Poltava State Medical University

Department of Microbiology, Virology and Immunology

General Virology. Methods of Viruses cultivation and identification

Connection

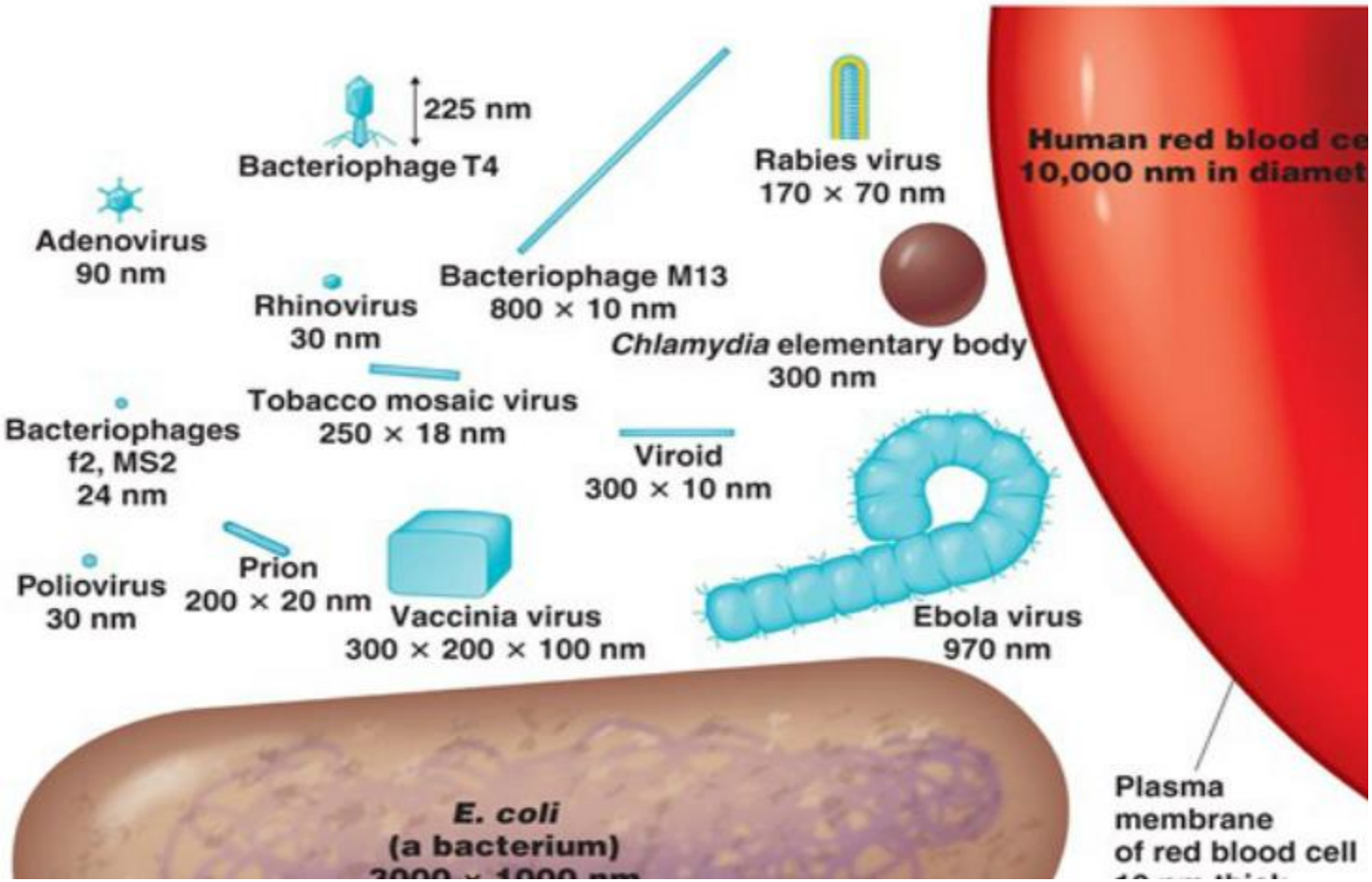
- **For two-way communication between the lecturer and students during the lecture, please contact o.hancho@pdmu.edu.ua**

General Characteristics of Viruses

- **Obligatory intracellular parasites**
- Contain DNA or RNA
- Contain a protein coat
- Some are enclosed by an envelope
- Some viruses have spikes
- Most viruses infect only specific types of cells in one host
- **Host range** is determined by specific host attachment sites and cellular factors

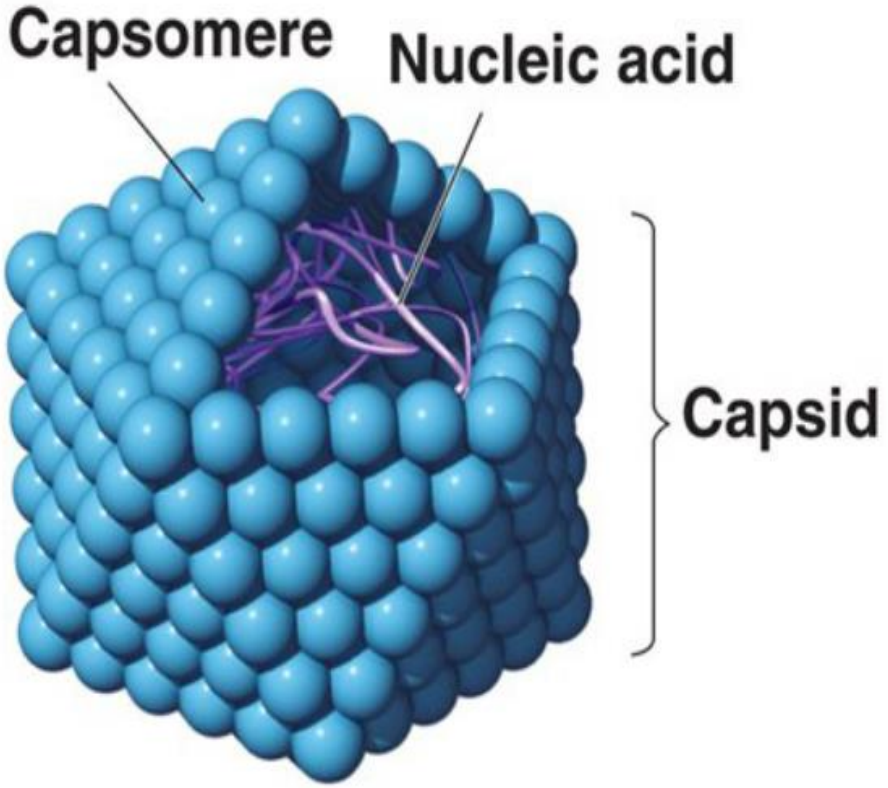
Справка
Virus, Viroids and Prions ...
Viruses, Viroids and Prions
https://mdc.blackboard.com/bbcswebdav/pid-158325-dt-content-rid-650629_1/courses/MCB2010-808035/iSpring
Google

