LESSON 22

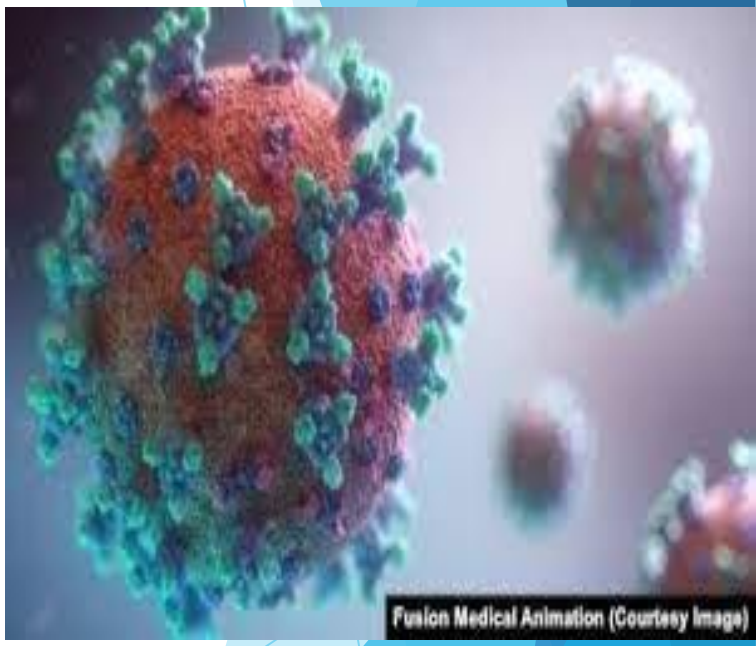
Introduction to virology. Pathogenesis and laboratory diagnostic principles of viral infections. Anti-virus

Virology

- The term “virus” was also used by L. Pasteur to denote an infectious principle. Currently, a virus refers to the smallest replicating microorganisms that are found everywhere where there are living cells.
- Viruses are a diverse and heterogeneous group of microorganisms that can infect most groups of more complex organisms, including bacteria, blue-green algae, fungi, plants, insects, and vertebrates.
- Virology studies morphology, structure, ecology, genetics, molecular biology of viruses, mechanisms of their replication, directions and mechanisms of evolution; epidemiology, methods of laboratory diagnostics, prevention and treatment the diseases they cause.

Viruses

- Viruses have fundamental differences from other prokaryotic microorganisms:
- 1. They do not have a cellular structure. These are non-cellular forms of biological life.
- 2. They have submicroscopic dimensions, varying in human viruses within 15–250 nm or more
- 3. Characterized by only one type of nucleic acid: either DNA or RNA as a genome.
- 4. Viruses do not have their own metabolic and energy systems.
- 5. Viruses replicate in cells using their protein-synthesizing and energy systems, so they are obligate intracellular parasites.
- 6. Viruses are not capable of progressive growth and division. They are formed in the form of mature forms (virions) by self-assembly from ready-made, i.e., preformed components (proteins, nucleic acids).

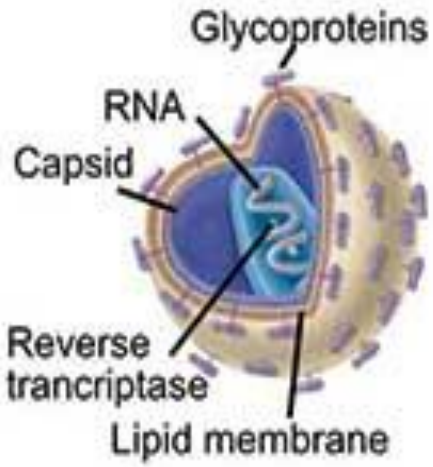


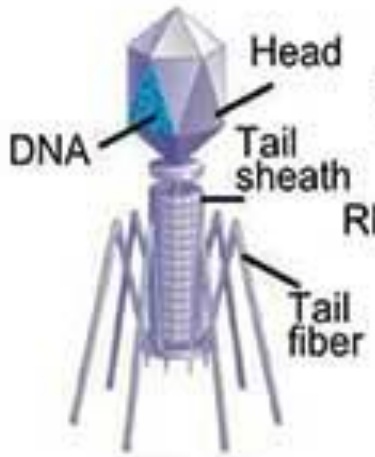
- To date, the classification of viruses has undergone nine editions. The ratified version of 2016 includes 4404 species, 735 genera, 35 subfamilies, 122 families, 8 orders of viruses.
- The universal system used is based on a conditionally chosen hierarchy corresponding to the species-genus-subfamily-family-order.

