Monitoring Virus Replication

Virus Inoculation

Adsorption Technique:

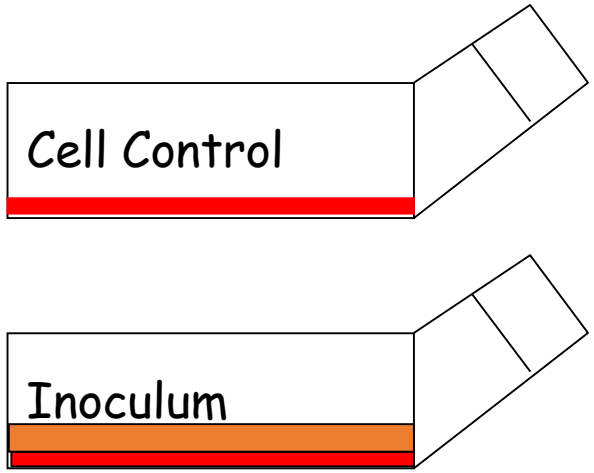
- Following virus inoculation, there is an additional incubation period of 1 hour at 37^{o}C.

Non-adsorption technique:

- After virus inoculation, the virus production medium is placed without any additional waiting time.

Monitoring Virus Replication

- Daily microscopic checks are made to see if there is any virus replication.
- First, control (uninfected) cells are examined under the microscope. In parallel, cytopathological findings in the infected cells are examined.



Cytopathic Effect=CPE

- Cytopathic effect or cytopathogenic effect (abbreviated CPE) refers to structural changes in host cells that are caused by viral invasion. In other words, changes that occur in cells due to virus infection and can be detected microscopically are called CPE.
- CPE occurs as a result of the toxic effect of viral proteins accumulating inside the cell and the effects of viral proteins on cellular protein, cellular DNA, RNA and mRNA synthesis.

1. Cell cytoplasm
2. Cell nucleus

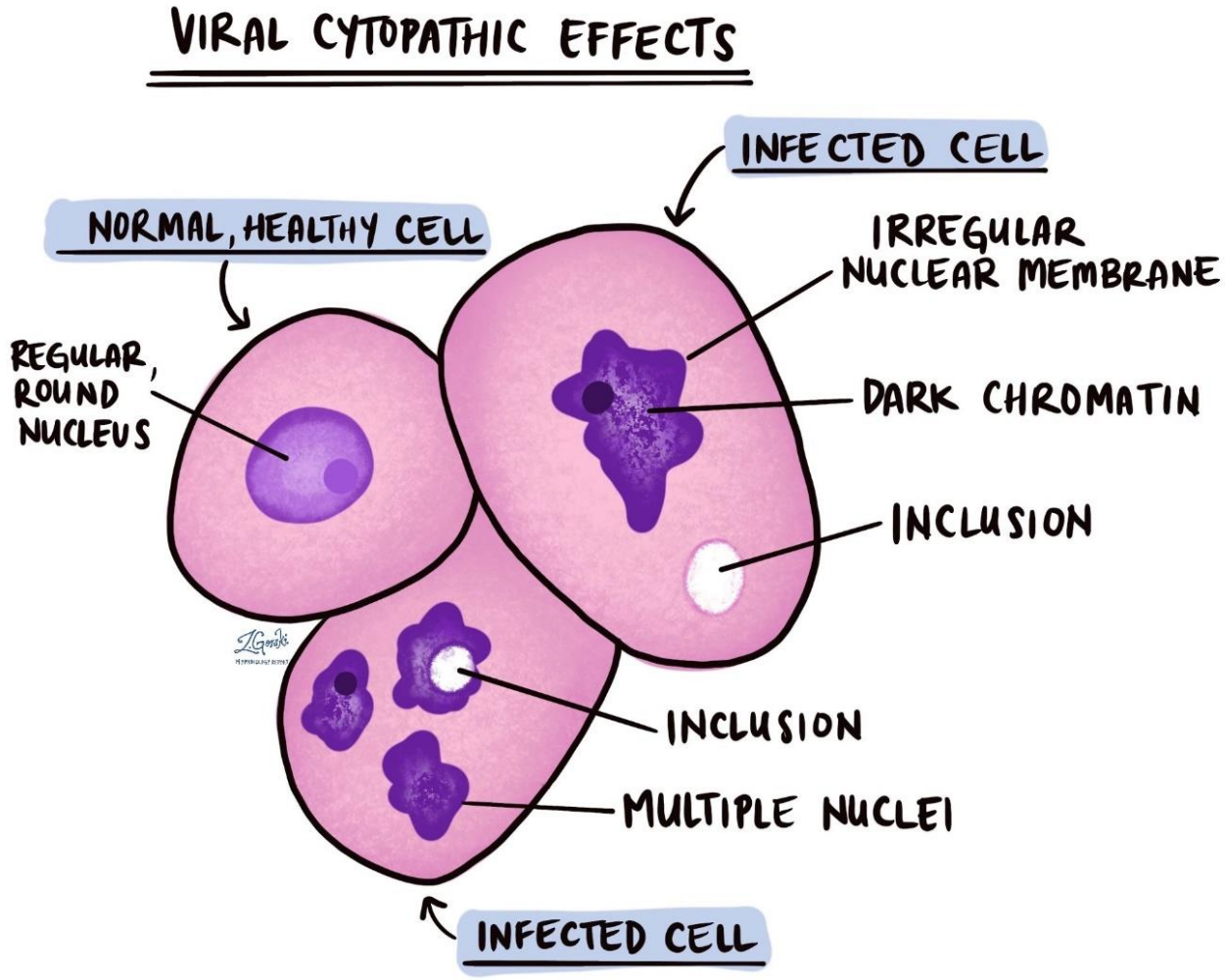
Types of CPE

Cytoplasm

- Rounding
- Granulation
- Vacuolization
- fusion with adjacent cells to form syncytia
- Giant cells
- cytoplasmic inclusion body

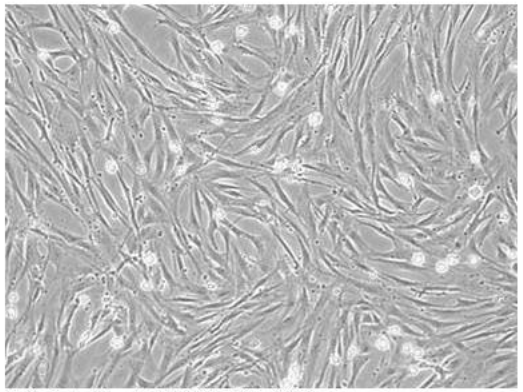
In the nucleus

- pyknosis
- karyorrhexis
- Nucleolus growth
- nuclear inclusion Bodies

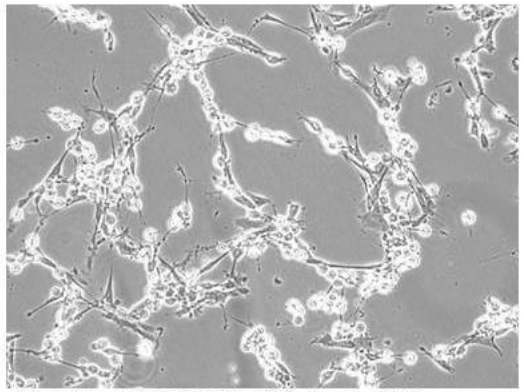


https://www.mypathologyreport.ca/pathology-dictionary/viral-cytopathic-effects/

ATCC® Number: **VR-188™**
Agent: **Bovine herpesvirus 1**
Strain: **Los Angeles**

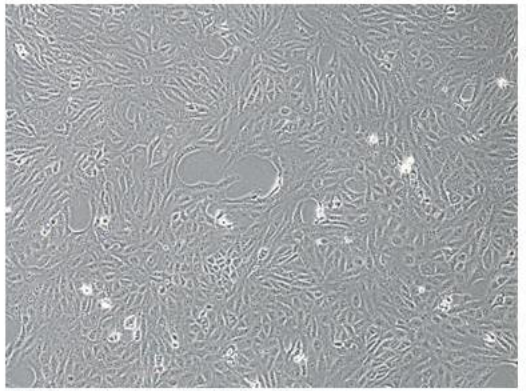


Uninfected host (ATCC® CCL-44™)

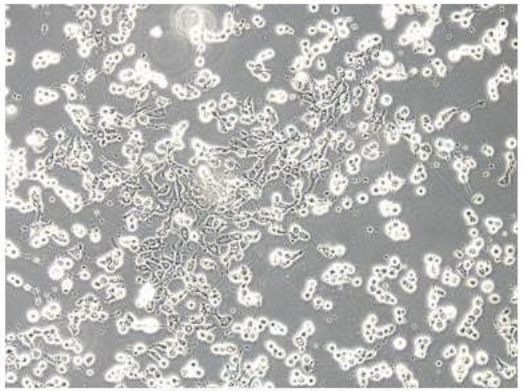


Infected host (ATCC® CCL-44™) showing CPE

ATCC® Number: **VR-1789™**
Agent: **Human herpesvirus 1**
Strain: **ATCC-2011-9**

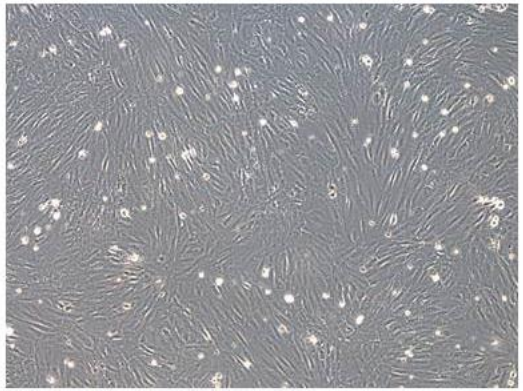


Uninfected host (ATCC® CCL-81™)

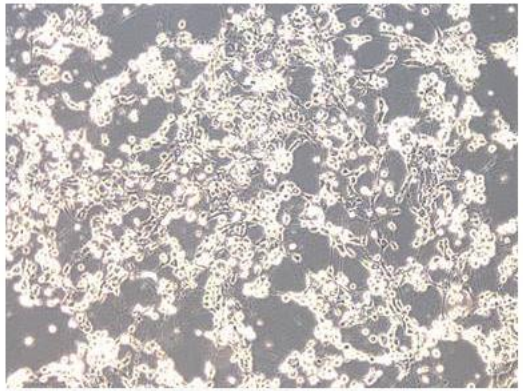


Infected host (ATCC® CCL-81™) showing CPE

ATCC® Number: **VR-1787™**
Agent: **Felid herpesvirus 1**
Strain: **FVRm (modified)**

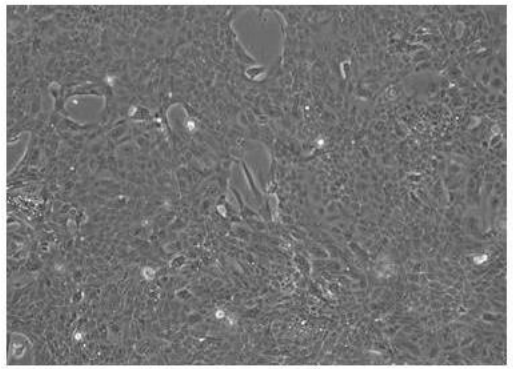


Uninfected host (ATCC® CCL-94™)

