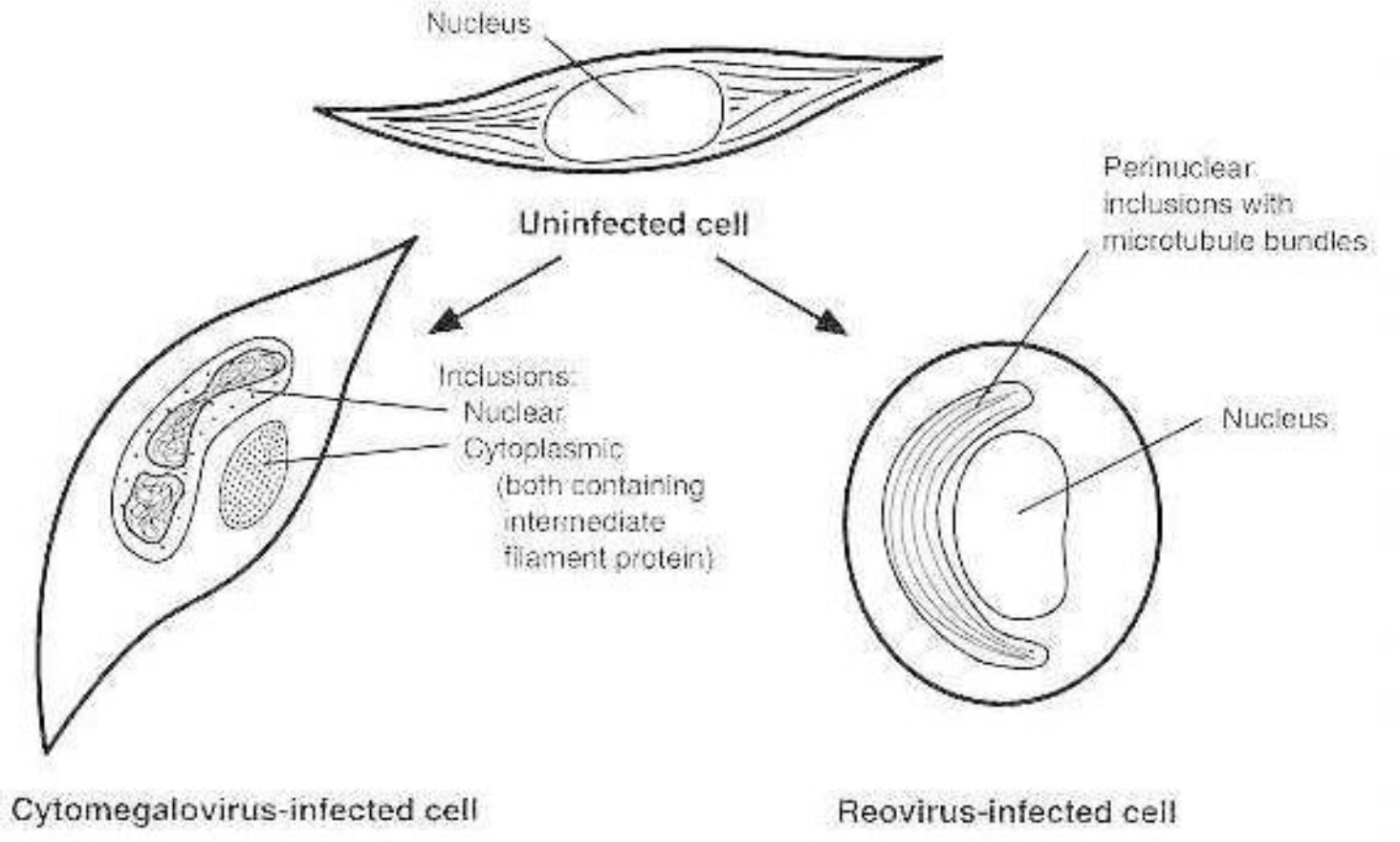
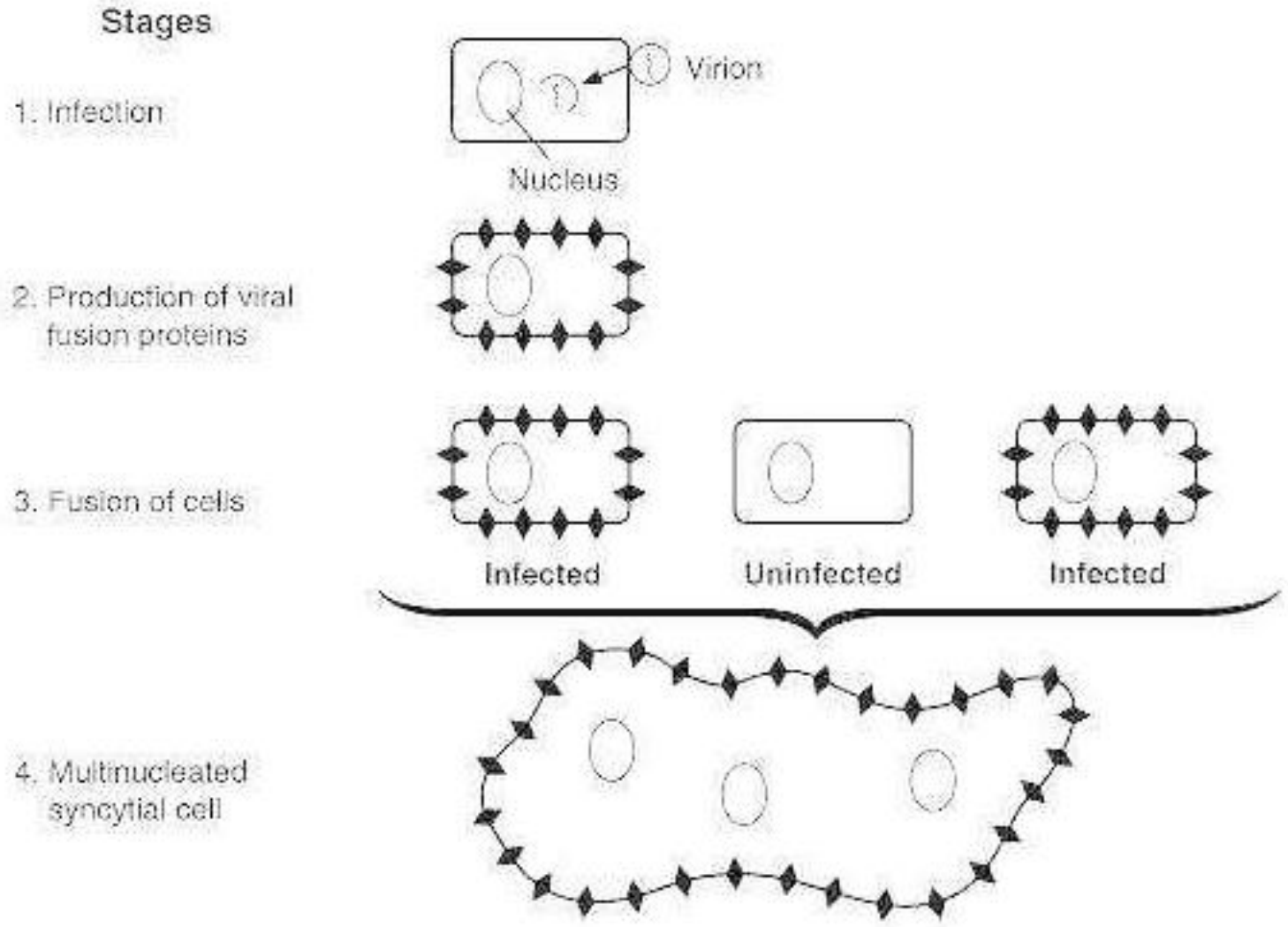
Shell vial assay