Virus Sizes



Virion Structure

- Nucleic acid
  - DNA or RNA
- Capsid
  - Capsomeres
- Envelope
- Spikes



(a) A polyhedral virus

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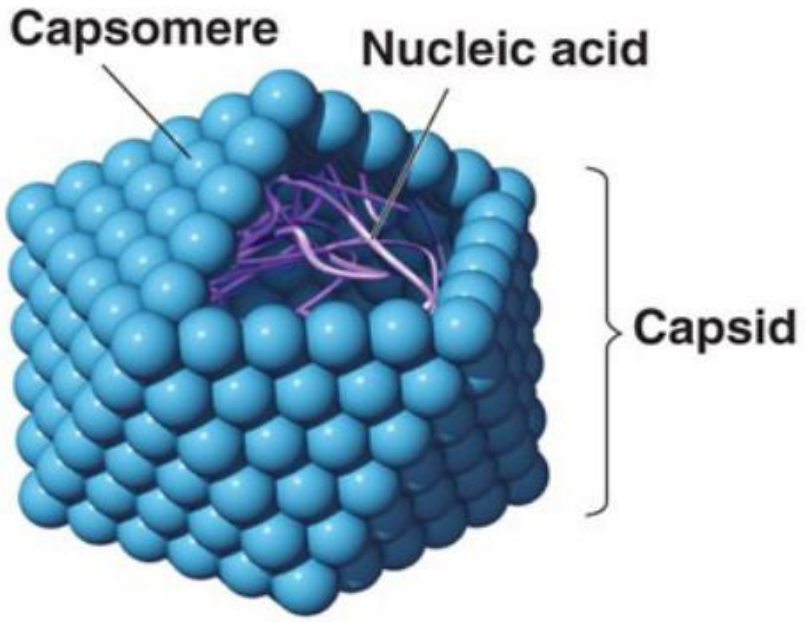
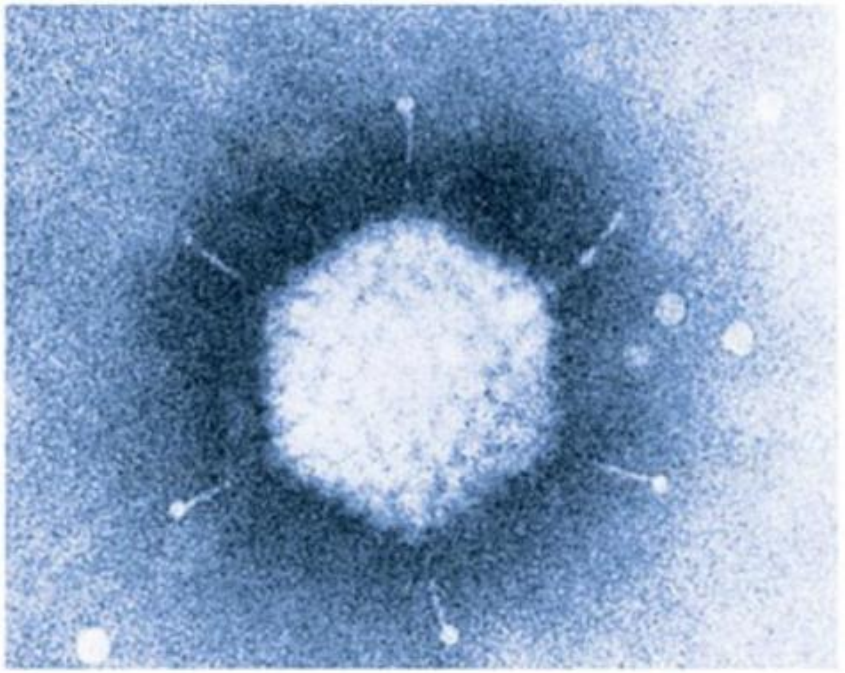
Figure 13.2a