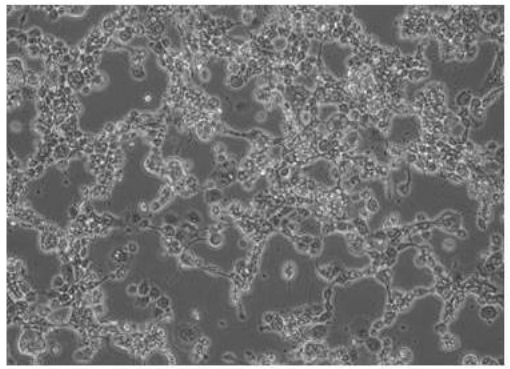


Infected host (ATCC® CCL-94™) showing CPE

ATCC® Number: **VR-552™**
Agent: **Canid herpesvirus 1**
Strain: **D 004**

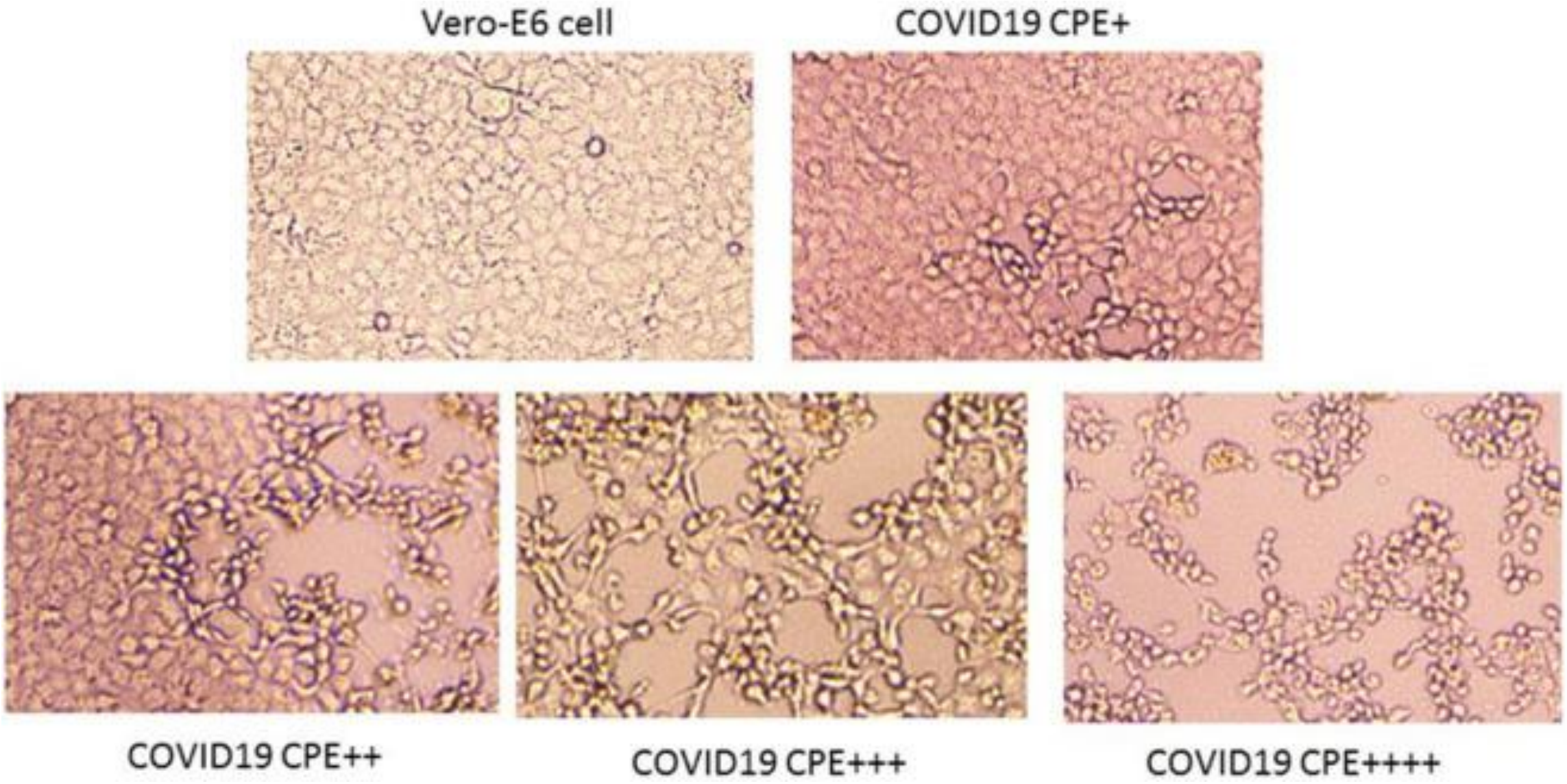


Uninfected host (ATCC® CCL-34™)

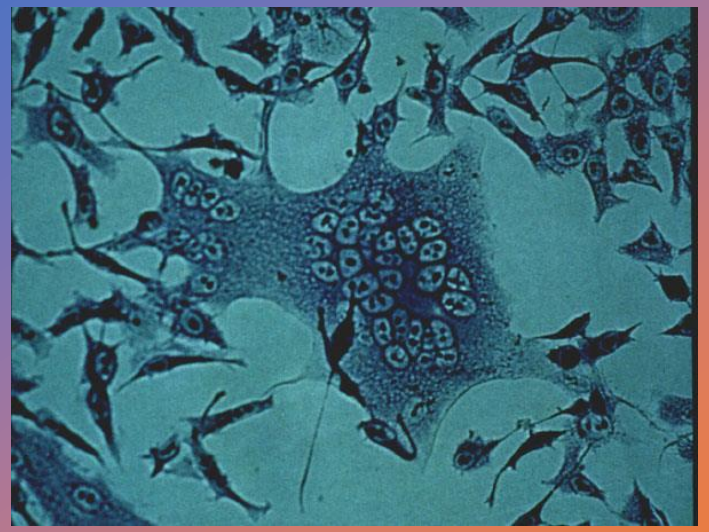


Infected host (ATCC® CCL-34™) showing CPE

https://www.atcc.org/products/vr-188 https://www.atcc.org/products/vr-1787 https://www.atcc.org/products/vr-552 https://www.atcc.org/products/vr-1789

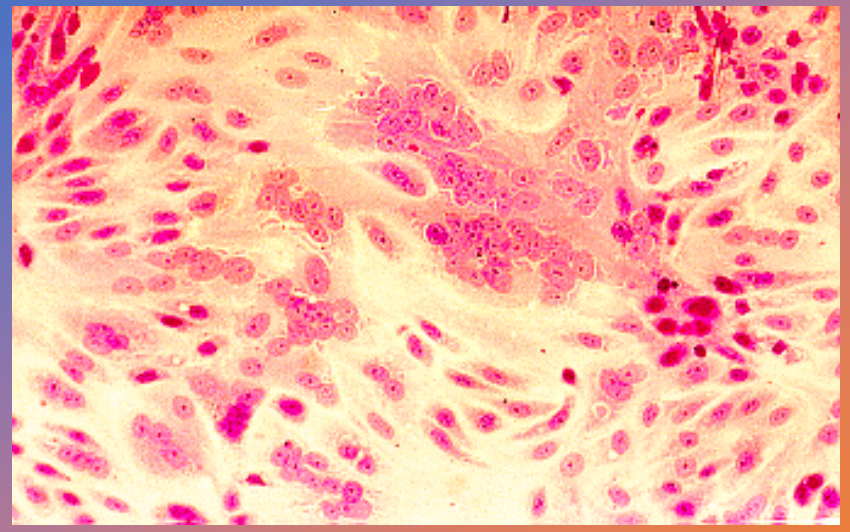


Lijun Liang et al 2021 Low infectious risk of re-positive COVID-19 patients: a single-center study https://doi.org/10.1016/j.ijid.2021.08.019

