

Laboratory Diagnosis of Viral Infections

G. Jamjoom 2005

Five Main Techniques:

- Virus Culture and Isolation
- Serology
- Rapid Detection of Viral Antigens
- Detection of Viral Nucleic Acid
- Electron Microscopy

Virus Culture and Isolation

- Which cell lines
- How many cell lines

Virus Culture and Isolation

- Cytopathic Effects (CPE)

Cell Lines

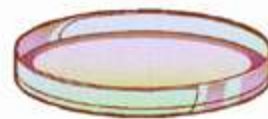
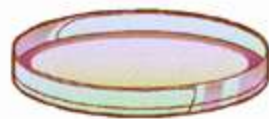
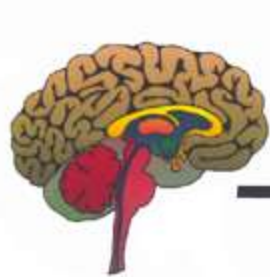
- Primary
- Continuous

Examples of Commonly Used Cell Lines :

- Primary Cell Lines :
 - monkey kidney cells
 - human foreskin fibroblasts

Examples of Commonly Used Cell Lines :

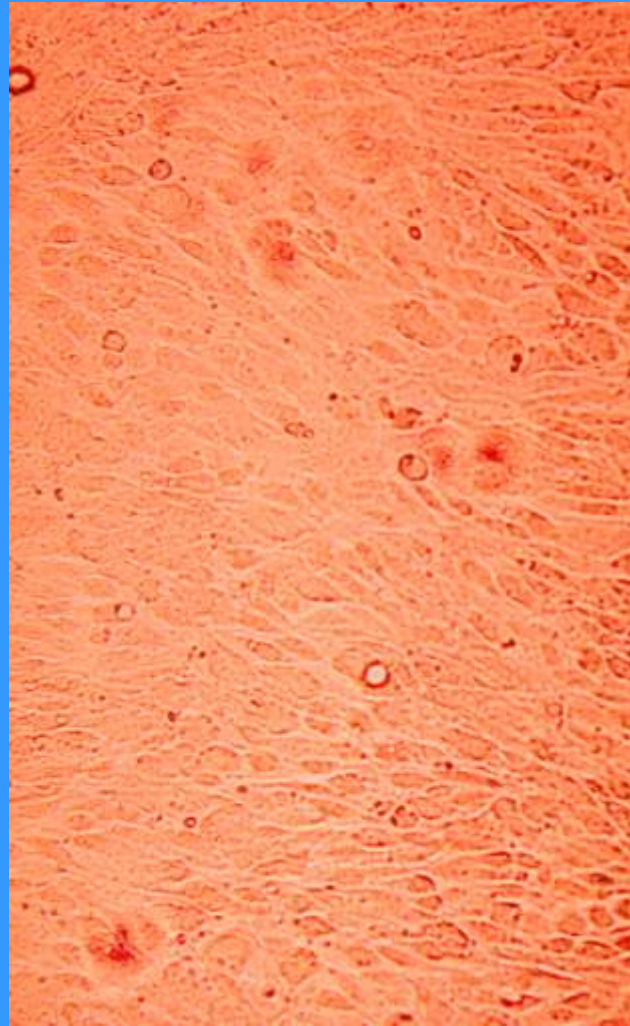
- Continuous Cell Lines :
 - VERO
 - HEP-2
 - MDCK



tissue culture cells



epithelial



epithelioid

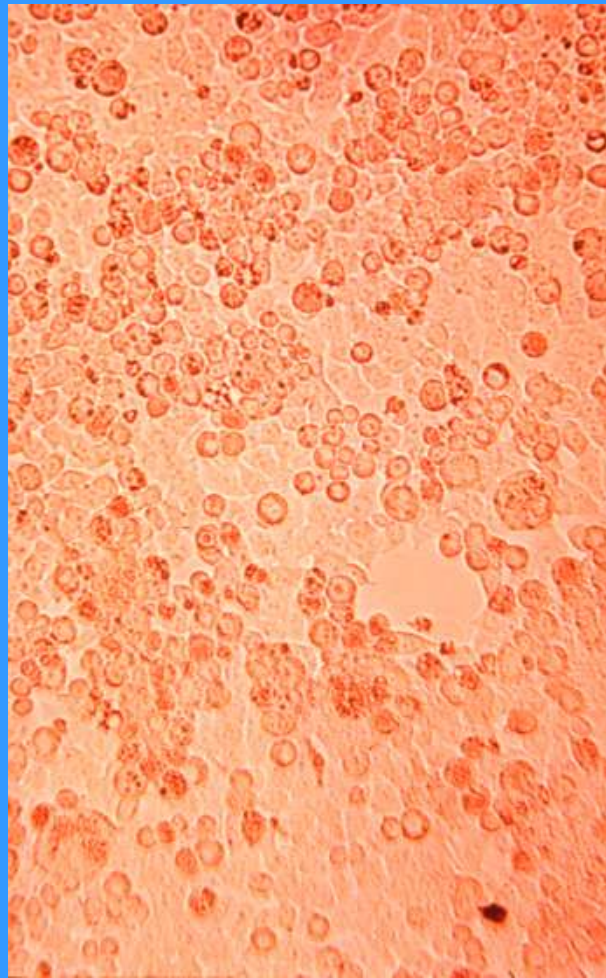


fibroblastic

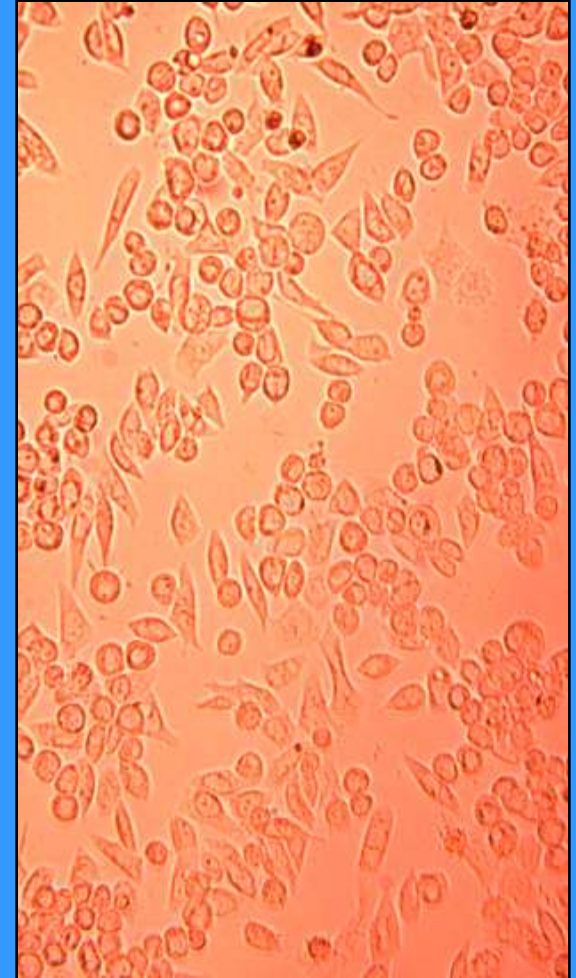
epithelial cells - adenovirus



uninfected



early infection



late infection

fibroblastic cells - poliovirus



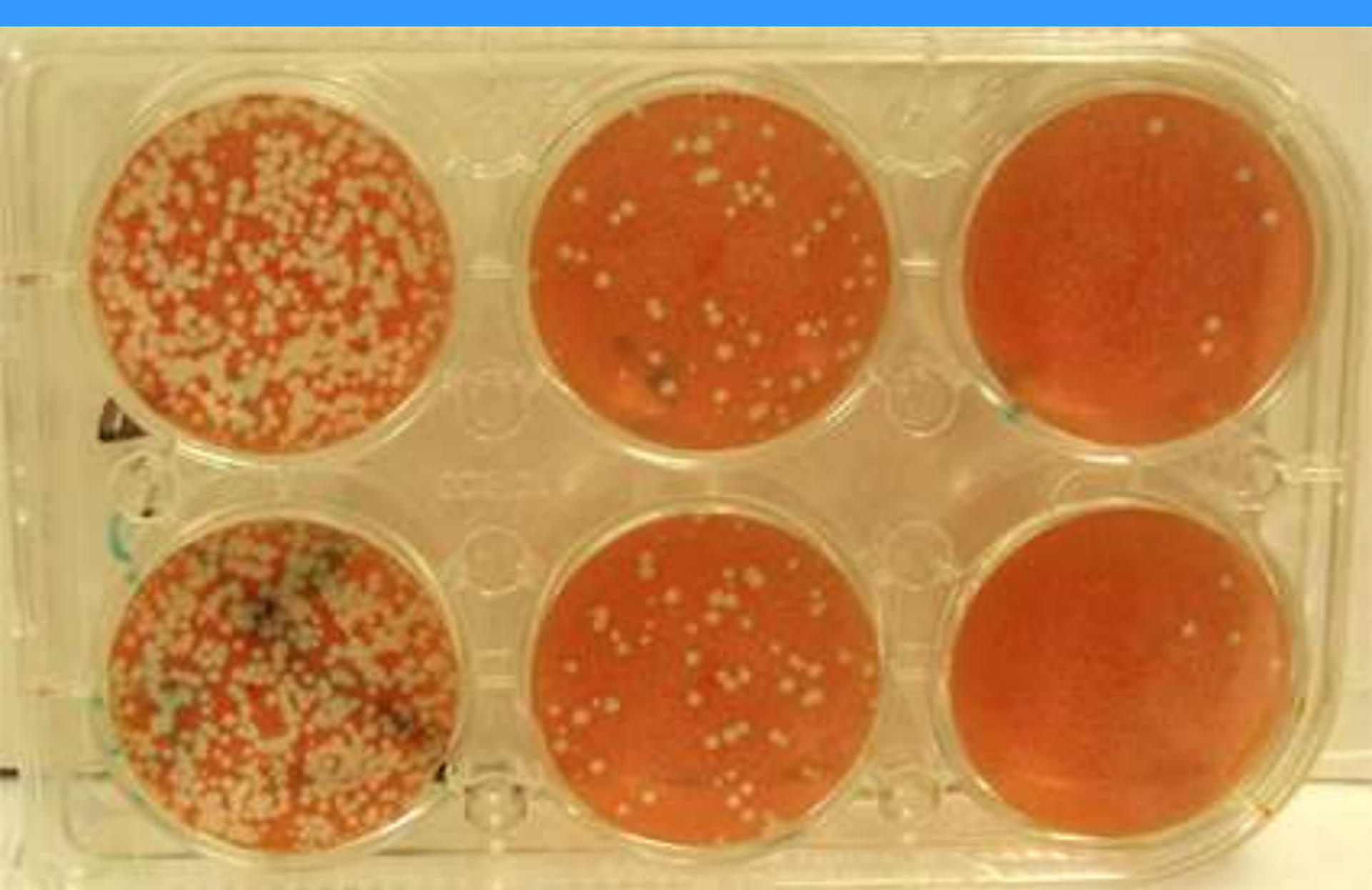
uninfected



early infection



late infection

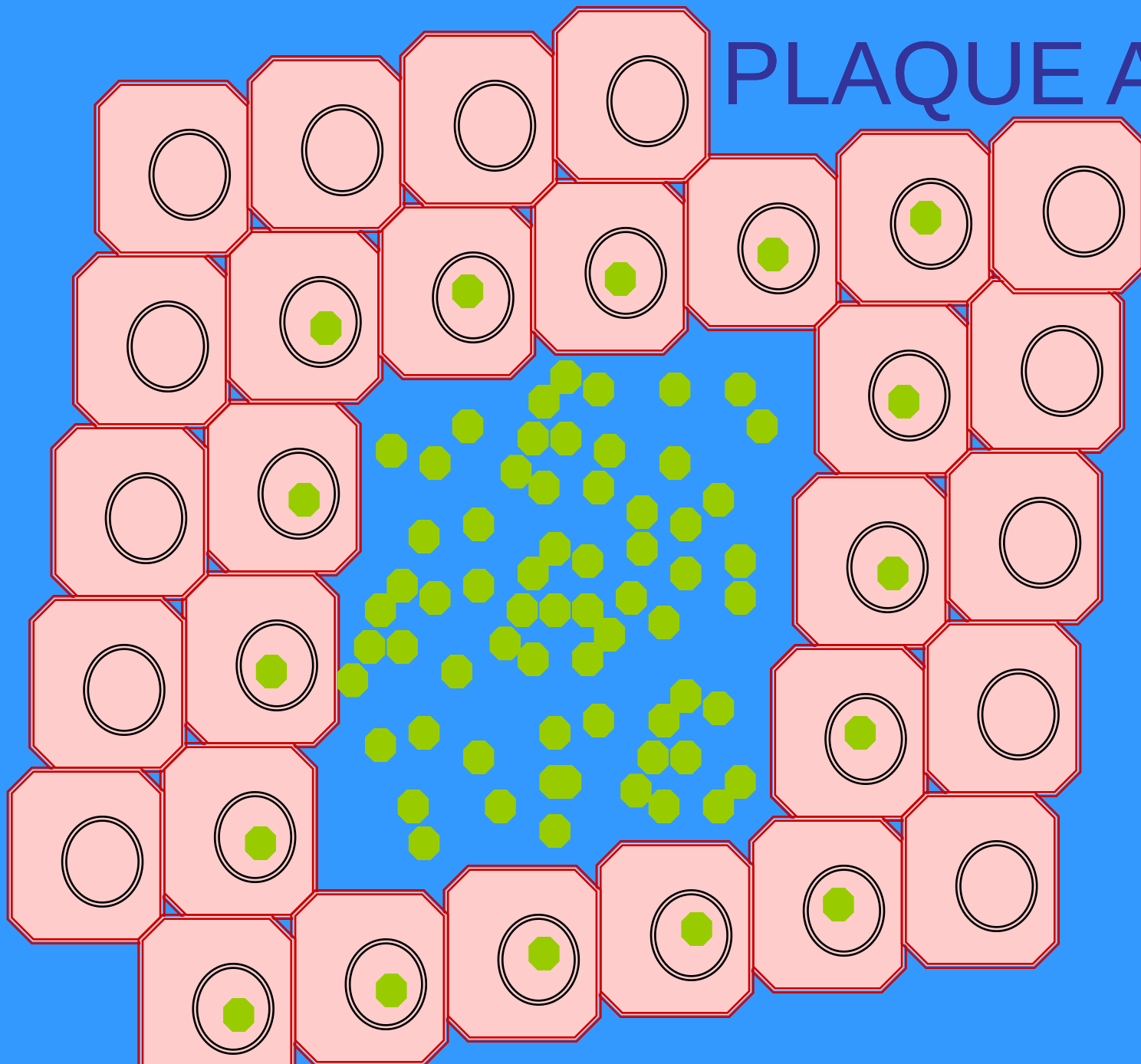


Diluted 10 fold

Diluted 100 fold

Diluted 1000 fold

PLAQUE ASSAY



PLAQUE FORMING UNIT

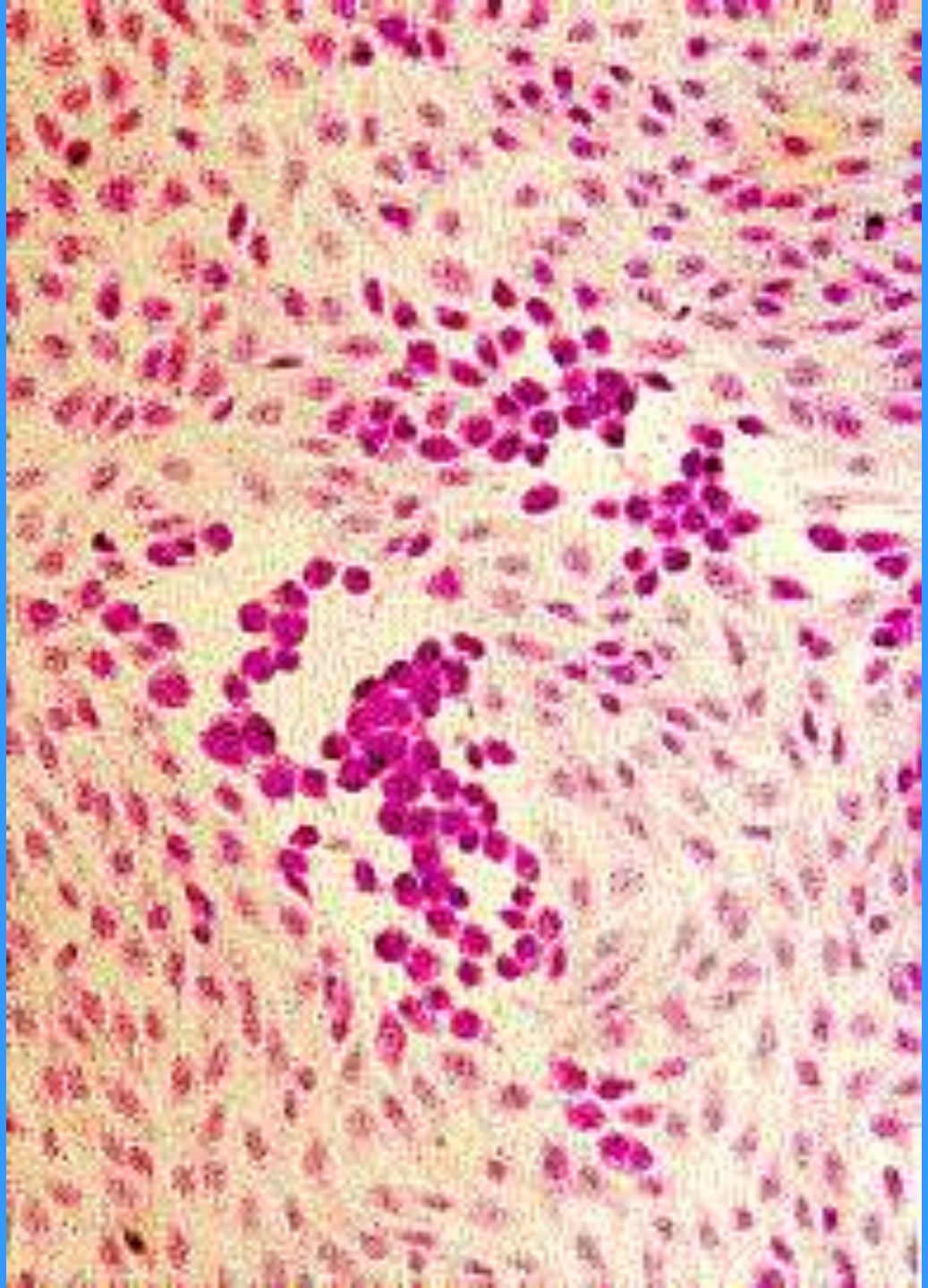
P.F.U.