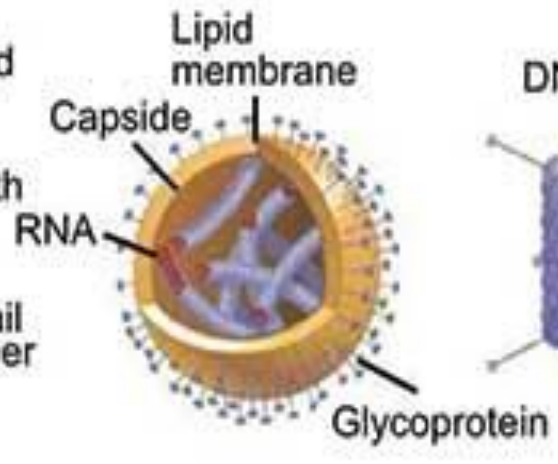
The following criteria are used in the classification of viruses:

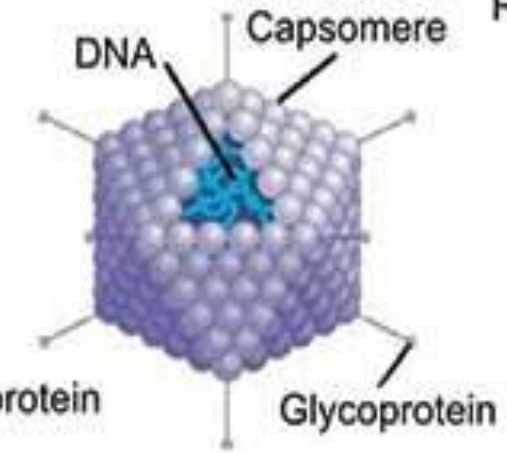
- – type of nucleic acid (RNA or DNA), its structure (single- or double-stranded, linear, circular, continuous or fragmented);
- – the strategy of the viral genome (i.e., the transcription, translation, replication pathway used by the virus);
- – the structure of the virion (presence of a lipoprotein envelope (supercapsid), size and morphology, type of symmetry, number of capsomeres, etc.);
- – antigenic and physico-chemical properties;
- – phenomena of genetic interactions;
- – ecological interactions (circle of susceptible hosts, geographic range);
- – mechanisms of pathogenicity (character of changes in cells, formation of intracellular inclusions, changes in gene expression of host cells, apoptosis and cell transformation);
- - methods of transmission and resistance to environmental factors (γ-radiation, temperature, the action of detergents, ether, antiviral drugs);
- – features of the infectious process and its epidemiology

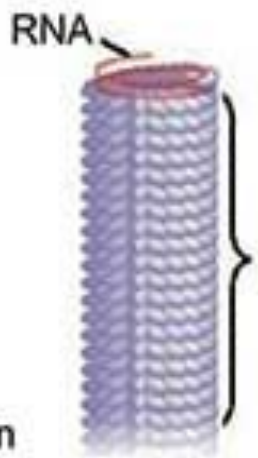
Virus Classification on the basis of morphology and replication

