

VIROLOGY

TECHNIQUES AND CULTIVATION

- ✓ History
- ✓ Techniques in virology
- ✓ Virus cultivation method

By,
A. Raihanathus Sahdhiyya
I M.Sc. Microbiology

HISTORY OF VIROLOGY

□ late 19th century → the independent work of Dimitri Ivanofsky and Martinus Beijerinck marked the beginning of the field of virology.

□ Causative agent in tobacco plant disease → able to pass through the filter retaining Bacteria

In 1898,

Friedrich Loeffler and Paul Frosch applied the filtration criteria to a disease in cattle known as foot and mouth disease.

LABORATORY TECHNIQUES

1. Detection of a meaningful immune response by immunologic assay
2. Identification of the agent by staining (light and electron microscopy)
3. Isolation and identification of the agent (cell culture or fertile eggs)
4. Detection of viral nucleic acid (probes or amplification).

1. IMMUNOLOGIC ASSAYS

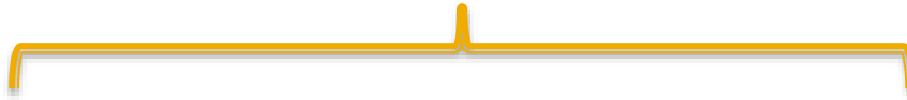
- Virus neutralization (VN)
- Hemagglutination inhibition (HI)
- Enzyme linked immunosorbent assay (ELISA)
- Indirect fluorescent antibody (IFA)
- Complement fixation (CF)
- Agar-gel immunodiffusion (AGID)
- Agar-gel precipitin (AGP)
- Latex agglutination (LA).

Virus neutralization (VN)

sample is incubated with the target virus



changes in cell culture are observed
(cytopathic effect)