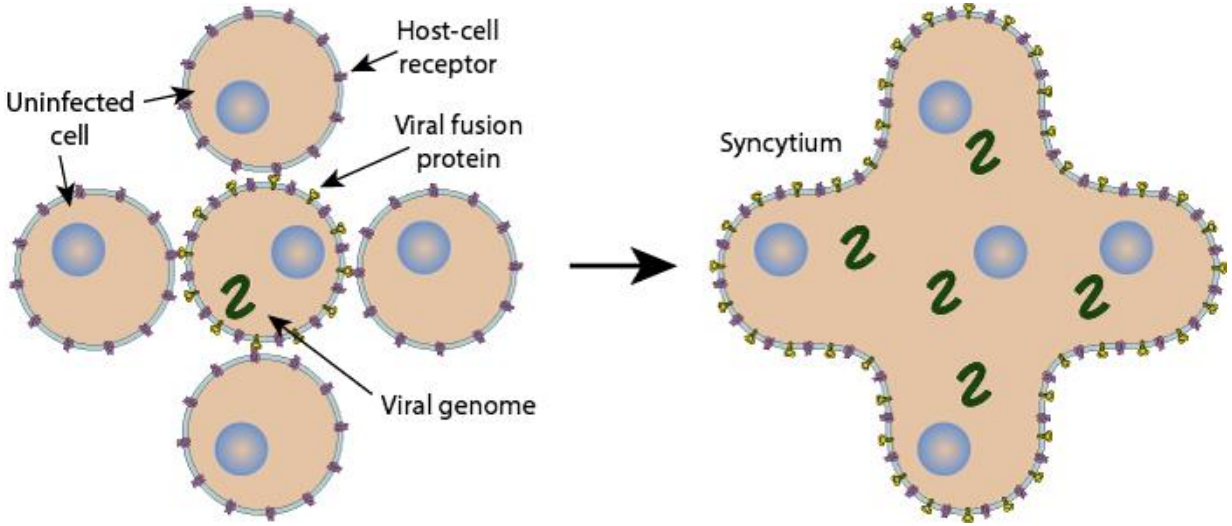


measles virus www.tau.ac.il

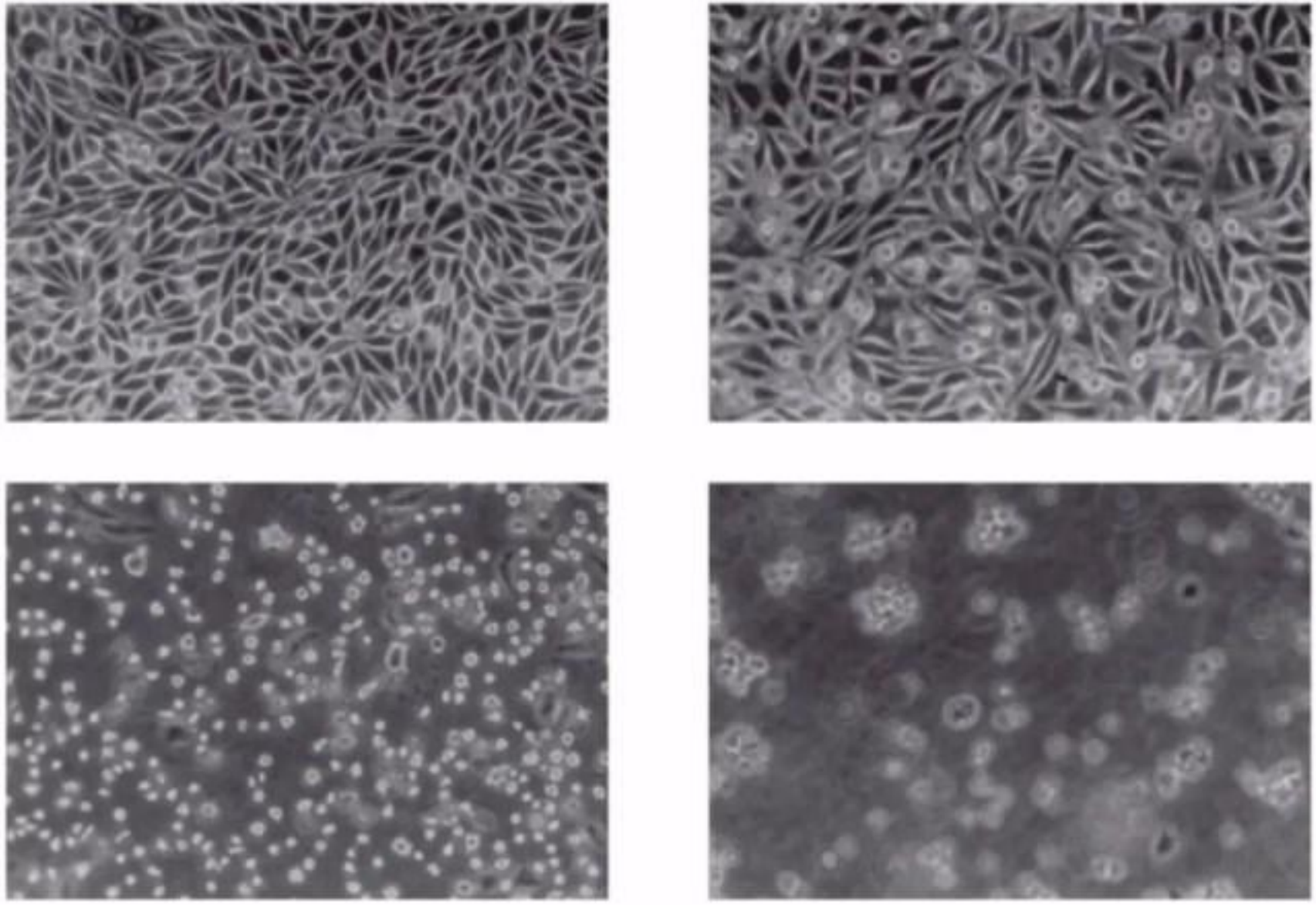
- Syncytia and giant cell formation: Infected cells fuse with neighboring cells through cytoplasmic bridges (syncytium). Infected cells appear as a single multinucleated cell (giant cell).



RSV (Courtesy of Linda Stannard, University of Cape Town, SA)



https://viralzone.expasy.org/5957?outline=all_by_species



HeLa cell lines infected with poliovirus
(From top left, normal cells, 4 hours, 8 hours and 12 hours after infections)

https://microbeonline.com/cytopathic-effect-cpe-viruses-examples/

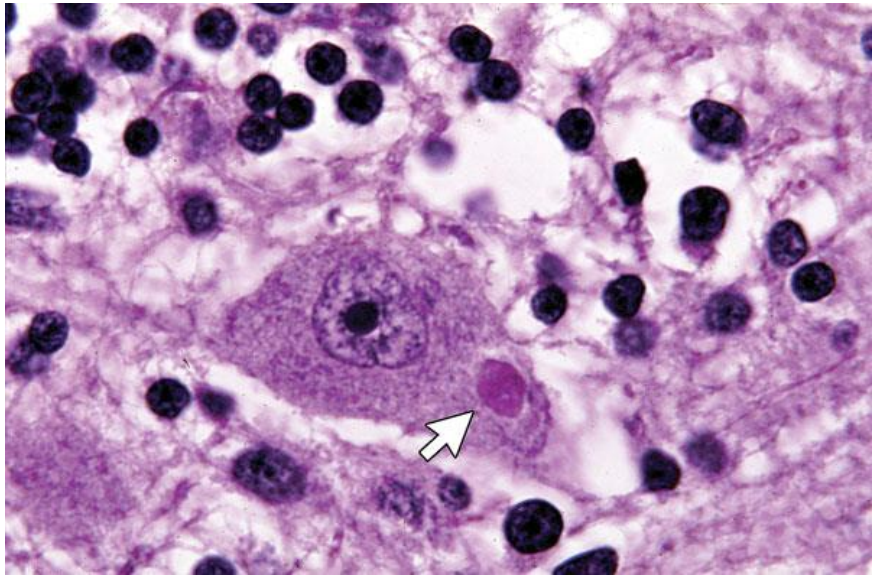
Inclusion Bodies

- Inclusion bodies are aggregates of virus particles or virus-induced proteins or special structures characteristic of infection by viruses either in the cytoplasm or the nucleus. It consists of accumulation of viral proteins, viral nucleic acid, degenerated chromatin, etc.

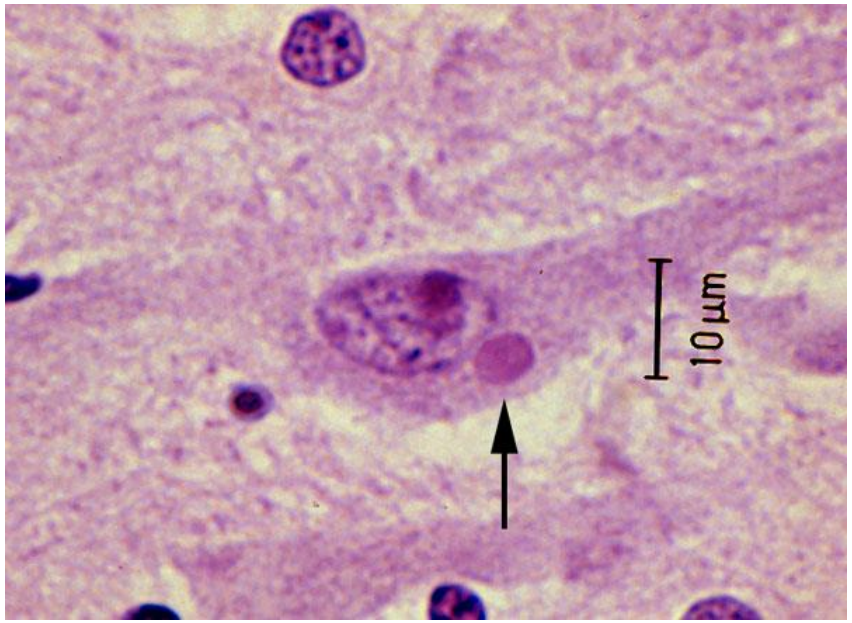
Viral inclusion bodies

Intracytoplasmic	Henderson-Peterson bodies (Molluscum Contagiosum)		
	Negri bodies (Rabies)		
	Guarnieri bodies (Small pox)		
	Paschen bodies (Small pox)		
	Bollinger bodies (Fowl pox)		
	Borrel bodies (Fowl pox)		
Intranuclear	Acidophilic	Cowdry type A	Varicella zoster virus
			Herpes simplex virus
			Yellow fever virus
		Cowdry type B	Polio virus
	Basophilic	Cowdry type B	Adeno virus
			Cytomegalo virus

V. Celia Chalam et al 2020 Modern technologies for the diagnosis and assay of plants viruses in Applied Plant Virology,

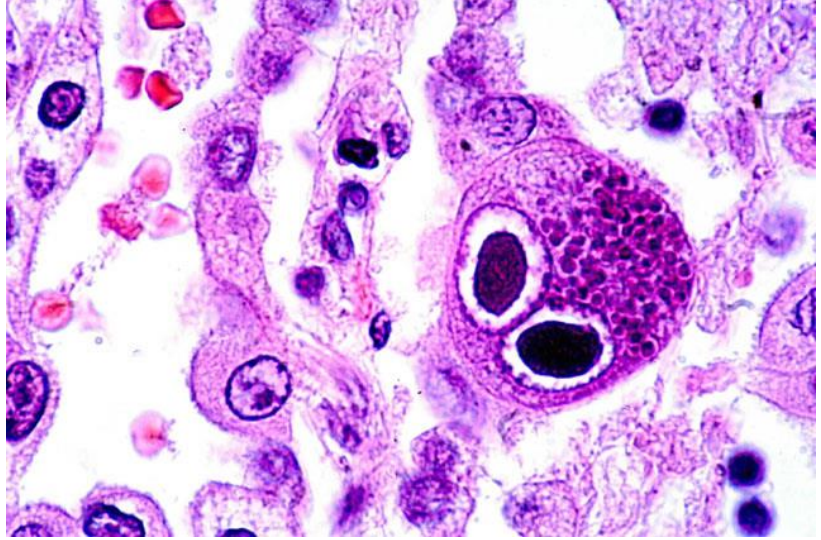


NEGRI BODIES

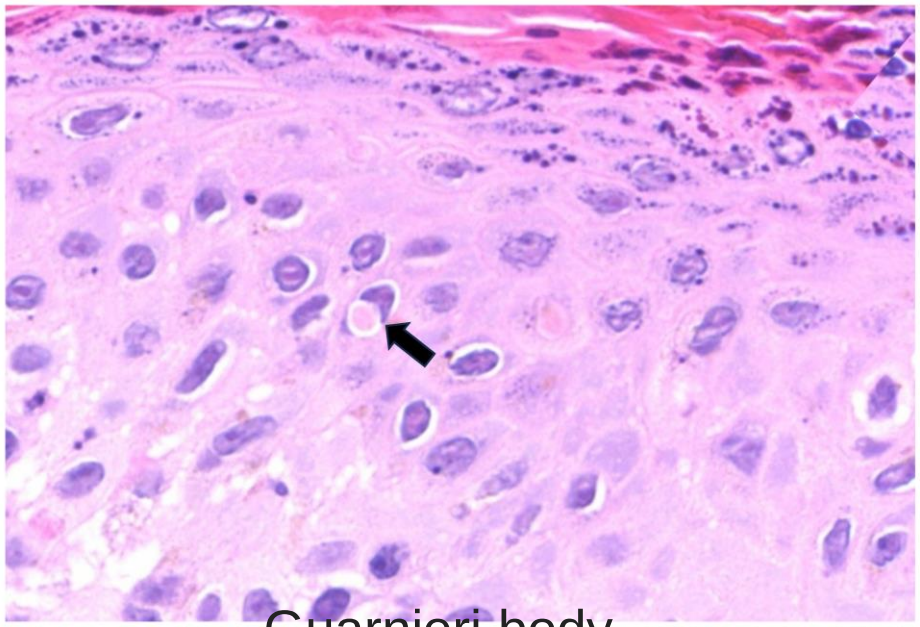


A Negri body in a cell affected by rabies (arrow). Source: Nethealthbook

Henry, R., & Murphy, F. A. (2017). Etymologia: Negri Bodies. *Emerging Infectious Diseases*, *23*(9), 1461. https://doi.org/10.3201/eid2309.et2309.