Morphology of a Polyhedral Virus



(a) A polyhedral virus



(b) *Mastadenovirus*

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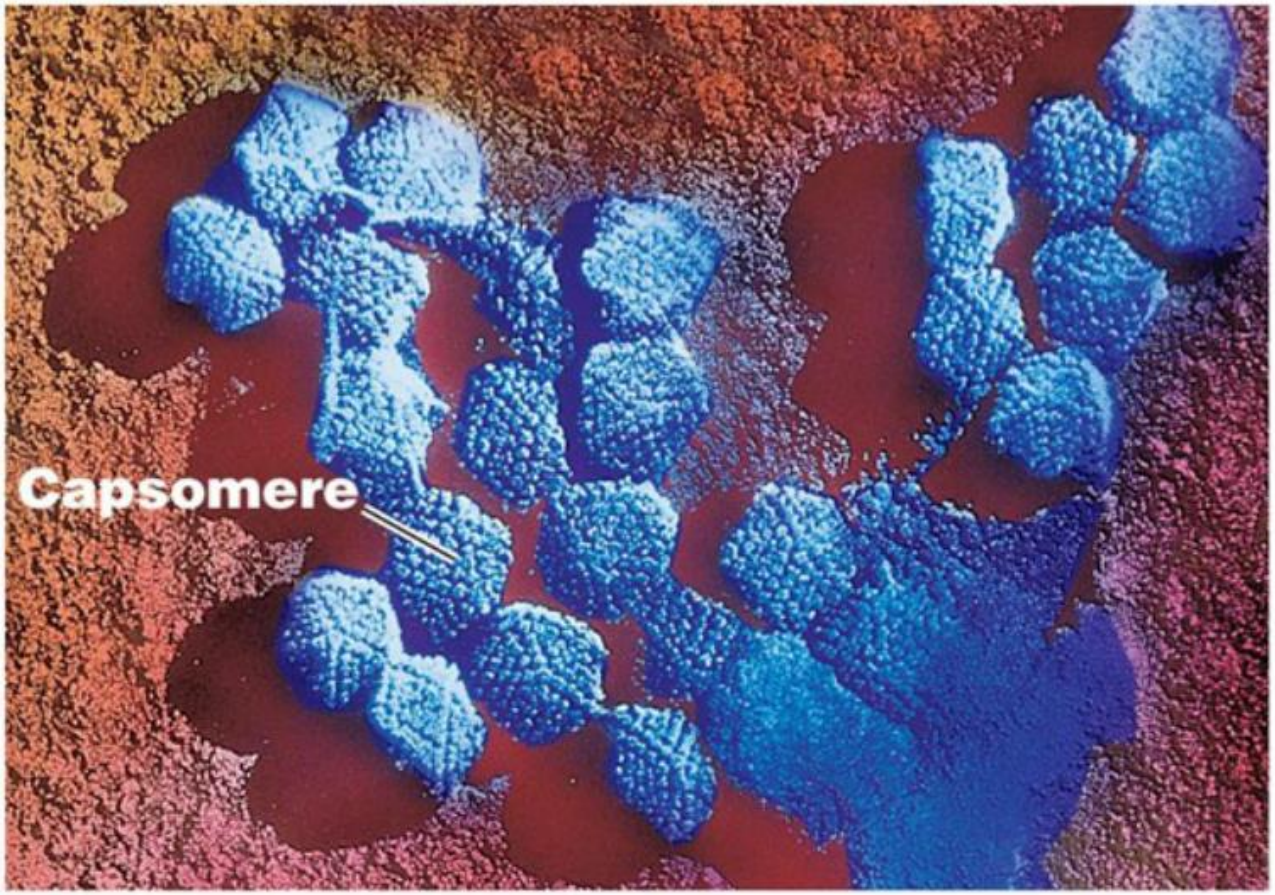
Figure 13.2