- Acute stage of infection
- convalescent stage of infection

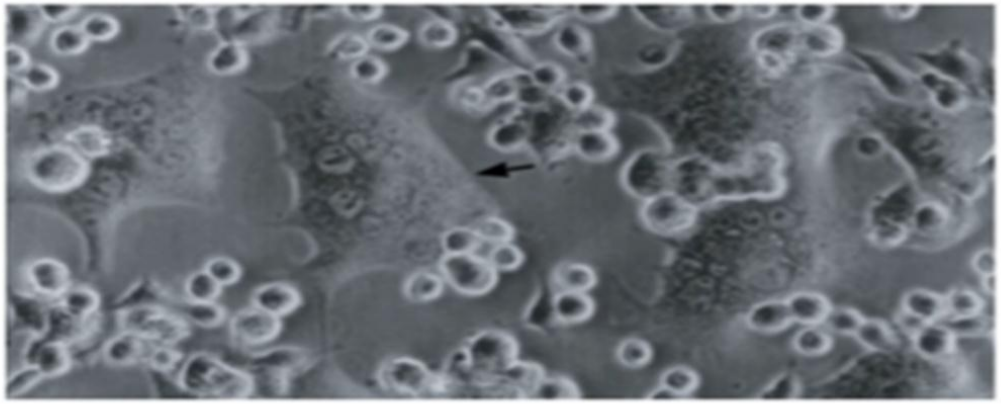
Cytopathic effects (CPE) – morphological changes in cultured cells, seen under microscope, characteristic CPE for different groups of viruses.