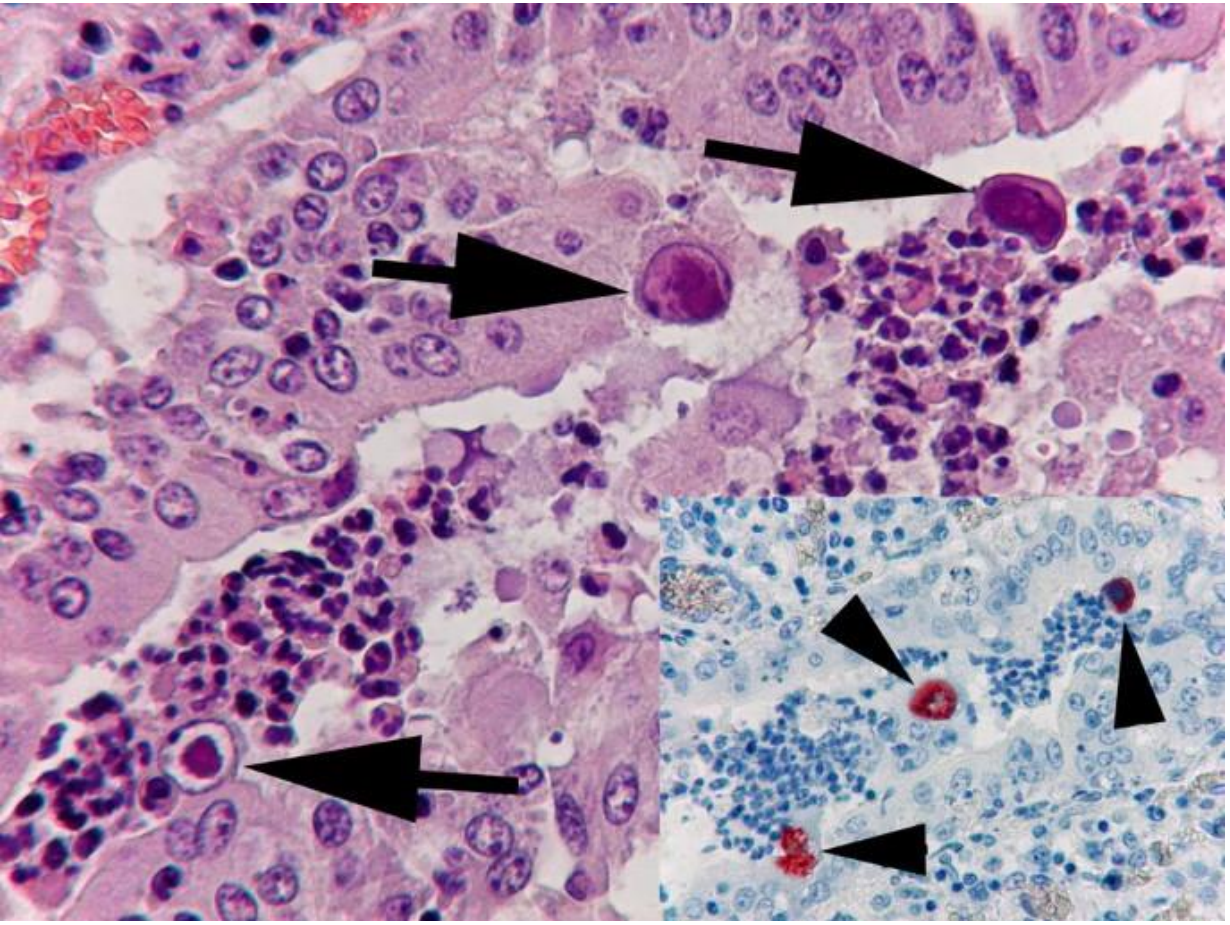


Owl eye CMV



Guarnieri body

Moltrasio C, et al 2023. Monkeypox: A Histopathological and Transmission Electron Microscopy Study. *Microorganisms*. https://doi.org/10.3390/microorganisms11071781



Enlarged bronchiolar epithelial cells containing intranuclear inclusion bodies (arrows) Vidovszky, *et al.* Isolation and complete genome sequence analysis of a novel ovine adenovirus type representing a possible new mastadenovirus species. *Arch Virol* https://doi.org/10.1007/s00705-019-04299-6

Examples;

Negri bodies- Rabies

Guarnieri bodies -Small pox

Bollinger bodies - fowlpox

Henderson-Peterson bodies - Molluscum contagiosum

Cowdry type A -Herpes simplex virus ve Varicella zoster virus

Torres bodies - Yellow fever

Cowdry type B - Polio

Cowdry type B - Adenovirus

"owl eyes" - cytomegalovirus

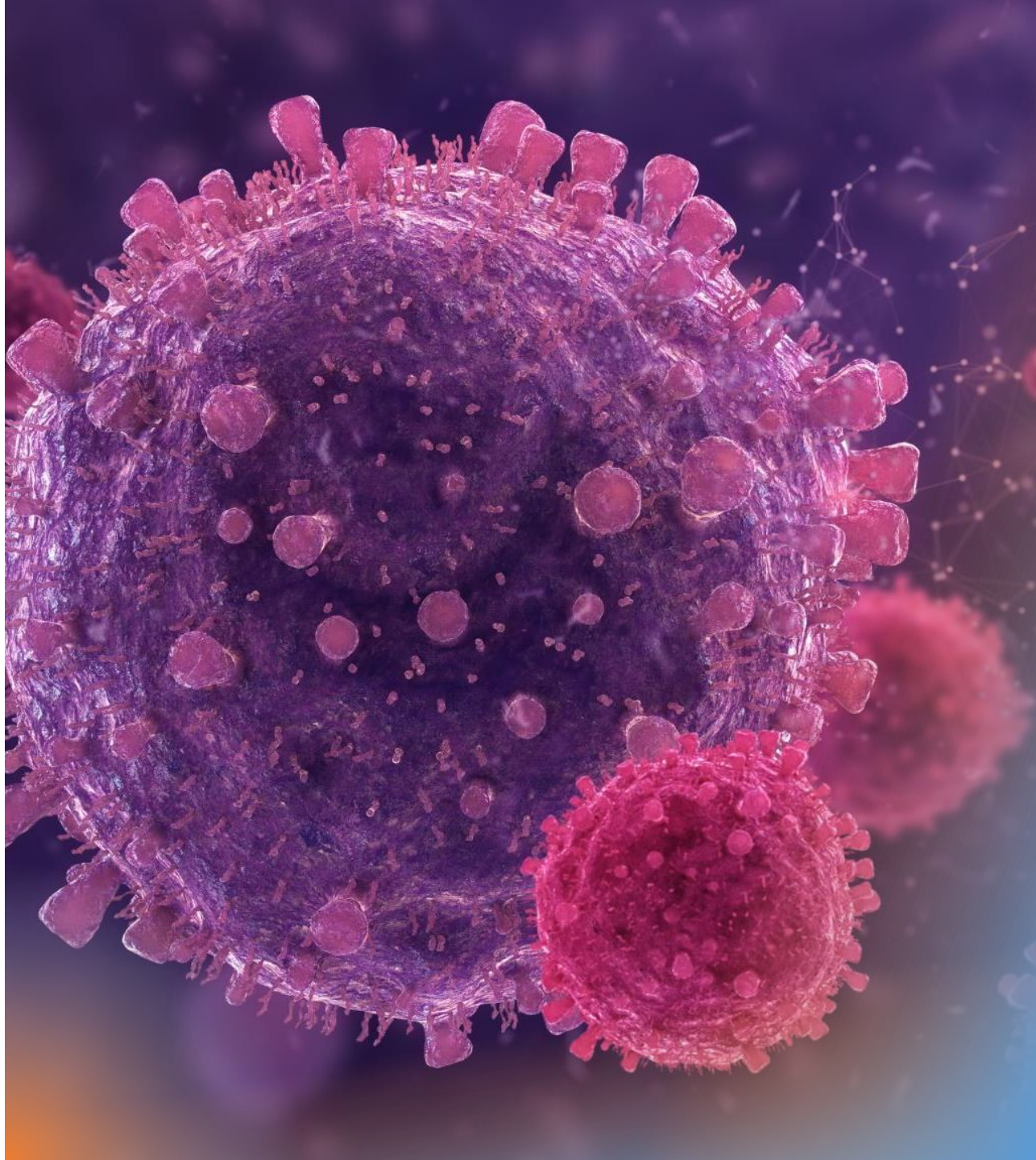
Warthin finkeldey bodies - Measles

- Physio-chemical, immunocytochemical, serological tests and molecular methods (such as PCR, sequence analysis) are performed to identify viruses after their isolation in cell culture.


Does every virus cause CPE?


NO!!!!

Then how can the presence of the virus be detected?



Detection of non-CPE viruses in cell culture

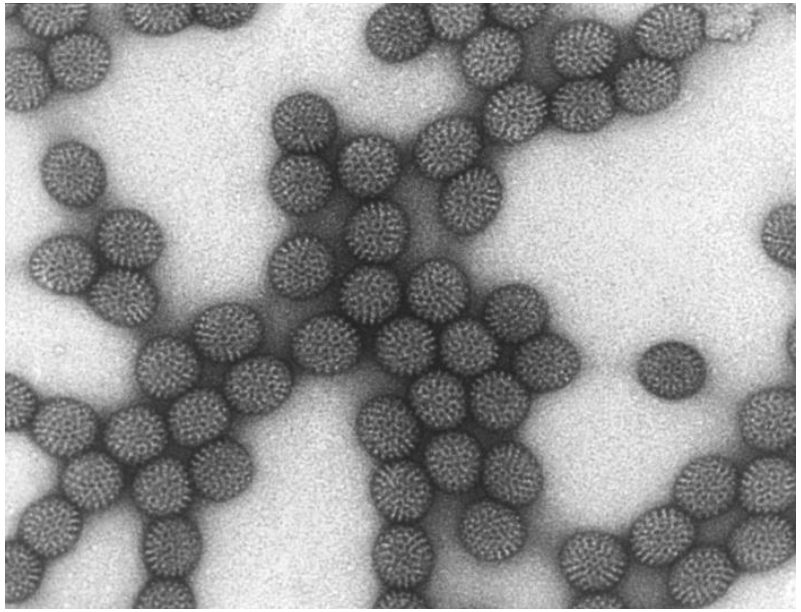
- In cells infected with non-cytopathogenic viruses, metabolic activity is reduced to a very limited extent. These cells continue to live and divide.
- One of the most striking examples of this type of cellular infection is pestivirus infections. Cytopathogenic pestivirus strains cause cytopathological changes such as vacuole formation and lysis in sensitive cells. Non-cytopathogenic BVD virus strains do not cause any morphological changes, although they grow at high titers in the cells they infect.