Polyhedral Viruses



(a) ***Mastadenovirus***

SEM

100 nm

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Figure 13.16a

Morphology of an Enveloped Virus

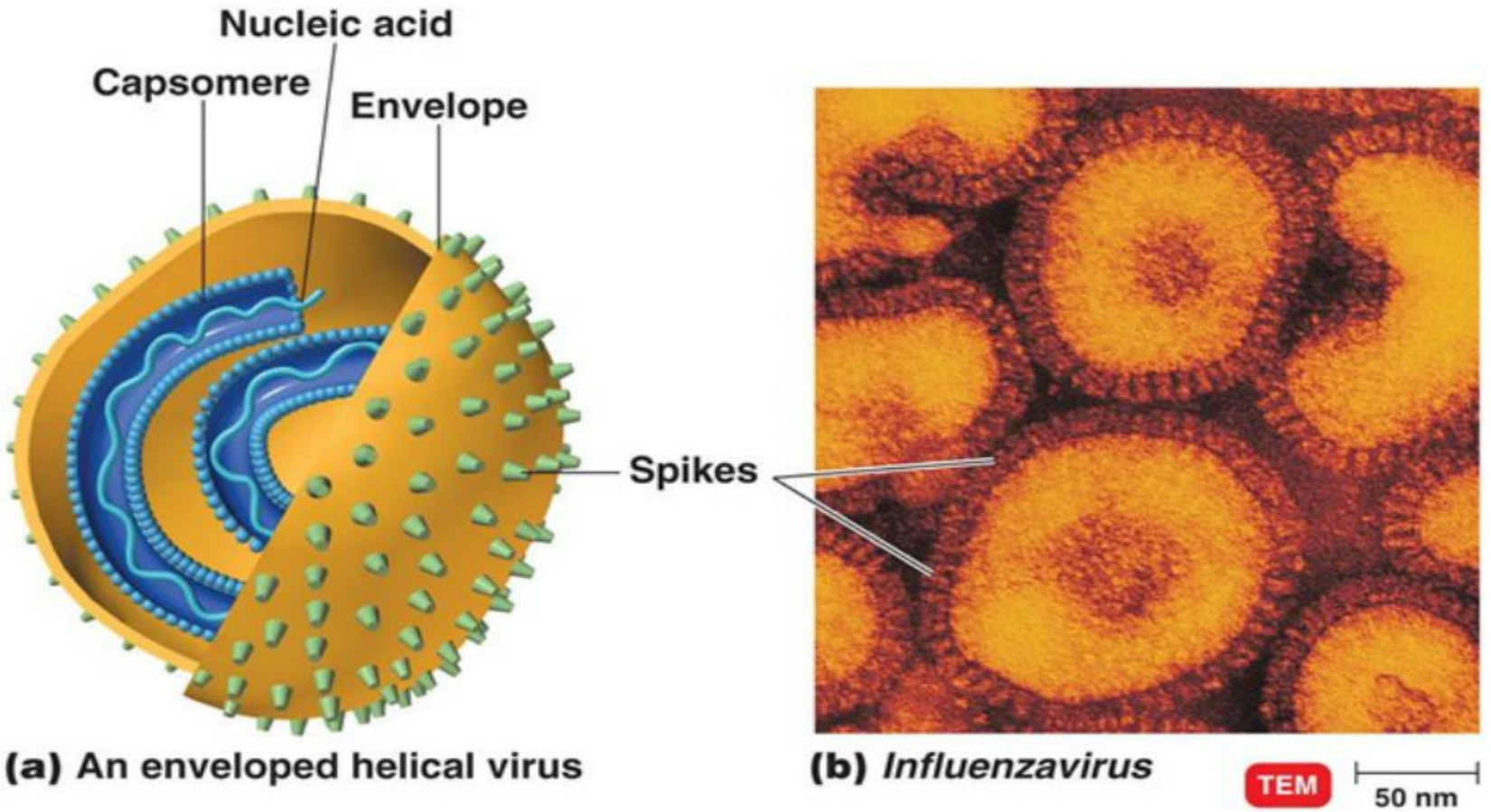

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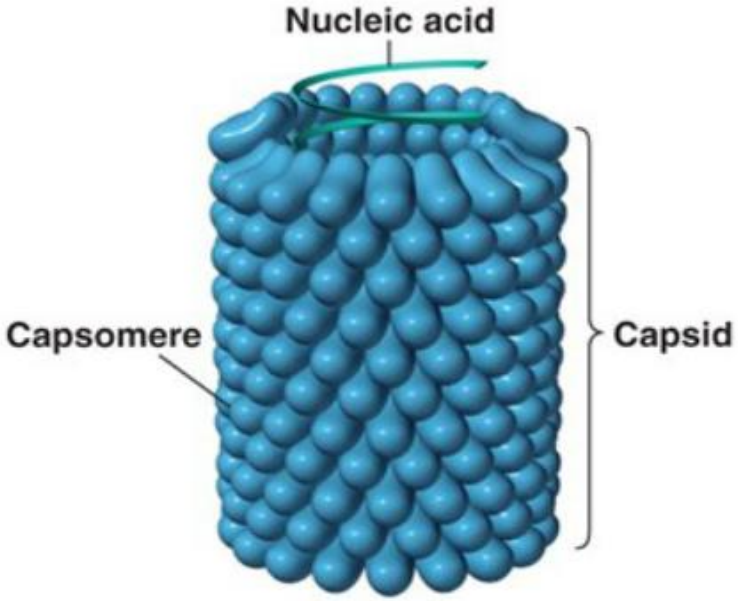
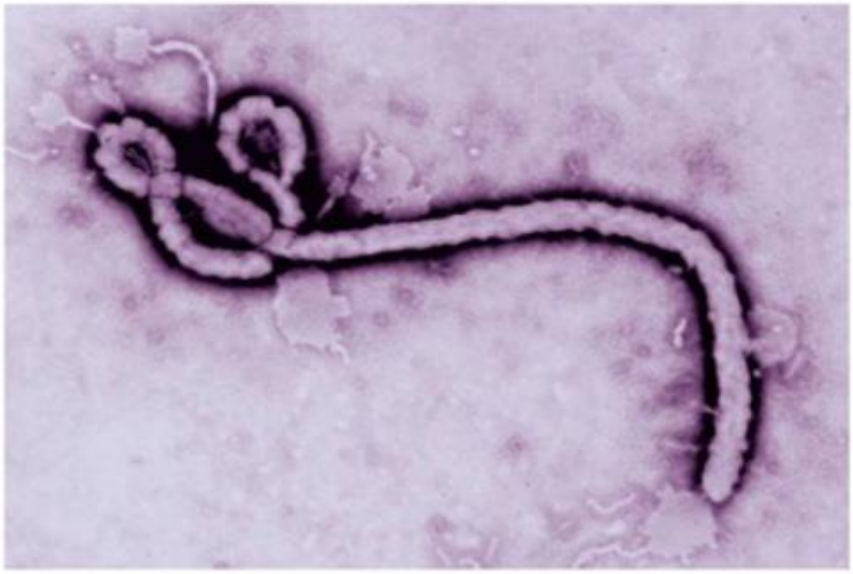
Figure 13.3

Morphology of a Helical Virus



(a) A helical virus



(b) Ebola virus

TEM

100 nm

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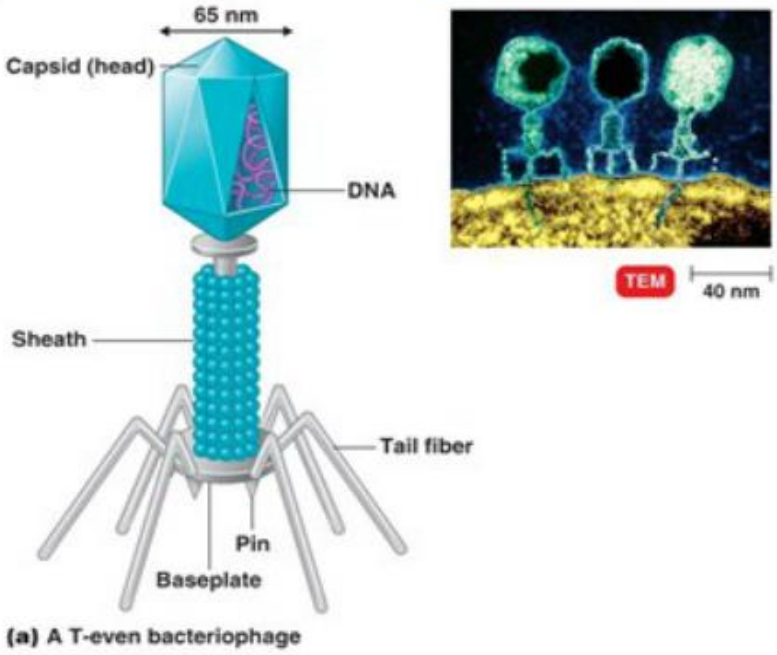
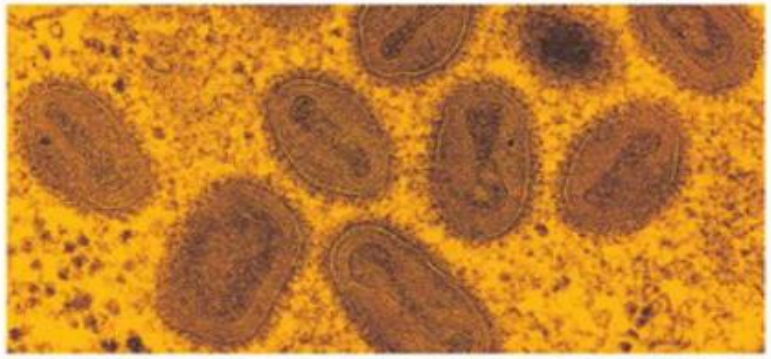
Figure 13.4