pfu

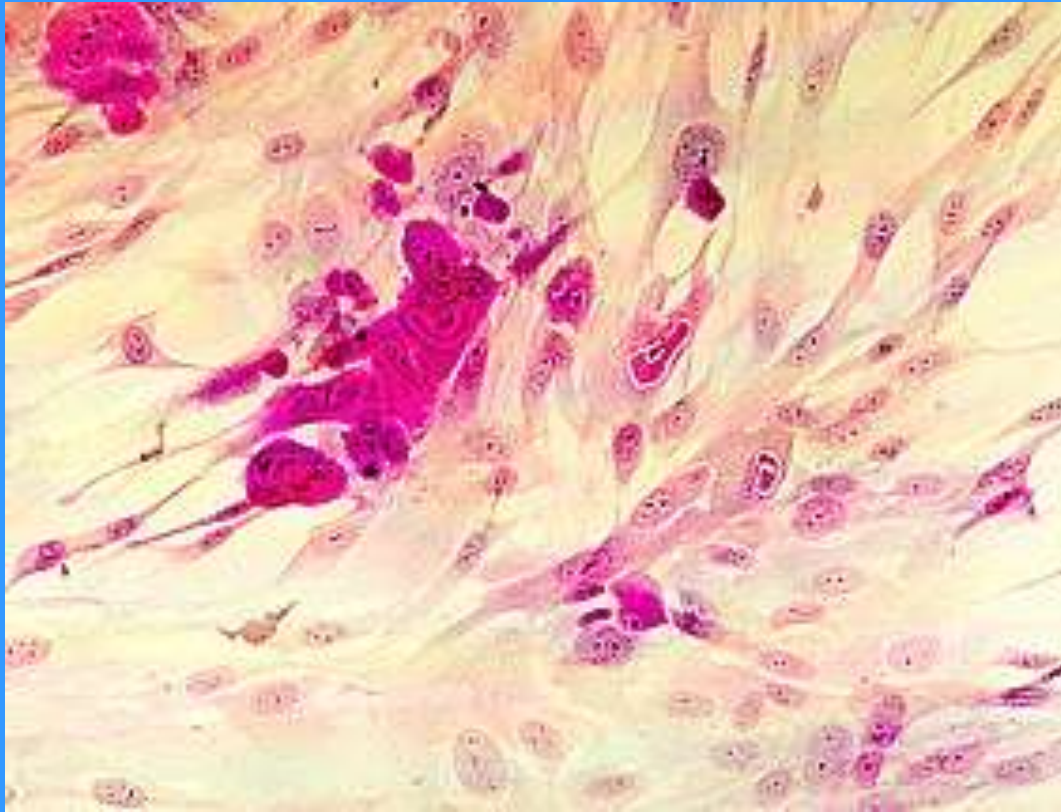
Cytopathic Effects :

- Rounding
- Detachment (plaques)
- Clumping
- Ballooning (Giant Cell)
- Fusion (Syncytium Formation)
- Inclusion Formation

**Cytopathic Effect of
HSV in cell culture:**
**(Note the ballooning
of cells).**

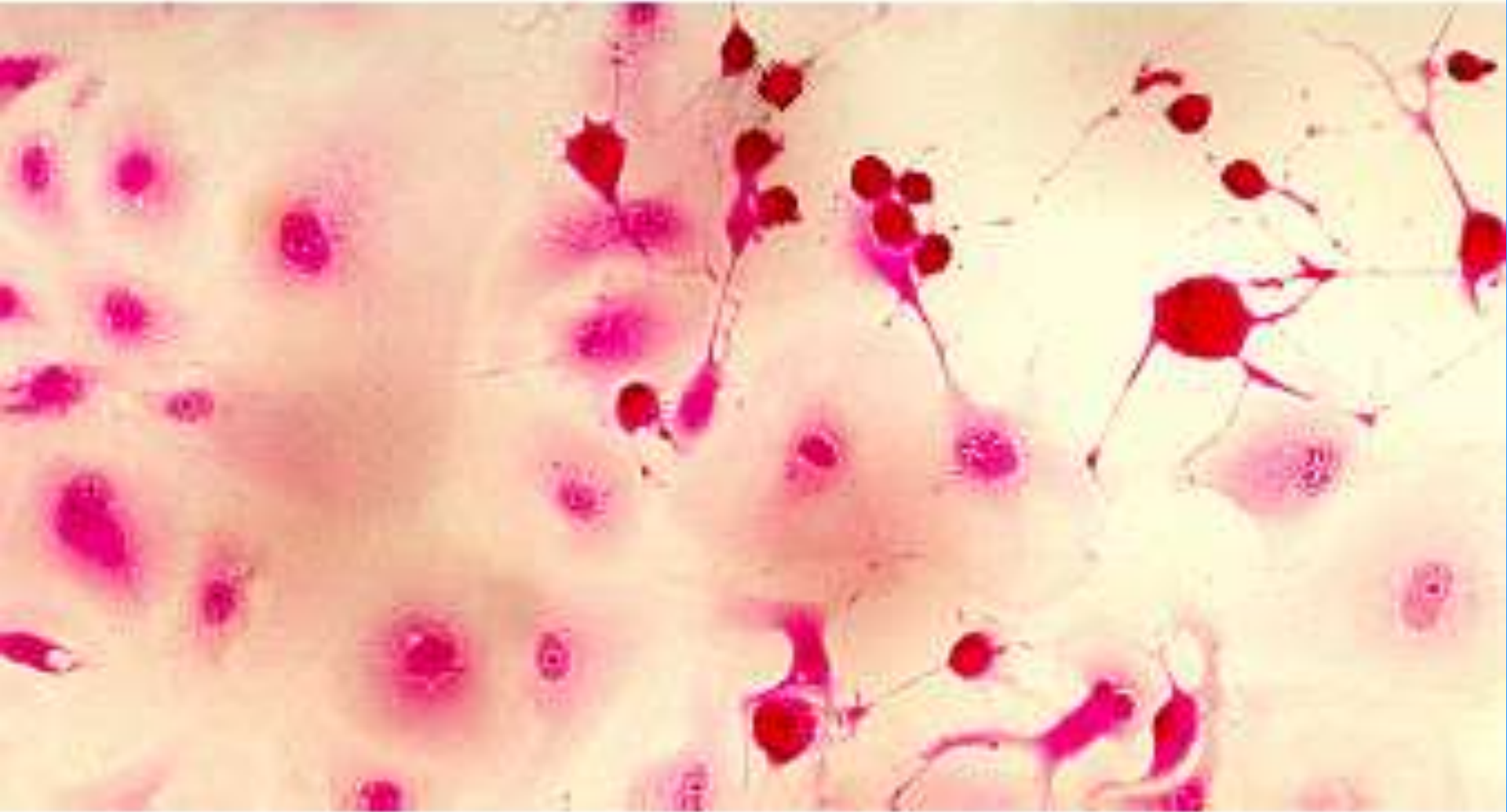


Cytopathic Effect of CMV

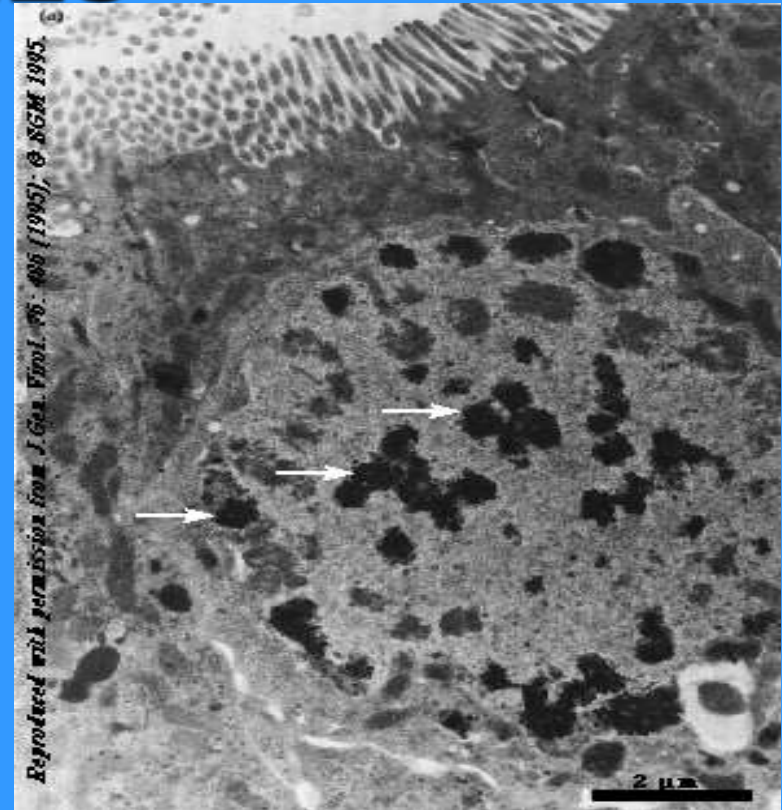
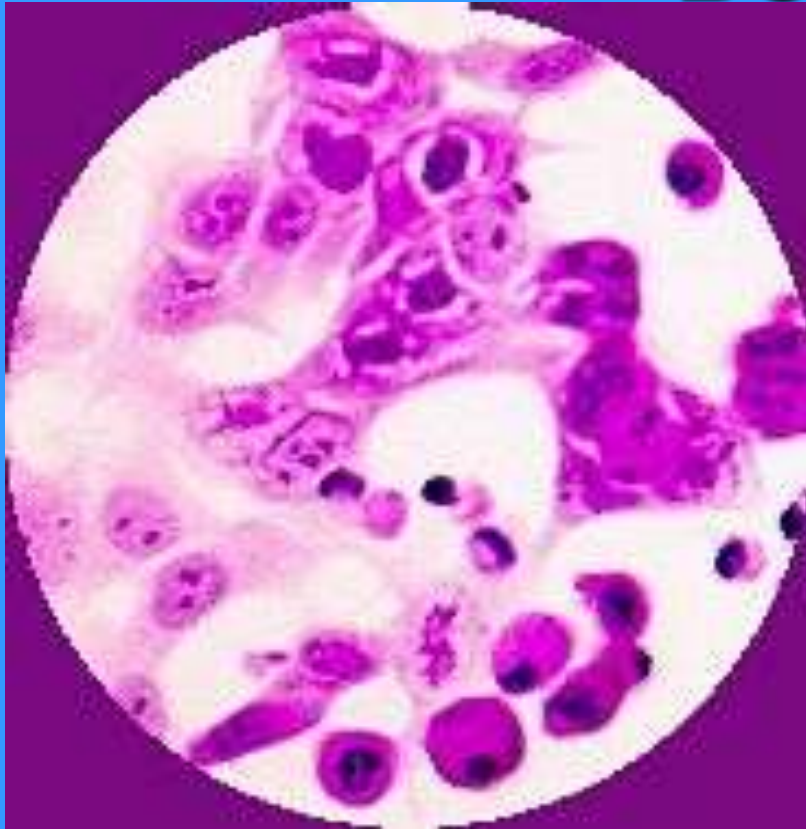


(Courtesy of Linda Stannard, University of Cape Town, S.A.)

Cytopathic Effect of VZV



ADENOVIRAL INCLUSION BODIES



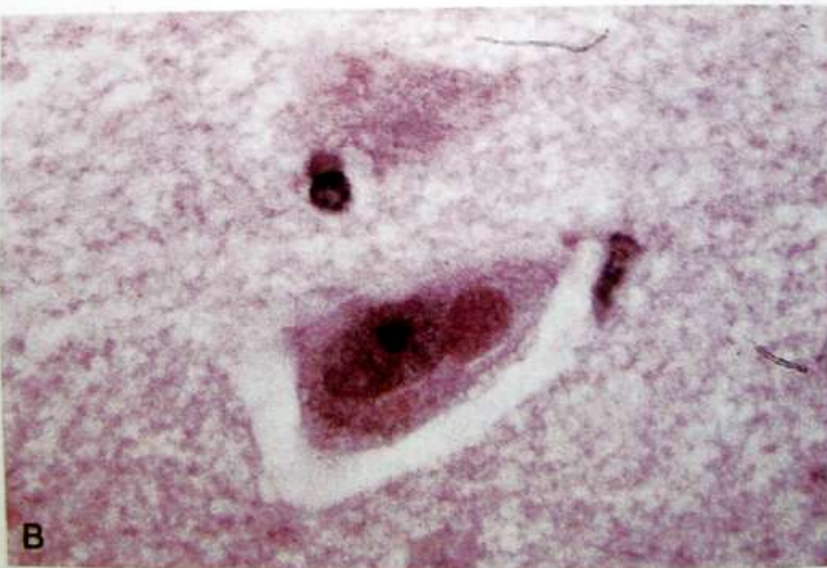


FIGURE 48-3. Negri bodies caused by rabies. *A*, A section of brain from a patient with rabies shows Negri bodies (arrow). *B*, Higher magnification from another biopsy specimen. (A from Hart C, Broadhead RL: *A color atlas of pediatric infectious diseases*, London, 1992, Wolfe.)

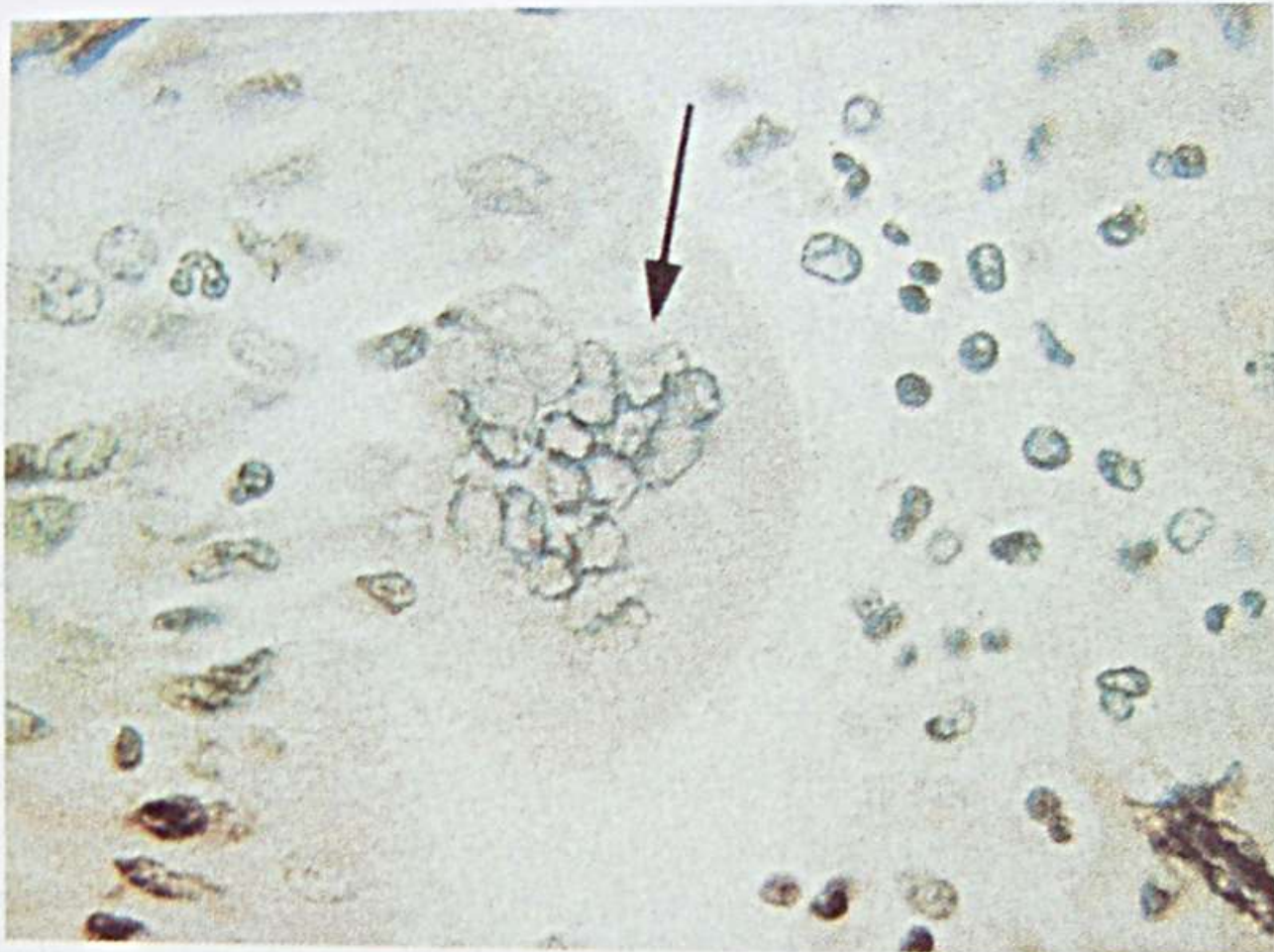
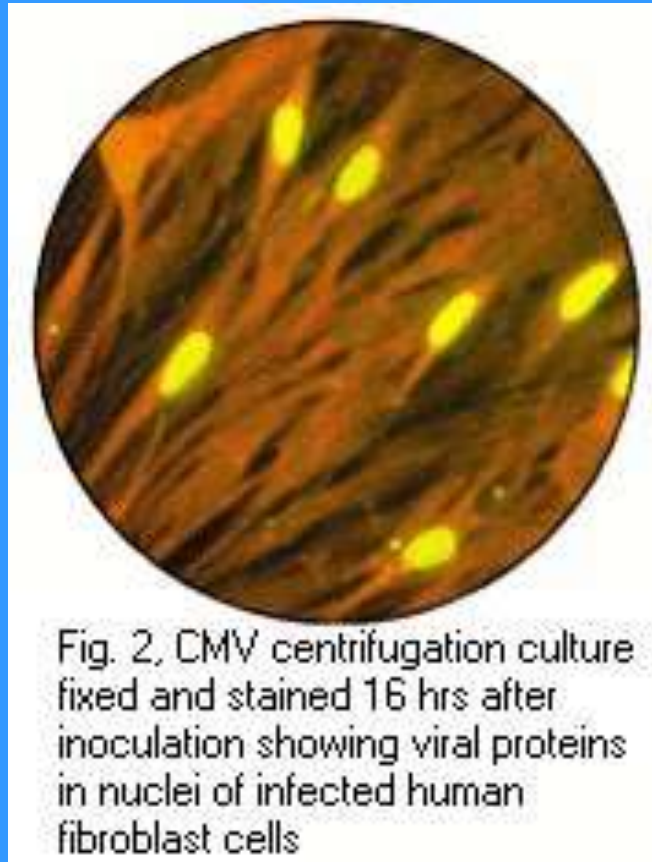


FIGURE 48–1. Syncytium formation by measles virus. Multi-nucleated giant cell (*arrow*) visible in a histologic section of lung biopsy tissue from a measles virus–induced giant cell pneumonia in an immunocompromised child. (From Hart C, Broadhead RL: *A color atlas of pediatric infectious diseases*, London, 1992, Wolfe.)