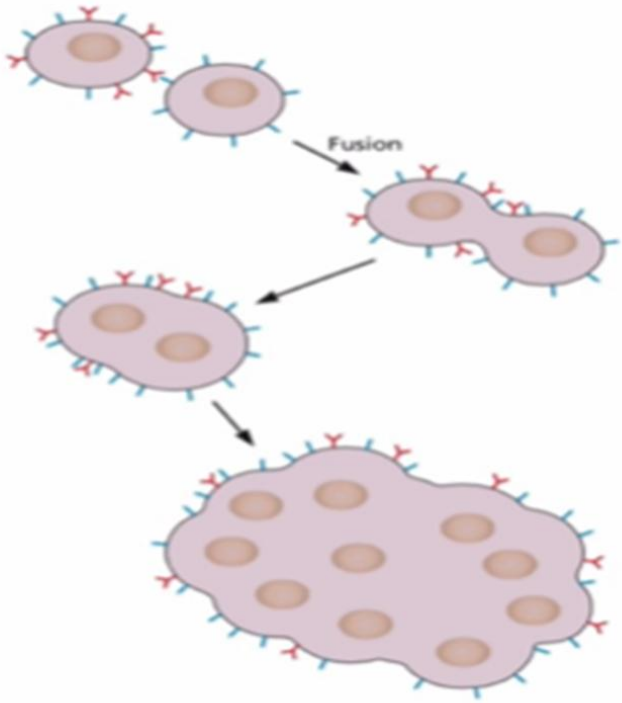


Formation of multinucleated cells



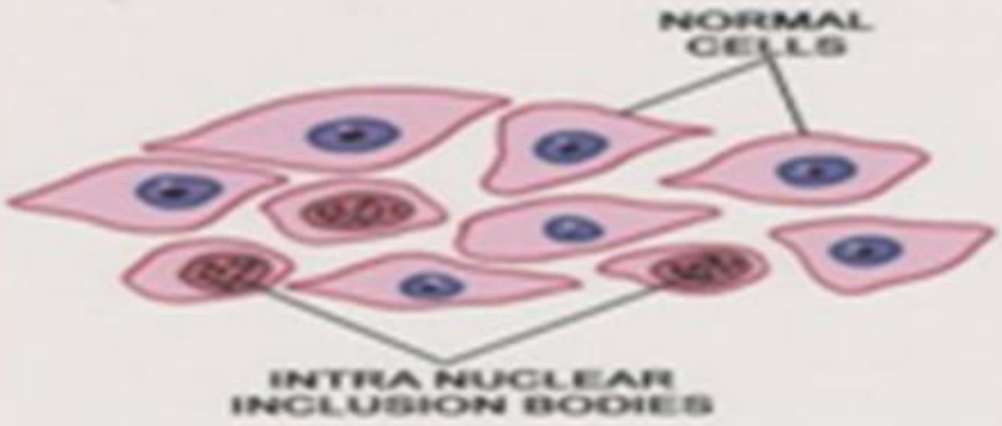
Formation of syncytia





INCLUSION BODIES :

The site of VIRAL multiplication and protien synthesis



INCLUSION BODIES :

