Diagnosis of Viral Infection

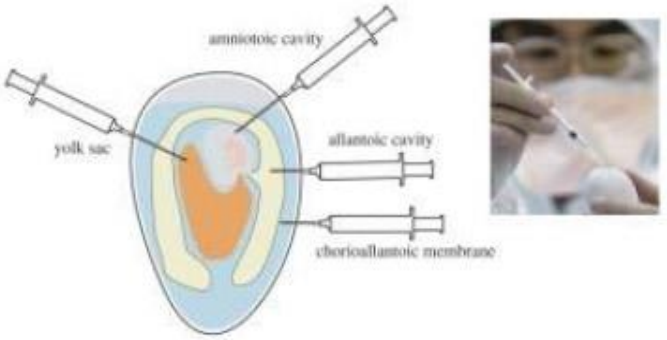
Virus Isolation

- Viruses require a living host cell for replication. Infected host cells (eukaryotic or prokaryotic) can be cultured and grown, and then the growth medium can be harvested as a source of virus.

- Virions in the liquid medium can be separated from the host cells by either centrifugation or filtration. Filters can physically remove anything present in the solution that is larger than the virions; the viruses can then be collected in the filtrate.

Methods for Cultivation of Virus

- Generally three methods are employed for the virus cultivation
  1. Inoculation of virus into animals
  2. Inoculation of virus into embryonated eggs
  3. Tissue culture