contains antibodies



Prevent viral growth



no CPE

no antibodies



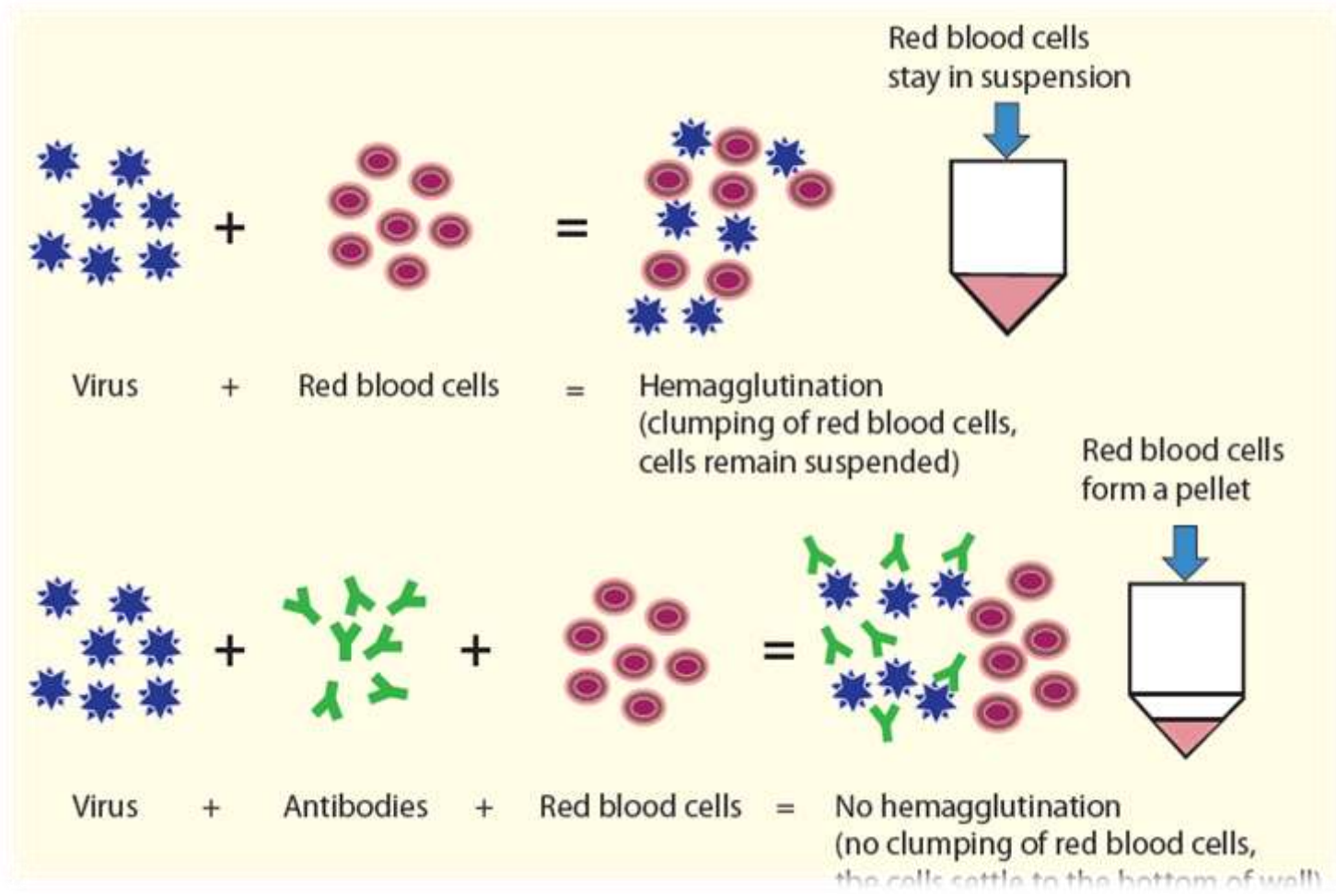
viral growth



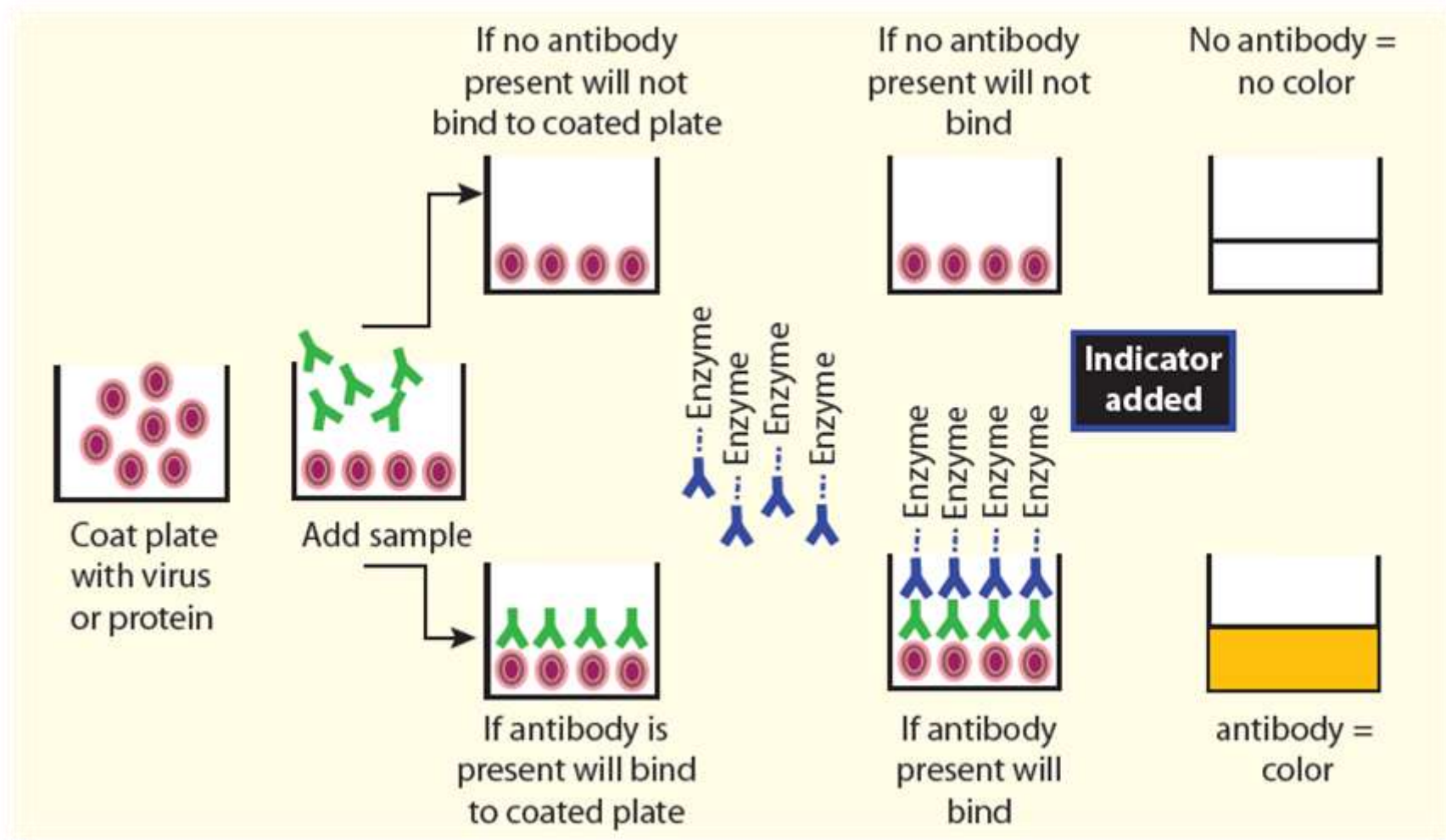
CPE

Hemagglutination inhibition

(HI)



Enzyme linked immunosorbent assay (ELISA)



2. LIGHT & ELECTRON MICROSCOPY

Light Microscopy

Viruses - too small to be seen using a standard light microscope

Antibodies labeled with an **indicator**



visualization of the virus cluster

peroxidase