Morphology of a Complex Virus



(a) A T-even bacteriophage



(b) *Orthopoxvirus*

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Figure 13.5

Growing Viruses

- Viruses must be grown in living cells
  - Bacteriophages form plaques on a lawn of bacteria

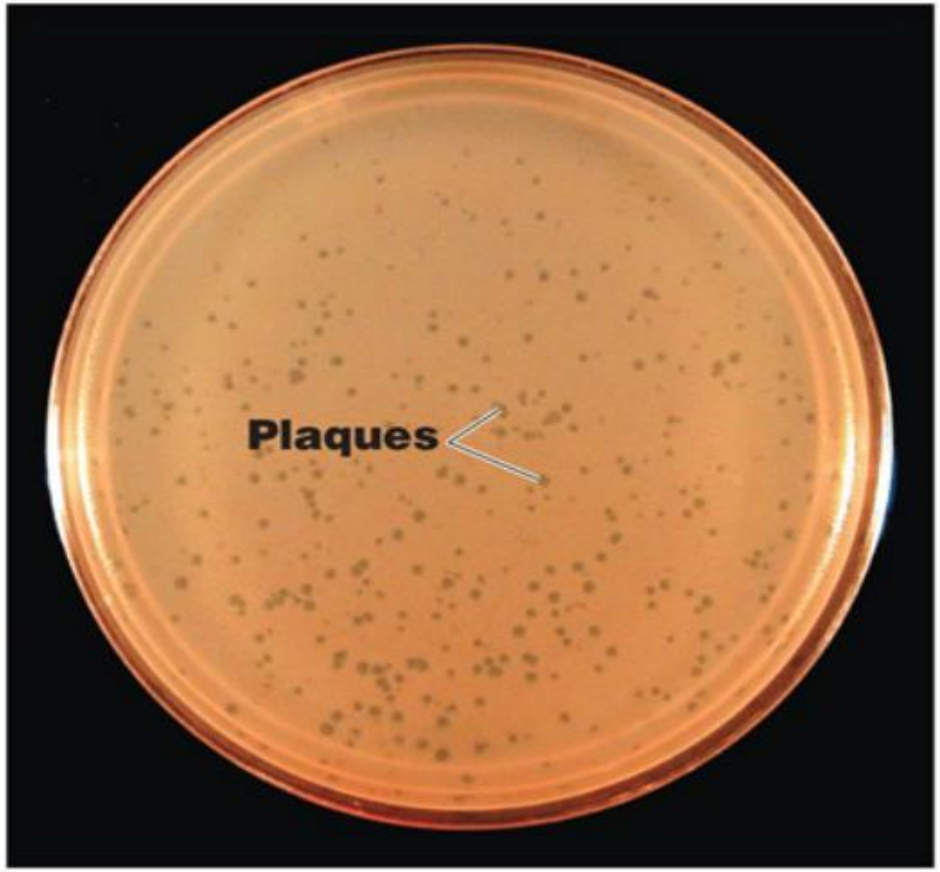

Figure 13.6

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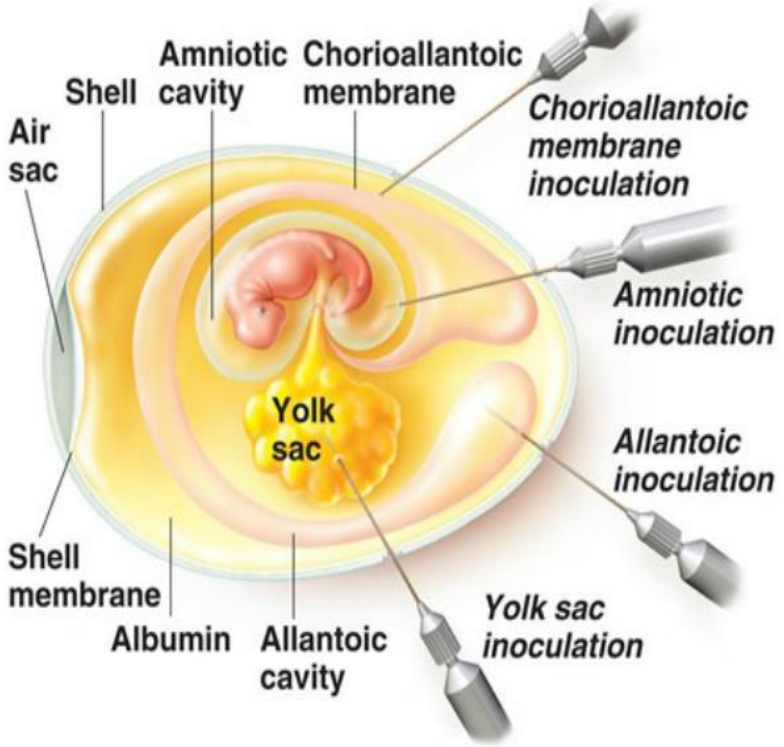
viruses - Microsoft Powe...
22:46