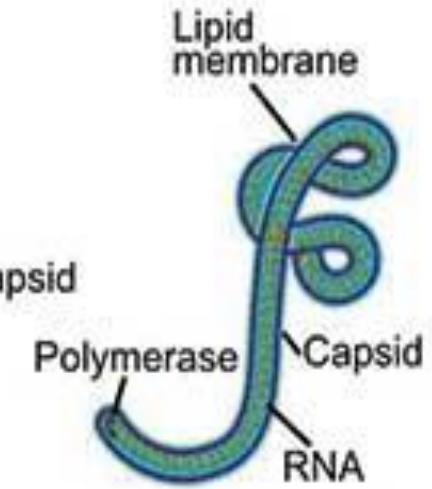


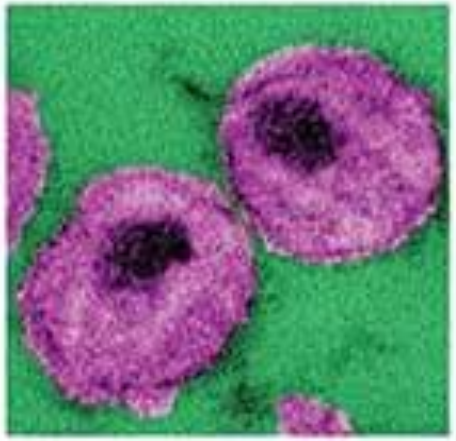








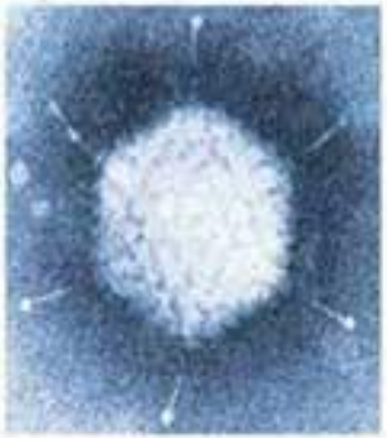




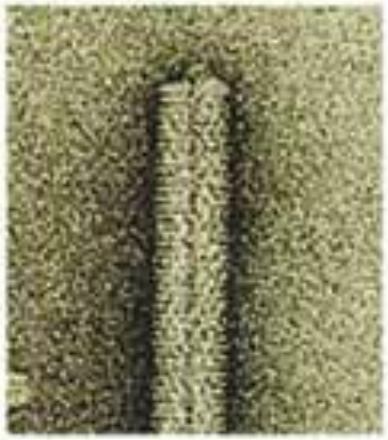
Human immudeficiency virus

Bacteriophage

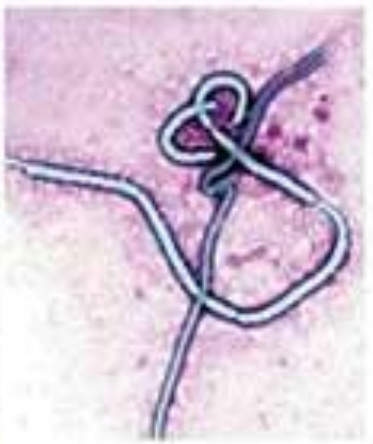
Influenza virus



Adenovirus



Tobacco mosaic virus



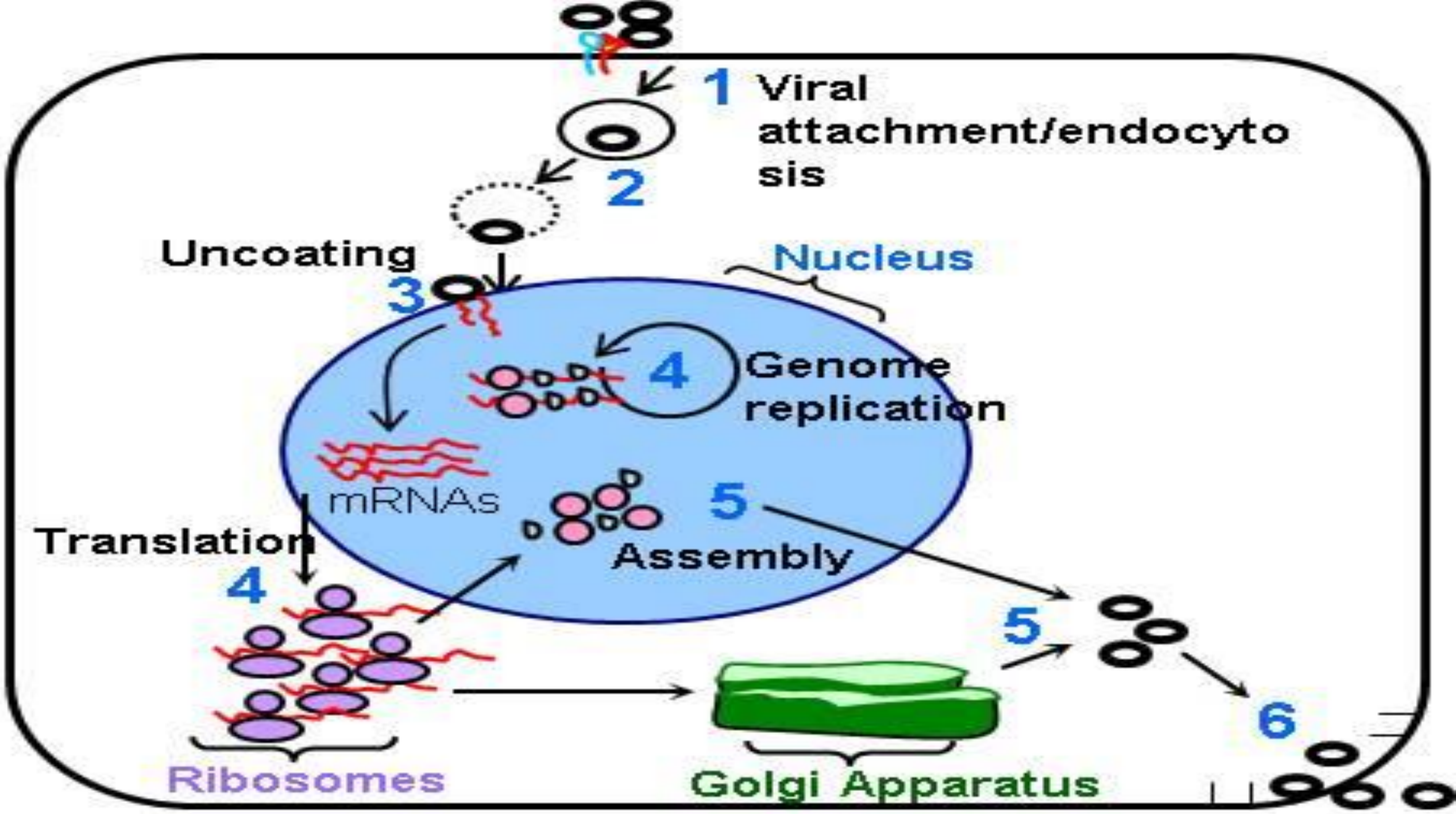
Ebola virus

VIRUS INTERACTION WITH A CELL REPRODUCTION (PRODUCTION) OF VIRUSES

- Viruses are obligate intracellular parasites that can reproduce only in living cells. Unlike prokaryotic and eukaryotic microorganisms, viruses do not reproduce by binary fission.
- An increase in the quantitative content of viruses in a cell occurs through reproduction (eng. reproduce - reproduce, make a copy), that is, through the biosynthesis of many molecules of nucleic acids and proteins, followed by their self-assembly in the form of virions.
- Synthesis of nucleic acids and proteins of the virus occurs in different parts of the cell (nucleus and cytoplasm). This method of reproduction is called disjunctive (separated)

The process of intracellular reproduction of viruses is conditionally divided into 2 phases

- The first phase includes 3 stages:
- 1) adsorption of the virus on the receptors of certain cell types;
- 2) penetration of the virus into the cell;
- 3) deproteinization of the virion.
- The second phase is synthetic, it includes the stages of implementing the strategy of the viral genome:
- 1) transcription,
- 2) broadcast,
- 3) replication,