Virus Culture and Isolation

- Shell Vial

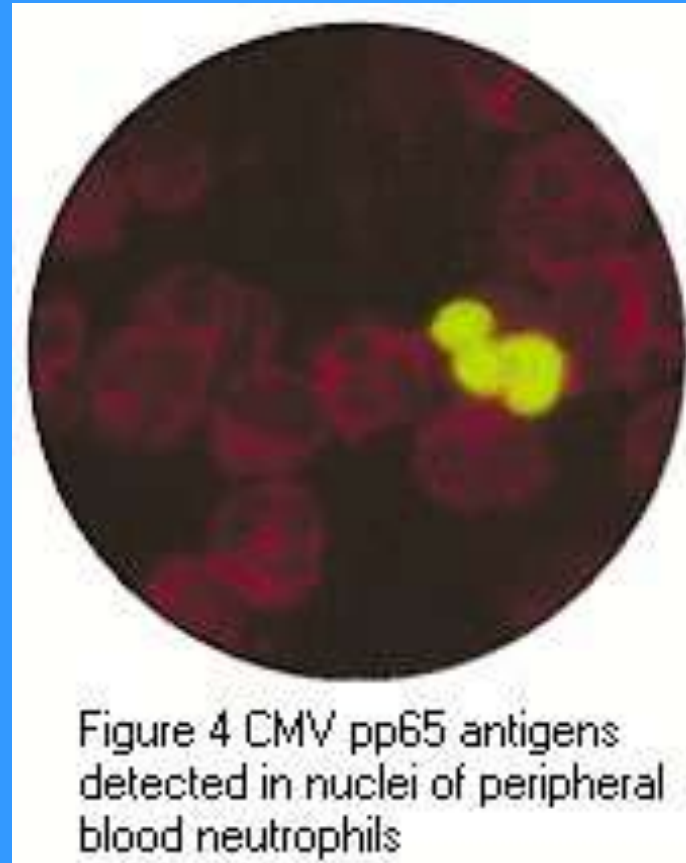
DEAFF test for CMV



(Virology Laboratory, New-Yale Haven Hospital)

Rapid Detection of Viral Antigens

CMV pp65 antigenaemia test



(Virology Laboratory, New-Yale Haven Hospital)

**Positive
immunofluorescence
test for HSV antigen in
epithelial cell.**

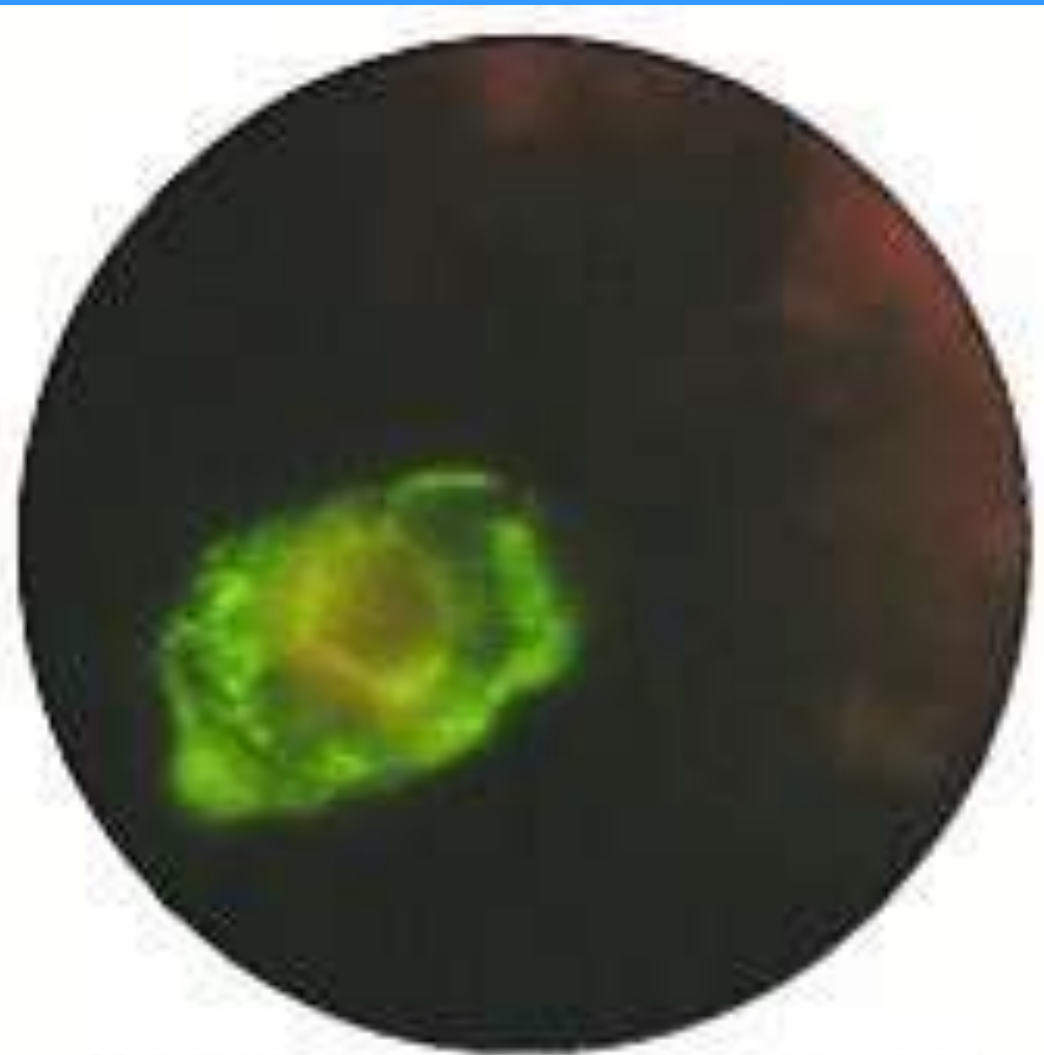
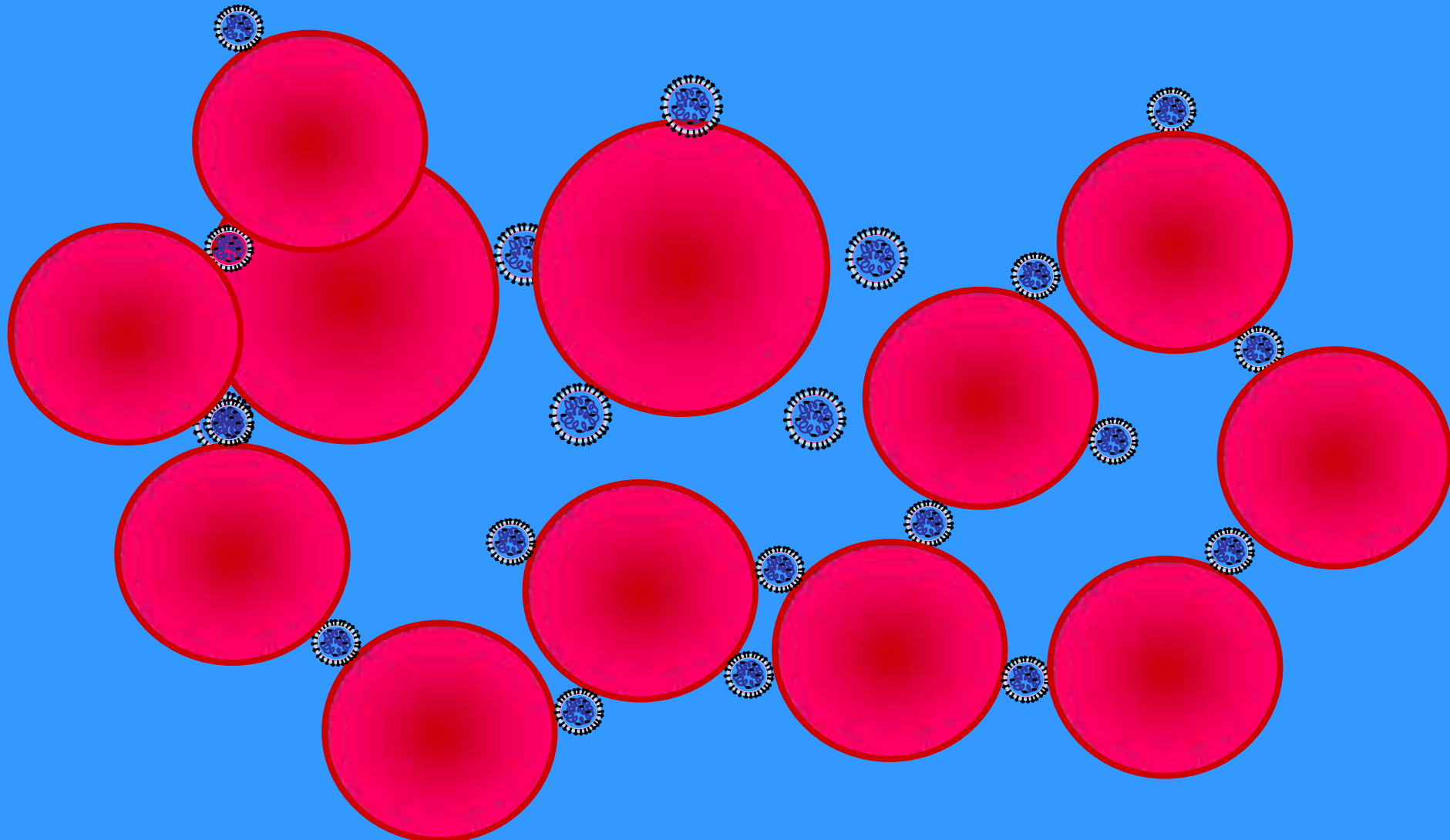


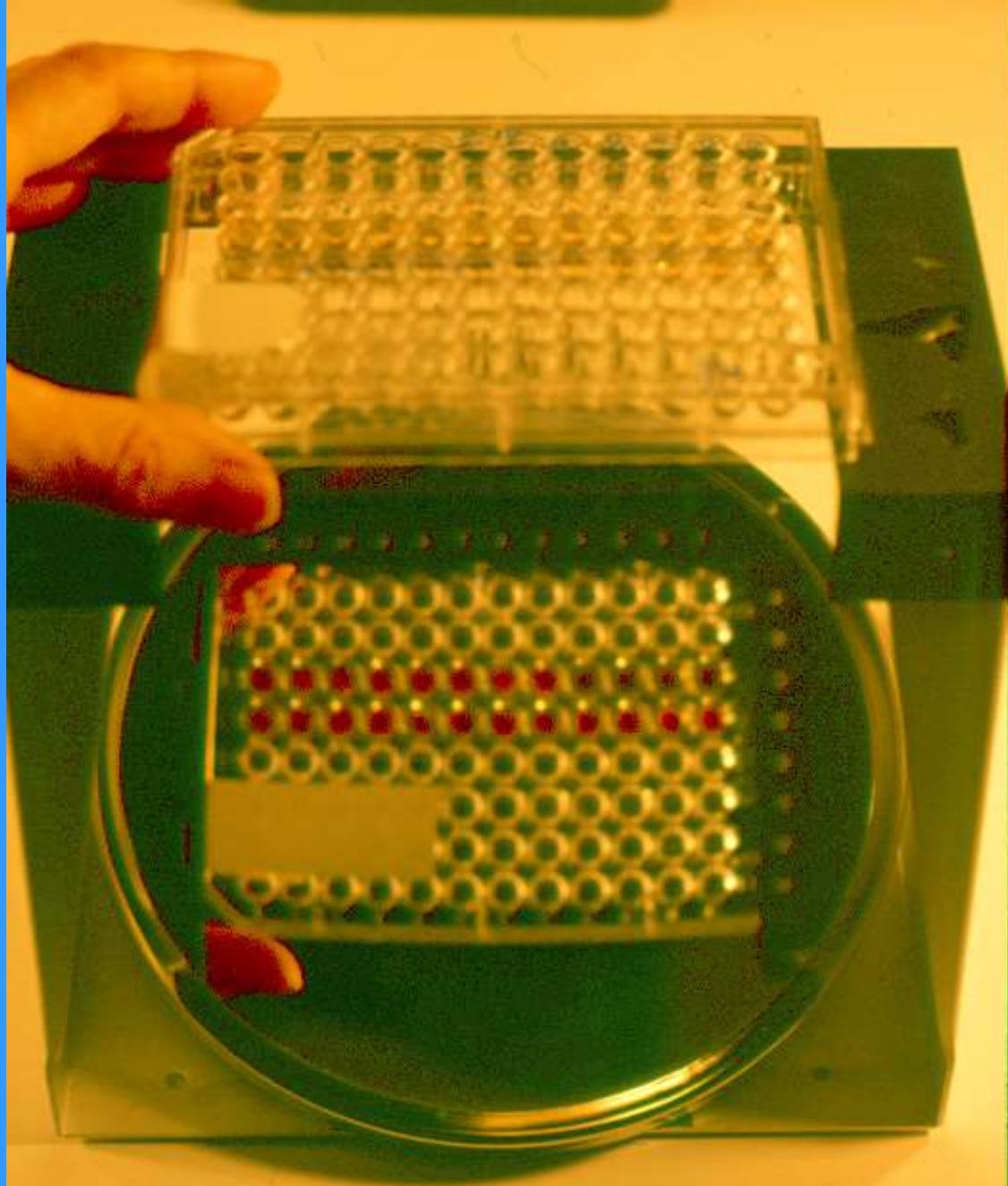
Fig. 3, HSV-infected epithelial cell from skin lesion (DFA)

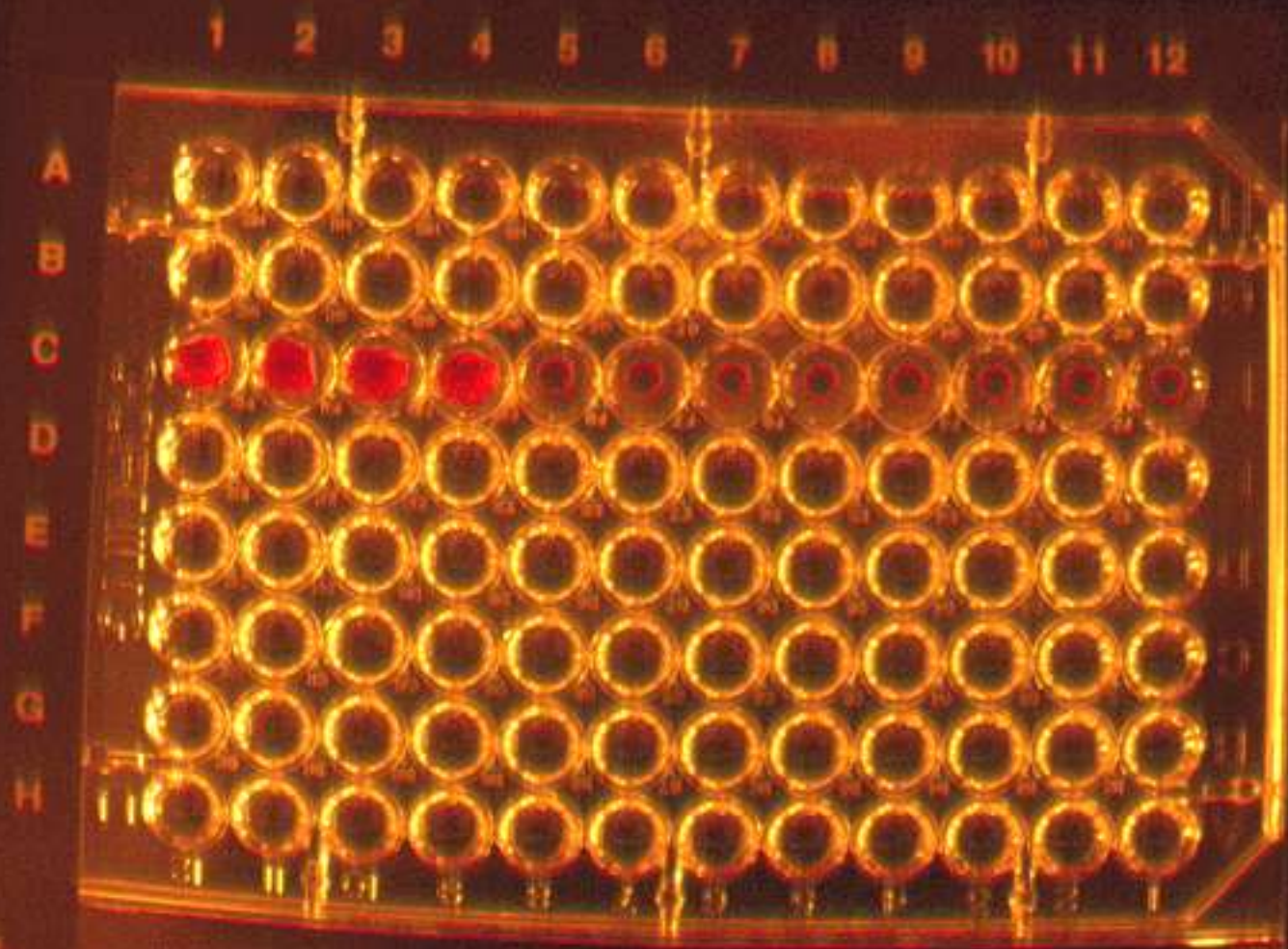
Serology :

- Hemagglutination Inhibition

hemagglutination

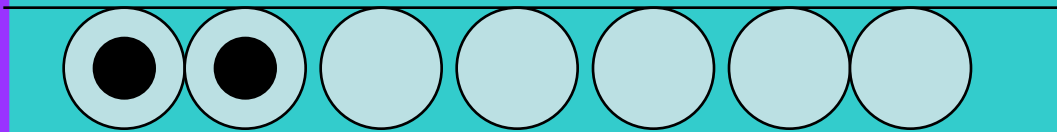




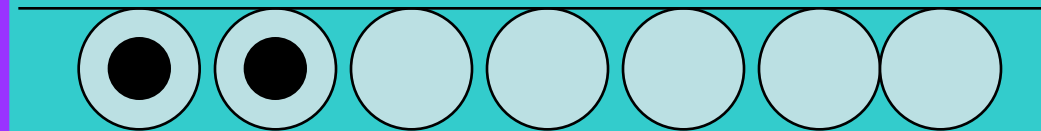


Serology :

- No rise



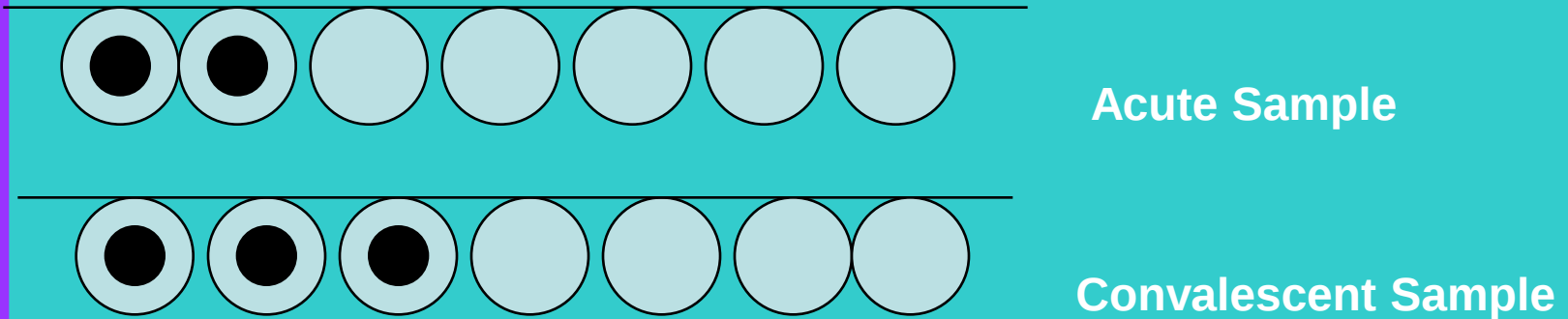
Acute Sample



Convalescent Sample

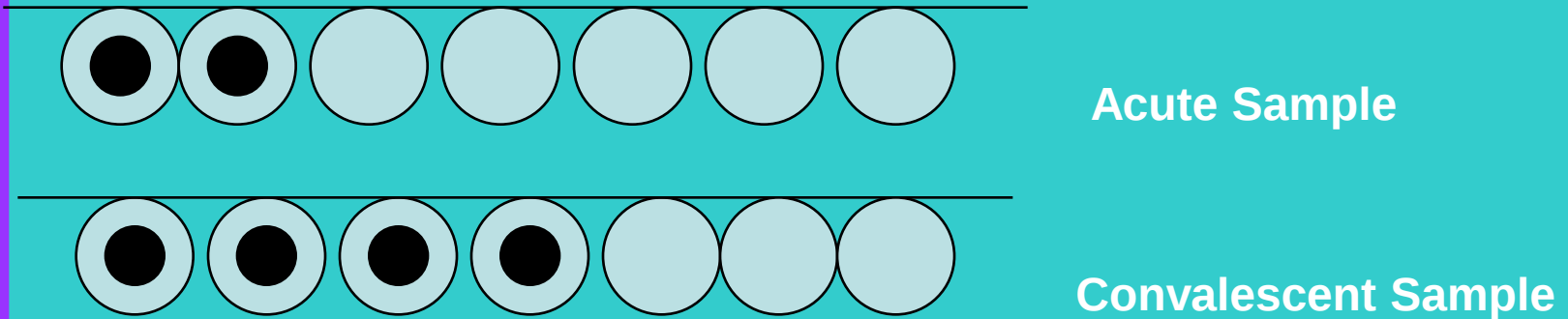
Serology :