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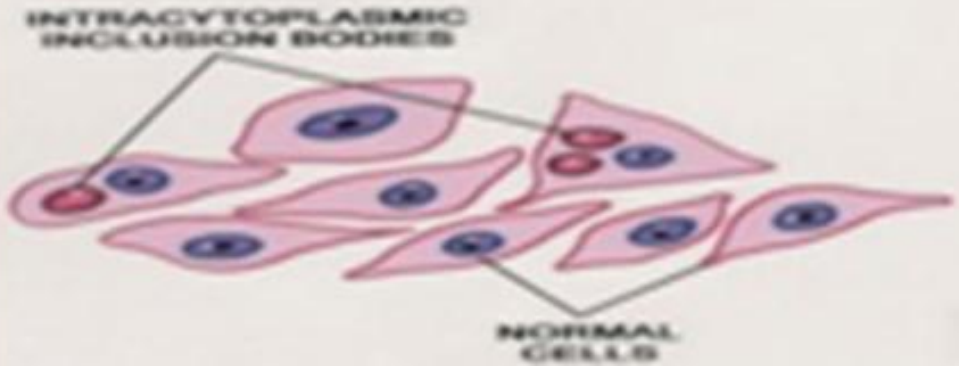
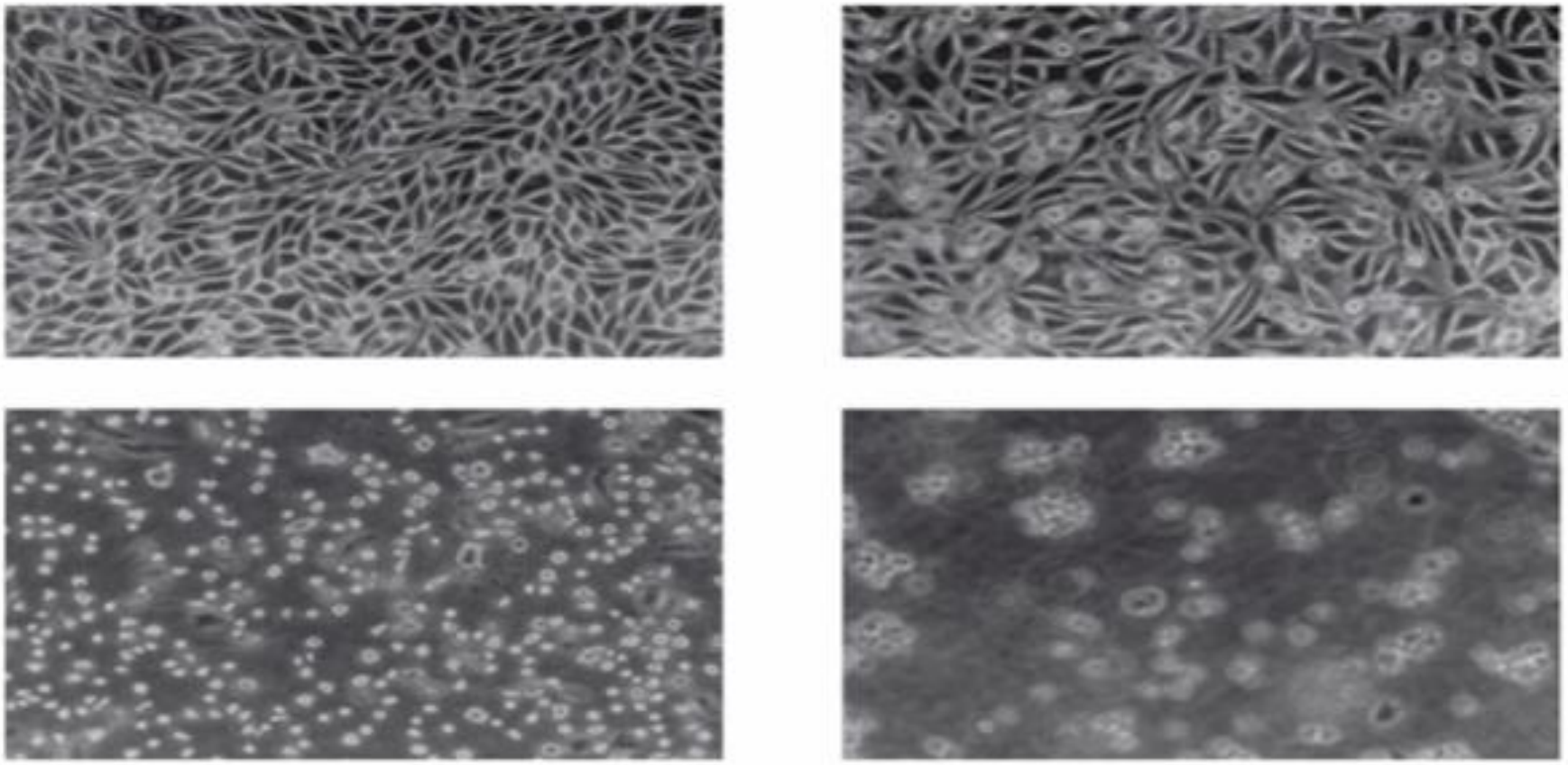
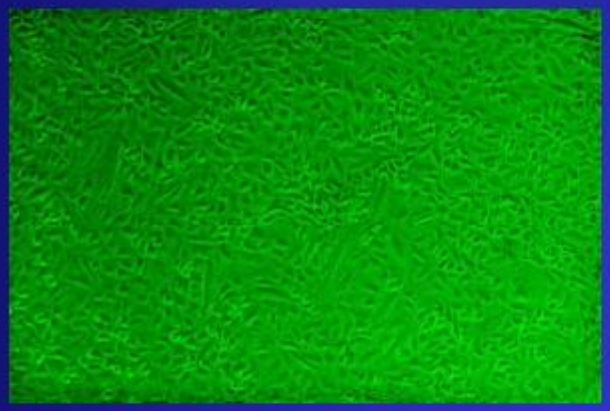




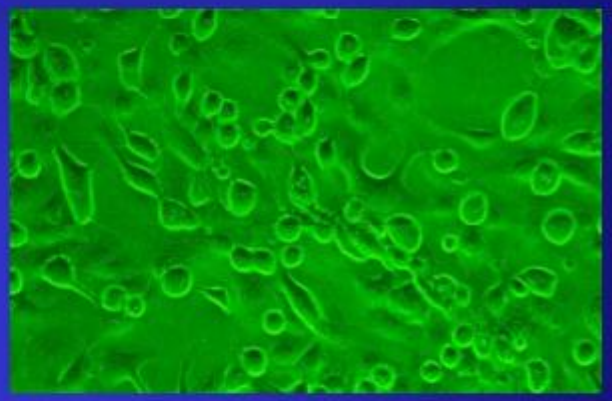
cytopathic effect (CPE)