- 4) assembly, maturation of viral particles;
- 5) release of viral particles from the cell.
- The interaction of a virus with a cell begins with the process of adsorption, i.e., with the attachment of the virus to the surface of the cell.


1 Viral attachment/endocytosis
2
Uncoating
3
Nucleus
4 Genome replication
mRNAs
5
Assembly
Translation
4
5
6
Ribosomes
Golgi Apparatus

The implementation of the genome strategy in DNA-containing viruses proceeds in the same way as in host cells:

DNA transcription and RNA translation protein.

U-RNA viruses, i.e., viruses with a negative genome (influenza, parainfluenza, etc.), the genome implementation path is as follows:

-RNA transcription and -RNA translation protein.

In +RNA viruses, i.e. with a positive genome (togaviruses, picornaviruses), there is no transcription step. The plus-strand of the RNA genome performs the function of mRNA, respectively, the way to implement the genome is simpler:

+RNA translation protein.

Hepadnaviruses (hepatitis B virus) have circular double-stranded DNA as their genome. Their genome replicates via an RNA intermediate:

DNA transcription RNA reverse transcription DNA transcription and RNA translation protein.

Retroviruses (they have a genome in the form of diploid + RNA and a reverse transcriptase enzyme) have a unique way of transmitting genetic information:

RNA reverse transcription DNA transcription and RNA translation protein. The DNA copy integrates with the genome of the host cell (provirus).