Detection of non-CPE viruses in cell culture

Therefore, some special techniques need to be applied to detect the growth of non-cytopathogenic viruses.

- Electron Microscopy
- Immunocytochemical Techniques (IFT, IPT)
- Hemadsorption
- Interference
- PCR

➢Electron Microscopy

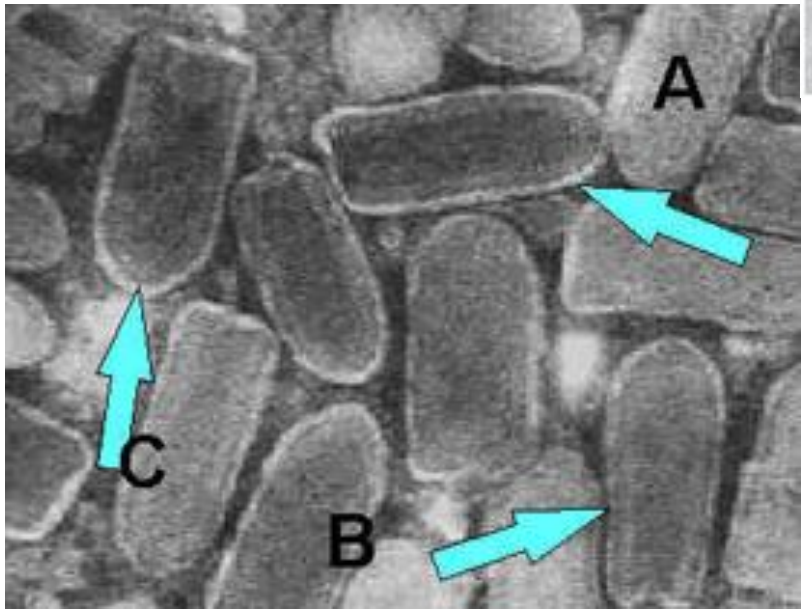


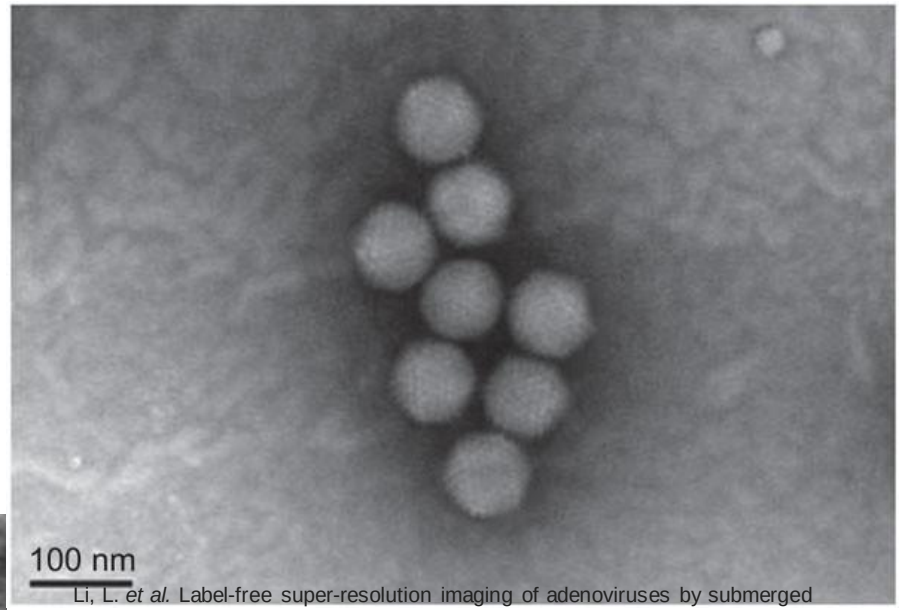
https://www.cdc.gov/rotavirus/images/disease_rotavirus_lg.jpg

Rotavirus

Rabiesvirus

https://www.cdc.gov/rabies/diagnosis/electron_microscopy.html



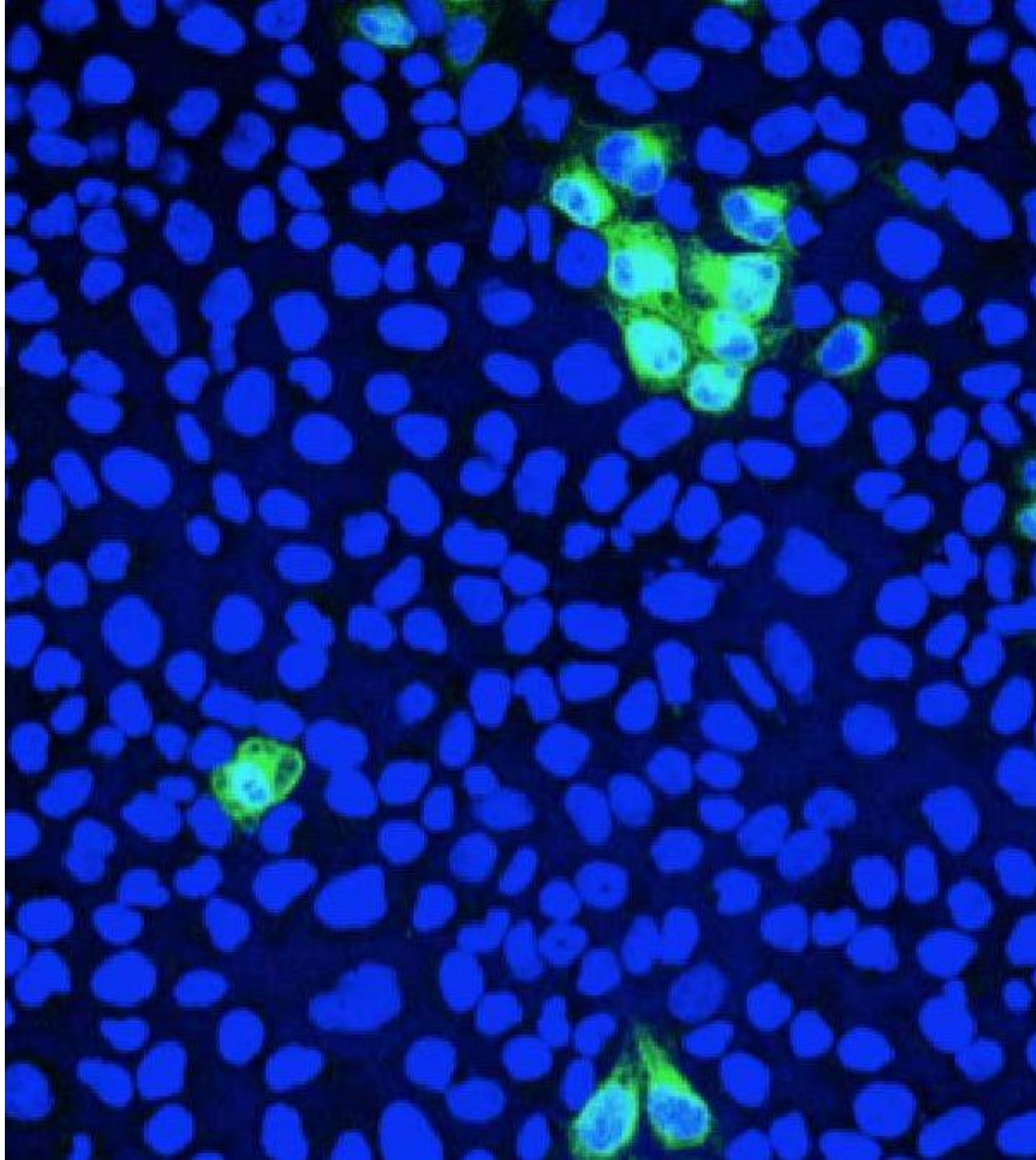


Li, L. *et al.* Label-free super-resolution imaging of adenoviruses by submerged microsphere optical nanoscopy. *Light Sci Appl*https://doi.org/10.1038/lsa.2013.60

Adenovirus

➢Immunofluorescence test (IFT)

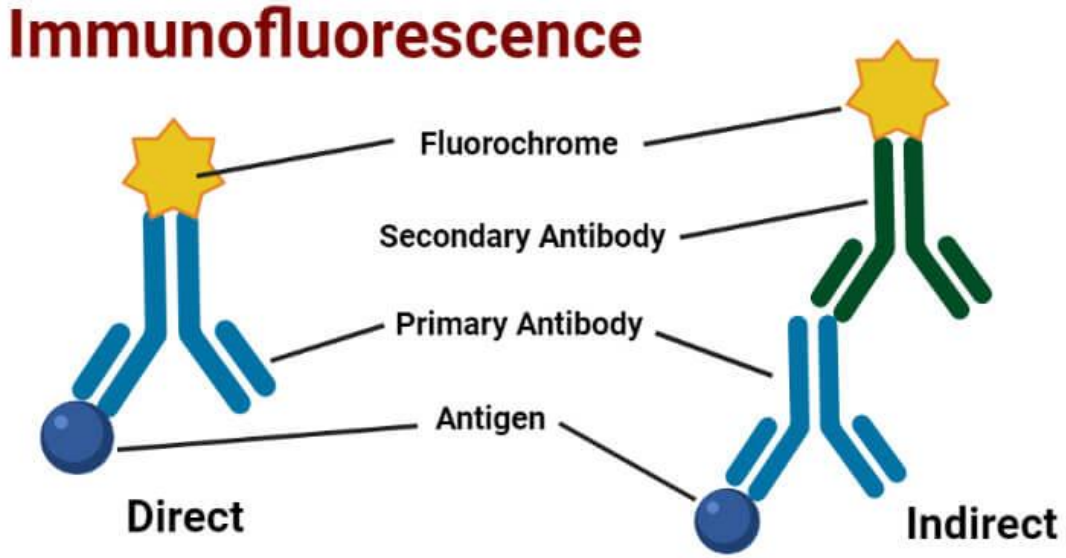
- This test aims to identify virus-infected cells through labeled antibodies.
- However, the conjugate used here is labeled with a fluorescent dye (fluorescein isothiocyanate or rhodamine) and binds to the specific antigen, causing a yellow-green or red glow in infected cells.
- This luminescence can be evaluated under an ultraviolet light source in a special fluorescent microscope.