Inculation of viruse in embryonated

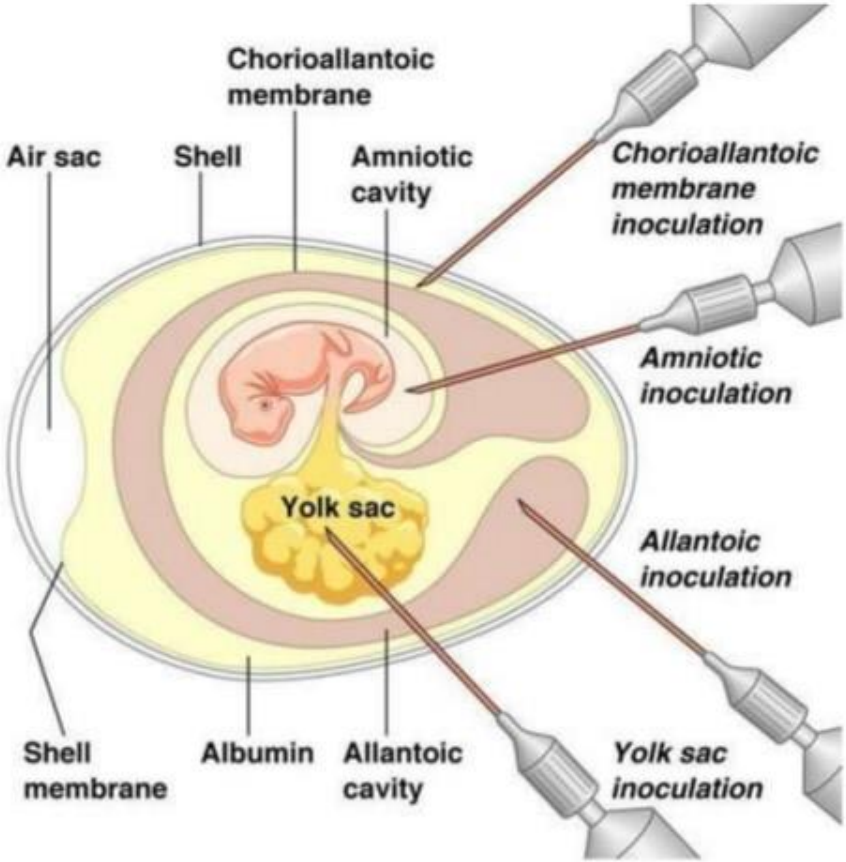
Inoculation of Virus

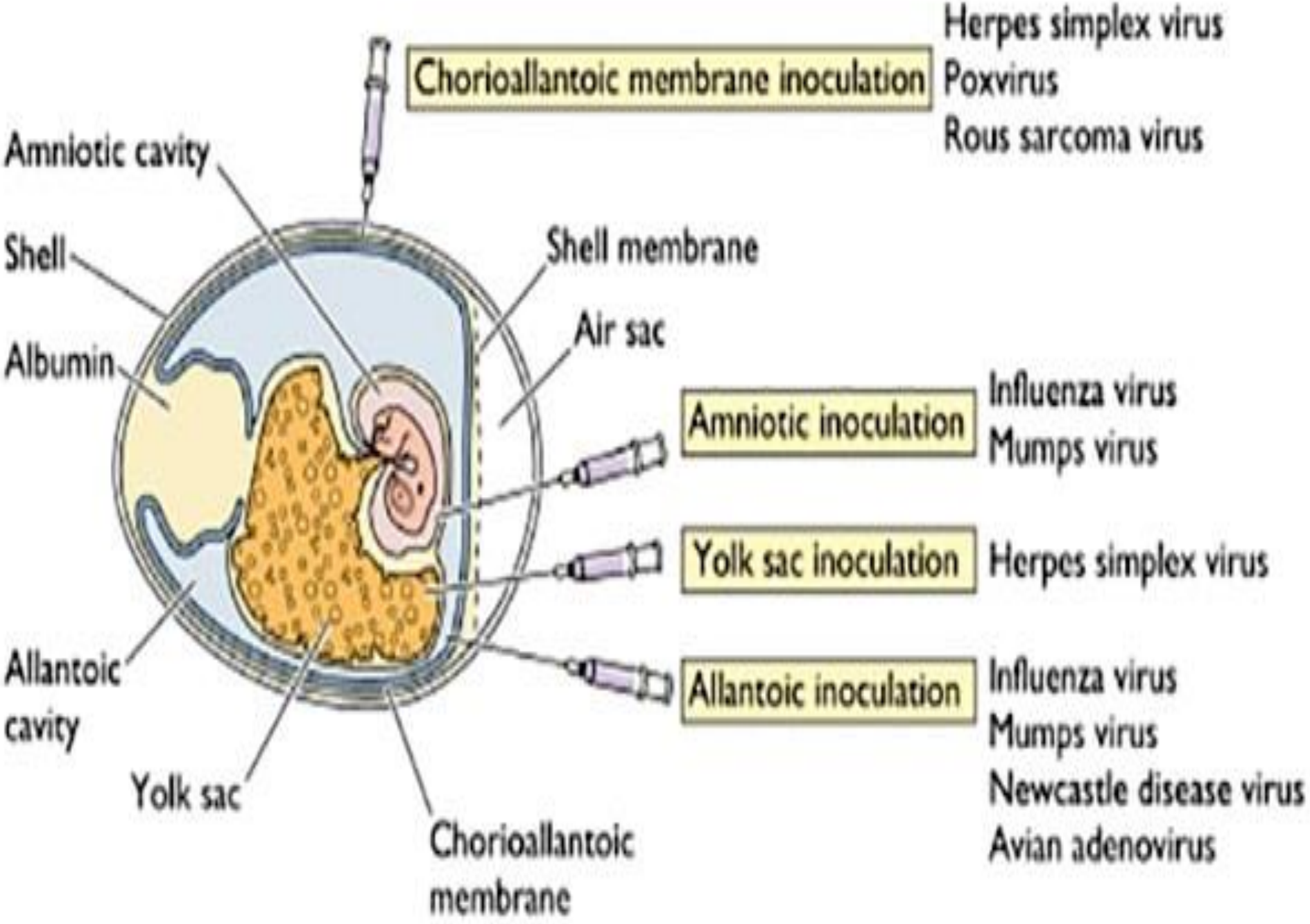
- Chicken, duck, and turkey eggs are the most common choices for inoculation
- The egg used for cultivation must be sterile and the shell should be intact and healthy
- Rigorous sterile techniques must be used to prevent contamination by bacteria and fungi from the air and the outer surface of the shell

- The embryo or host animal serves as an incubator for viral replication . Location within the embryo or host animal is important. Many viruses have a tissue tropism, and must therefore be introduced into a specific site for growth. Within an embryo, target sites include:.

Routes of Viral Inoculation

- An embryonated egg offers various sites for the cultivation of viruses
- The different sites of viral inoculation in embryonated eggs are:
  1. **Chorioallantoic membrane(CAM)**
  2. **Amniotic Cavity**
  3. **Allantoic Cavity**
  4. **Yolk sac**




Chorioallantoic membrane inoculation
Herpes simplex virus
Poxvirus
Rous sarcoma virus
Amniotic cavity
Shell
Albumin
Shell membrane
Air sac
Amniotic inoculation
Influenza virus
Mumps virus
Yolk sac inoculation
Herpes simplex virus
Allantoic cavity
Allantoic inoculation
Influenza virus
Mumps virus
Newcastle disease virus
Avian adenovirus
Yolk sac
Chorioallantoic membrane

USE OF FERTILE EGGS

- Three parts of the egg are of use

- ***A The amniotic cavity:***
- ***B The allantoic cavity:***

- Both are useful for the cultivation of viruses, particularly
  - orthomyxoviruses (e.g., influenza) and paramyxoviruses (e.g., mumps).

C The chorio-allantoic membrane : Dermatropic viruses (poxviruses and some herpes viruses) will grow on this membrane, and at low concentrations, will give discrete foci of infection which consist of centres of cell proliferation and necrosis (pocks). The membrane may therefore be used to assay these viruses. In addition, different viruses cause pocks of different colour and morphology, and this is of diagnostic value for distinguishing between different poxviruses.

cell culture

- Is the process by which cells are grown under controlled conditions, isolated from living tissue they can subsequently be maintained under carefully controlled conditions. consist of:

✓ Growth medium or culture medium is a solid, liquid, or semi-solid .

✓ Essential nutrients (amino acids, carbohydrates, vitamins, minerals), growth factors, hormones, and gases (CO2, O2)

Virus isolation and cultivation

In cell culture prepared as monolayer

Primary cultures –unable to grow for more than a few passages in culture; monkey, rabbit kidney, embryonic cells.

Diploid cell lines –semi –continuous, up to 50 passages, Embryonic tissue origin derived from human, mice fibroblasts, HSV, CMV, VZV, rhinoviruses.

Heteroploid cell lines (established, continuous)- Permanent cell lines Spontaneous mutation of diploid cell lines.