Virus cultivation methods

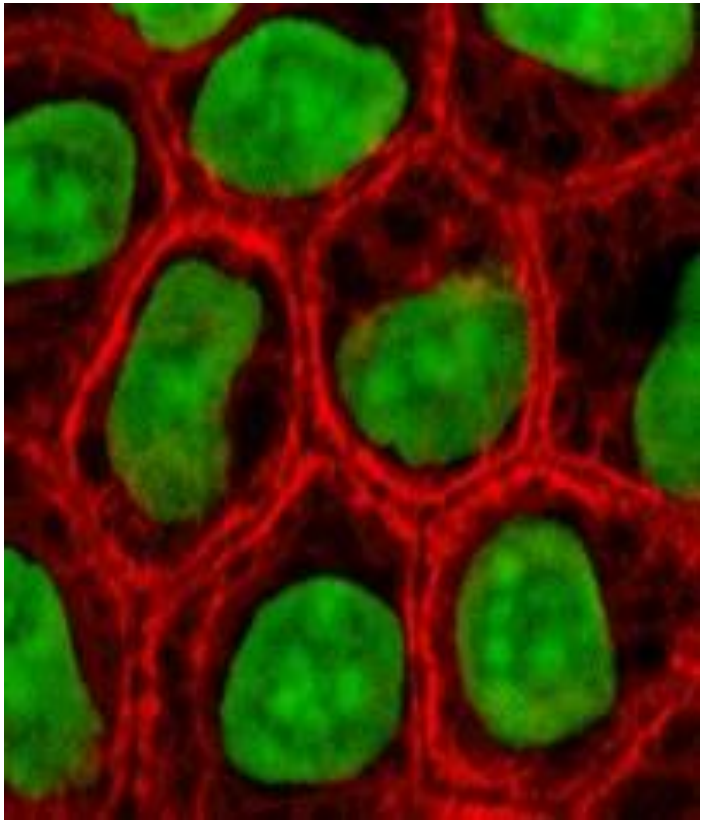
Laboratory animals are infected in various ways depending on the tropism of the virus to certain tissues. For the cultivation of neurotropic viruses, infection is produced mainly into the brain (viruses of rabies, tick-borne encephalitis, etc.). Cultivation of respiratory viruses is carried out during intranasal infection of animals (influenza viruses), dermatotropic viruses (smallpox virus) - by transdermal and intradermal infection. Intradermal, intramuscular and intracerebral infection are most often used

Virus cultivation methods



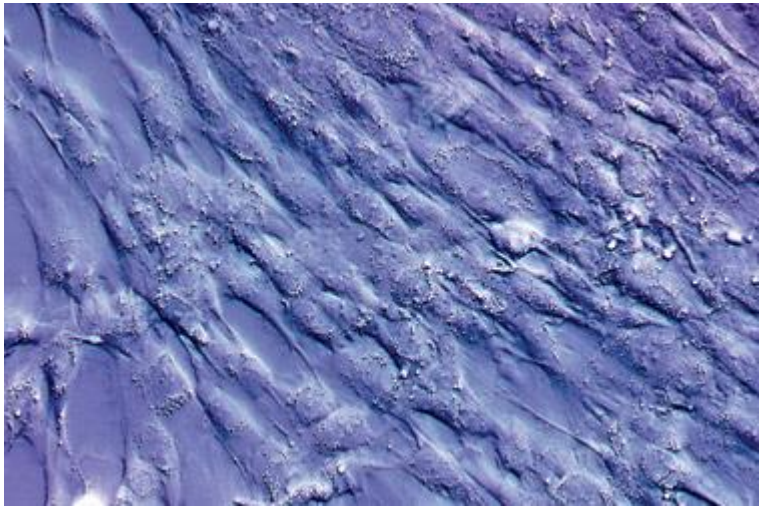
Since 1931, chicken embryos have been used in virological practice The structure of the chicken embryo and methods of its infection: 1 - in the amnion; 2 - in the allantoic cavity; 3 - in the yolk sac; 4 – on the chorionallantoic membrane

Cell cultures



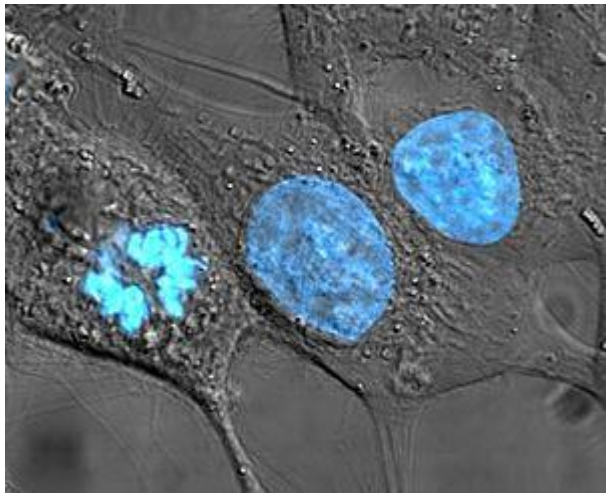
- Primary cell culture - obtained by enzymatic disintegration: primary treatment with trypsin; this treatment disrupts intercellular connections, resulting in the release of individual cells. The source is any organs and tissues, most often - embryonic (have high mitotic activity) Able to undergo about 10 divisions

Cell cultures



- Diploid cells are cultures of cells of the same type that can withstand about 100 divisions. Flasks for culturing viruses on tissue culture in synthetic media: 199, Igla

