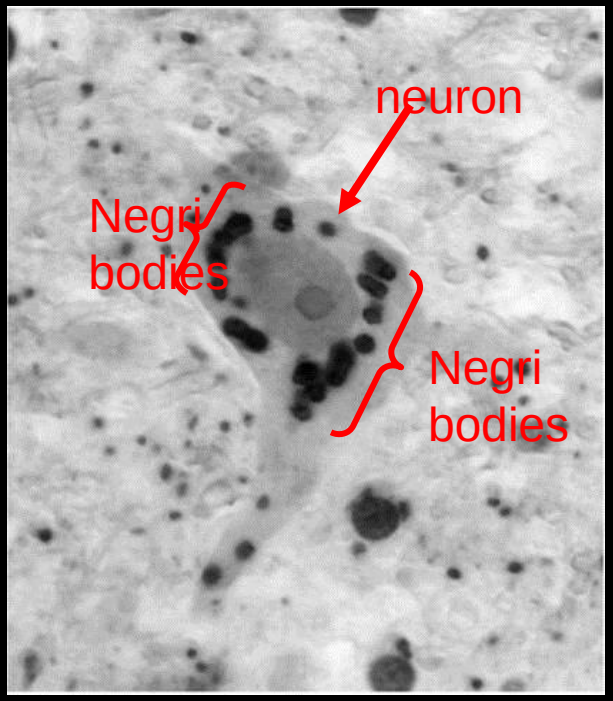
Adeno virus



Cell fusion

Formation of multinuclear giant cells

(e.g. Measles, H S V)