light microscope

fluorescence



ultraviolet (UV) light microscope

ELECTRON MICROSCOPY

- The magnification -50,000x magnified → provides the ability to see the viral particles

- Problem :

lack of sensitivity (1,000,000 virus particles/ ml of fluid is required)

To improve this, **immune electron microscopy**



sample is incubated with an antibody



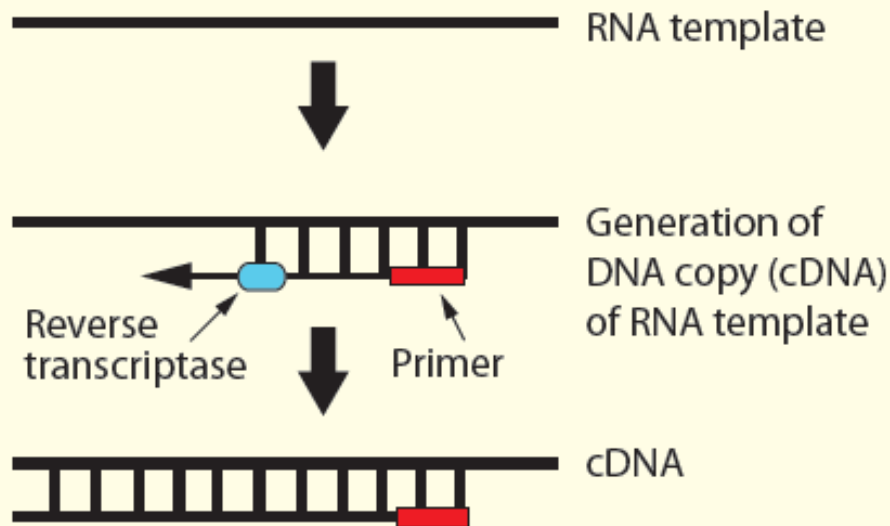
antibody-antigen reaction results in clumps
of the virus