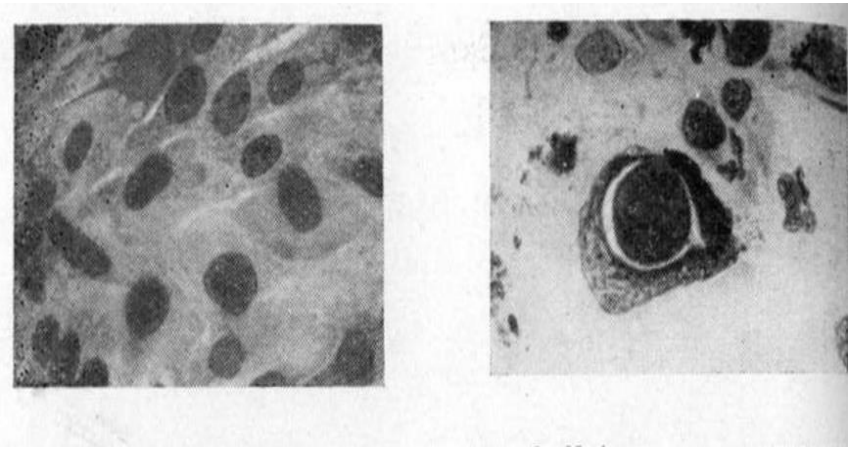
Cell cultures



- HeLa cell culture stained by Hoist. Altered nuclei are colored blue

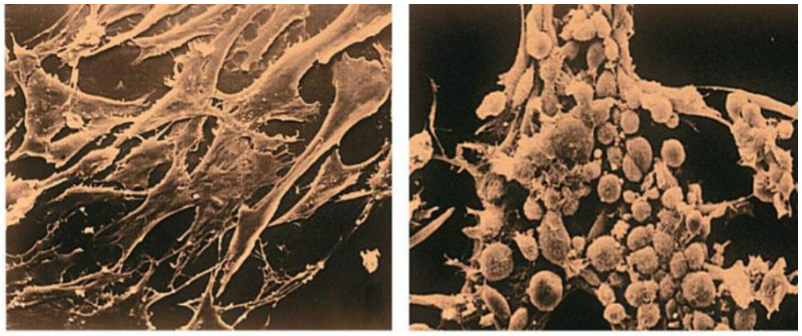
Continous (transplanted) - cultures of tumor cells; do not have a separation limit according to Hayflick (HeLa, HEp 2, KB, Vero, etc.). One of the earliest human HeLa cell cultures was obtained from Henrietta Lacks, who died of cervical cancer.

Virus detection methods

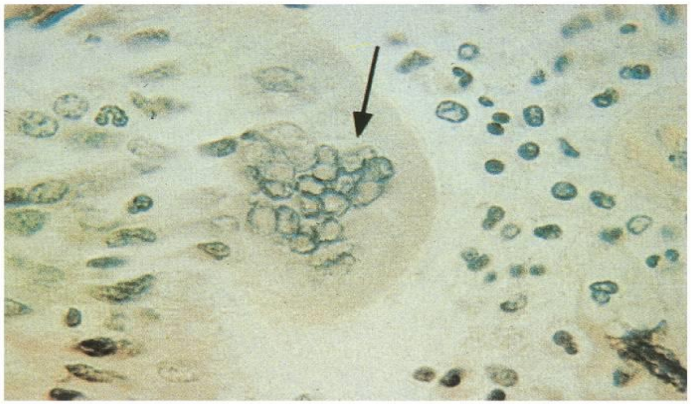


- By cytopathic action
  According to the formation of plaques
  Hemadsorption reaction
  Hemagglutination reaction
- By the formation of intracellular inclusions

The main manifestations of the cytopathic effect of viruses



- Cytopathic effect of adenovirus in cell culture - cluster formation



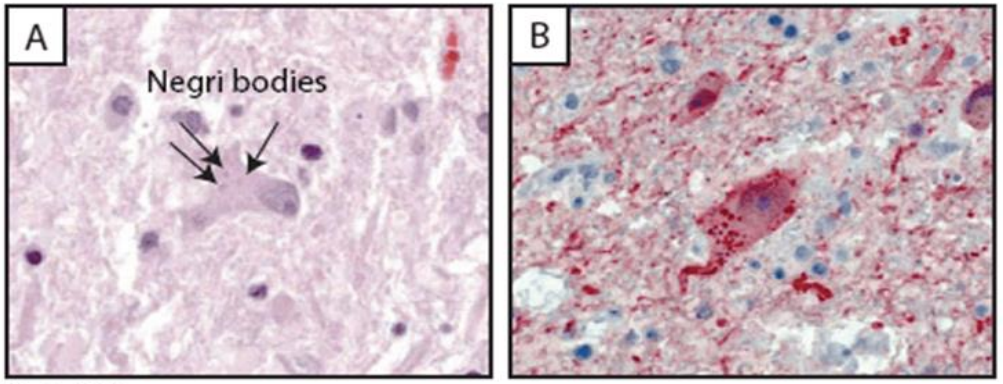
- Formation of multinucleated syncytium by measles virus

1) Virus reproduction may be accompanied by cell death or morphological changes they; 2) some viruses cause the fusion of cells and the formation of a multinucleated syncytium; 3) cells can grow but divide, resulting in giant cells;

4) inclusions (nuclear, cytoplasmic, mixed) appear in the cells. Inclusions can be stained pink (eosinophilic inclusions) or blue (basophilic inclusions); 5) if viruses containing hemagglutinins multiply in the tissue culture, then in the process of reproduction the cell acquires the ability to adsorb red blood cells (hemadsorption).