Cytopathic Effect (CPE) of Herpes Simplex Virus Type 1 In Vero Cells

A



B

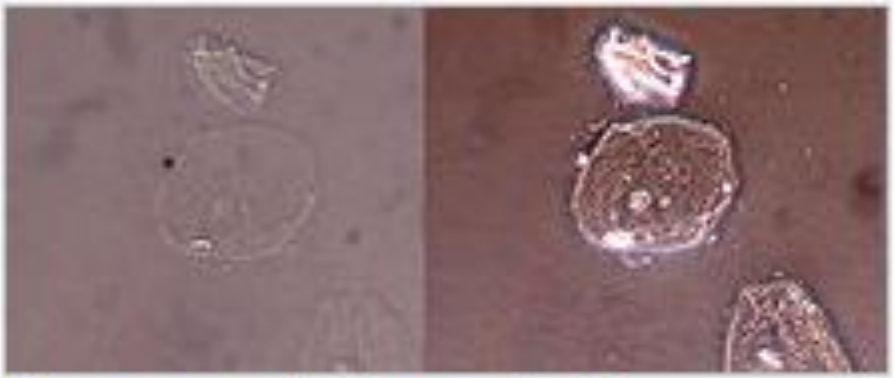


A, Tube culture of uninfected monolayer of vero cells. Phase contrast microscopy, × 40. **B,** Tube culture inoculated with a clinical specimen of HSV-1 showing the presence of CPE after 5 days of incubation. Phase contrast microscopy, × 200.

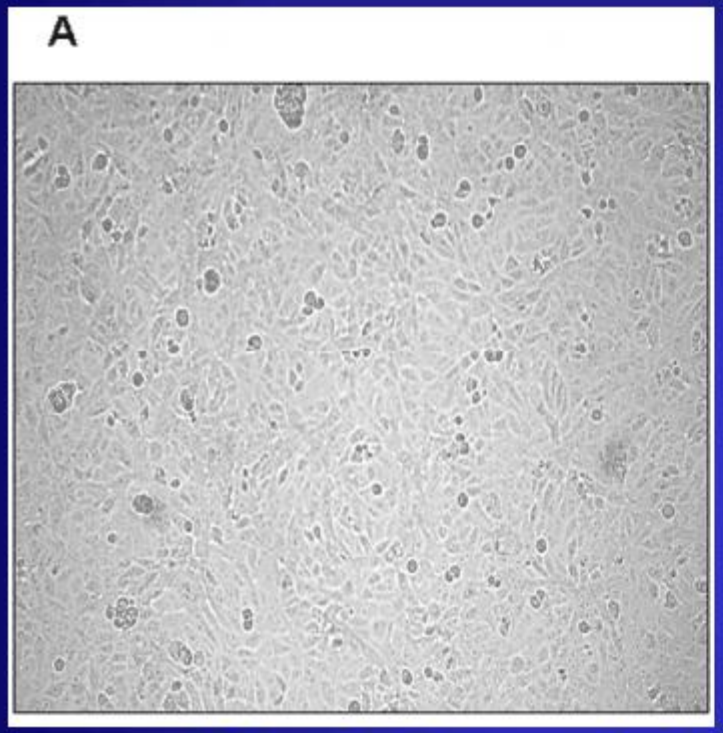
Phase-contrast microscope

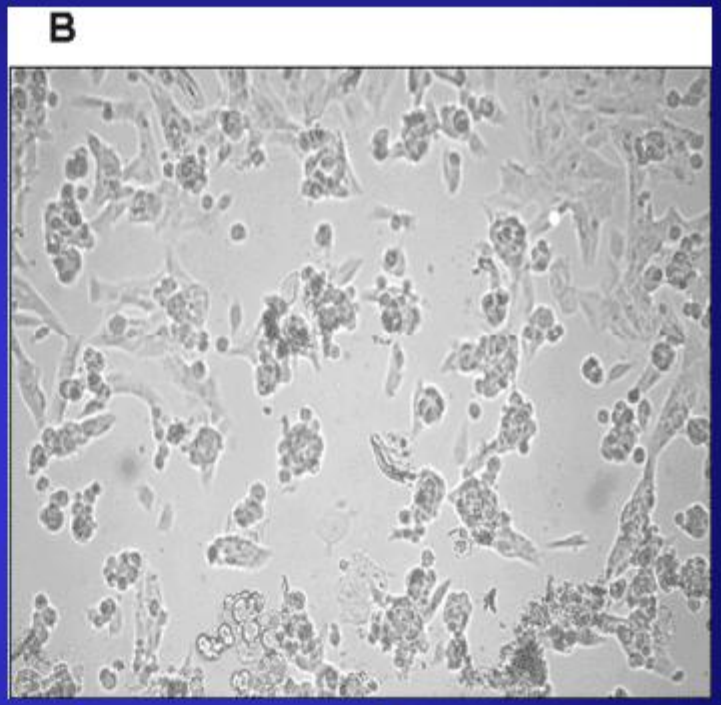
A phase-contrast microscope

Uses Microscopic observation of unstained biological material



The same cells imaged with traditional bright-field microscopy (left), and with phase-contrast microscopy (right)