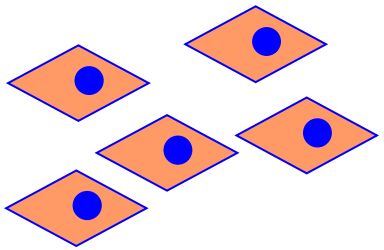
Oncogenic cell lines suspension immortal derived from malignant tissue;(HeLa -human cervix carcinoma and Hep-2 - human larynx carcinoma)

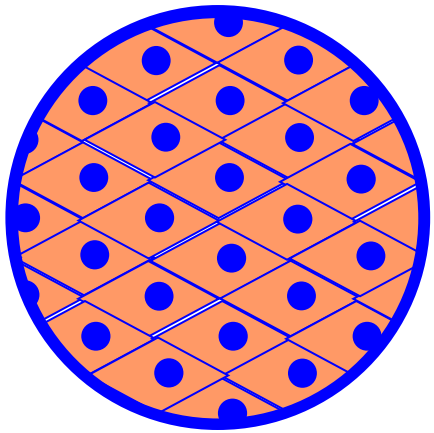
Virus Isolation

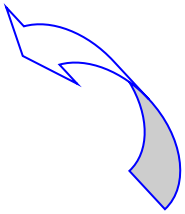
Primary cell culture are widely acknowledged as the best cell culture systems available **since they support the widest range of viruses**. However, they are **very expensive** and it is often **difficult to obtain** a reliable supply. Continuous cells are the most easy to handle but the range of viruses supported is **often limited.**