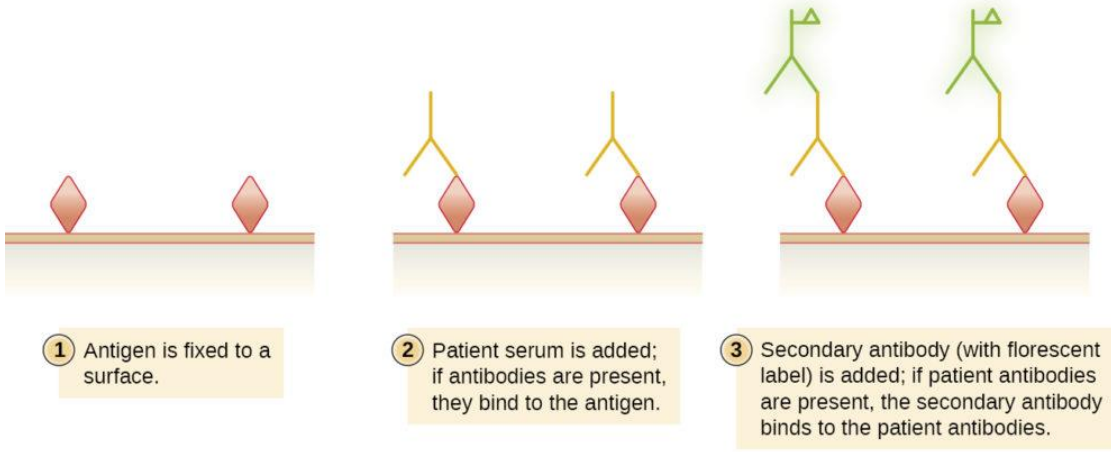
https://thenativeantigencompany.com/visualising-viruses-with-immunofluorescence-microscopy/

Green: Zika virus infected cells; Blue: cell nuclei

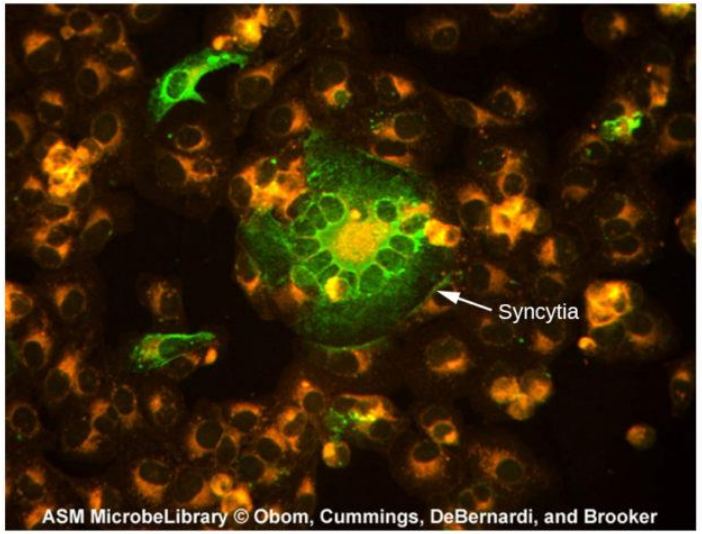
➢Immunofluorescence test (IFT)



https://microbenotes.com/immunofluorescence/



(a)



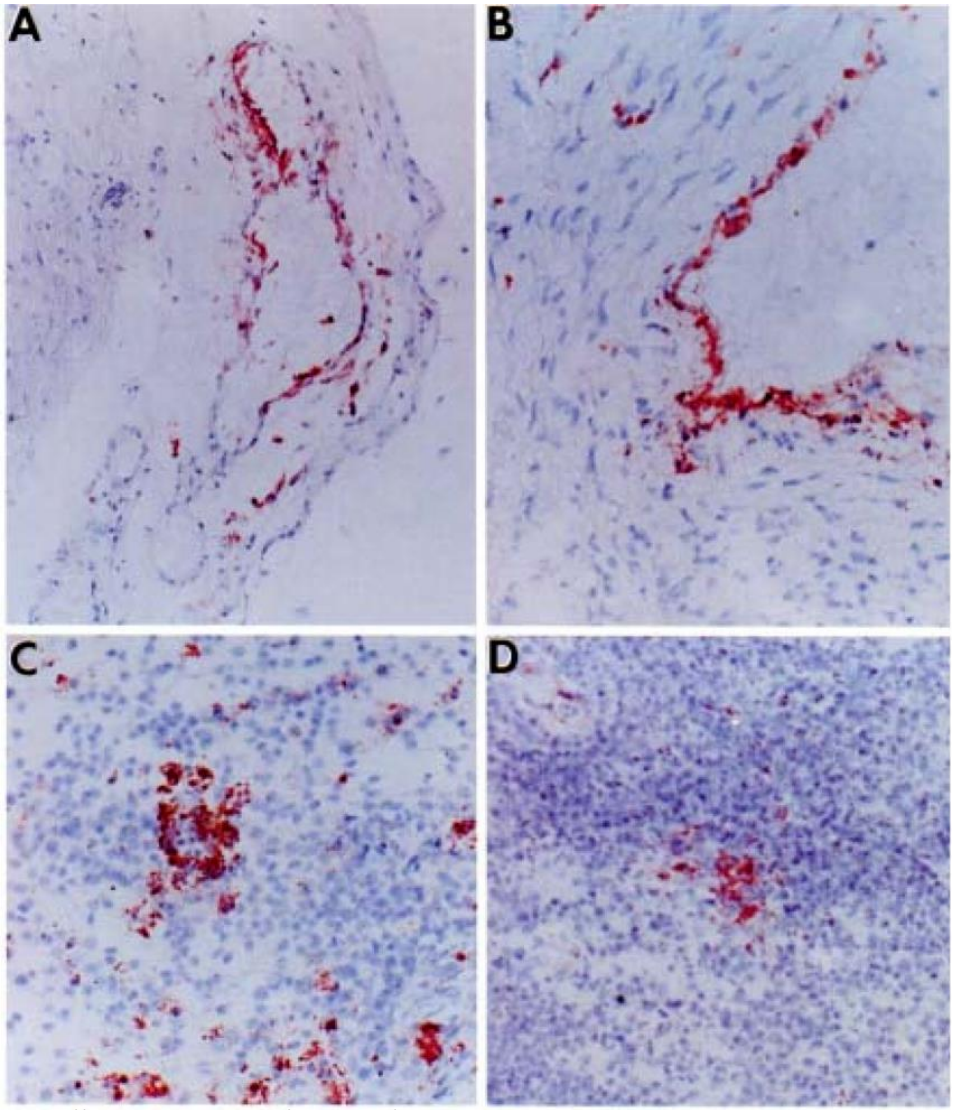
(b)

https://courses.lumenlearning.com/suny-microbiology/chapter/fluorescent-antibody-techniques/

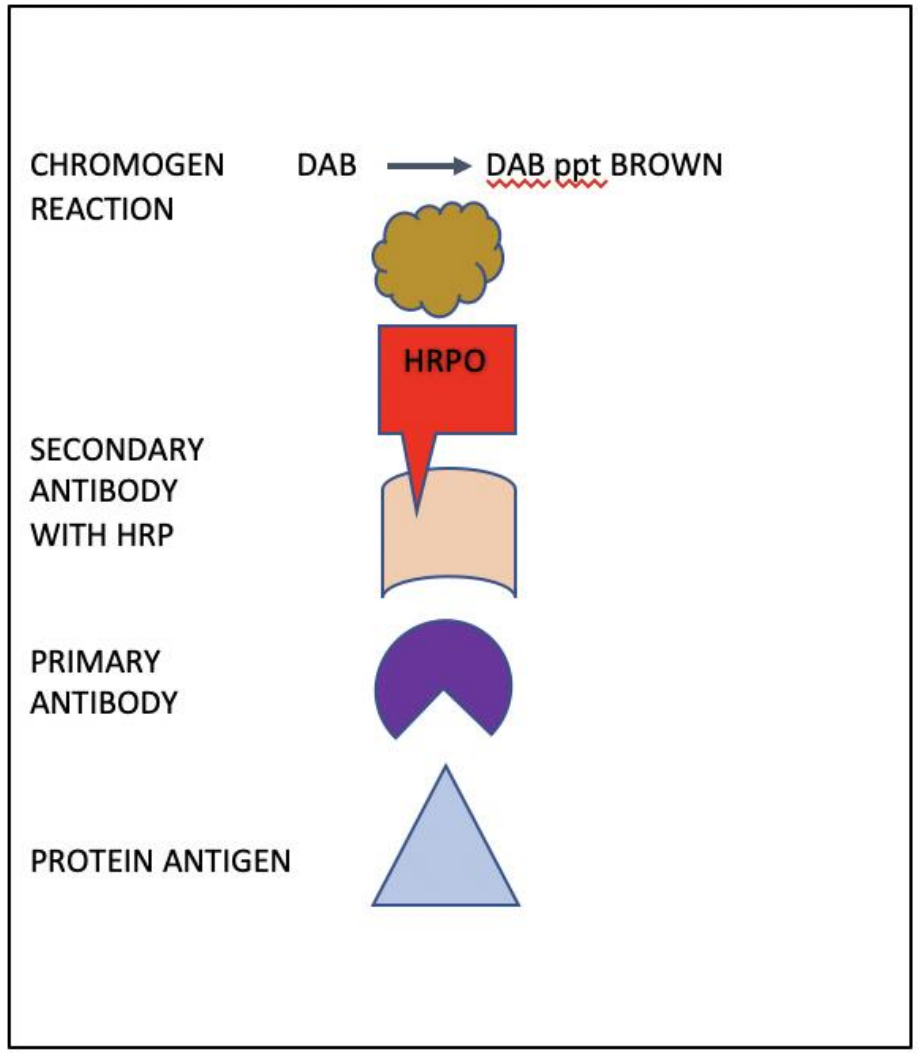
➢Immunoperoxidase test (IFT)

- In this test, antibodies obtained against the virus and marked with peroxidase enzyme are used. These labeled antibodies, called conjugates, bind to specific antigenic surfaces (epitopes) of the virus propagating on infected cells.
- Staining of infected cells is then achieved by adding enzyme substrate and chromogen substance to the medium. Virus replication is diagnosed by observing these stained cells under a light microscope.