The process of interaction between the virus genome and the host cell genome is complex and far from being fully understood. At the same time, more than 200 host cell genes are known to be involved in this process. The function of more than 80% of them is inhibited, and approximately 20% of the genes are activated

Features of viral infections

- 1. Viral infections are widespread. Their share in the structure of infectious morbidity is 60-80%.
- 2. Cause a state of viremia.
- 3. Intracellular reproduction of viruses leads to mass death of cells of the affected organs and body systems.
- 4. Some viruses (influenza, herpes, HIV, measles, hepatitis B and C) cause infections of the immune system and induce the development of secondary immunodeficiency states.
- 5. Integration of some viruses with the genome of the host cell (HIV, hepatitis B virus, oncogenic RNA genomic viruses) affects the expression of its genes.
- 6. Teratogenic properties of some viruses (rubella, cytomegaly).
- 7. Chronic viral infections can induce the development of tumor transformation (adenoviruses, herpesviruses, hepatitis B, C, G viruses).
- 8. Can cause slow infections (HIV, measles, rubella, rabies, hepatitis B, herpes, etc.).
- 9. Immunoprophylaxis and chemotherapeutic drugs against many viral infections are not available.
- 10. Diagnosis of viral diseases is complex, expensive due to the mass nature of a number of them and is not used in all cases.
- 11. In most cases, the diagnosis of a viral infection is retrospective.

PATHOGENESIS OF VIRAL INFECTIONS

The pathogenesis of viral infections is a set of processes that cause a disease and determine its development and outcome.

The pathogenesis is determined by the following factors:

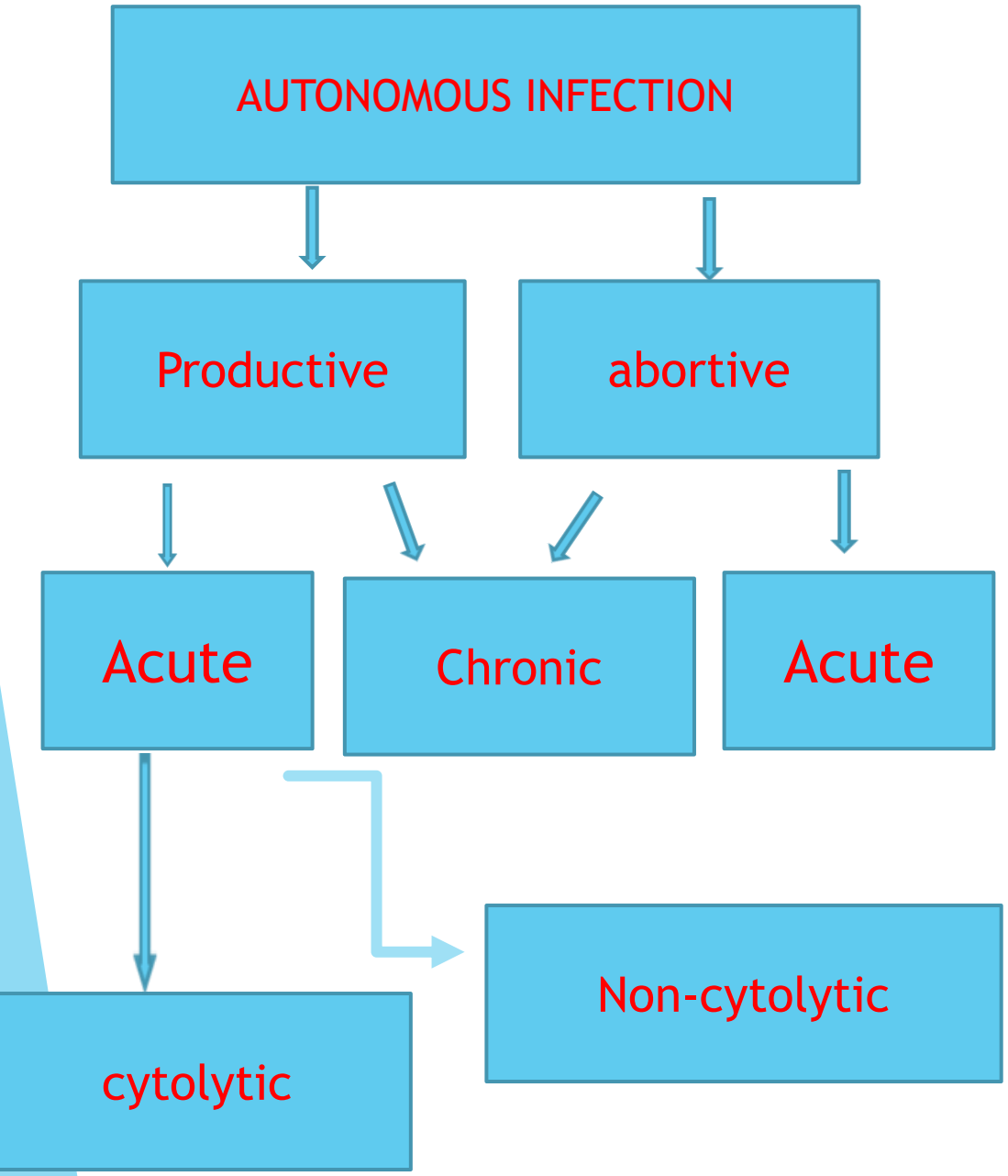
1. tropism of the virus (sensitivity to the virus of certain cells, tissues and organs of the macroorganism).
2. 2. the rate of virus reproduction and the number of infectious particles in the offspring;
3. 3. cell response to infection;
4. 4. reaction of the body to changes in cells and tissues caused by infection.