- Two-fold rise (not significant)



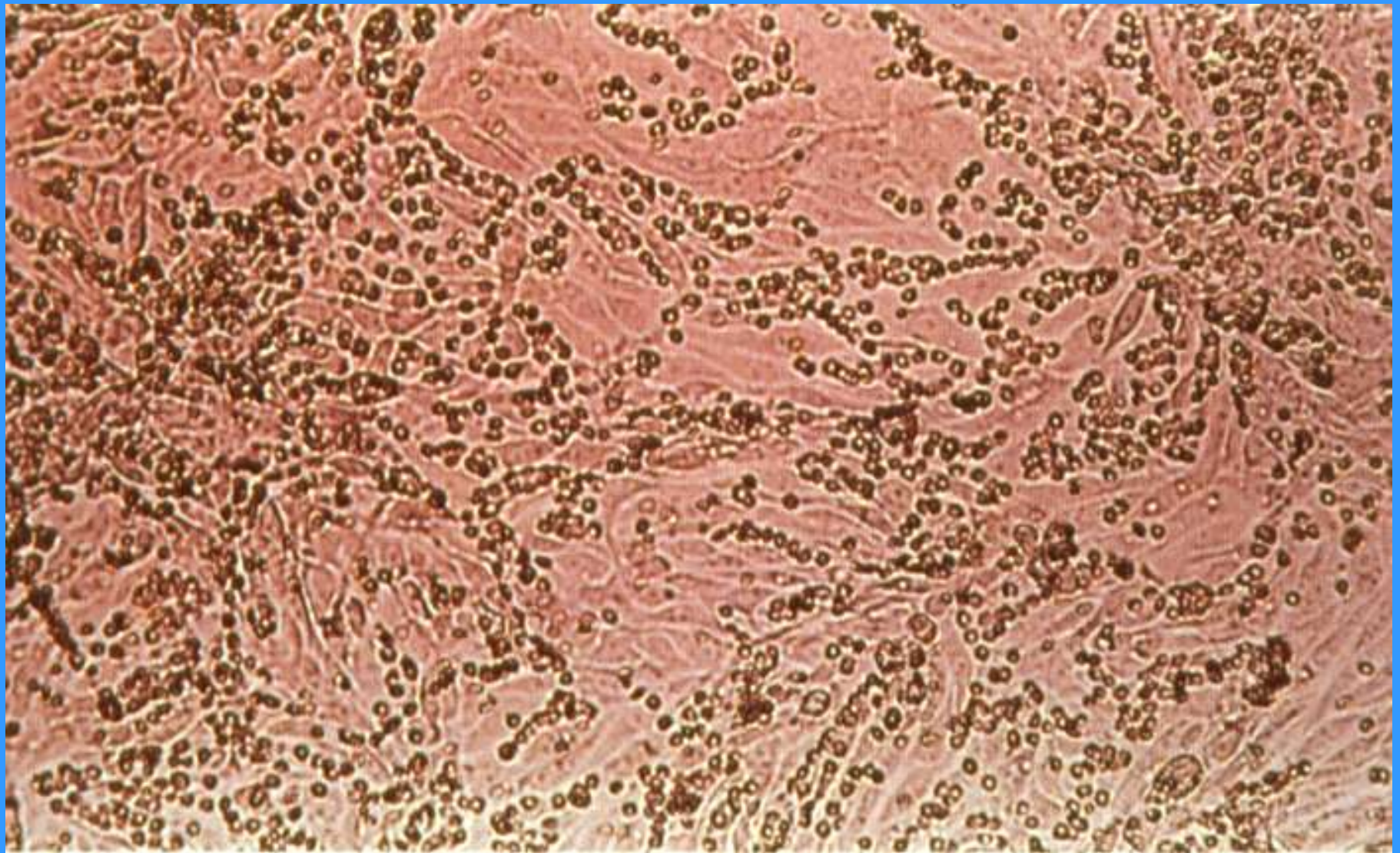
Serology :

- Four-fold rise (significant)



Serology :

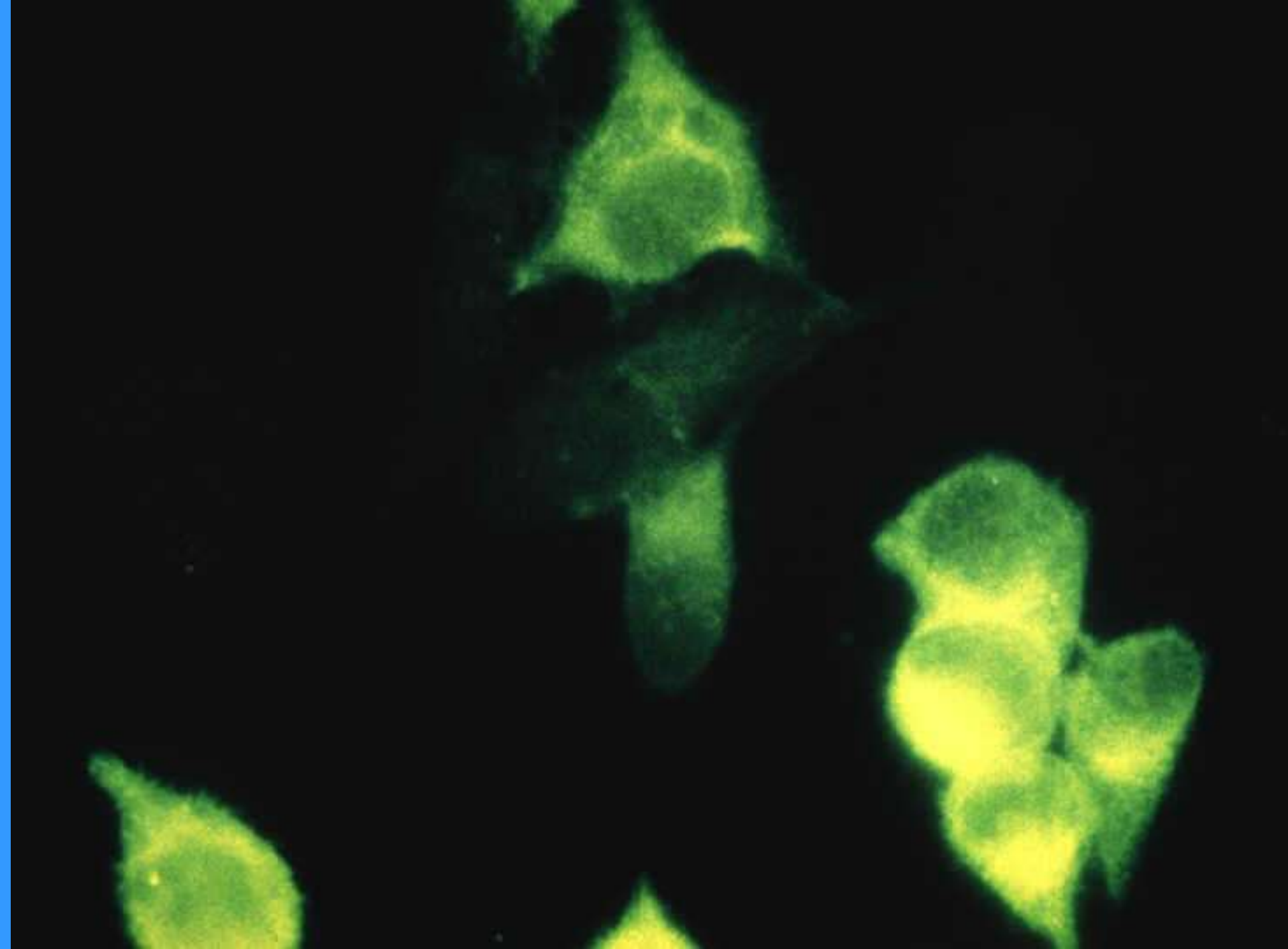
- Hemadsorption



Hemadsorption - influenza virus infected cells

Serology :

- Immunofluorescence



Serology :

- Enzyme Linked Immuno Sorbent Assay
(ELISA) (EIA)

HIV/AIDS: Diagnosis and monitoring

Serological tests for HIV infection-2

ELISA

The viral proteins can be derived from:

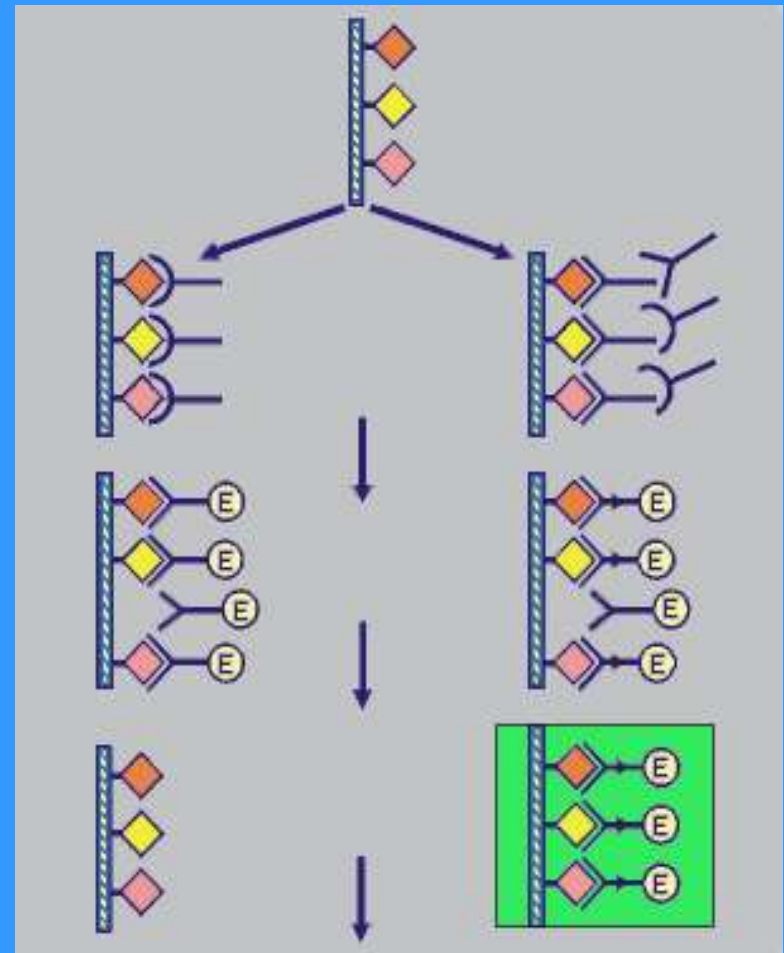
Gp160

Gp120

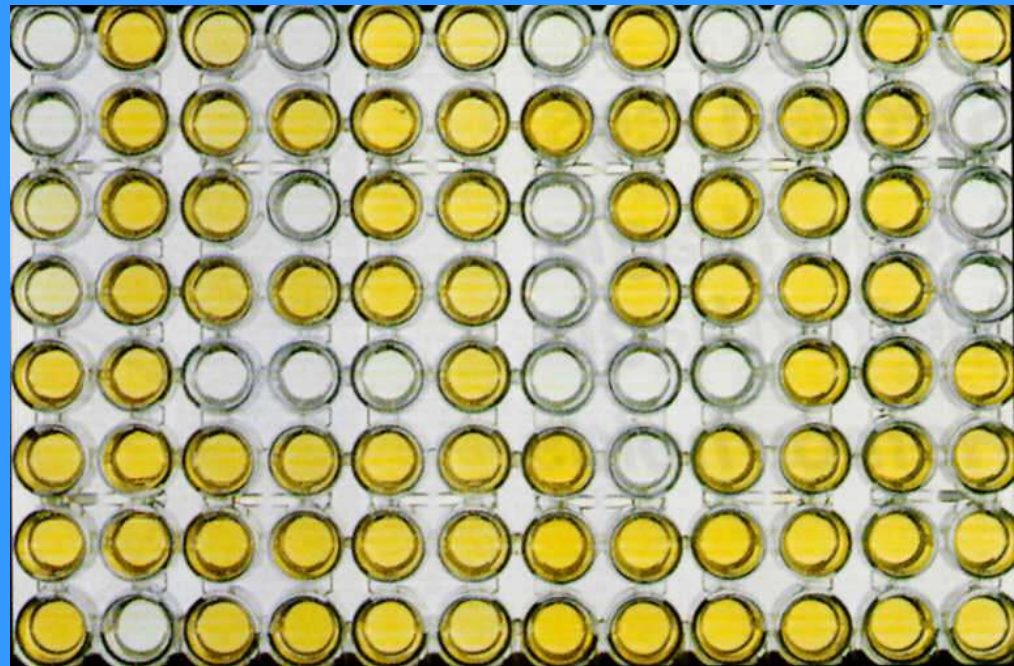
Gp24

Gp17/18

Synthetic peptides corresponding to viral proteins are also commonly used in ELISA