➢Immunoperoxidase test (IFT)



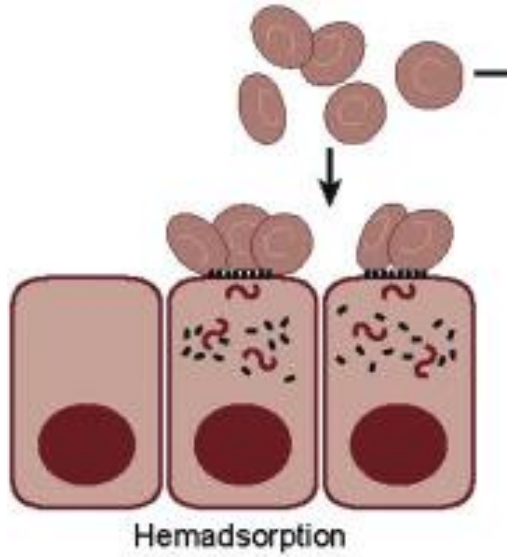
https://www.researchgate.net/publication/14360000_Fatal_Experimental_Equine_Arteritis_Virus_Infection_of_a_Pregnant_Mare_Immunohistochemical_Staining_of_Viral_Antigens



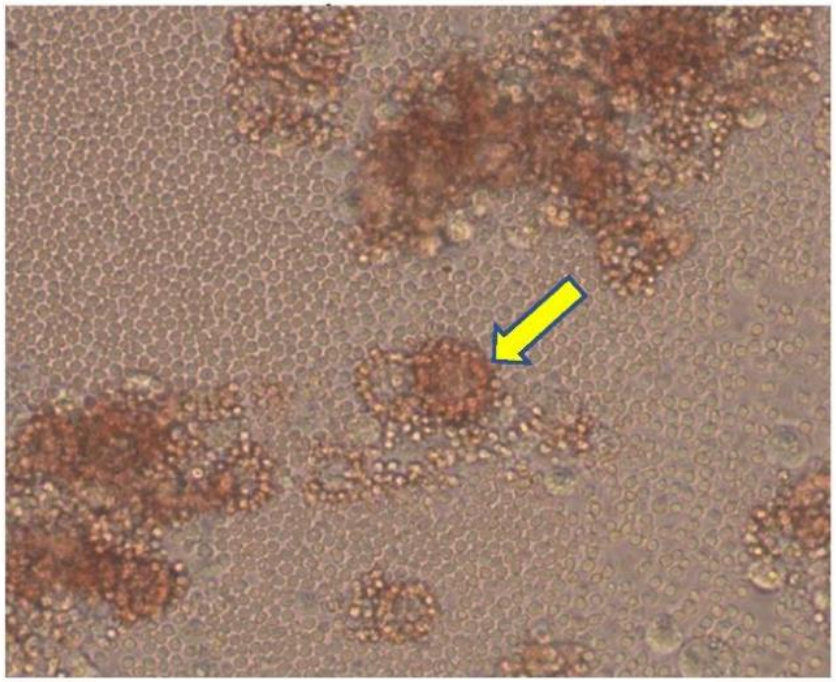
https://rankinbiomed.com/2019/10/07/immunohistochemistry/

➢ Hemadsorption

- When an erythrocyte suspension of the certain species is added to cell cultures infected with some viruses, it is observed that the erythrocytes adhere to the virus-infected cell surface and are not removed by washing.
- This phenomenon, called hemadsorption, occurs through the binding between receptors on erythrocytes and viral proteins that integrate into the host cell membrane during virus replication.
- The mechanism of this event is related to the envelopment process of the virus. Therefore, it is observed in enveloped viruses.
- parainfluenza-3, mumps, measles, influenza etc. can be detected.



Fenner's Veterinary Virology (Fifth Edition)
https://doi.org/10.1016/B978-0-12-800946-8.00003-9

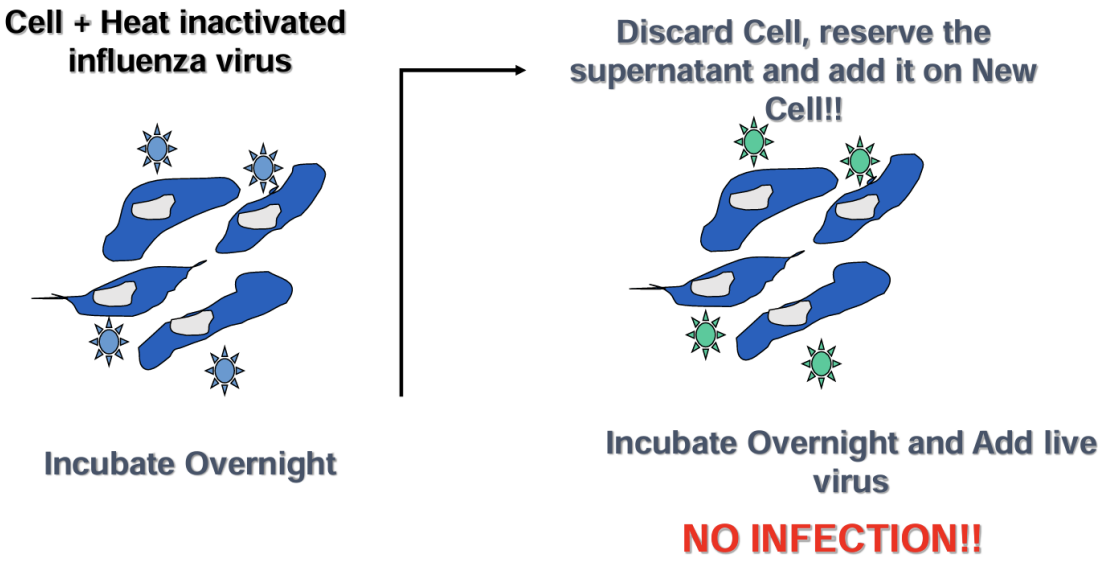


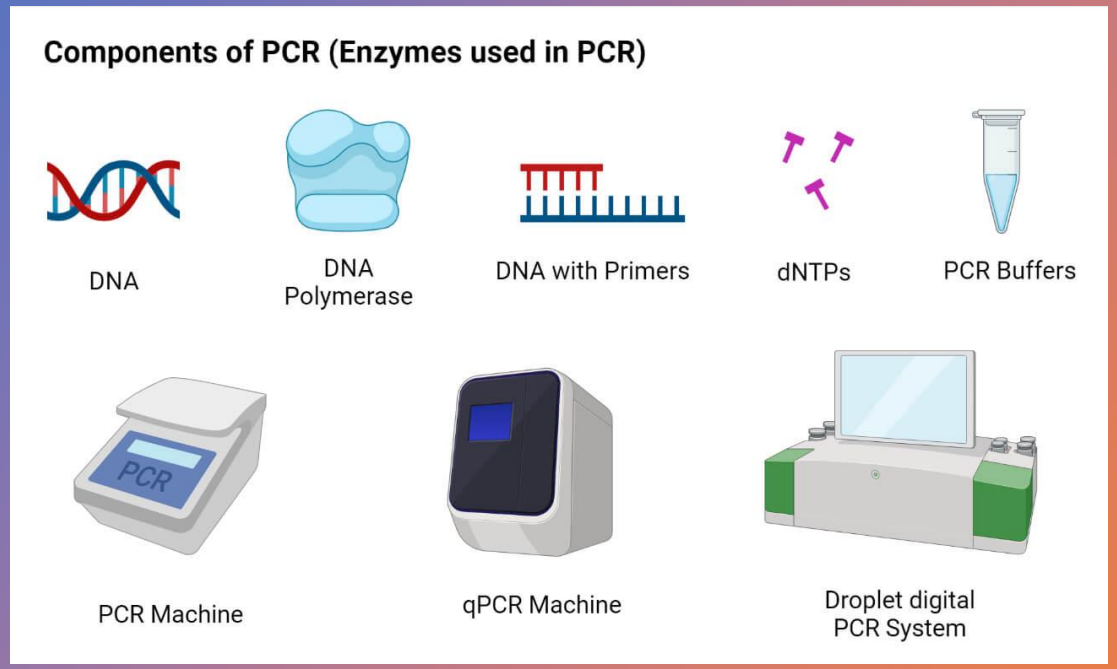
Hemadsorption in ASF virus-infected cells. Arrow indicates HAD

Hoang Minh Duc et al 2023 10.3390/pathogens12040628

➢ Interference

- Interference is defined when a virus infecting a cell partially or completely prevents that cell from being re-infected with a second virus of the same or different type. So, interference is an event.
- Accordingly, if cells infected with a virus that replicates without forming CPE are superinfected with a virus that causes CPE, it is expected that the second virus will not replicate and form CPE due to the interference effect.
- Thus, it can be indirectly demonstrated that the first virus reproduced.
- By taking advantage of this relationship, Newcastle disease virus can be used to detect non-cytopathogenic BVD virus strains (END test).





➢ PCR

- Polymerase Chain Reaction (PCR) is a nucleic acid amplification technique used to amplify the DNA or RNA in vitro enzymatically.
- t is a temperature-dependent enzymatic process where either a specific targeted region of DNA or the whole DNA is replicated to quickly make millions of copies of the target DNA or DNA segment.

https://microbenotes.com/pcr-principle-enzymes-steps-types-uses/#types-of-pcr

Kary Banks Mullis

(1944 - 2019)

Kary Banks Mullis
The Nobel Prize in Chemistry 1993

"For his invention of the polymerase chain reaction (PCR) method."