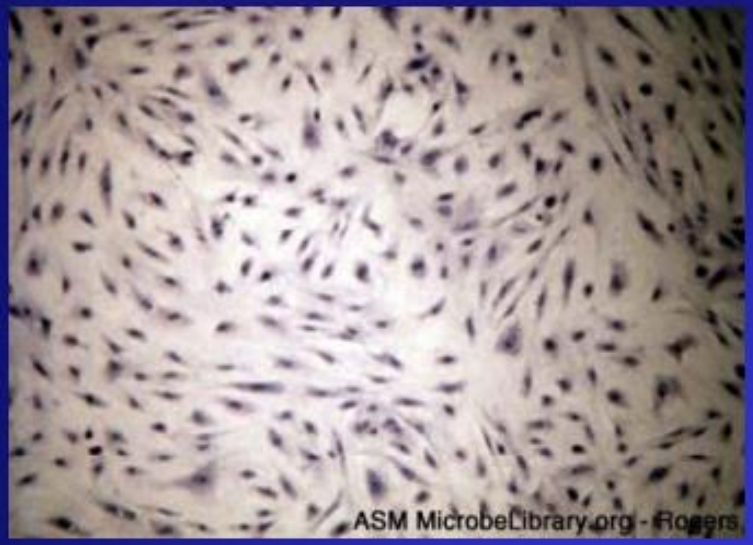




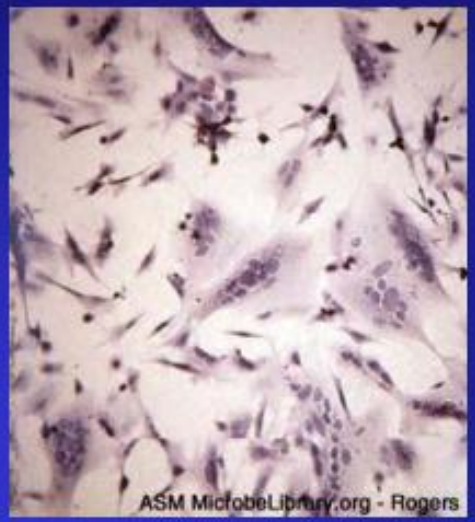
A, uninfected Vero cells form a continuous monolayer of spindle-shaped cells. B, a strong CPE was observed after 24 hours of incubation of Vero cells with the patient sputum sample (primary isolate).

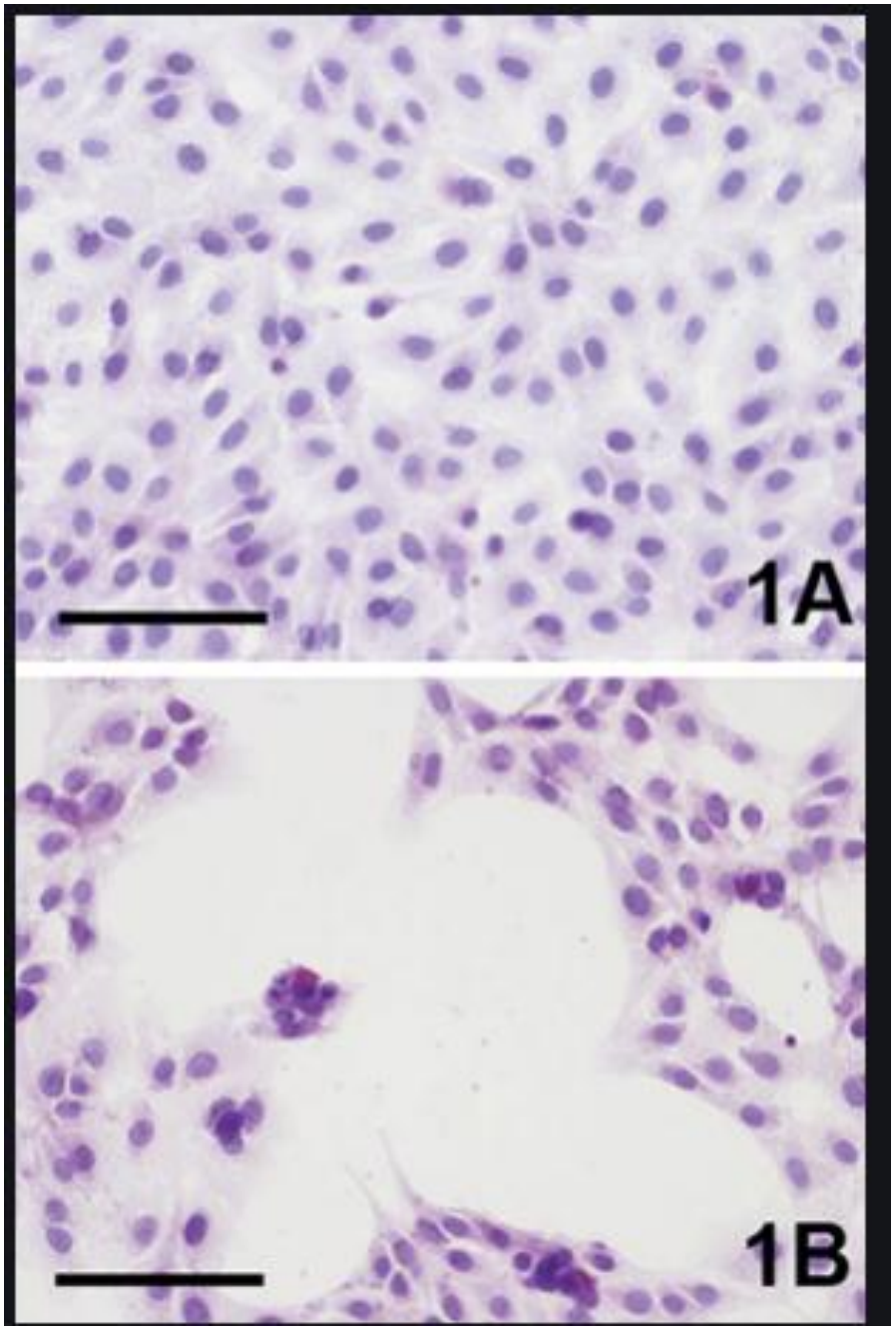
Cytopathic Effect (CPE) of Herpes Simplex Virus Type 1 In Primary Rabbit Kidney Cells