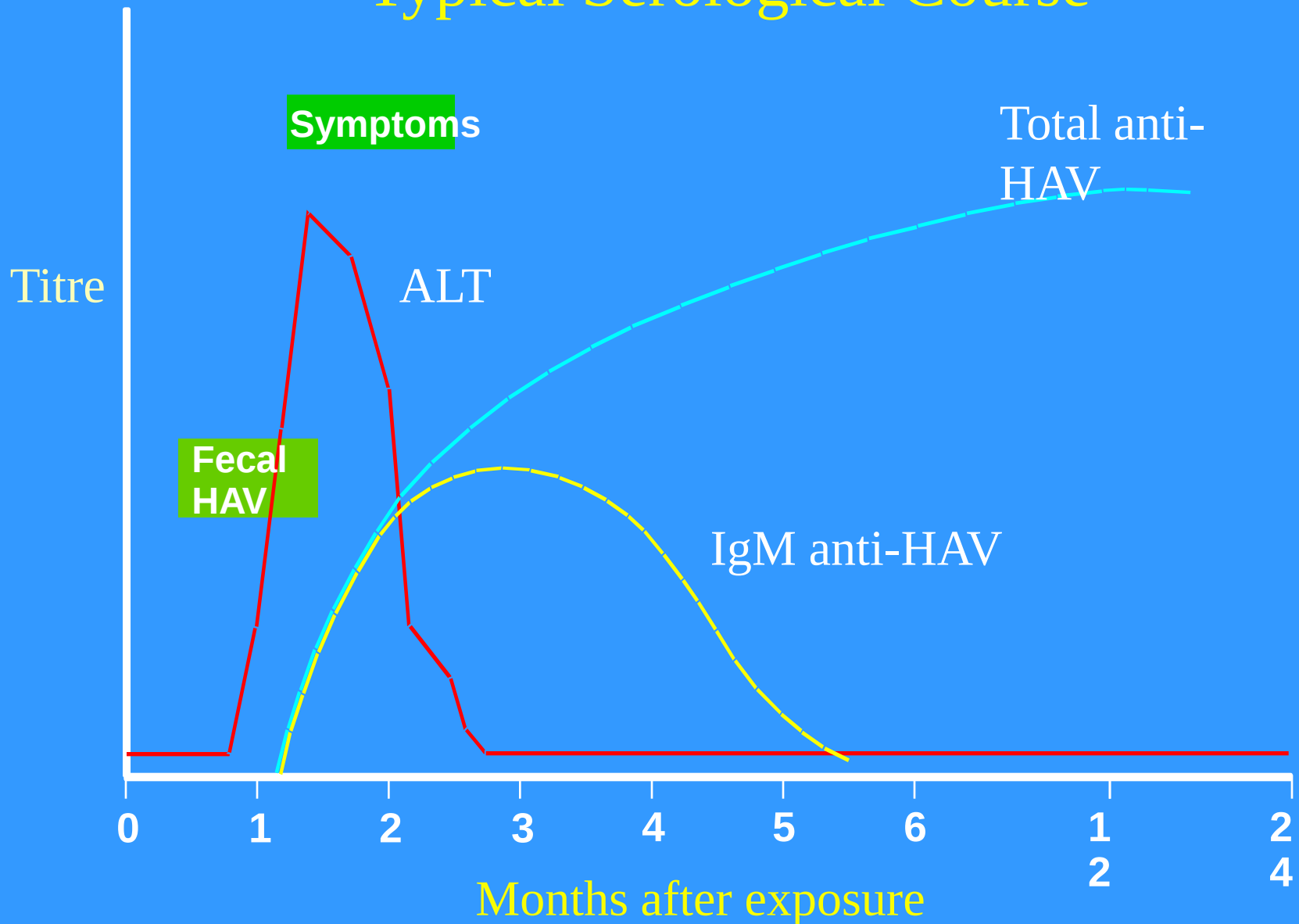


- ELISA

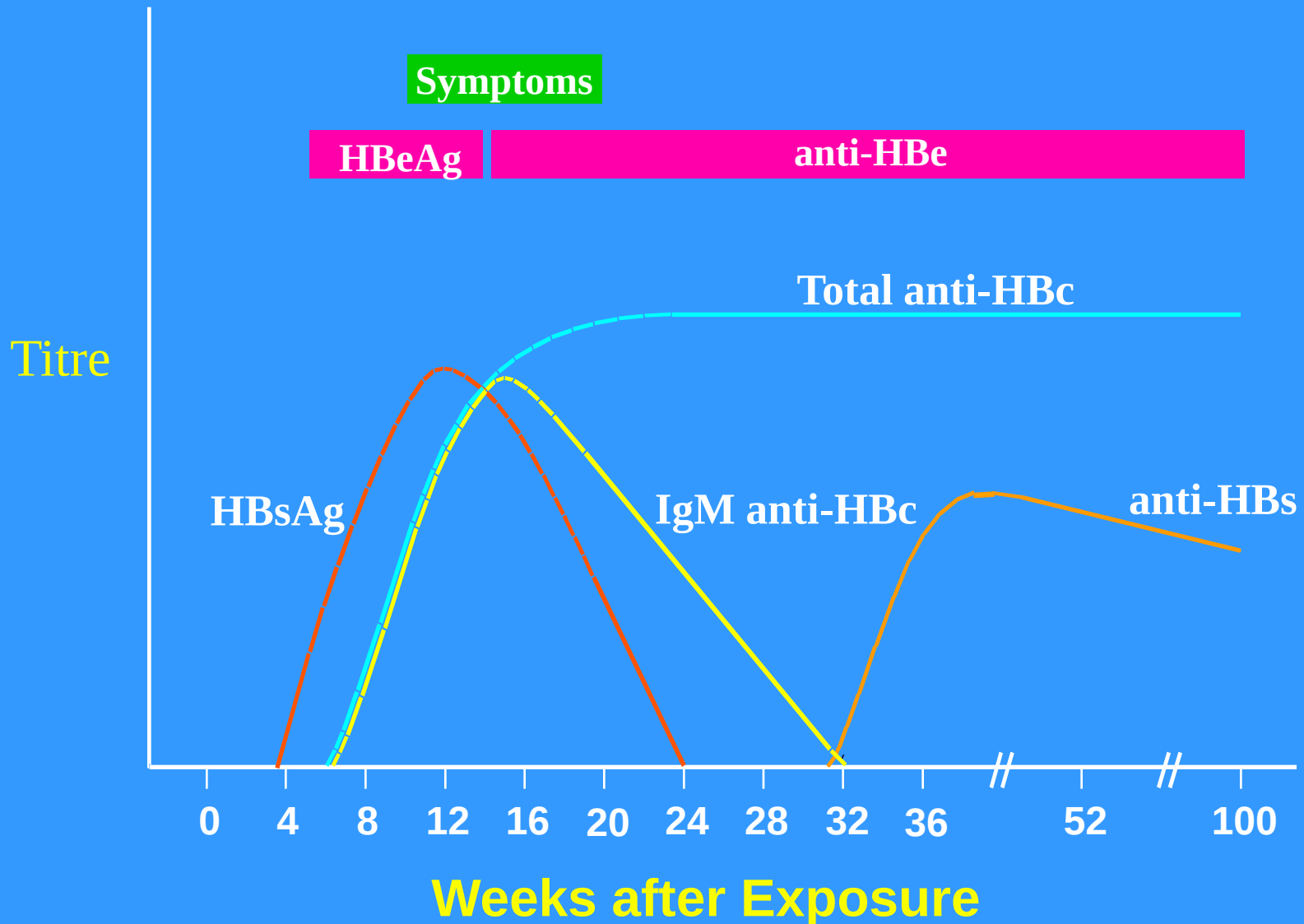


Hepatitis A Infection

Typical Serological Course

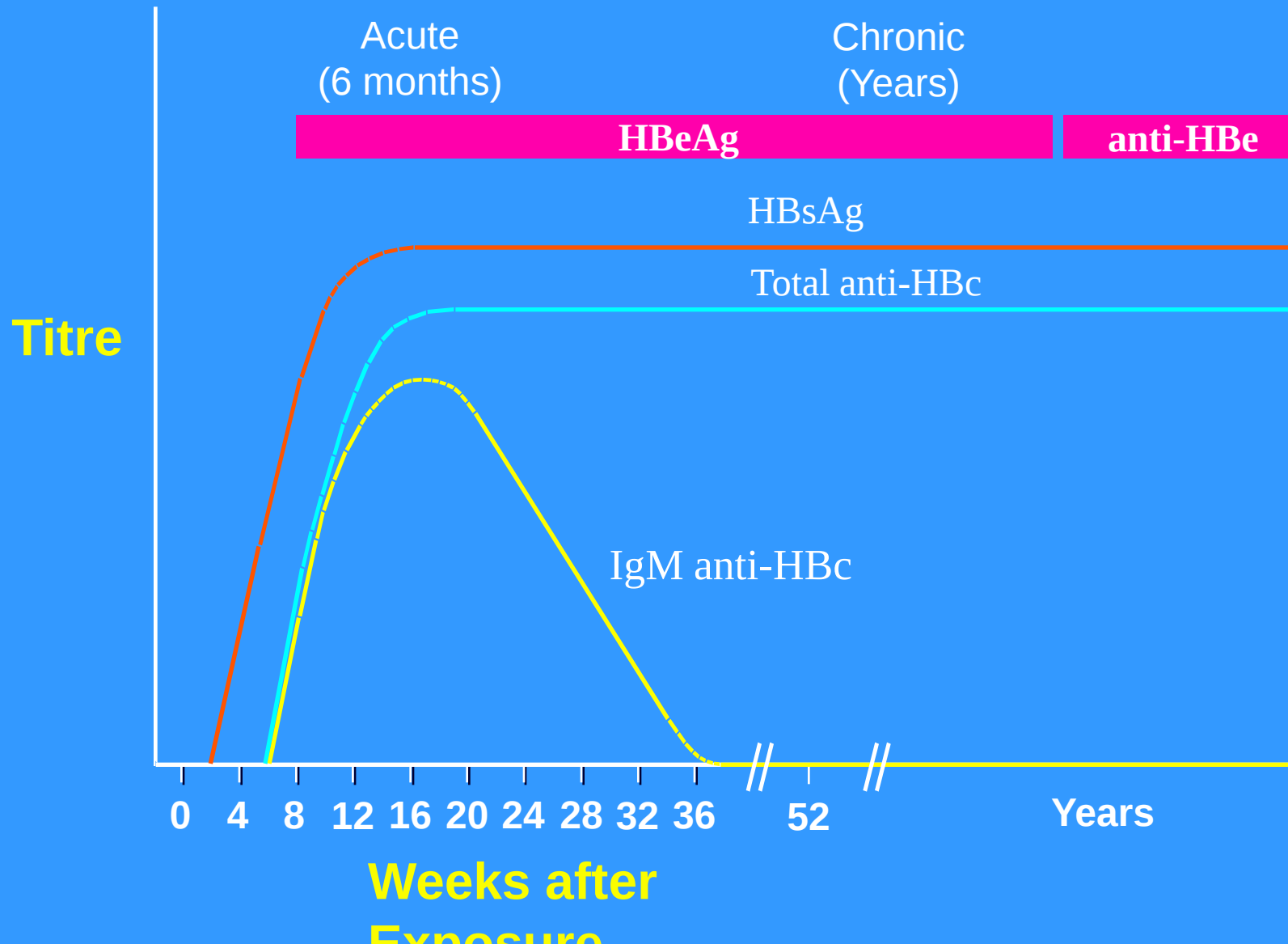


Acute Hepatitis B Virus Infection with Recovery Typical Serologic Course

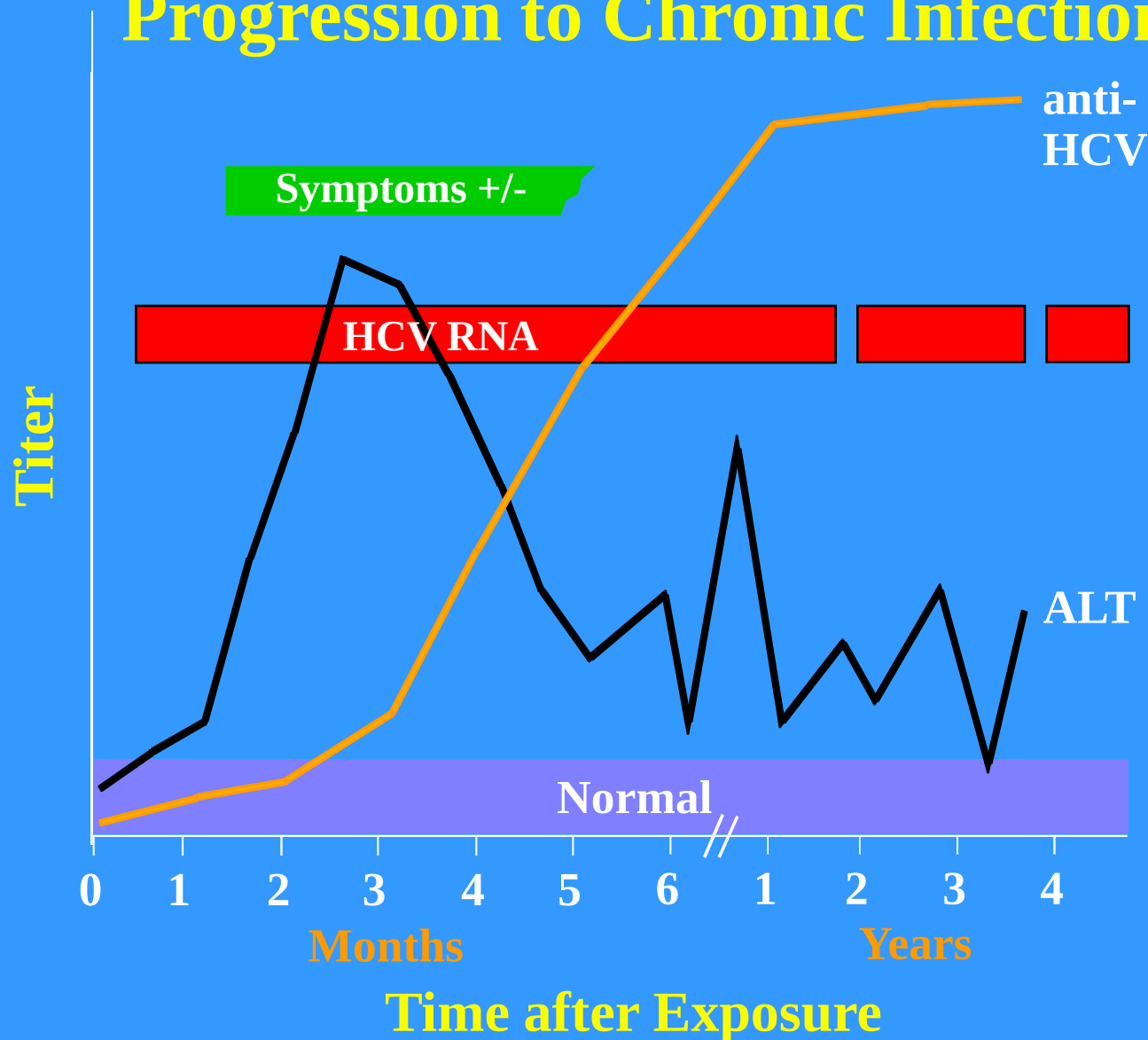


Progression to Chronic Hepatitis B Virus Infection

Typical Serologic Course



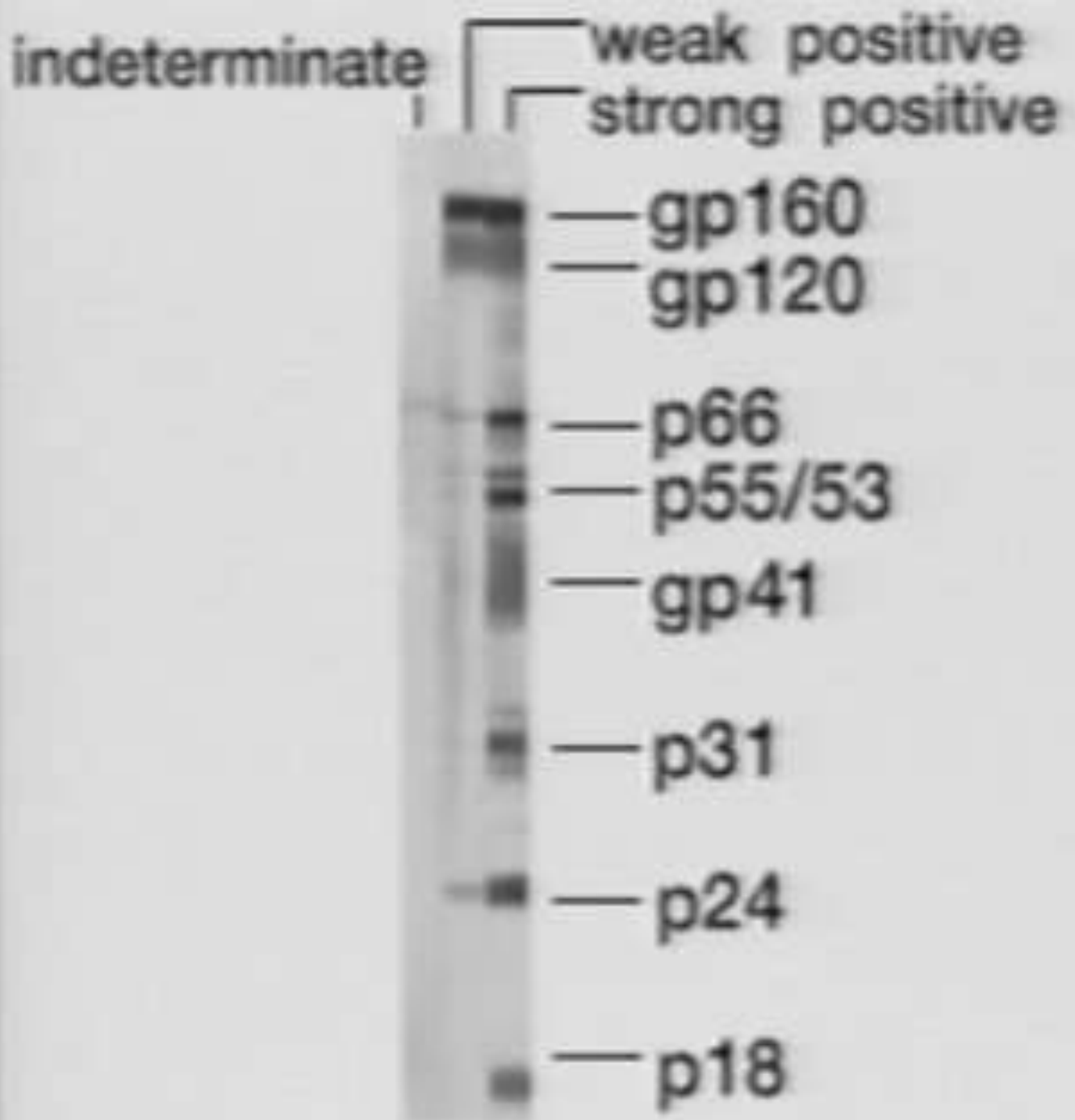
Serologic Pattern of Acute HCV Infection with Progression to Chronic Infection



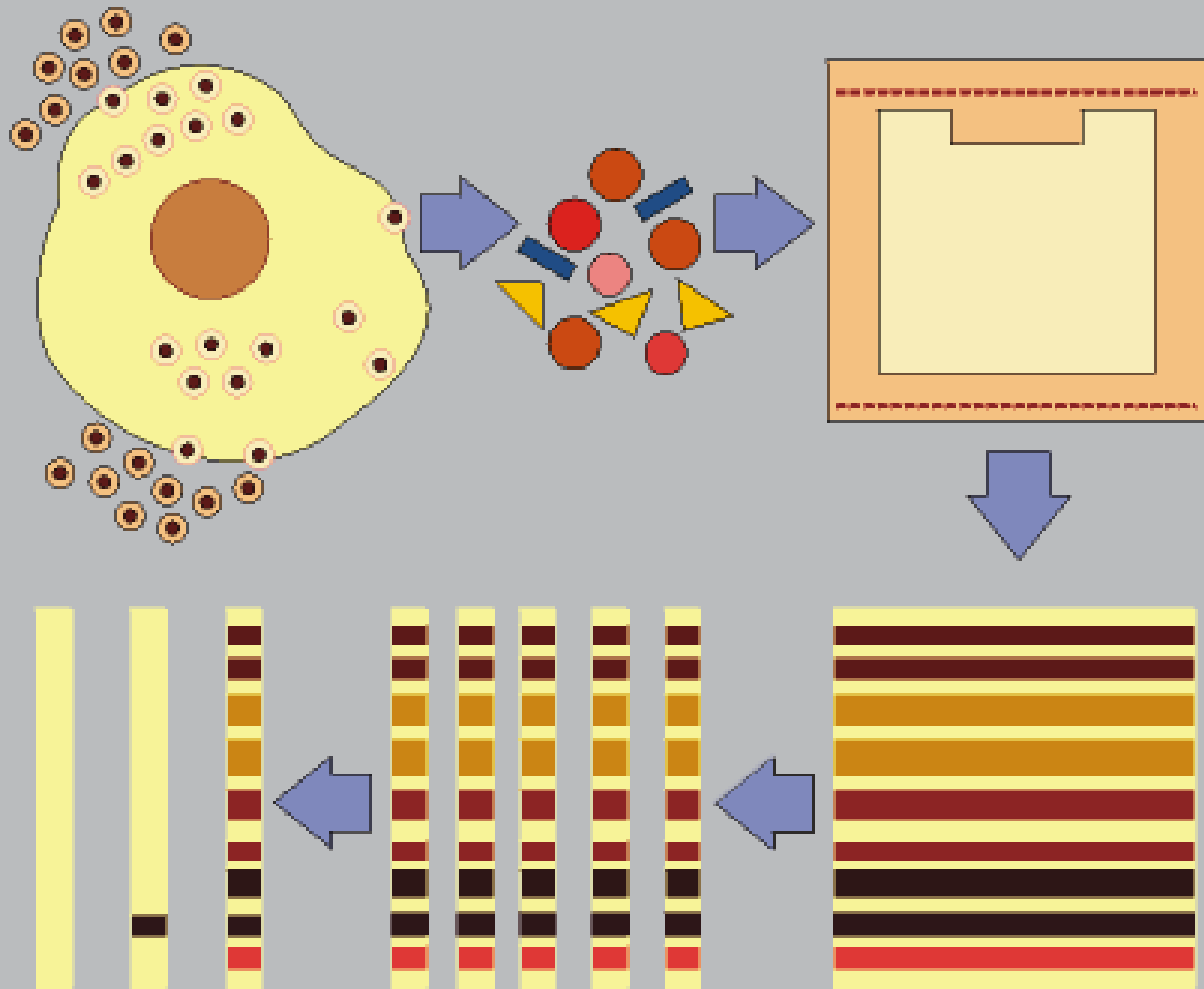
Serology :

- Western Blot

The Western Blot Confirmatory Test (HIV)



How to
prepare
strip
for the
Western
blot



Western blot for HIV antibody

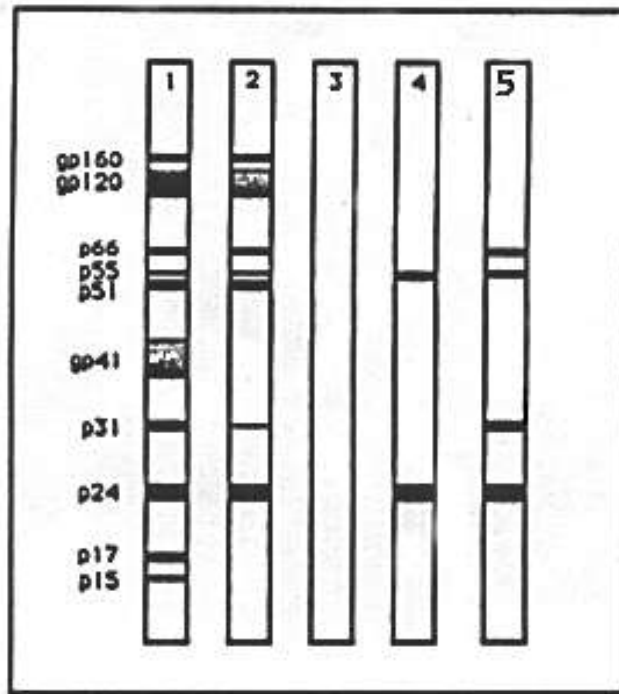


Figure:

Examples of reactions by an HIV-1 Western blot:

1. Positive control (strong)
2. Positive control (weak)
3. Negative control
4. Indeterminate profile
5. Indeterminate profile (highly suggestive)