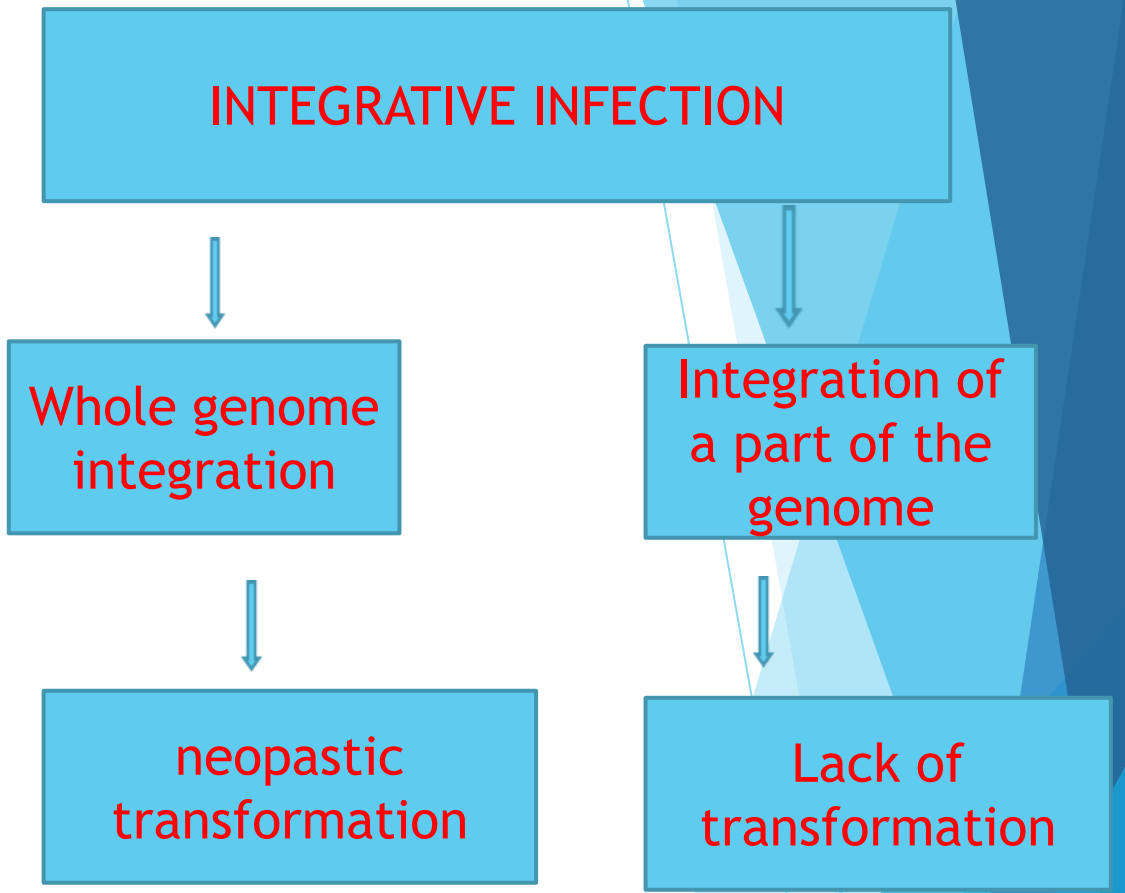
Types of human viral infections depending on the tropism of pathogens

- Respiratory: influenza, parainfluenza, respiratory gestational infection, adeno-, rhino-, reo-, coronovirus infections, measles, mumps, rubella, smallpox, chicken pox, etc.
- Intestinal: poliomyelitis, Coxsackie, ECHO, rotavirus infection, hepatitis A and E, etc.
- Blood (transmissible): HIV infection, hepatitis B, C and D, tick-borne and Japanese encephalitis, yellow fever, dengue fever, hemorrhagic fevers, etc.
- Infections of the external integument: rabies, herpes, cytomegaly, foot and mouth disease, etc.