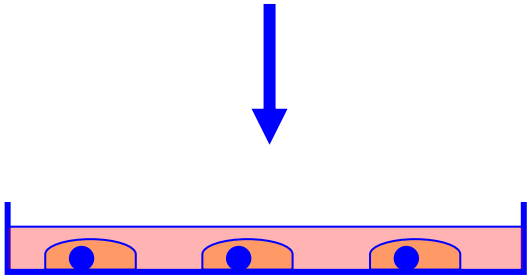
Primary cell culture

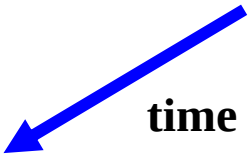
+ enzymes



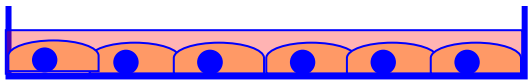




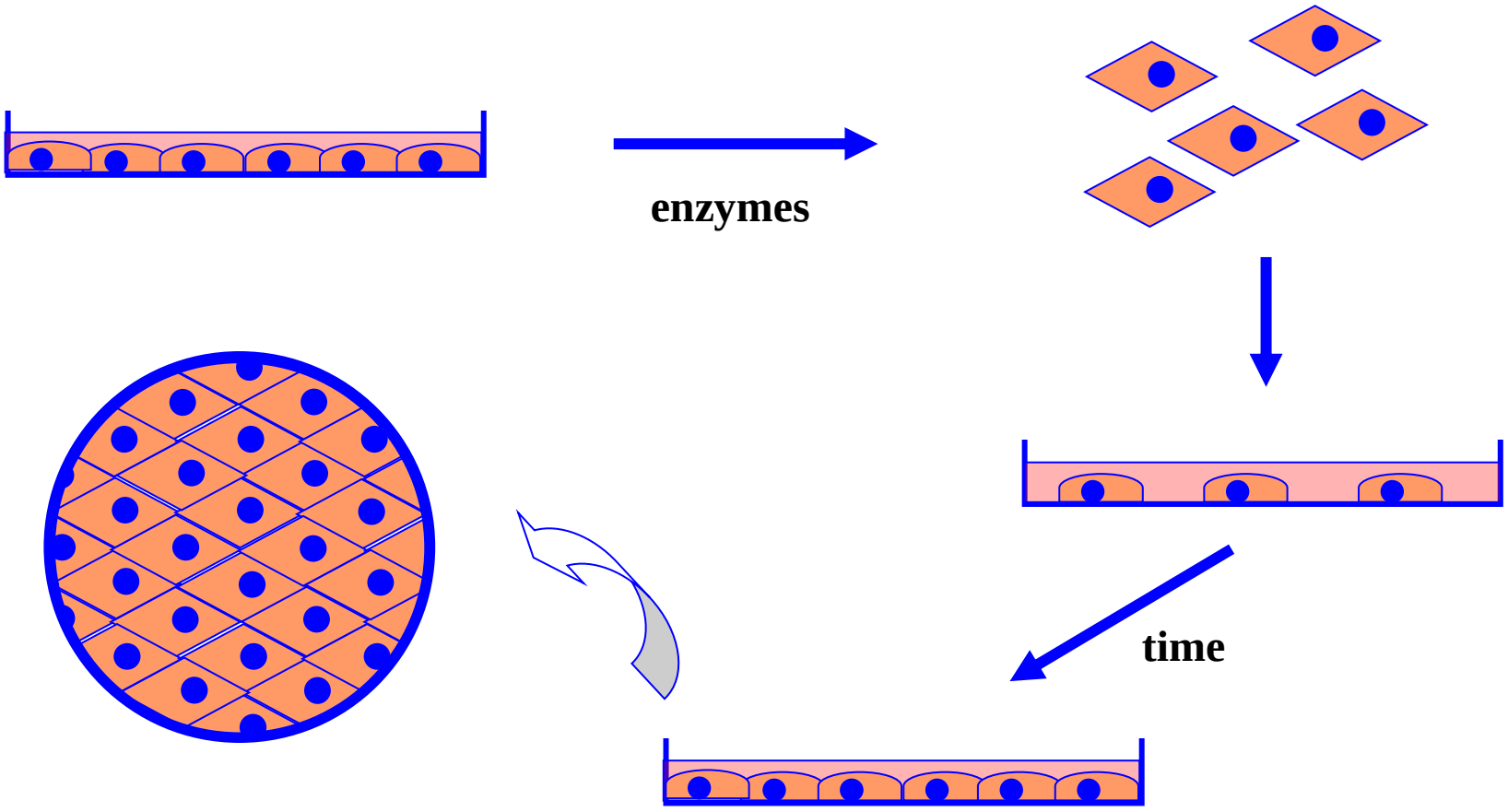




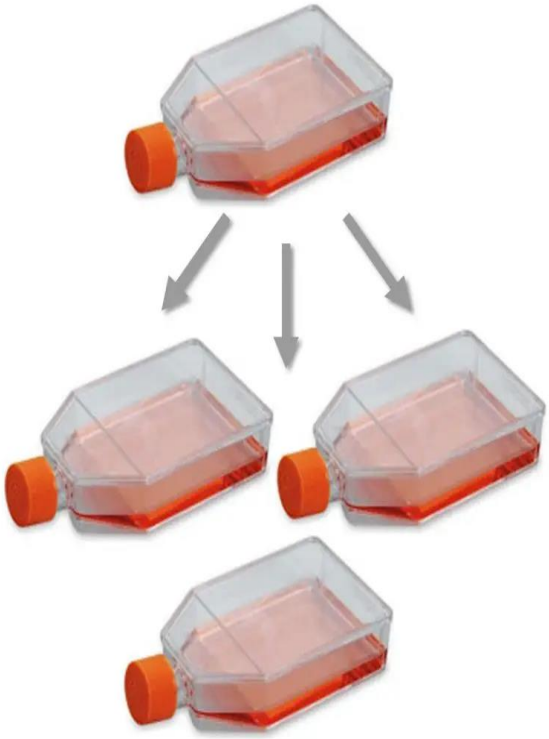
time

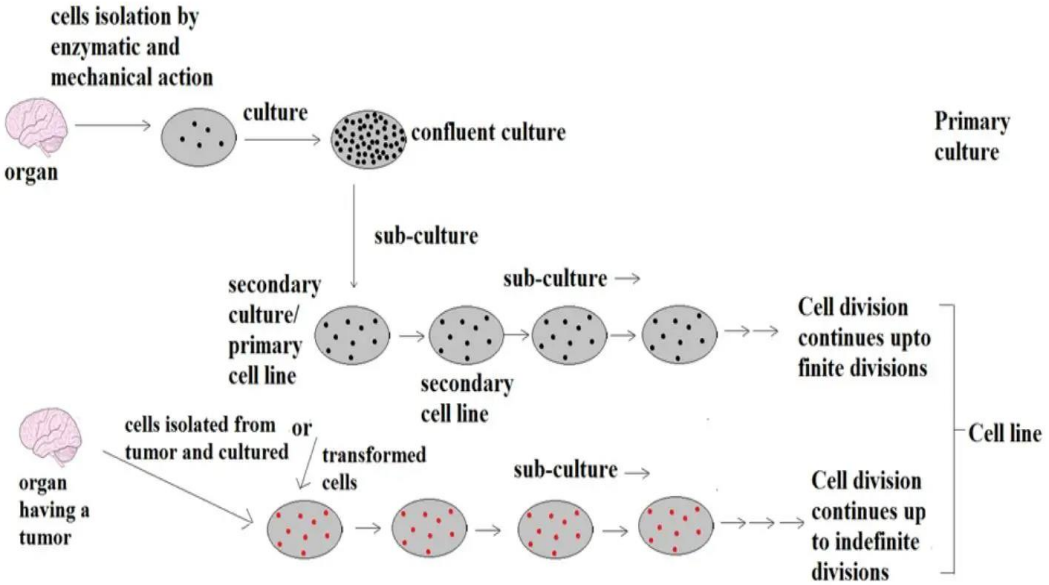


Subculture



Animal Cell Culture

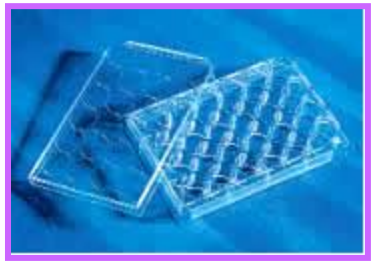






Culture flask



Culture Plate

- virus cultivation is important for :
- 1) identification and diagnosis of pathogenic viruses in clinical specimens
- 2) production of vaccines.
- 3) basic research studies.

In vivo host sources can be a developing embryo in an embryonated bird's egg (e.g., chicken, turkey) or a whole animal. For example, most of the influenza vaccine manufactured for annual flu vaccination programs is cultured in hens' eggs.

Medium Constituents

* **Balance salt solution : Phosphate buffer, Mg^{2+}, Ca^{2+}**

- **Inorganic ions and trace elements**

for membrane potential and osmotic pressure

- **Buffer**

* **Energy source : glucose, glutamine**

* **Amino acid : metabolism and biological synthesis**

Role of Serum

- Buffer, Chelator, Carrier proteins
- Bind to toxin
- Protease inhibitor
- Promotes attachment of cell to substratum
- Source of Intermediate metabolites,

  hormone and growth factor

• Preparation of cell suspension from intact tissue (cont.)

๑. Removal of tissue and place in Isotonic

(or growth medium with antibiotic)

๒. Trim off unwanted parts and cut into smaller pieces

๓. Dissociate cells using enzyme

๔. Remove large debris and wash cells

• Preparation of cell suspension from intact tissue (cont.)

৫. Suspend cells in growth medium accordingly

৬. Pipette into culture vessel and incubate

Common Cell line (cont.)