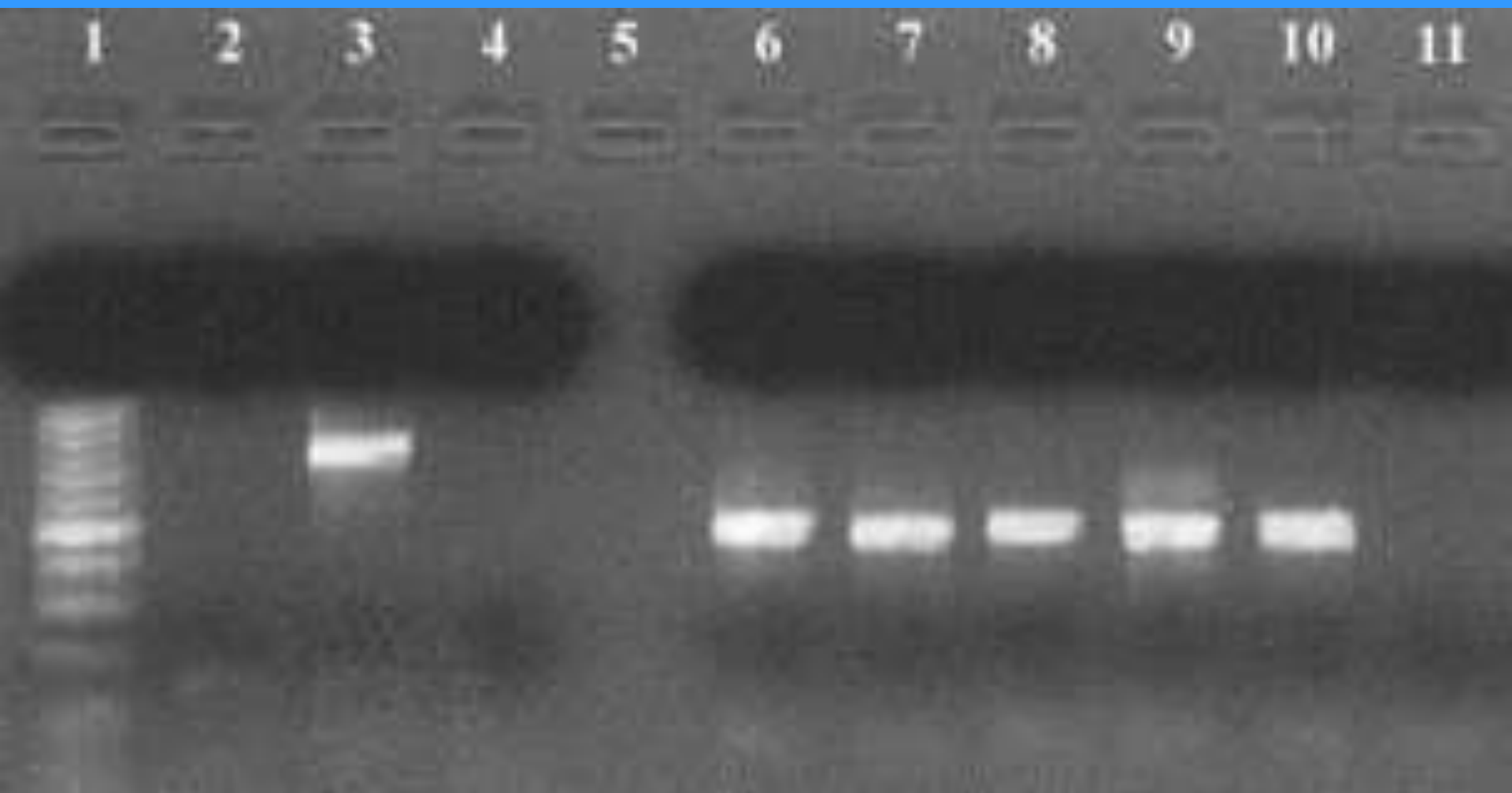
- There are different criteria for the interpretation of HIV Western blot results e.g. CDC, WHO, American Red Cross.

- The most important antibodies are those against the envelope glycoproteins gp120, gp160, and gp41

- p24 antibody is usually present but may be absent in the later stages of HIV infection

Detection of Viral Nucleic Acid

- Polymerase Chain Reaction (PCR)



The Polymerase Chain Reaction (PCR)

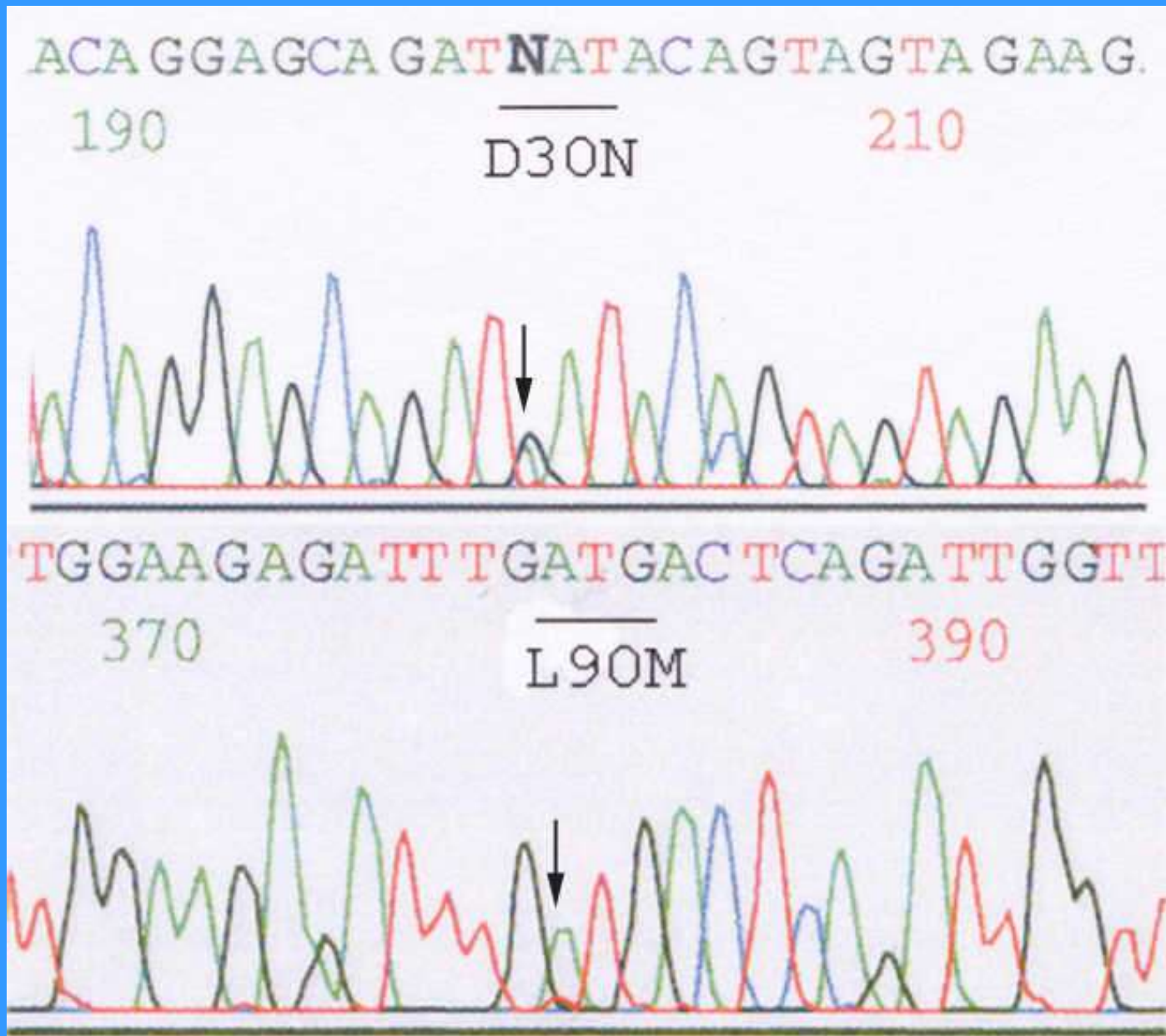
DNA Sequencing



Thermal Cyclers for PCR



DNA sequencing



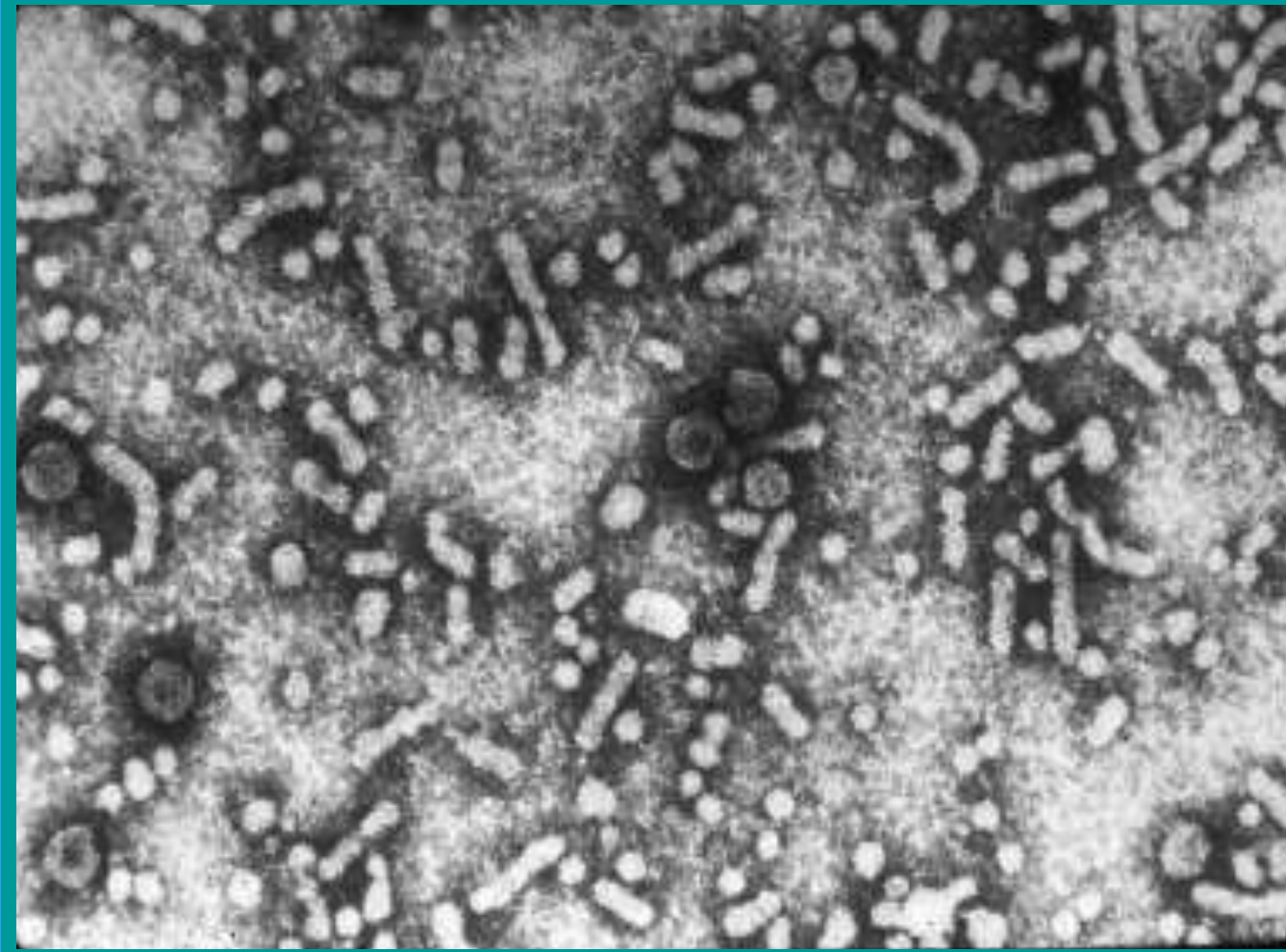
Electron Microscopy :

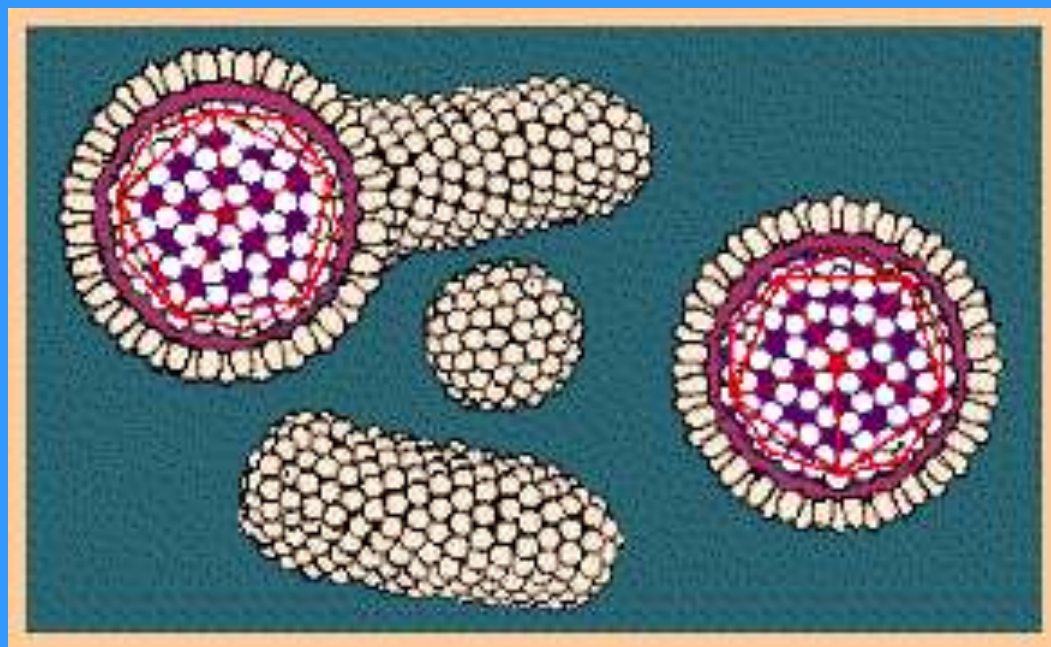
- Used to be very important before virus isolation and identification was developed
- Still important with viruses that cannot be cultured and with new viruses:

Electron Microscopy :

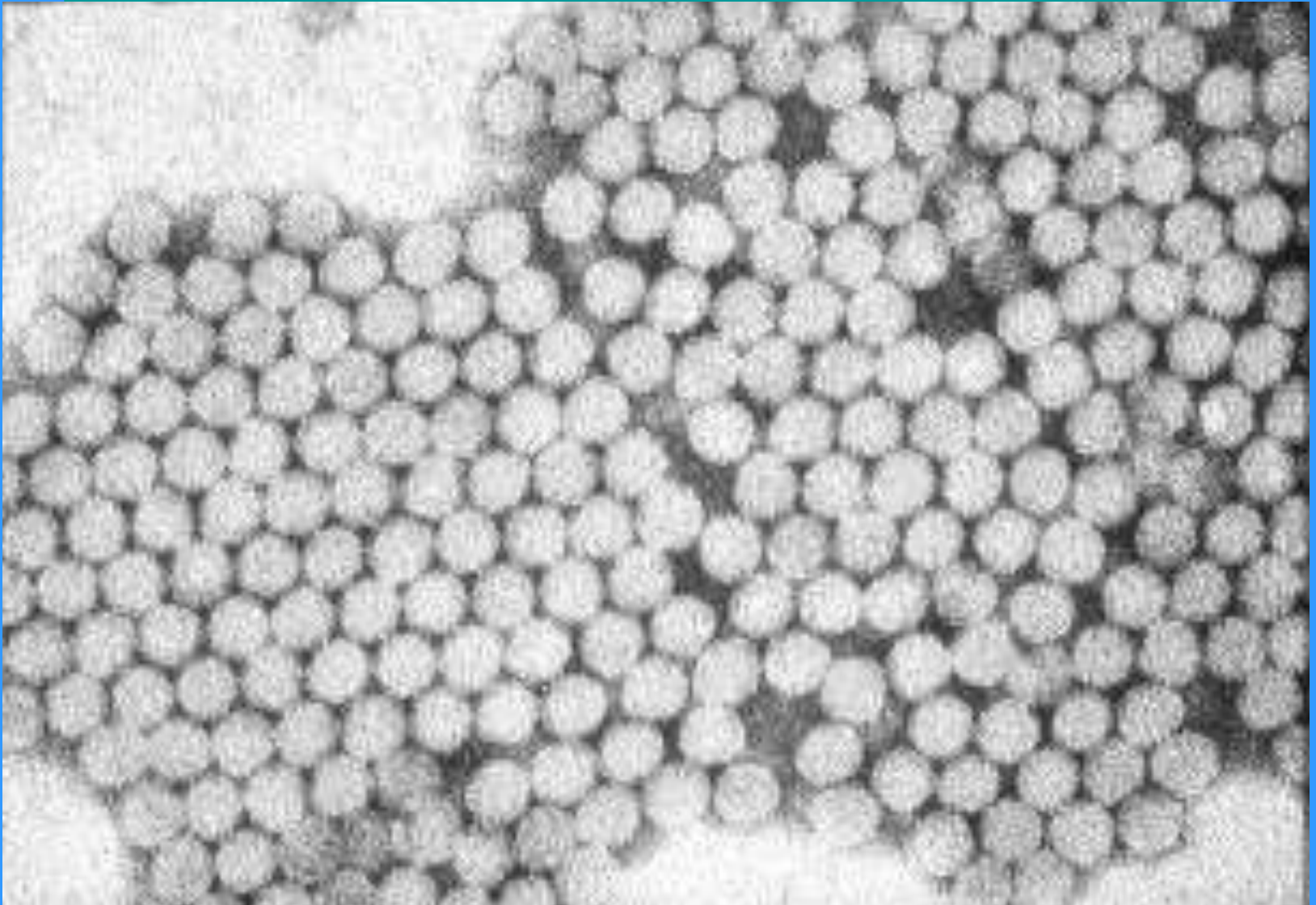
- Examples :
 - Adenovirus
 - Papillomaviruses
 - Enterovirus
 - Rotavirus