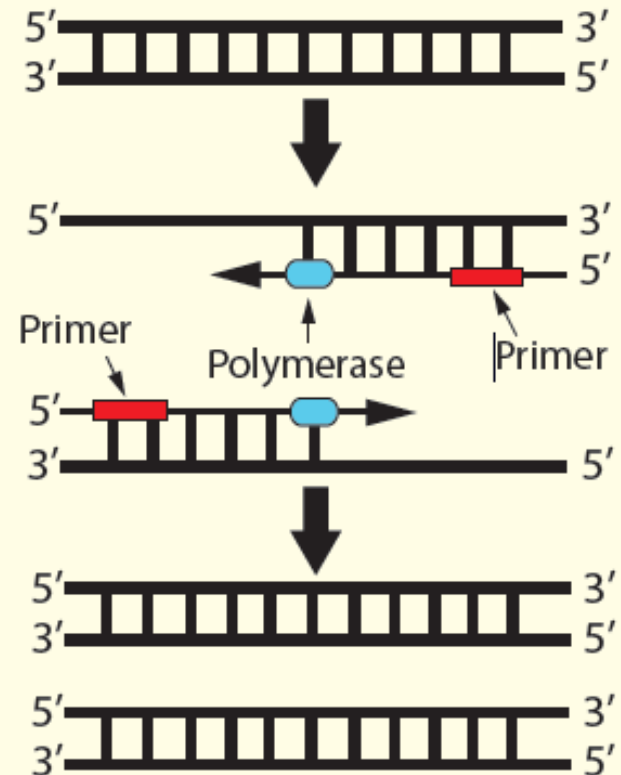
easier visualization.

3. MOLECULAR METHODS

Reverse Transcription (RT)