- **HeLa** : ๑๙๕๒

  : from *Henrietta Lach*; cancer tissue

  : harbors HPV type ๑๘ genome

- **Vero** : ๑๙๖๒

  : from African green monkey kidney

  : preparation of Poliovirus vaccine

Work in Safety cabinet Class-II

: ๓๐ min UV

: ๗๐% ethanol for decontamination

Culture medium and Reagent

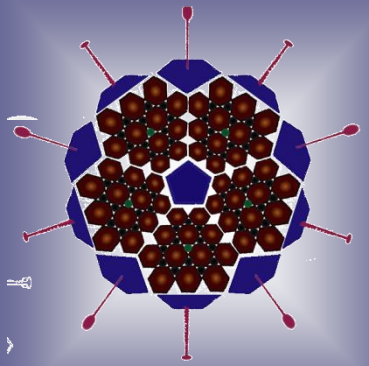
1. (๑x) PBS
2. Growth medium

   : ๕%FCS-DMEMfetal calf ser
3. (๐.๑%) Trypsin-Versene

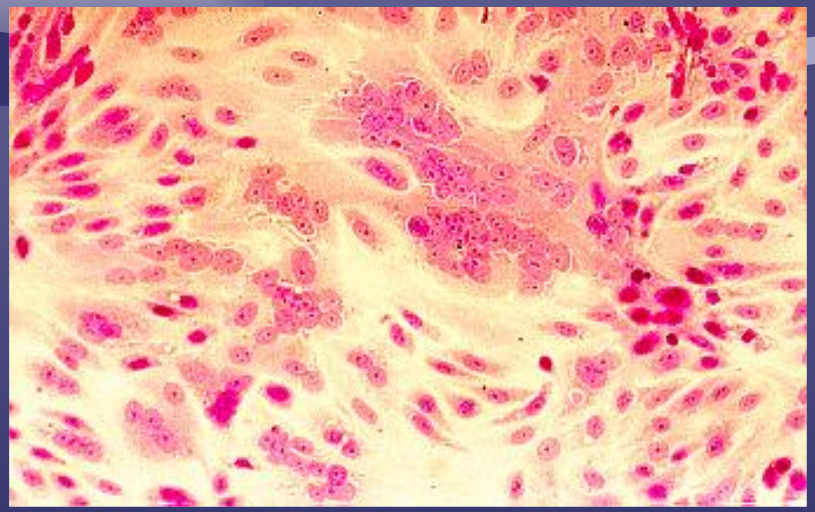
Detection of virus in cell culture:

- **Cytopathic effect -CPE:changed monolayer architecture, the entire cell/ nucleus Cytopathic effect -CPEthickening and shrinking (pycnosis), inclusion bodies formation, giant cell (syncytia)formation, nucleolar displacement,rearangement and margination of the nuclear chromatin,vacuolization,the type of CPE depends on virus specifity and the kind of cells, polio virus, adeno virus, HSV, CMV;**
- **Viral hemagglutination–direct reaction with red cells, influenza;**
- **Hemadsorption –adsorption of erythrocytes to infected cells;parainfluenza, influenza**
- **Plaques effect –clear areas appear in culture (PFU)due to cell lysis;**

- **Enzyme linked immunosorbent assay (ELISA)**
- **Indirect fluorescent antibody (IFA)**
- **Complement fixation (CF)**
- **Latex agglutination (LA)**
- **Chromatography technique:** Column chromatography is one of the most common methods of protein purification. Chromatography is based on the principle where molecules in mixture applied onto the surface or into the solid, and fluid stationary phase (stable phase) is separating from each other while moving with the aid of a mobile phase.