Hepatitis B Virus

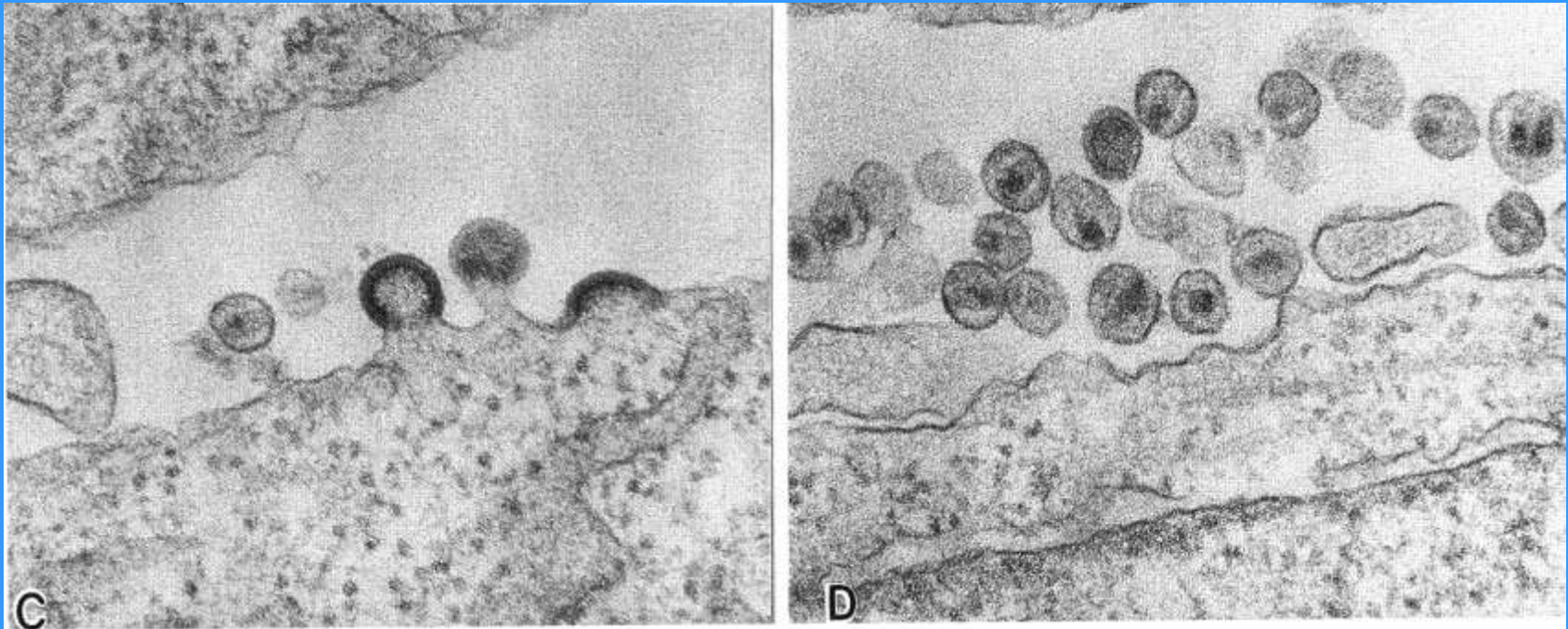




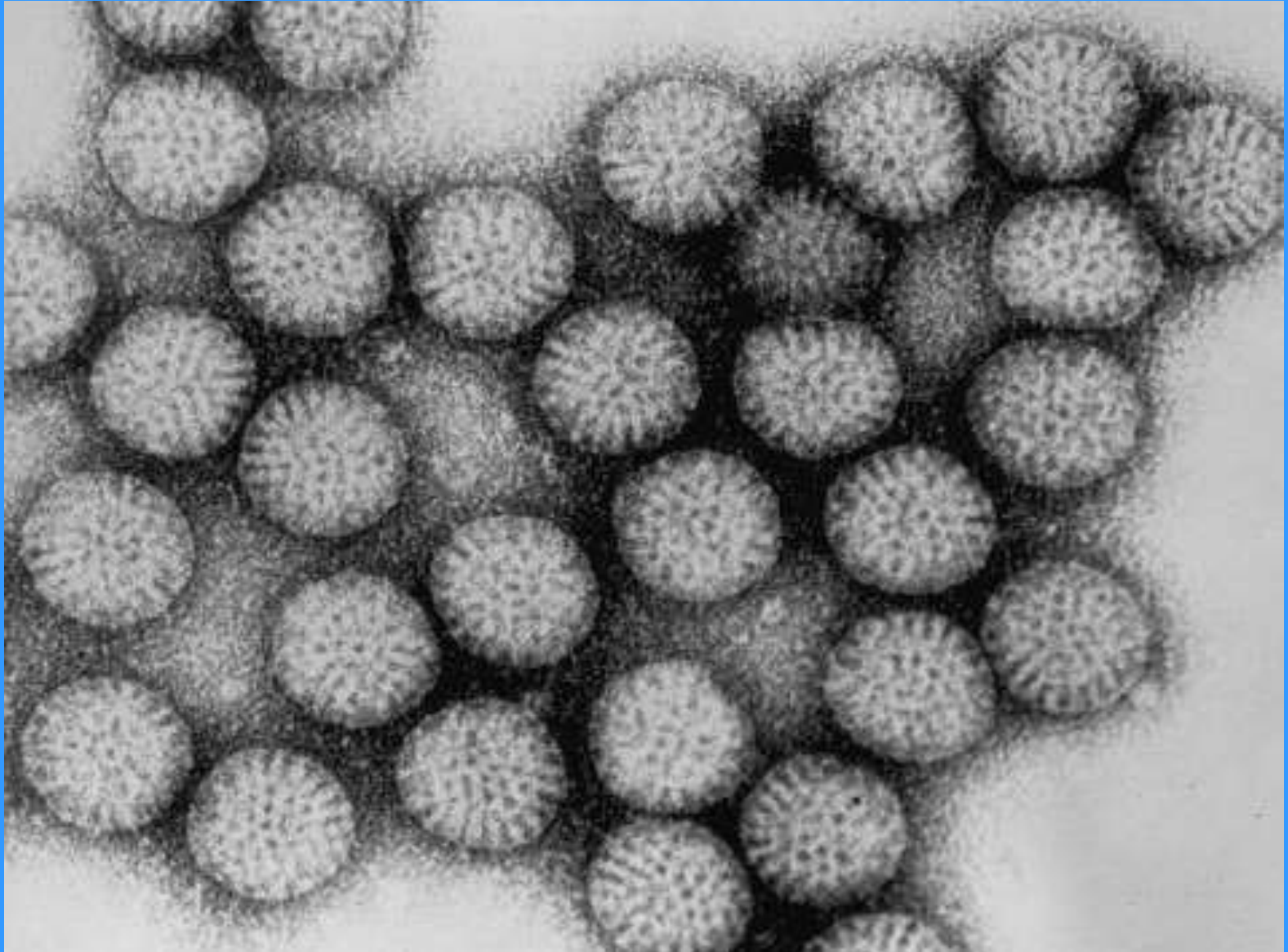
Hepatitis A Virus



HIV budding and maturation

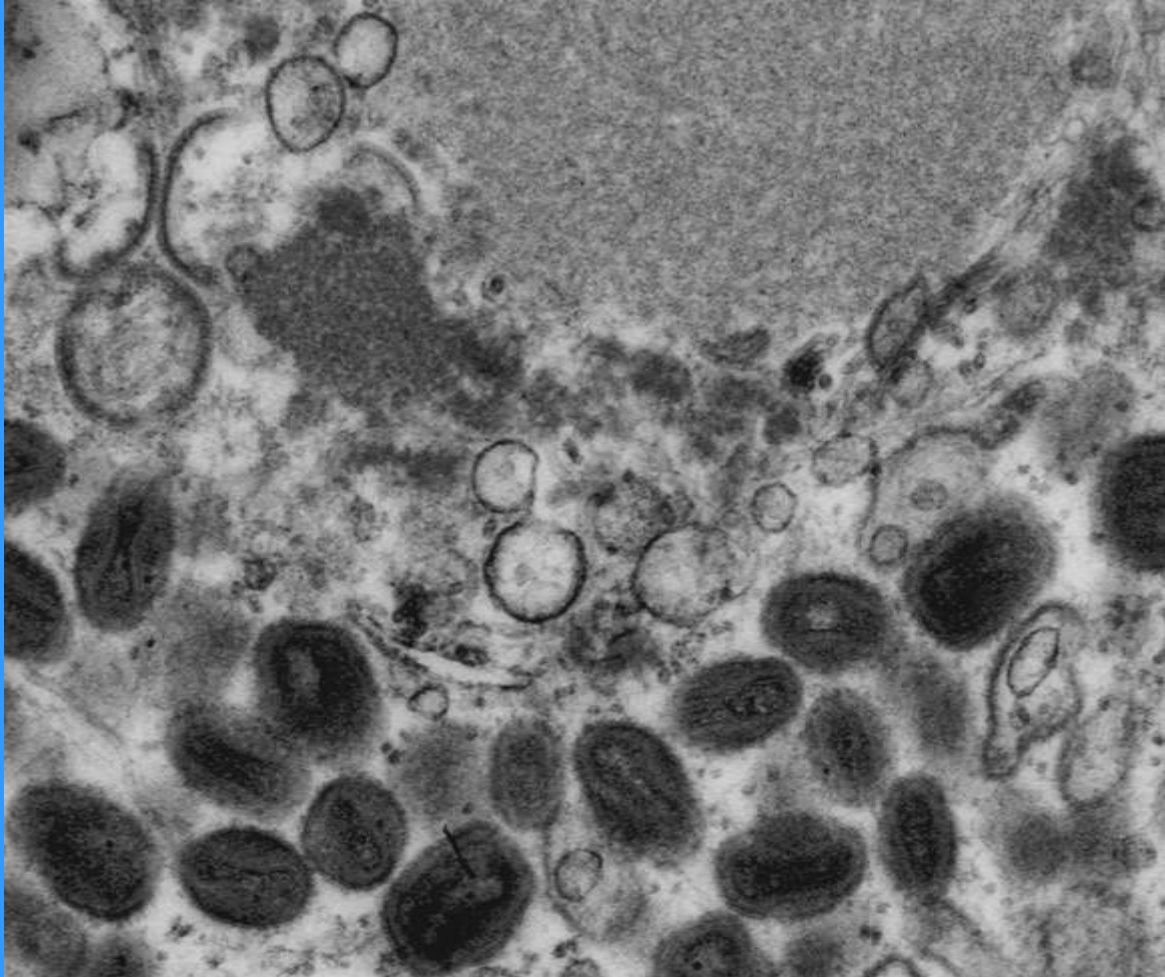


ROTAVIRUS

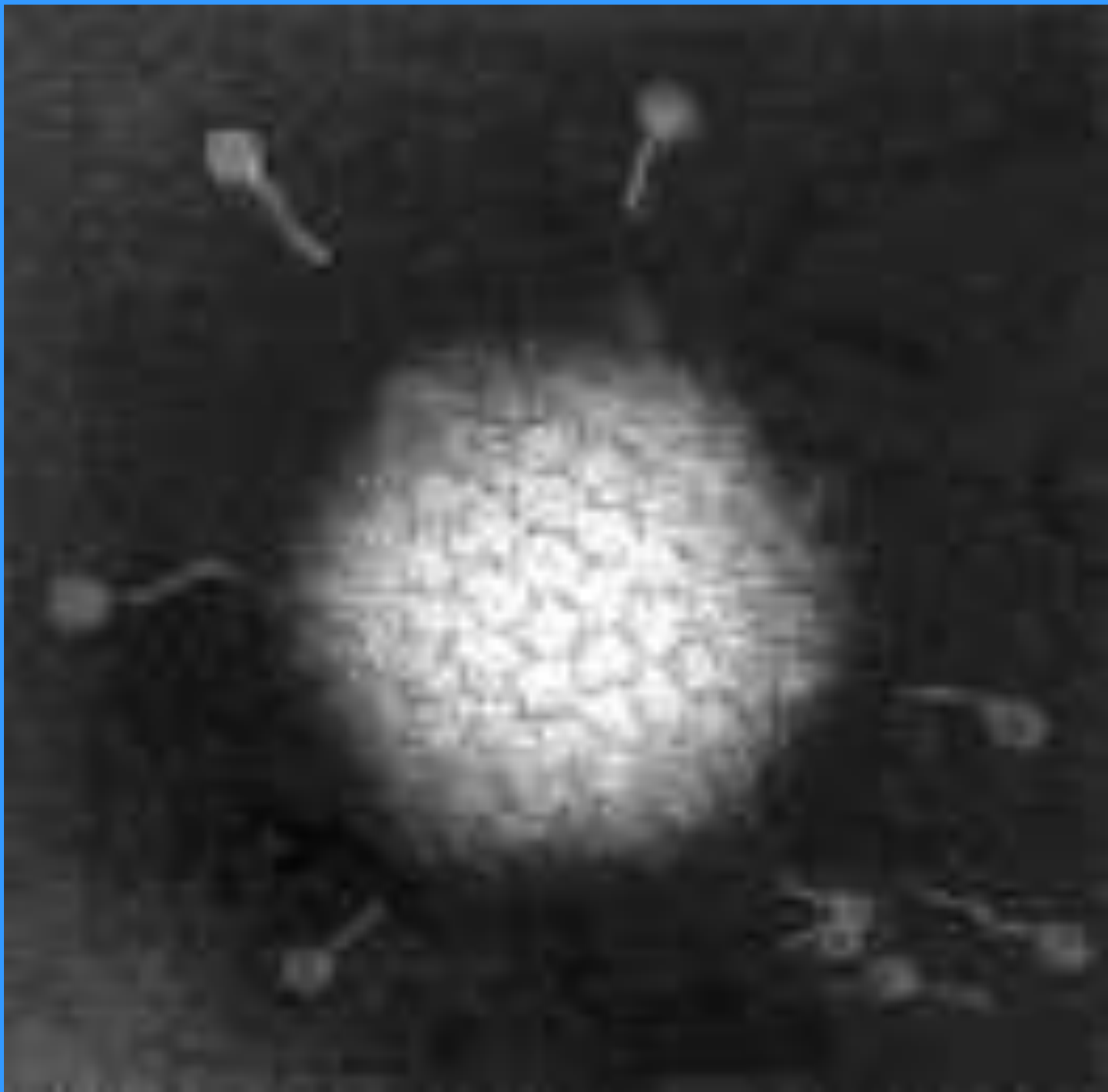


Erskine Palmer, CDC

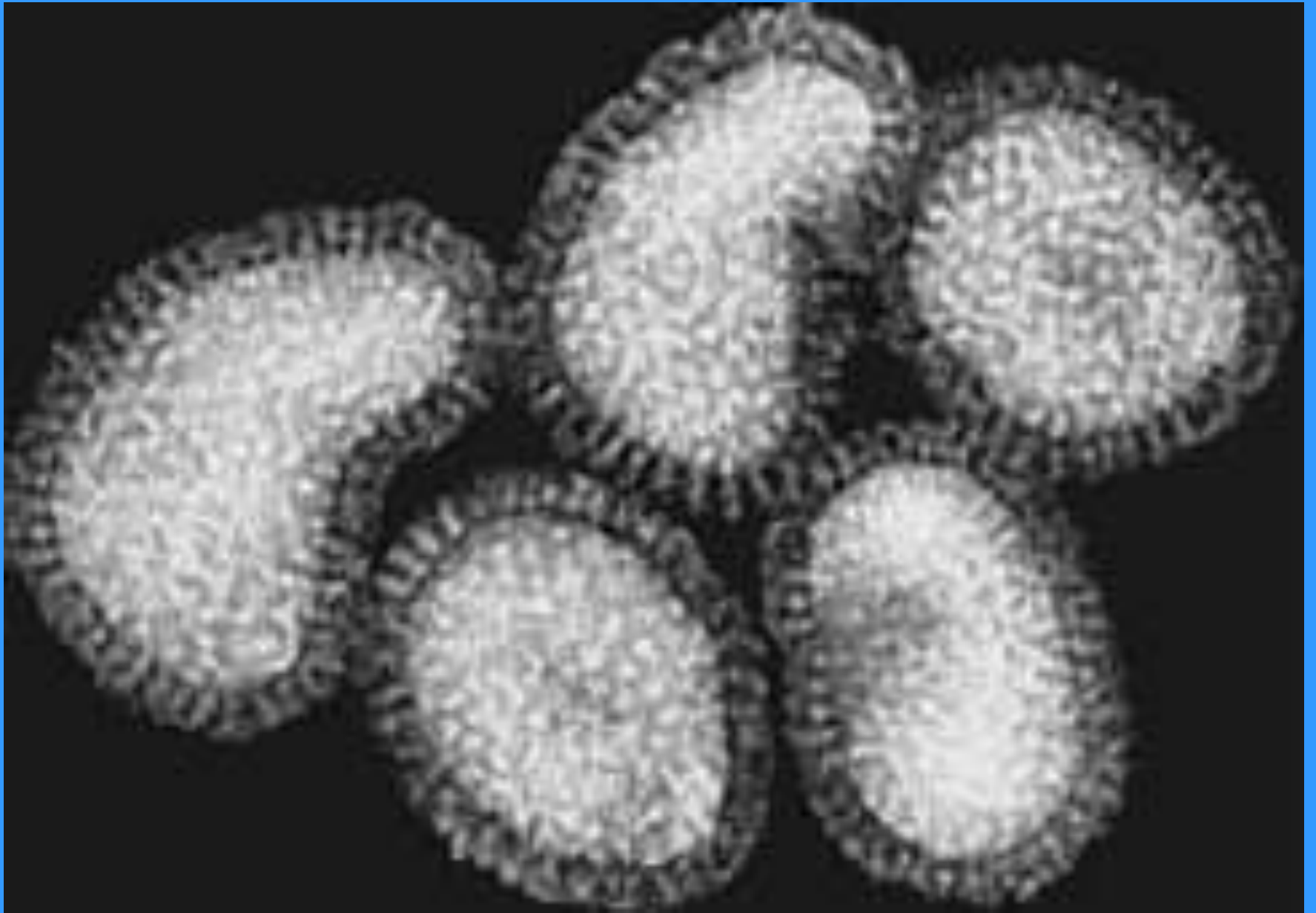
smallpox virus cytoplasmic assembly and maturation