Intracellular inclusions

Photo/CDC

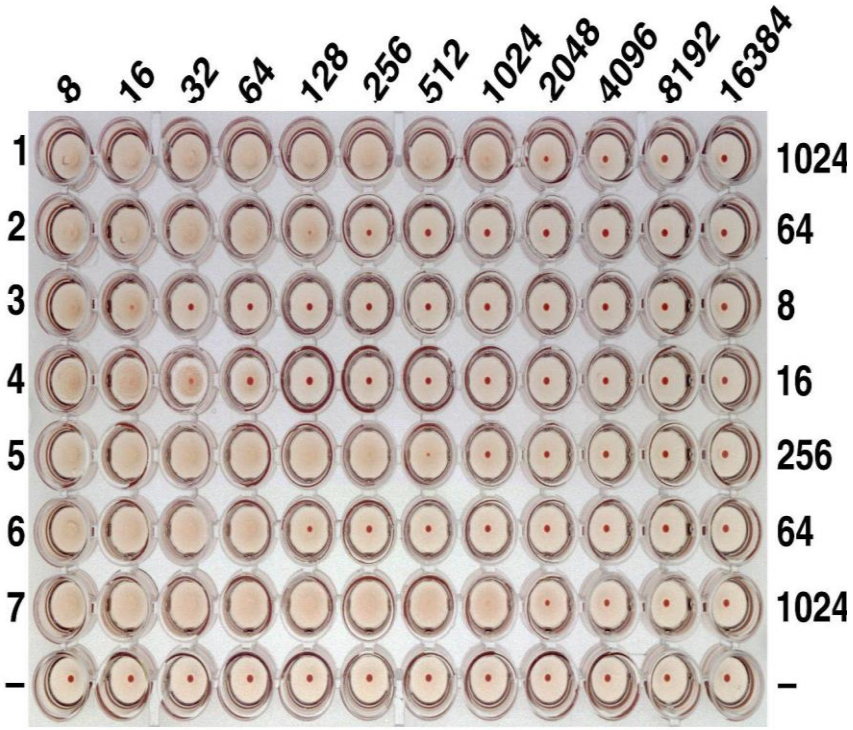


Photo/CDC

- Autopsy of hemorrhagic areas of the brain affected by the rabies virus

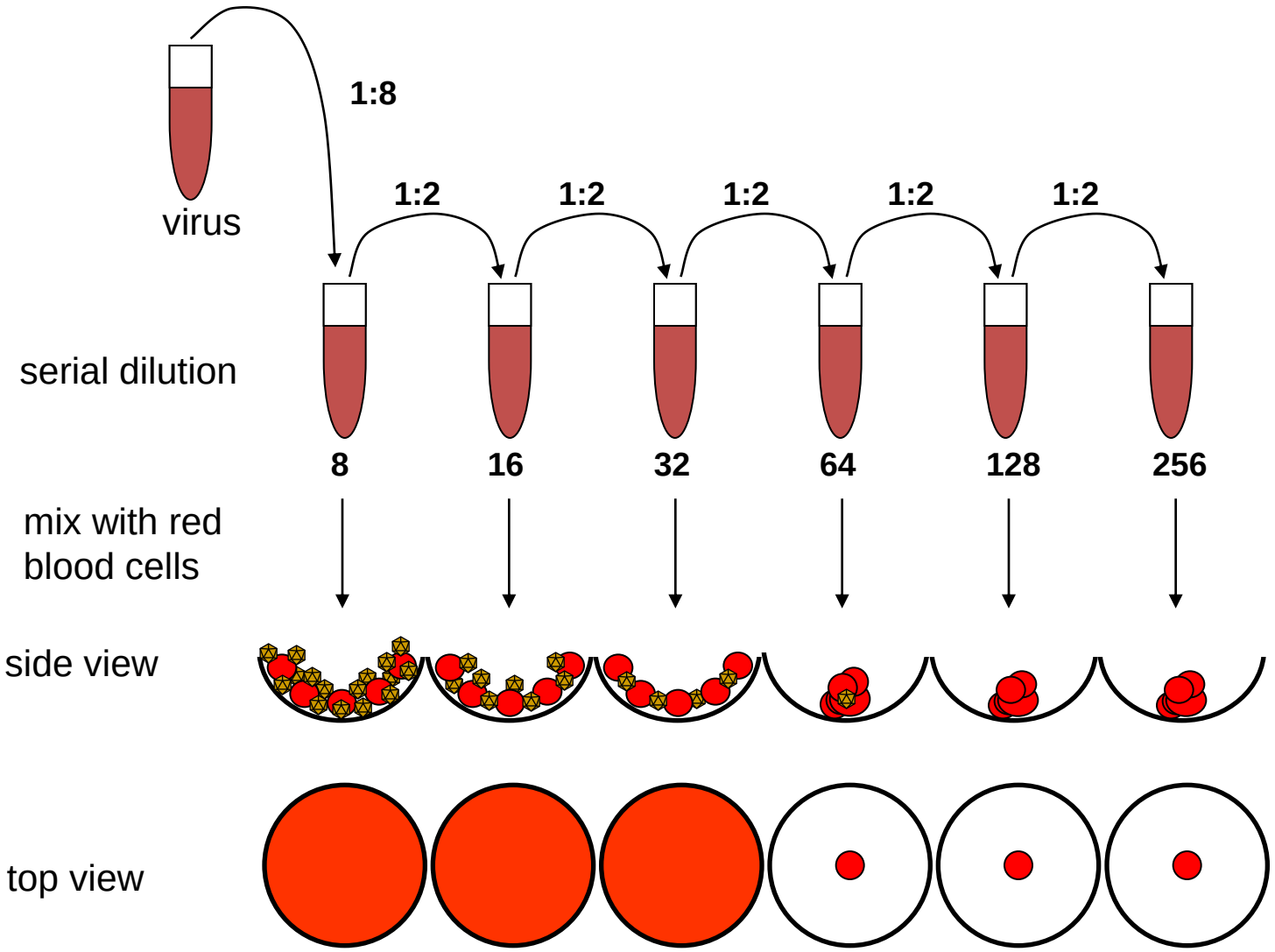
- Detection of inclusions - Babesh-Negri corpuscles, stained with hematoxylin (A) and by Mann (B)

Hemagglutination reaction



The indication is the detection of the fact of virus reproduction, established on the basis of hemagglutination (HAT). HAT is based on the ability of some viruses to cause agglutination (gluing) of erythrocytes of various species of animals, birds and humans due to the viral surface protein - hemagglutinin

Hemagglutination reaction



Titer of virus = 32

Virus identification methods

- Hemagglutination inhibition test (HAIT)
- Neutralisation test (NT)
- Immunodiffusion reaction
- Immune fluorescence (IF)
- Immune electron microscopy method
- Reactions of radial hemolysis