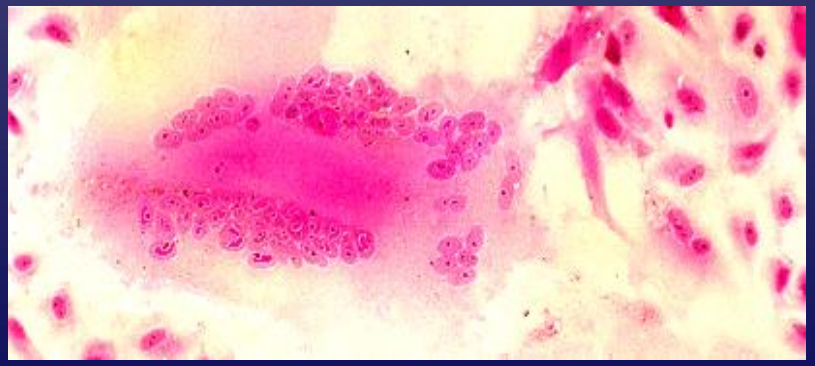


Cytopathic Effect

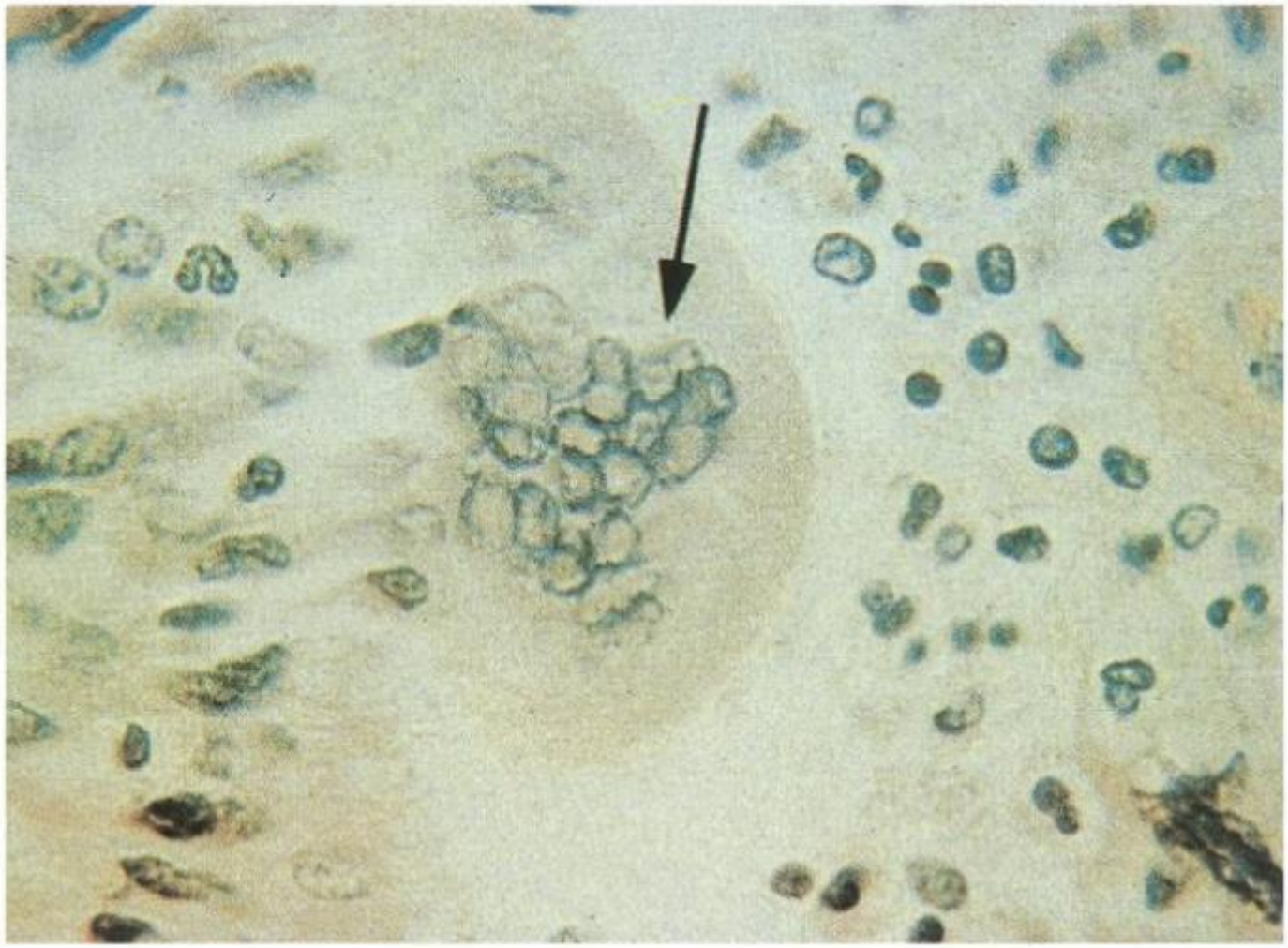


Syncytium formation in cell culture caused by RSV (top), and measles virus (bottom).

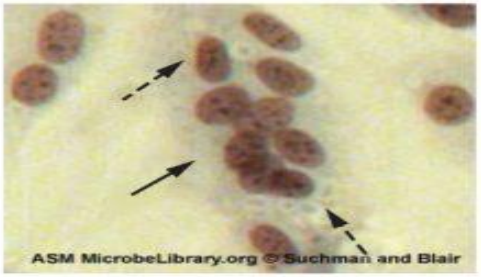
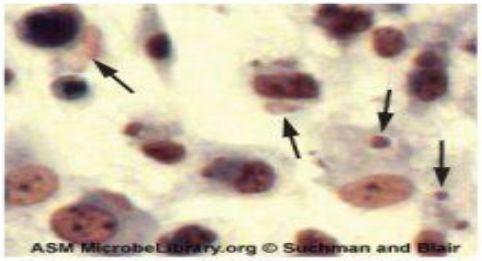
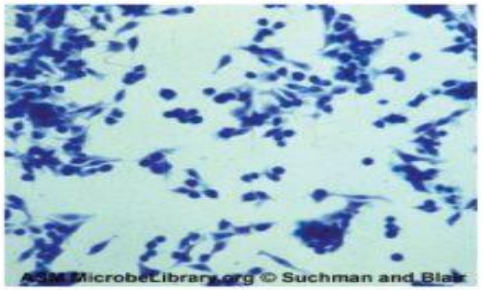
(courtesy of Linda Stannard, University of Cape Town, S.A.)



Cytolology: CPE

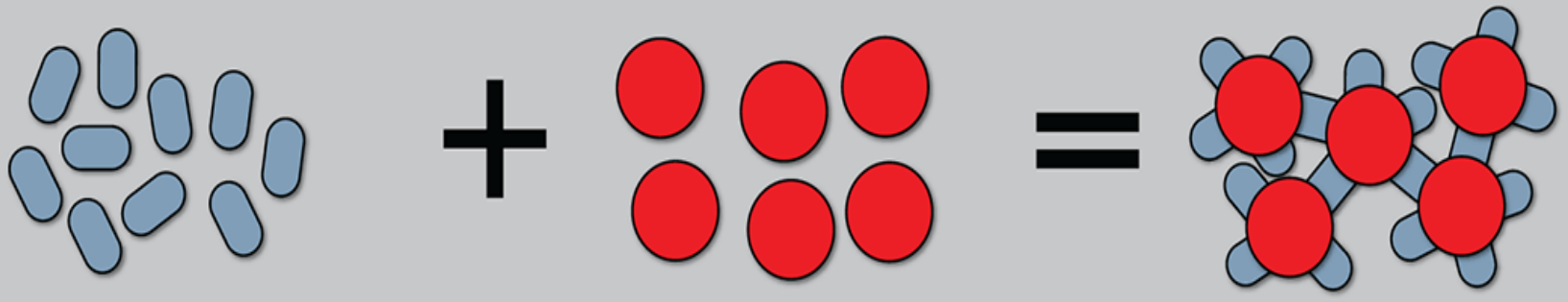


Syncytium formation by measles virus. Multinucleated giant cell (*arrow*) visible in a histologic section of lung biopsy tissue from a measles virus-induced giant cell pneumonia in an immunocompromised child.