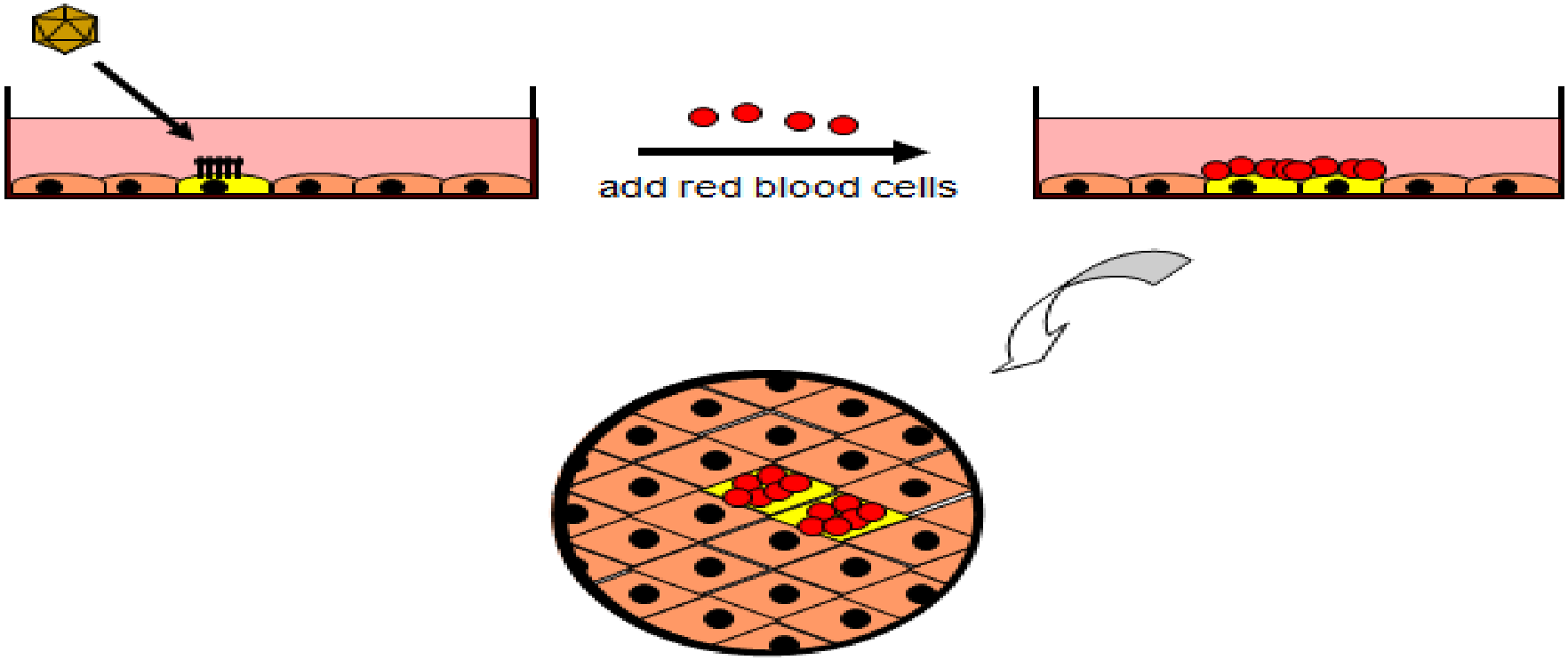


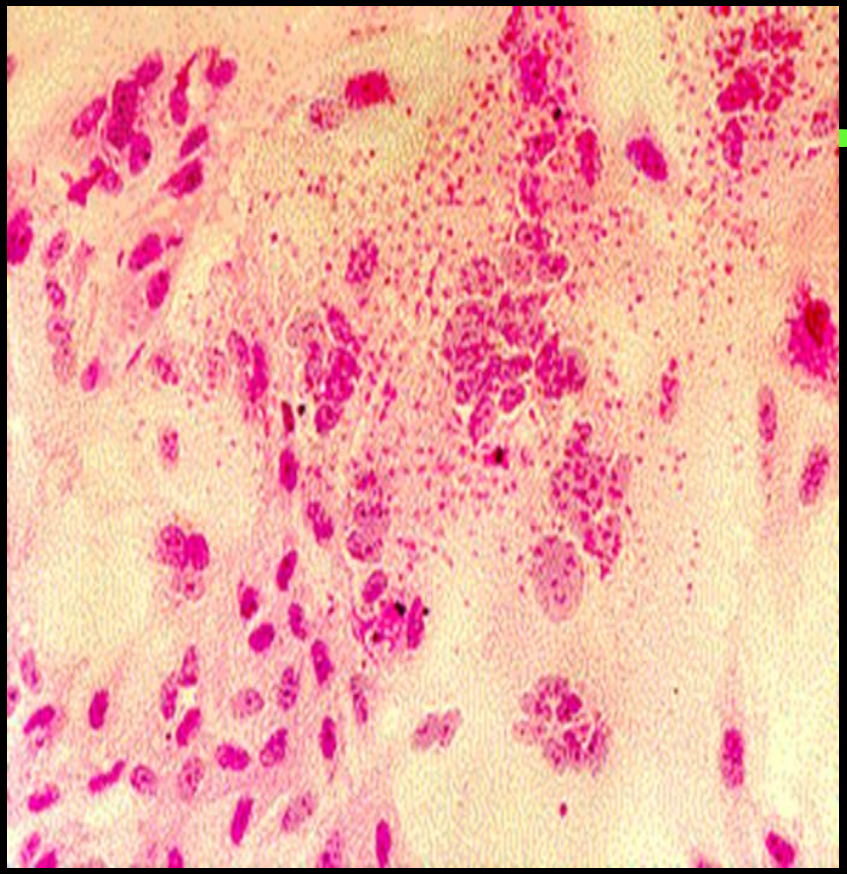
Inclusion bodies

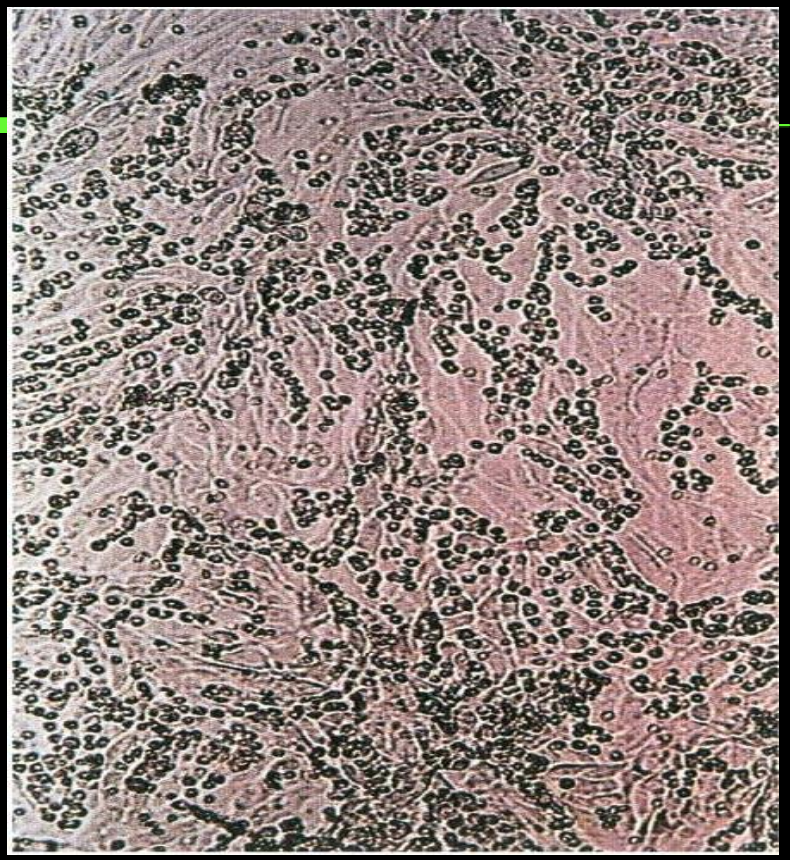
Rabies

Identification of growing virus

2. Haemadsorption – infected cells acquire the ability to stick to mammalian red blood cells.
Haemadsorption is mainly used for the detection of influenza and parainfluenza viruses.







Hemadsorption of erythrocytes to cells infected with influenza viruses

Limitations of cultures to identify viruses

1. *Absence of detection system for the agent.*
2. *Inappropriate culture systems.*
3. *Viruses that cannot be cultured.*
4. *A negative viral culture results does not mean that the agent is absent.*

- ***Need of other tests***
- ***PCR can detect the viral genome in absence of the complete virus***

Problems with cell culture

1. *The main problem with cell culture is the long period (up to 4 weeks) required for a result to be available. Also, the sensitivity is often poor and depends on many factors, such as the condition of the specimen, and the condition of the cell sheet.*
2. *Cell cultures are also very susceptible to fungal or bacterial contamination and toxic substances in the specimen.*
3. *Lastly, many viruses will not grow in cell culture at all e.g. Hepatitis B and C, Diarrhoeal viruses, parvovirus etc*

EVERY ENDING is really just a NEW BEGINNING