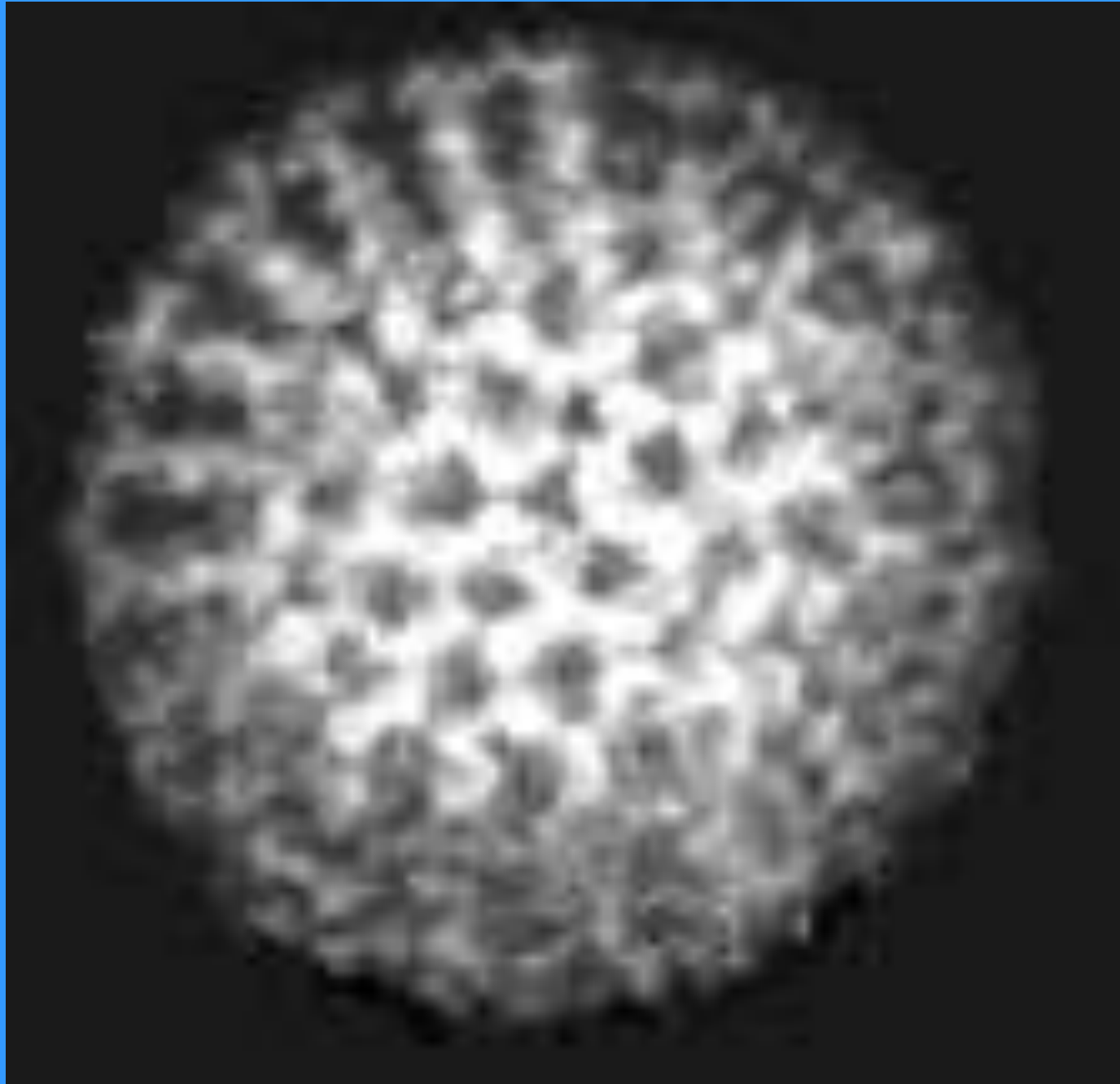
Adenovirus



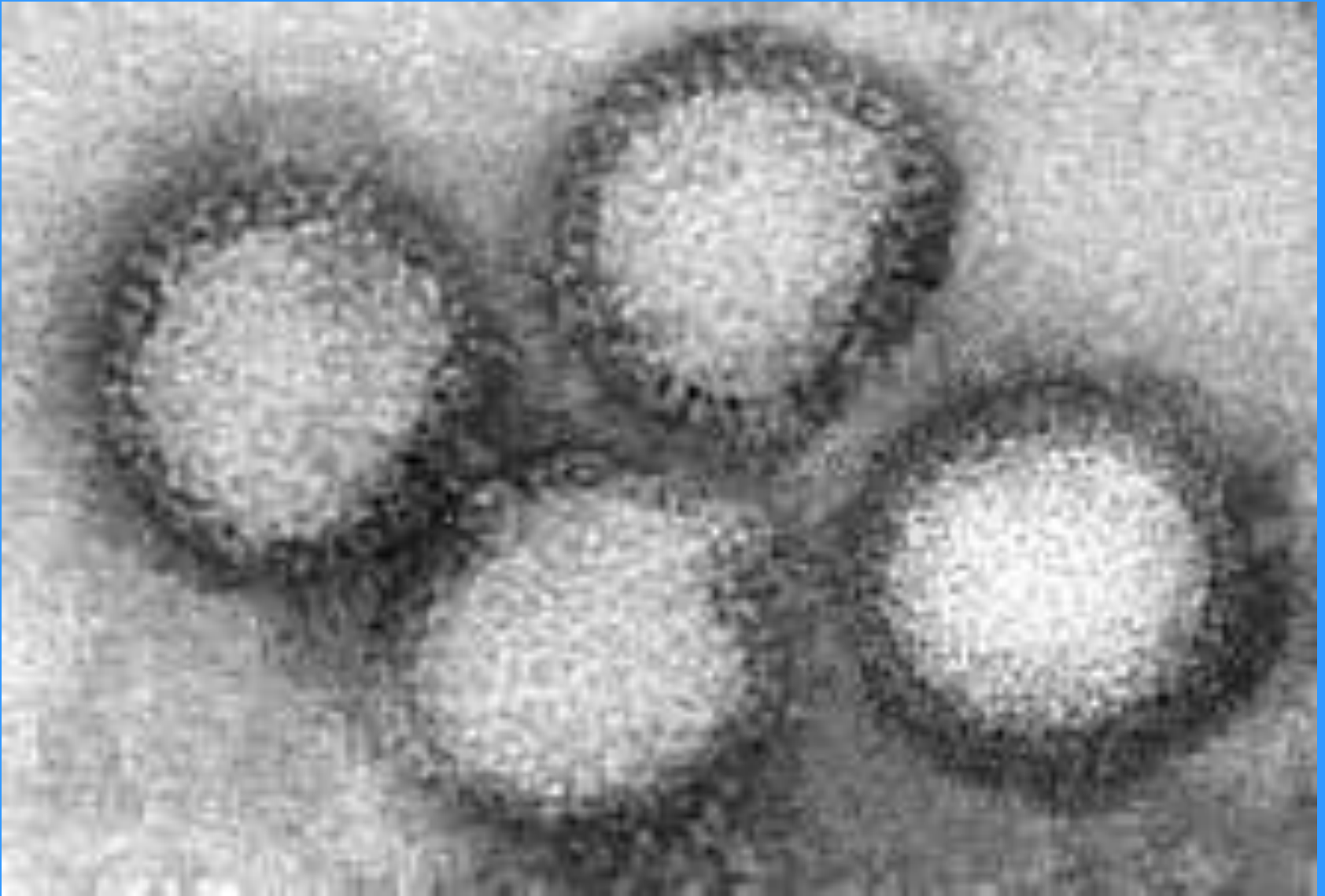
Influenza Virus



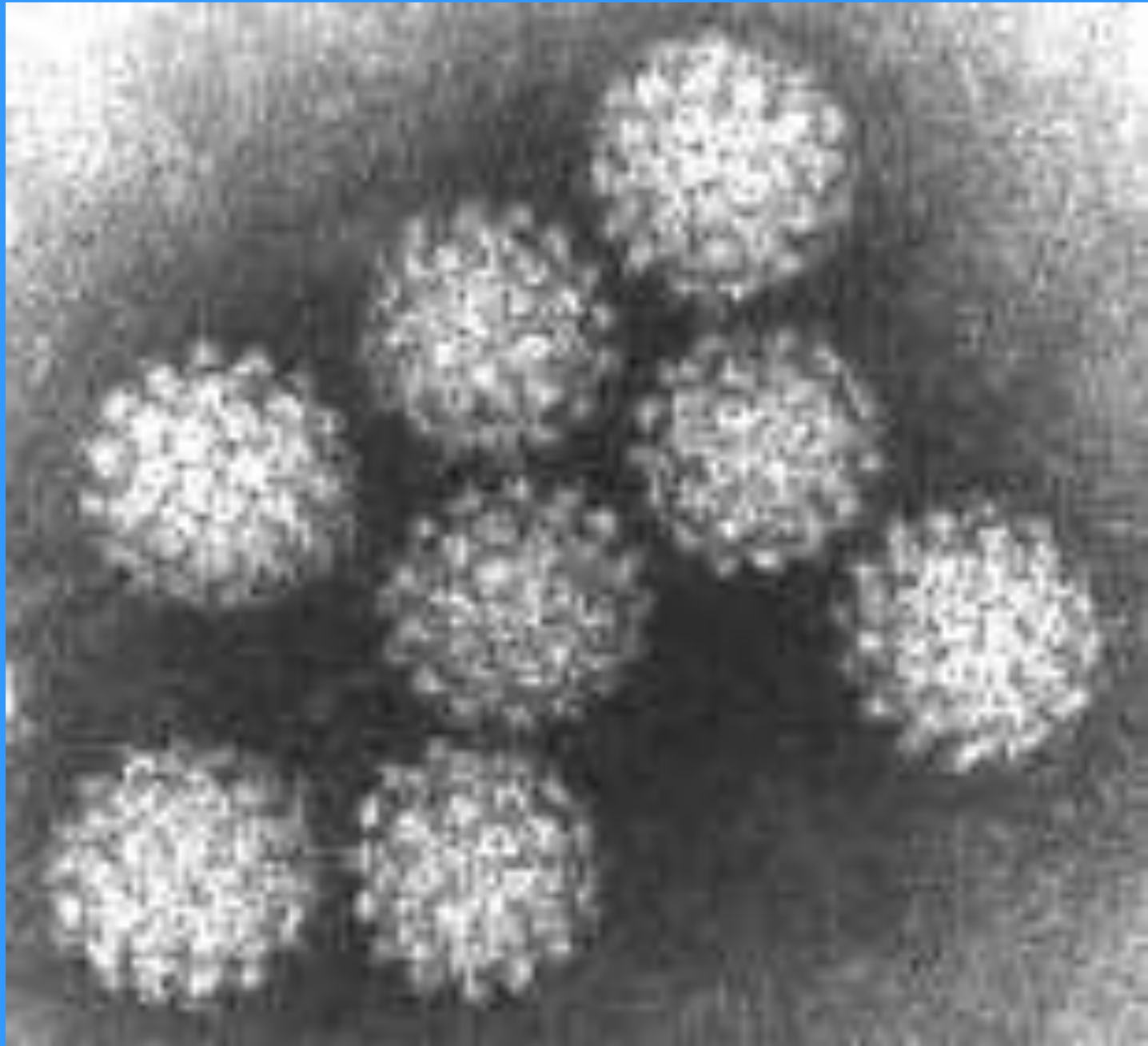
Rotavirus



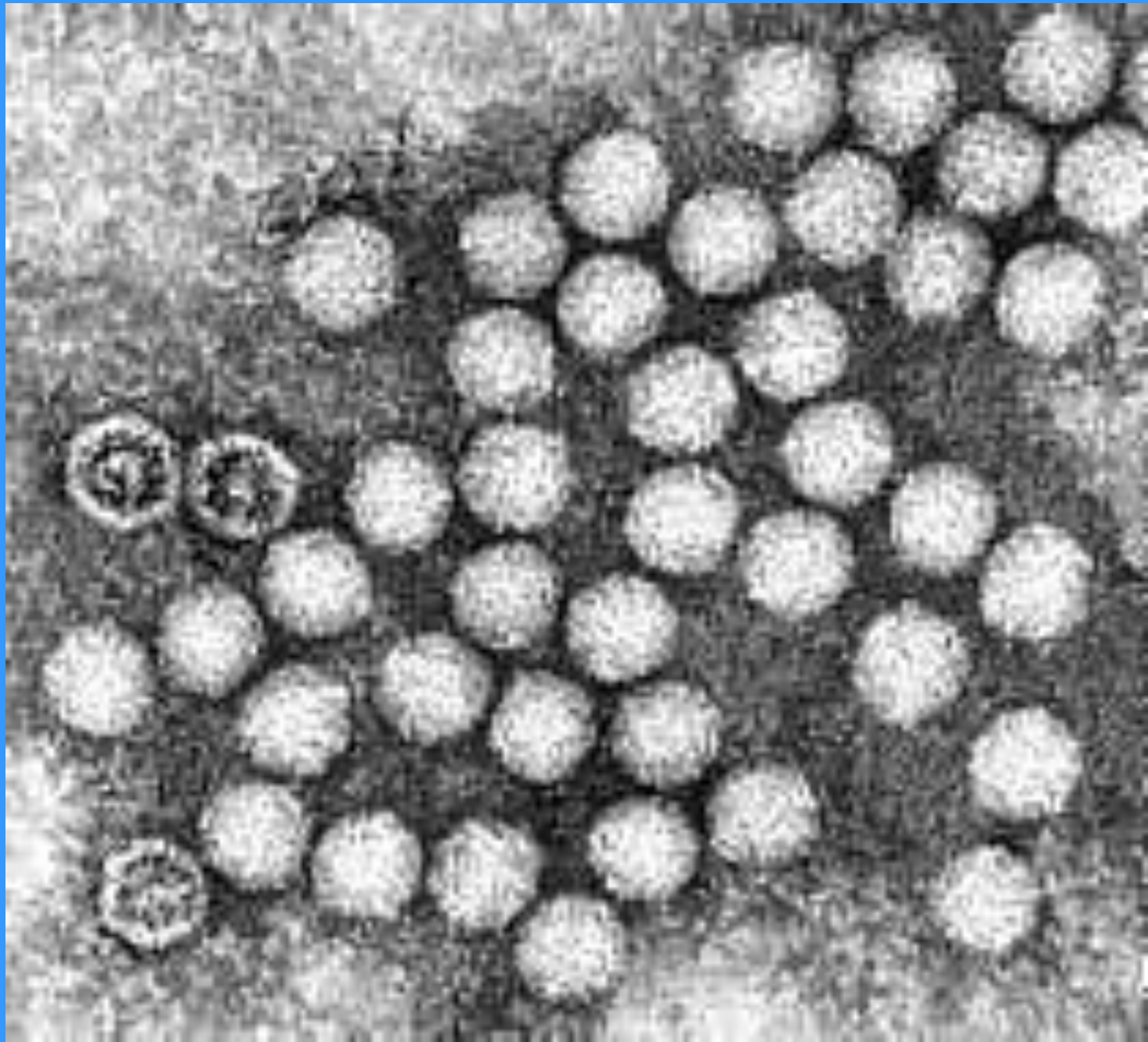
Rift Valley Fever Virus



Papilloma virus



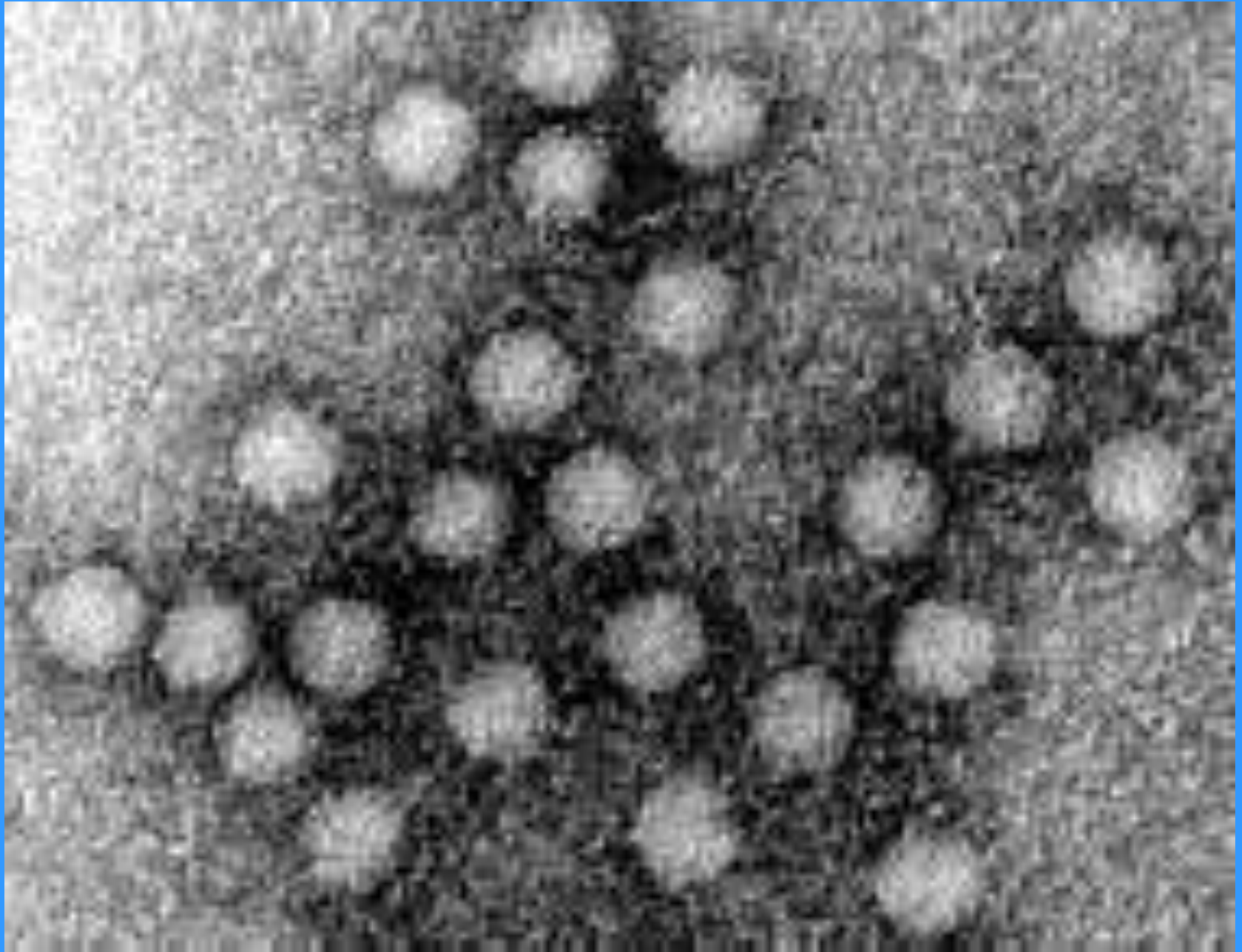
Enterovirus



Paramyxovirus

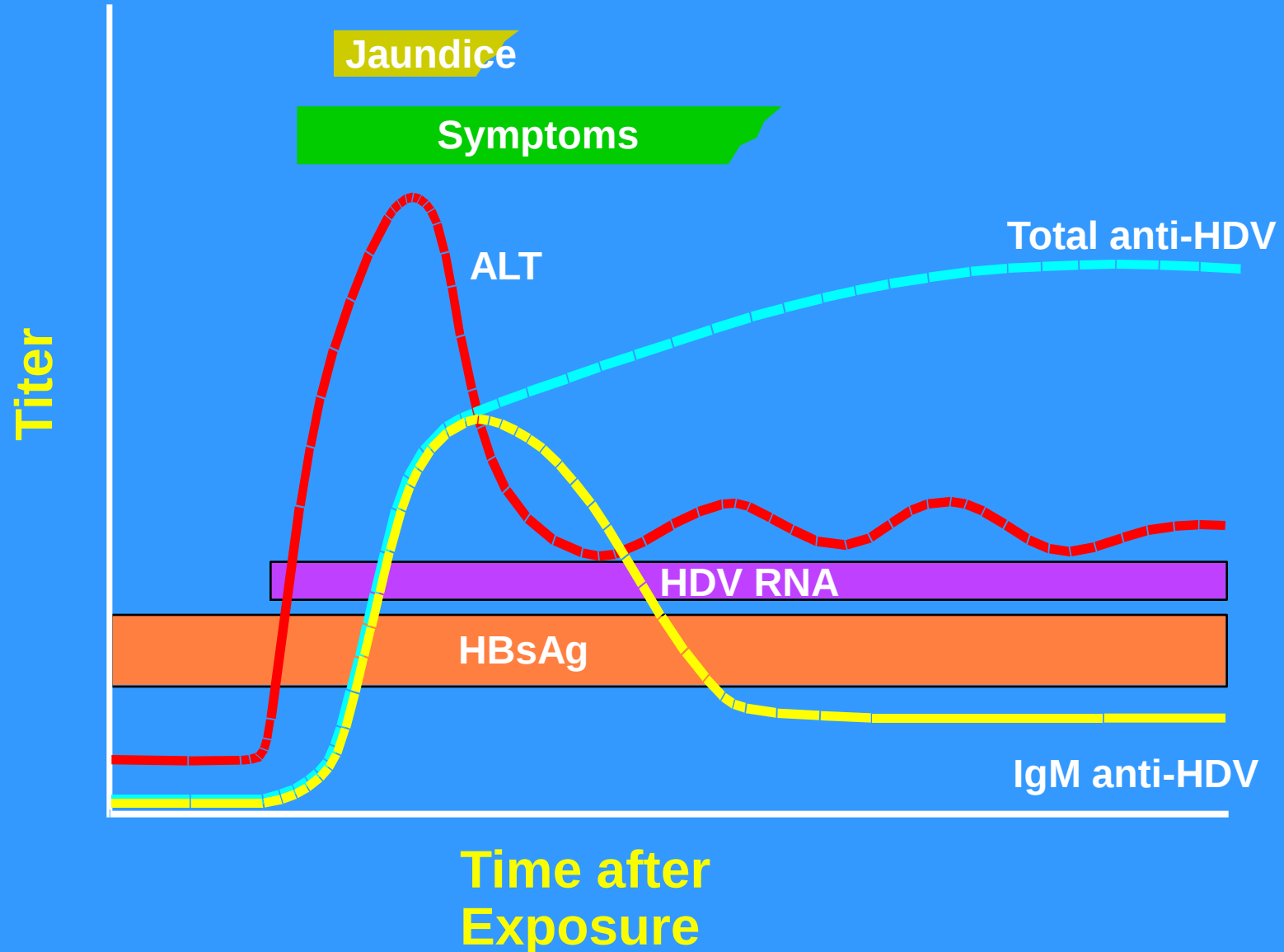


Parvovirus



HBV - HDV Superinfection

Typical Serologic Course



Hepatitis E Virus Infection

Typical Serologic Course

Symptoms