Cytopathic Effects of Specific Viruses		
Virus	**Cytopathic Effect**	**Example**
Paramyxovirus	Syncytium and faint basophilic cytoplasmic inclusion bodies	
Poxvirus	Pink eosinophilic cytoplasmic inclusion bodies (arrows) and cell swelling	
Herpesvirus	Cytoplasmic stranding (arrow) and nuclear inclusion bodies (dashed arrow)	
Adenovirus	Cell enlargement, rounding, and distinctive “grape-like” clusters	

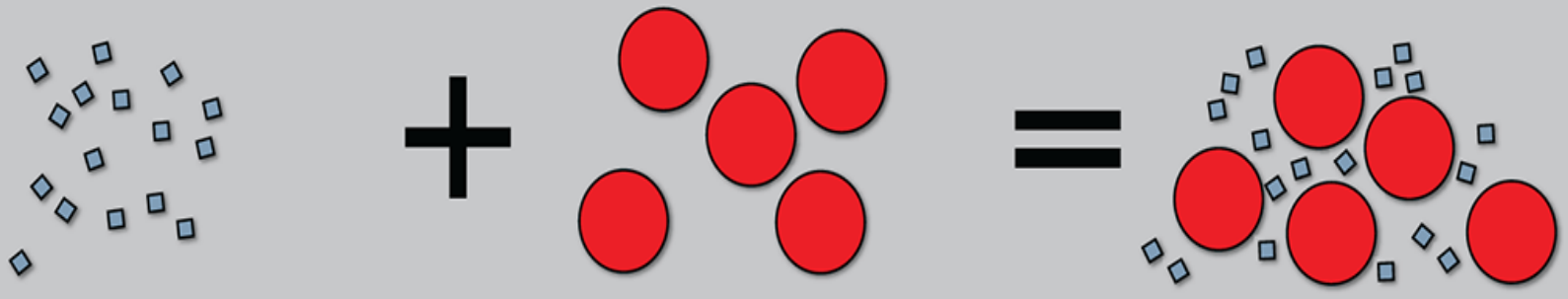
Direct Hemagglutination

Positive Reation:

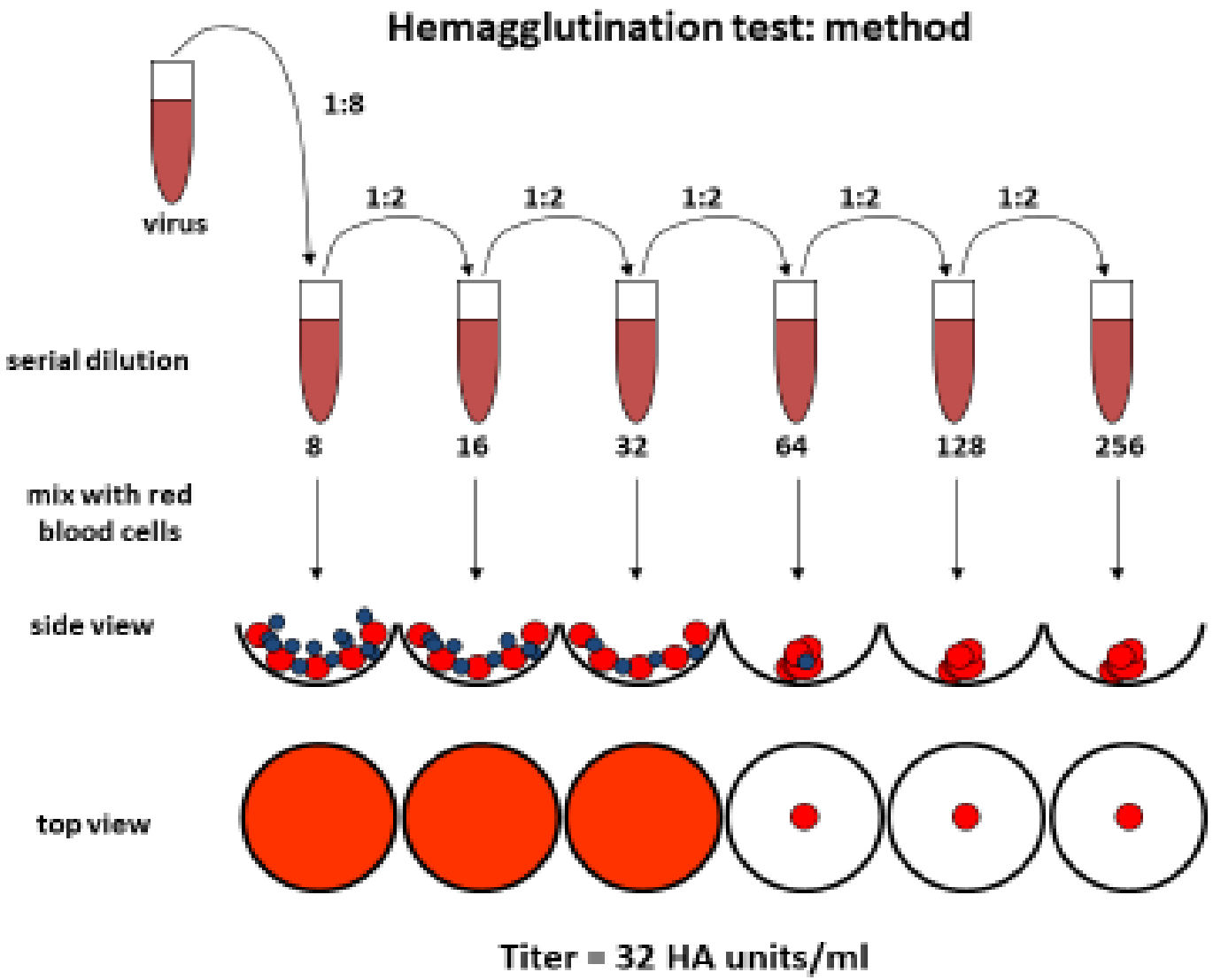


Hemagglutinating virus | Red blood cells | Agglutination

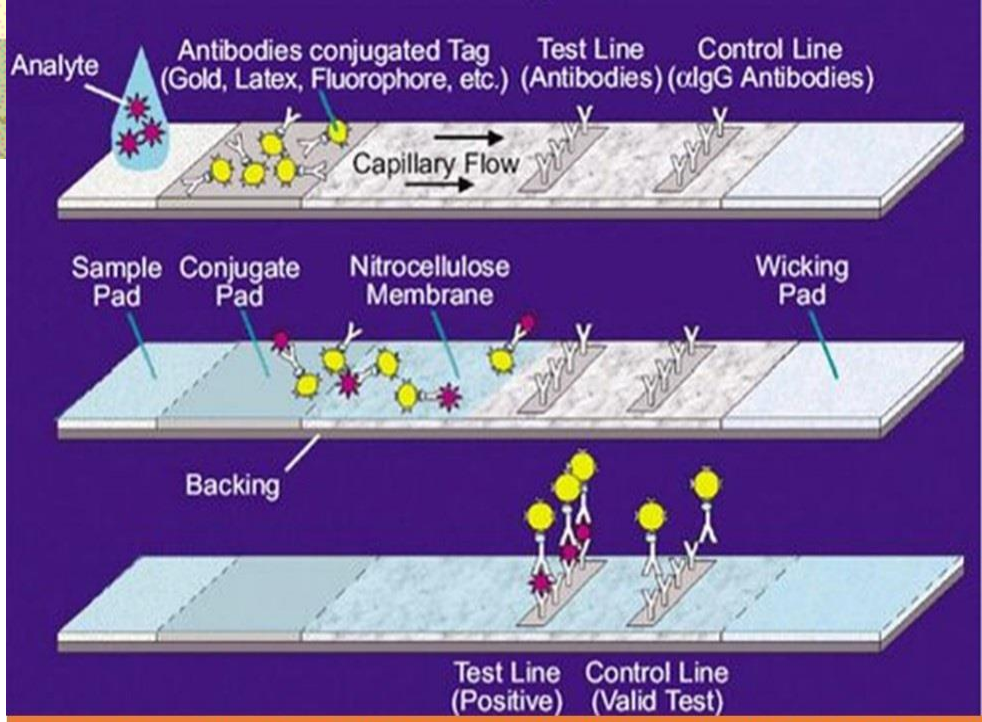
Negative Reation:



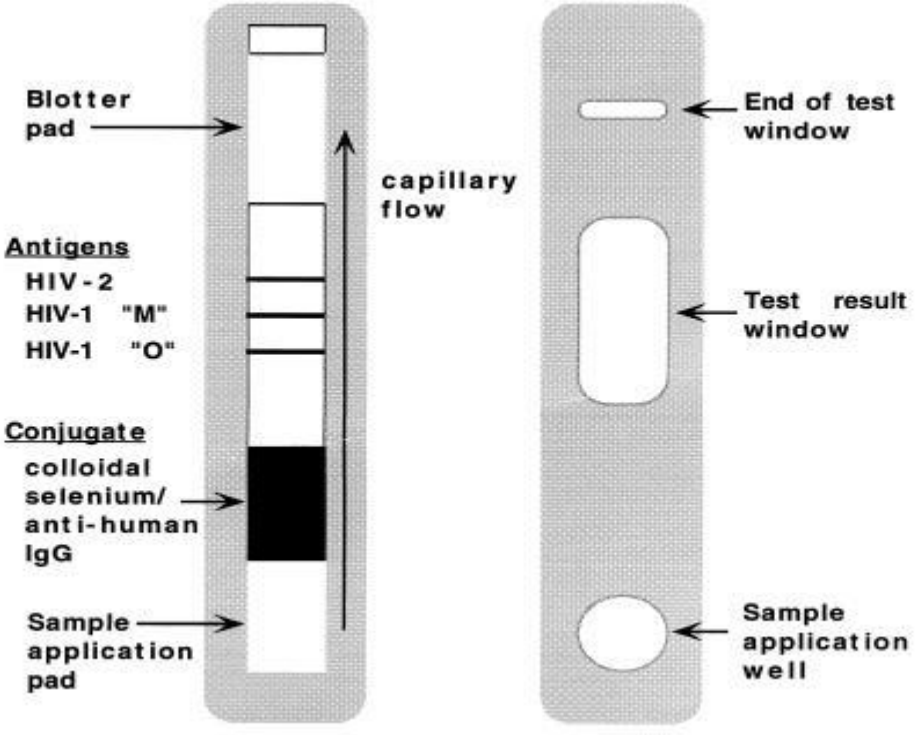
Non-hemagglutinating virus | Red blood cells | No agglutination


Hemagglutination test: method
1:8
virus
1:2
1:2
1:2
1:2
1:2
serial dilution
8
16
32
64
128
256
mix with red blood cells
side view
top view
Titer = 32 HA units/ml

Chromatography



Immunochromatographic Test (Image source: A NASA illustration of a lateral flow assay)




THE END