A



B



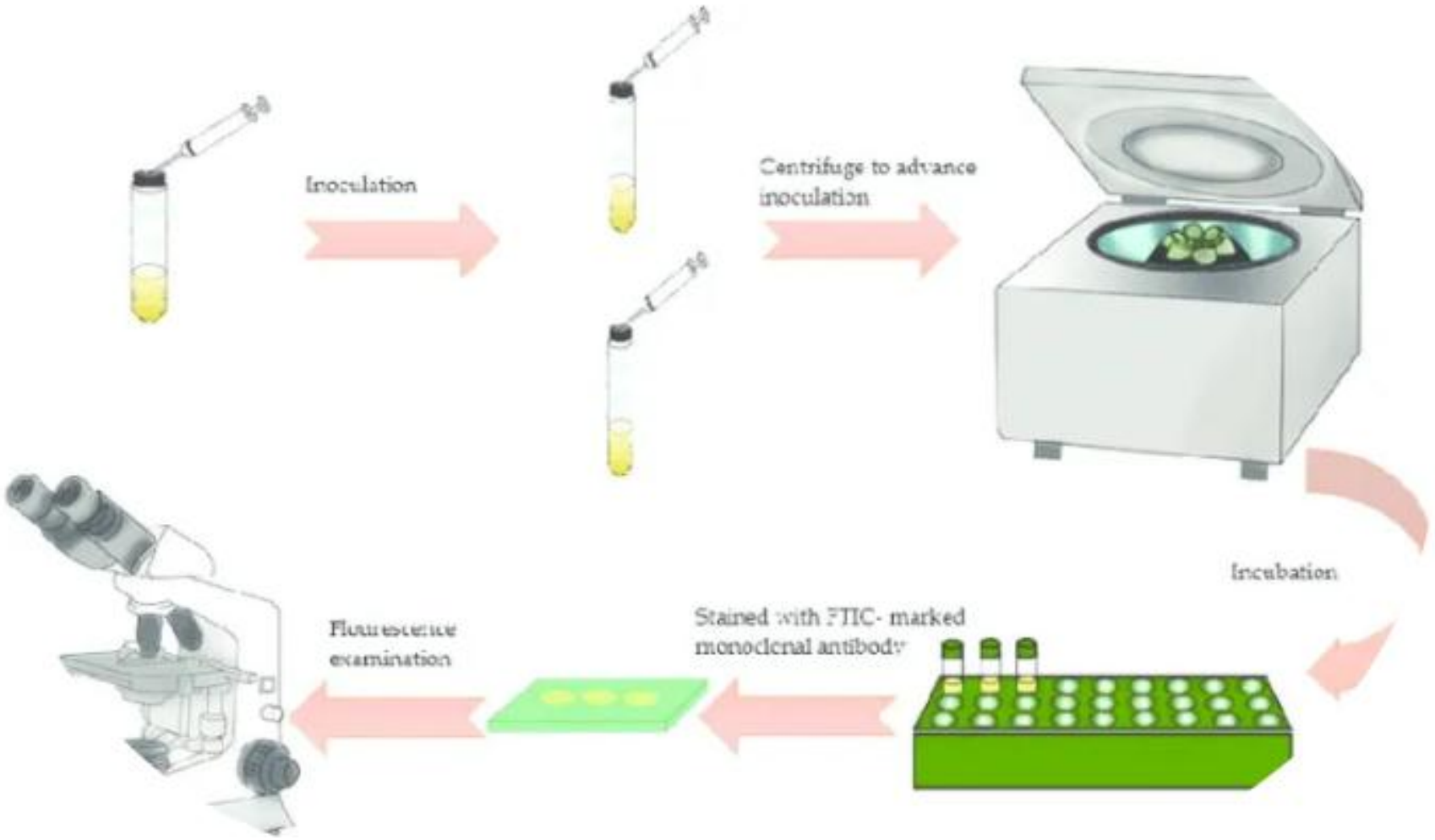

1A
1B

Examples of cytopathic effects

Cytopathic effect(s)	Virus(es)
Morphological alterations	
Nuclear shrinking (pyknosis), proliferation of membrane	Picornaviruses
Proliferation of nuclear membrane	Alphaviruses, herpesviruses
Vacuoles in cytoplasm	Polyomaviruses, papillomaviruses
Syncytium formation (cell fusion)	Paramyxoviruses, coronaviruses
Margination and breaking of chromosomes	Herpesviruses
Rounding up and detachment of cultured cells	Herpesviruses, rhabdoviruses, adenoviruses, picornaviruses
Inclusion bodies	
Virions in nucleus	Adenoviruses
Virions in cytoplasm (Negri bodies)	Rabies virus
"Factories" in cytoplasm (Guarnieri bodies)	Poxviruses
Clumps of ribosomes in virions	Arenaviruses
Clumps of chromatin in nucleus	Herpesviruses

Principle

The technique involves inoculation of the clinical specimen on to cell monolayer grown on a coverslip in a shell vial culture tube, followed by low-speed centrifugation and incubation.



Shell vial assay

Viral culture is a laboratory test in which samples are placed with a cell type that the virus being tested for is able to infect. If the cells show changes, known as cytopathic effects, then the culture is positive. Human and monkey cells are used in both traditional viral culture and shell vial culture.

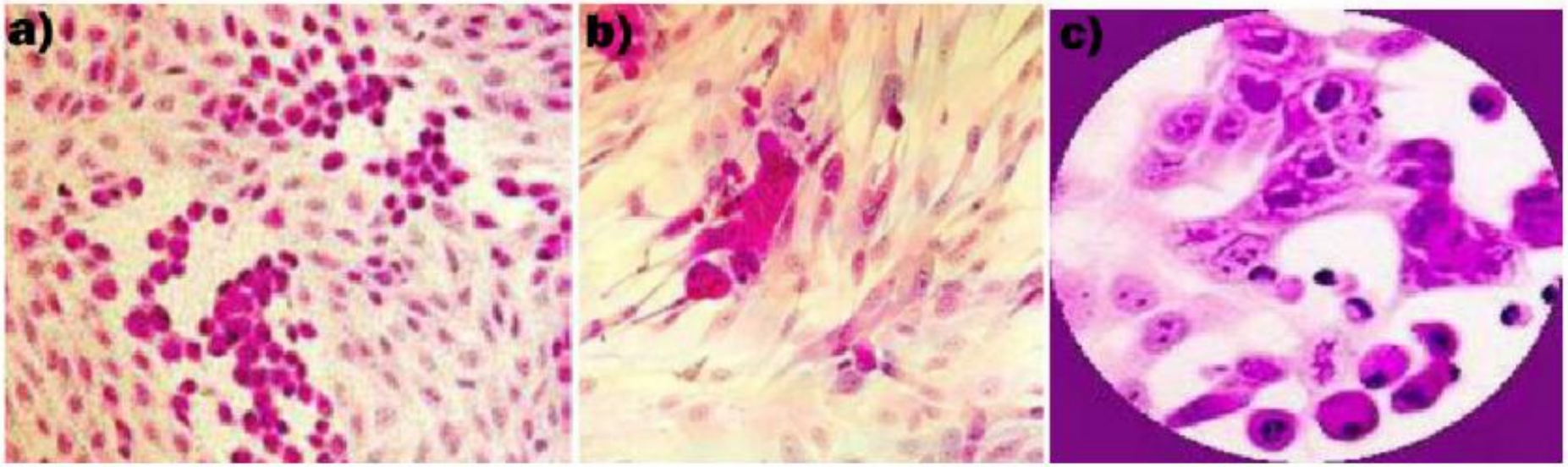
Human virus types that can be identified by viral culture include adenovirus, cytomegalovirus, enteroviruses, herpes simplex virus, influenza virus, parainfluenza virus, rhinovirus, respiratory syncytial virus, varicella zoster virus, measles and mumps.

Mechanism of action:

Traditional viral culture has been generally superseded by shell vial culture, in which the sample is centrifuged on to a single layer of cells and viral growth is measured by antigen detection methods. This greatly reduces the time needed to detect slow growing viruses such as cytomegalovirus, for which the method was developed. In addition, the centrifugation step in shell vial culture enhances the sensitivity of this method because after centrifugation, the viral particles of the sample are in close proximity to the cells.

For these, the final identification method is generally by immunofluorescence, with exception of cytomegalovirus and rhinovirus, whose identification in a viral culture are determined by cytopathic effects.

a) Cytopathic effect of HSV on cell culture b) Cytopathic effect of CMV c) Adenoviral inclusion bodies



- **Advantage**
- The advantage of shell vial is its speed; most viruses are detected within 24 hours.
- **Limitations**

1. Only one type of virus can be detected per shell vial. For example, a specimen that might contain influenza A and B, or adenovirus, would need to be inoculated to three separate virus-specific conjugates. This limitation can be overcome by using pool antibody followed by staining with individual antibody conjugates, if positive in pool antibody testing.

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