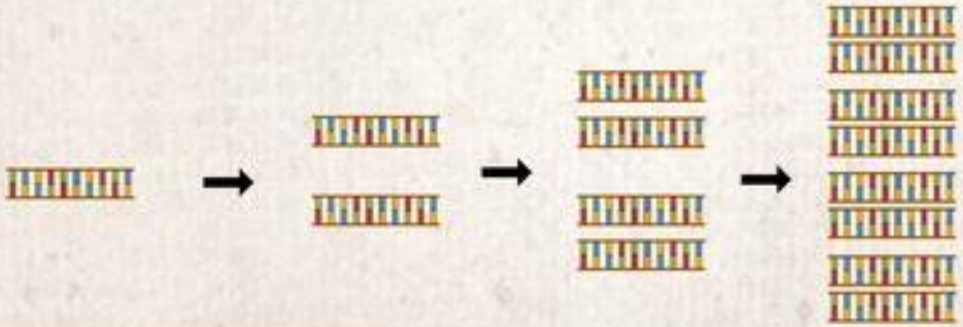
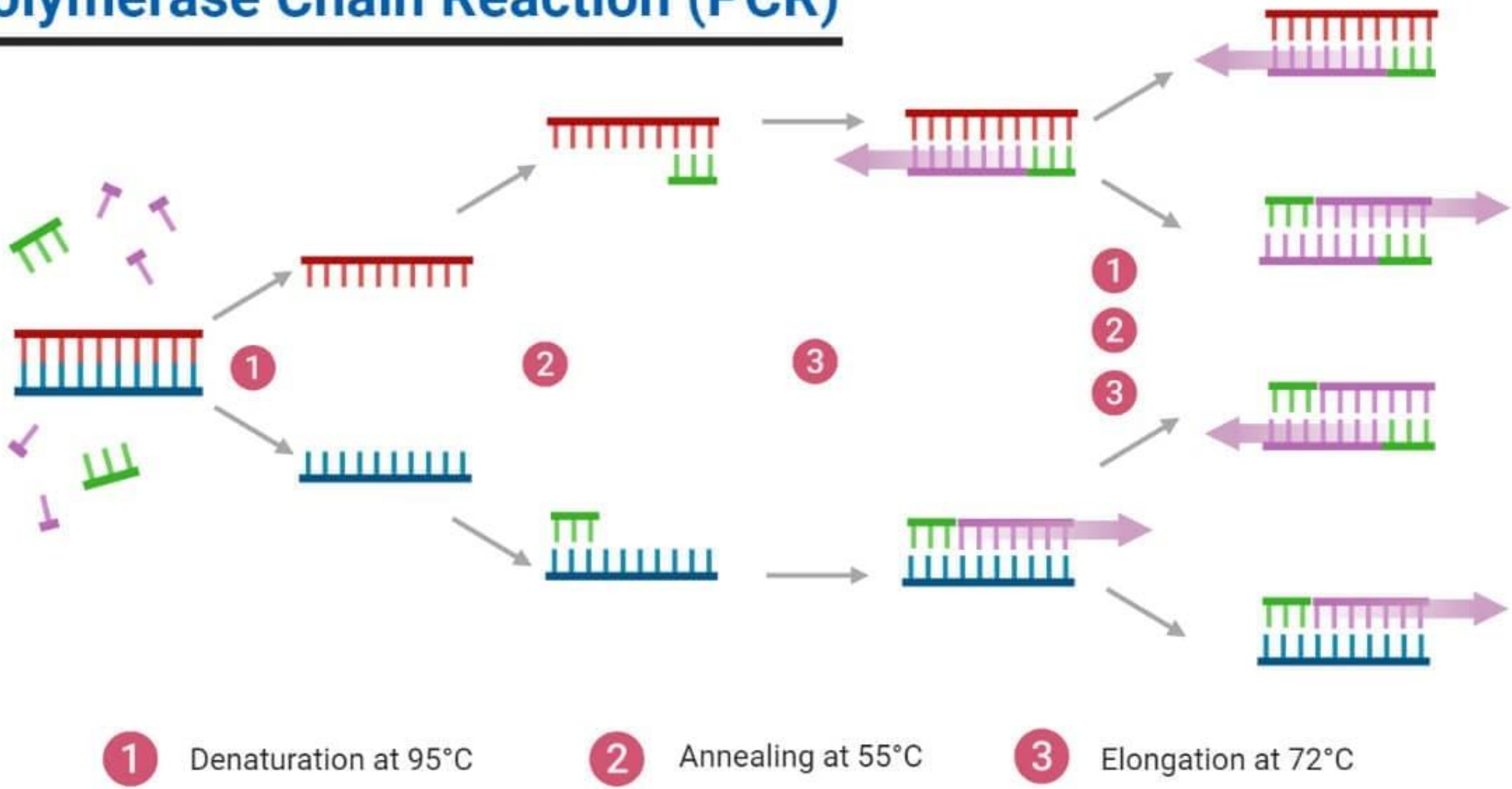


Image: The Nobel Foundation archive

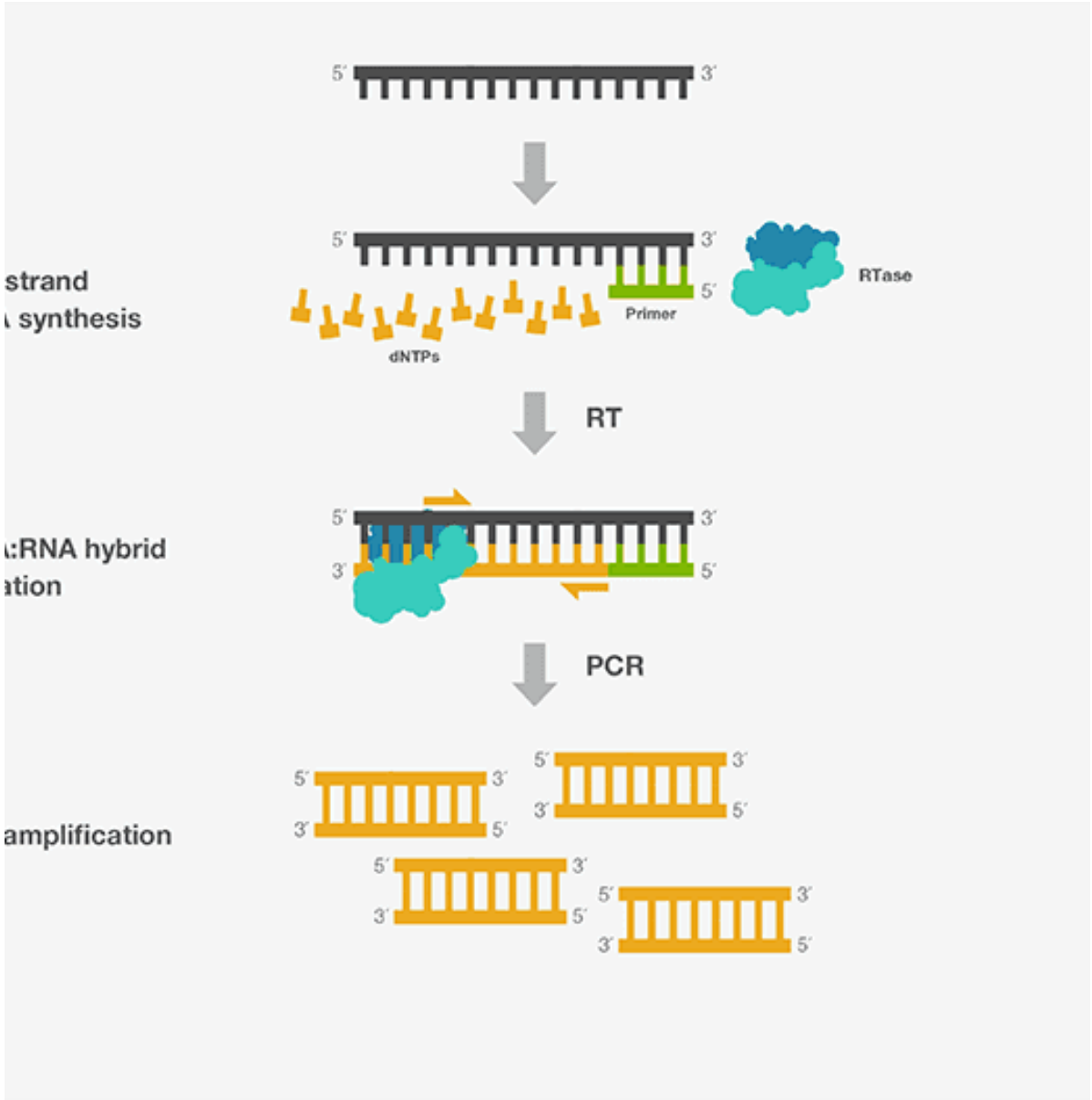
- PCR dates back to the mid-1980s when Kary Mullis and his associates developed this revolutionary technique. He was awarded the Nobel Prize in chemistry with Michael Smith in 1993.
- Since then, it has been the most important tool in molecular biology and genetics as a basic tool for DNA and RNA analysis.

https://microbenotes.com/pcr-principle-enzymes-steps-types-uses/#types-of-pcr

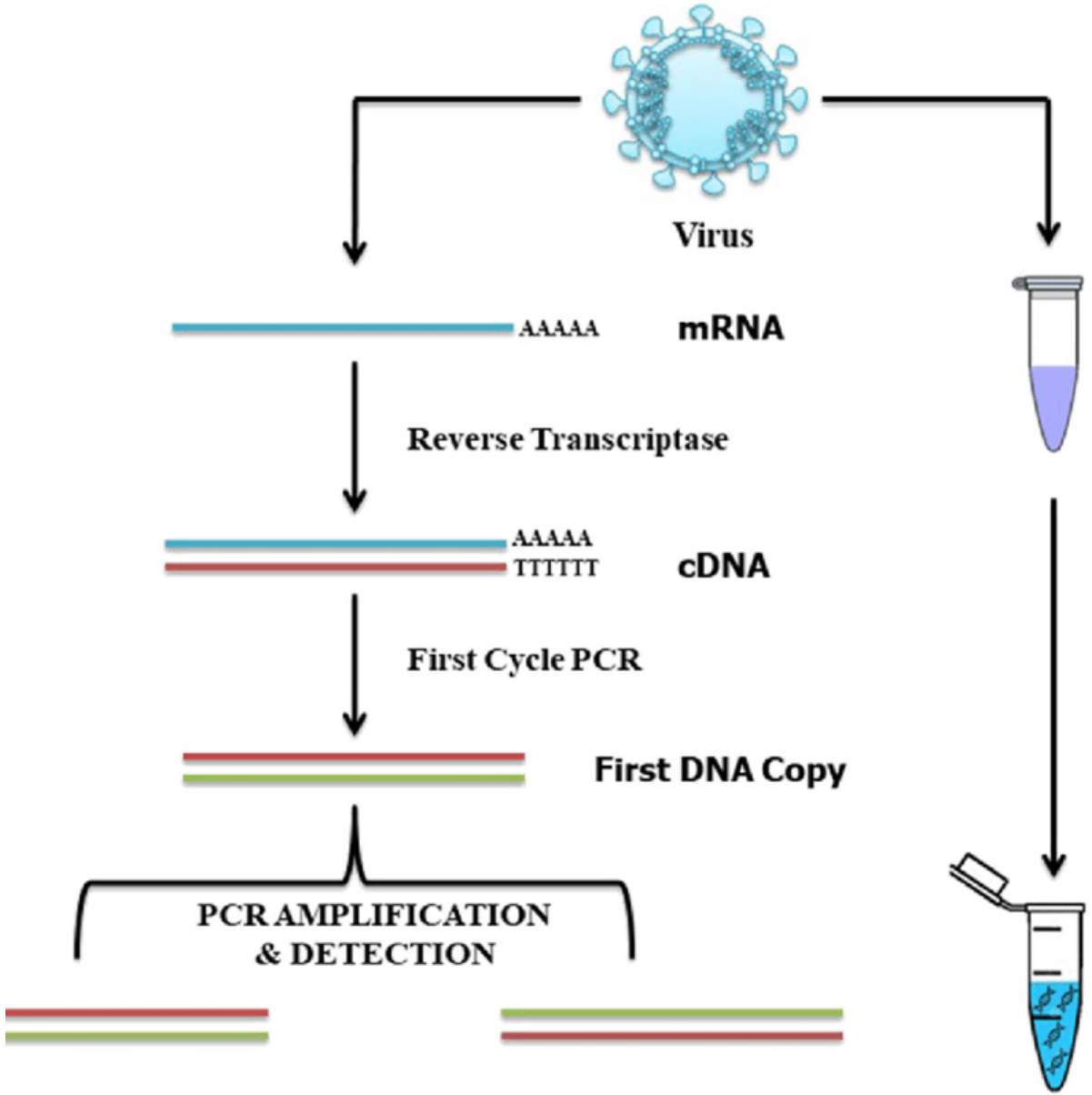
Polymerase Chain Reaction (PCR)



https://microbenotes.com/pcr-principle-enzymes-steps-types-uses/



https://microbiologynotes.org/reverse-transcription-polymerase-chain-reaction-rt-pcr/



Humzah Jamshaid et al. Diagnostic and Treatment Strategies for COVID-19 2020 DOI:10.1208/s12249-020-01756-3