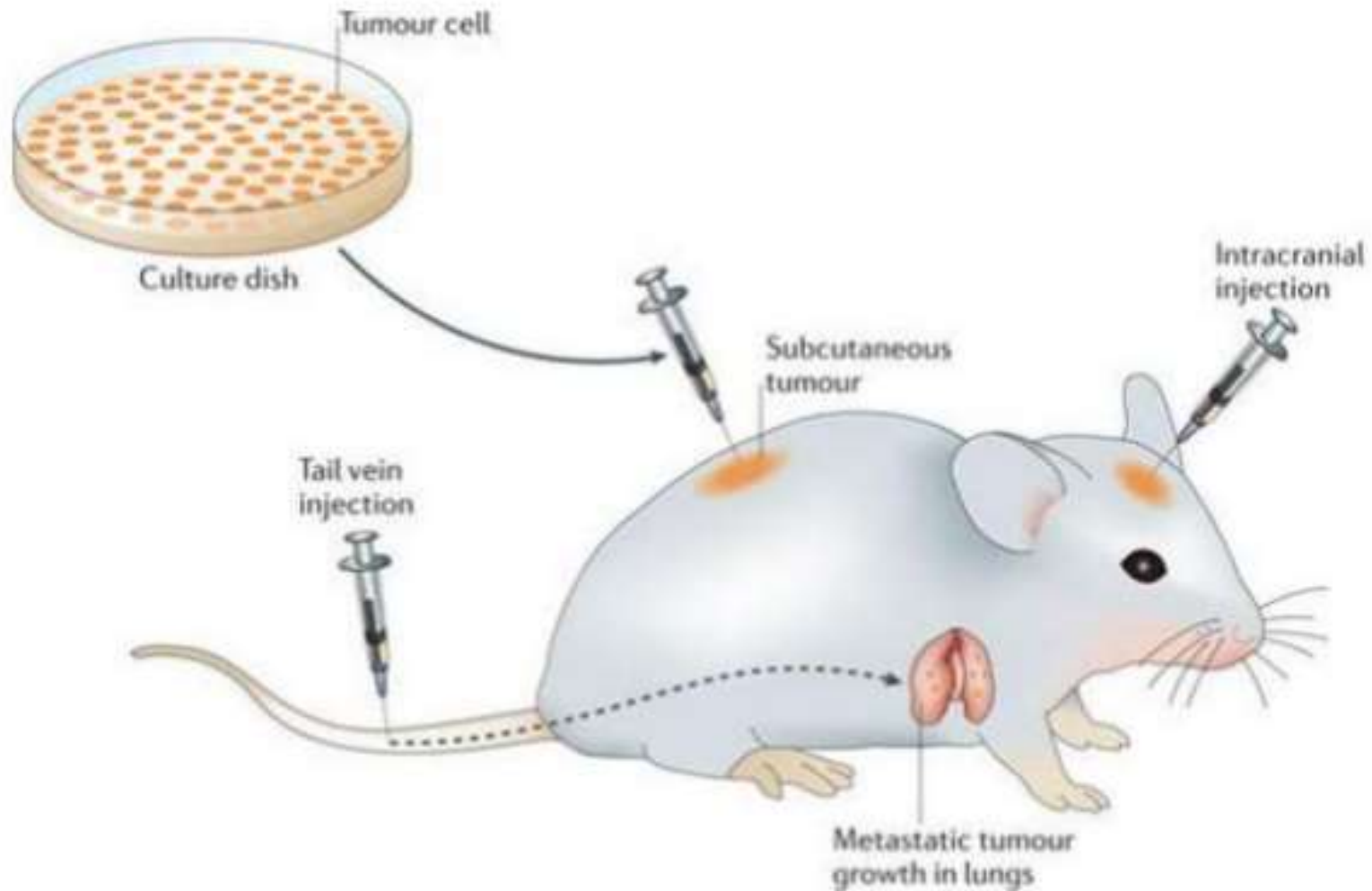


Polymerase Chain Reaction (PCR) Amplification

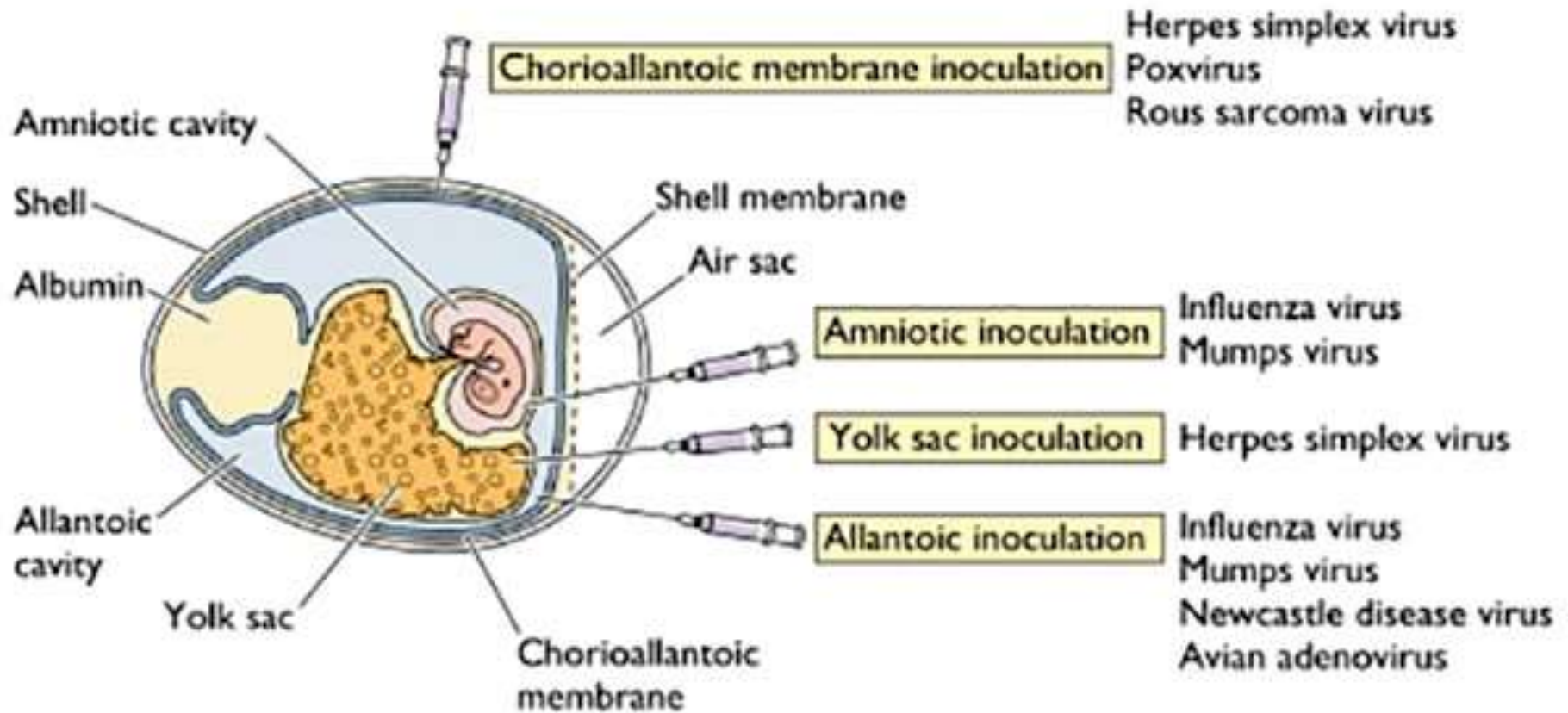


3. VIRUS CULTIVATION METHODS

ANIMAL MODEL

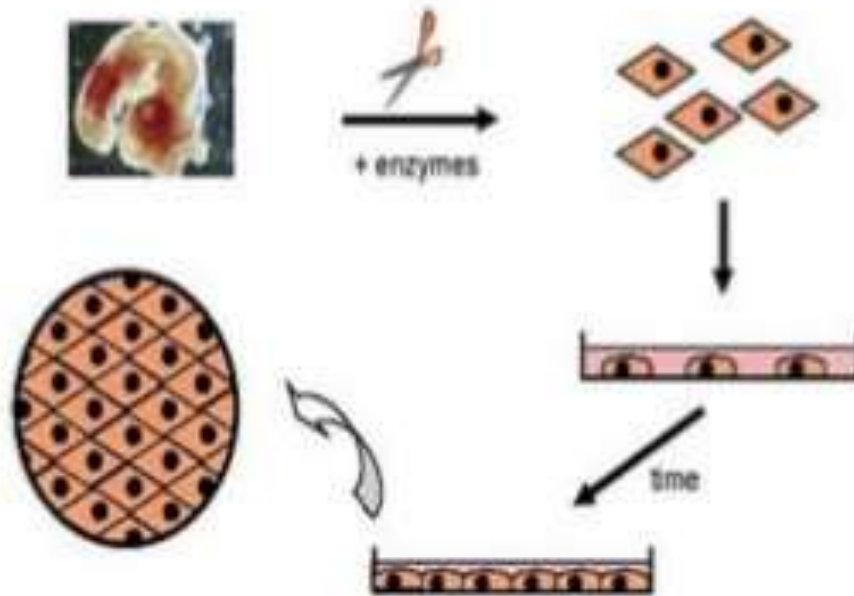


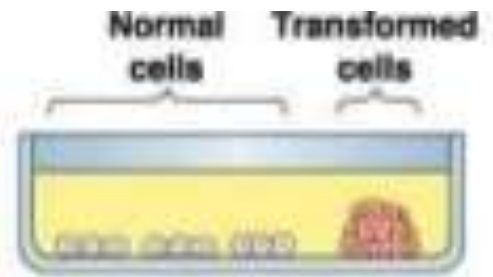
CHICKEN EMBRYO TECHNIQUE



CELL CULTURE

Primary cell culture





1 A tissue is treated with enzymes to separate the cells.

2 Cells are suspended in culture medium.

3 Normal cells or primary cells grow in a monolayer across the glass or plastic container. Transformed cells or continuous cell cultures do not grow in a monolayer.