Features of viral infections

- At the cell level, autonomous infections are distinguished if the viral genome replicates independently of the cellular one, and integrated infections if the viral genome is included in the cellular one.
- Autonomous infection is divided into productive, in which infectious offspring of virions are formed, and abortive, in which the infectious process is interrupted, and new viral particles are not formed at all or are formed in small quantities.
- Productive and abortive infections can be acute or chronic.
- Acute infection, depending on the outcome, is divided into cytolytic and non-cytolytic.
- Cytolytic infection ends with cell destruction, or CPP, and the virus that causes CPP is called cytopathogenic


AUTONOMOUS INFECTION
Productive
abortive
Acute
Chronic
Acute
cytolytic
Non-cytolytic

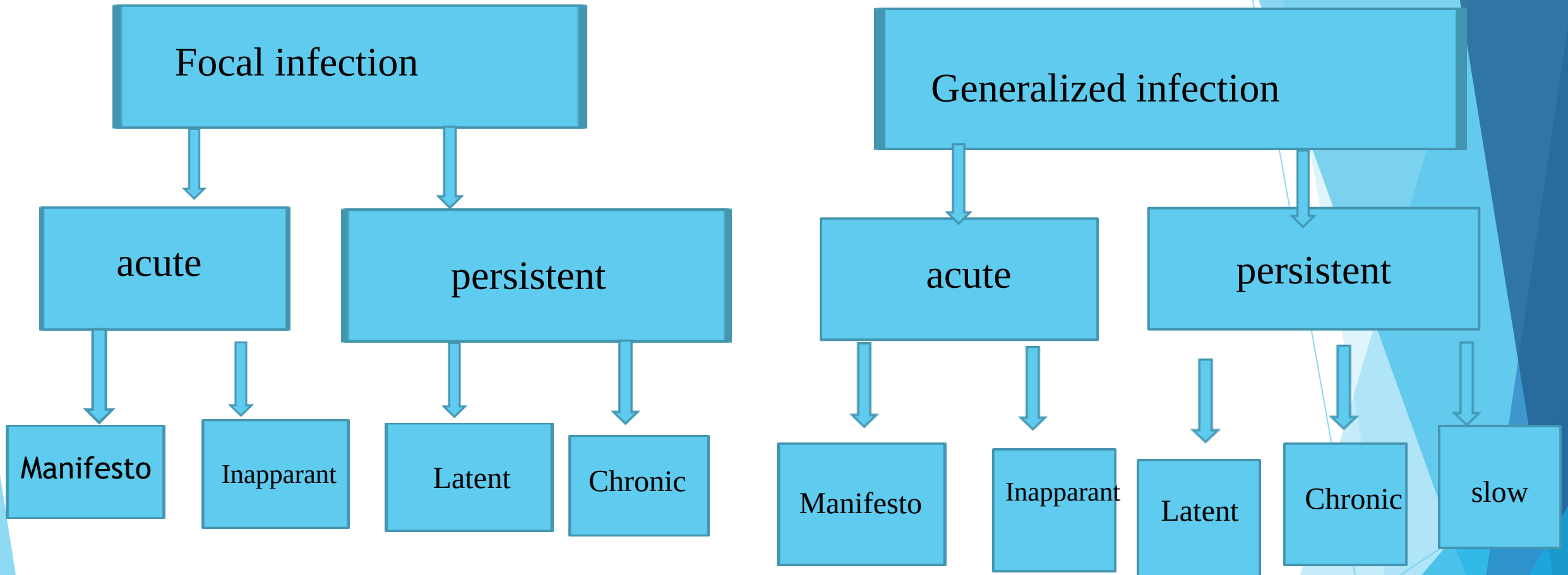

INTEGRATIVE INFECTION
Whole genome integration
Integration of a part of the genome
neopastic transformation
Lack of transformation

Features of viral infections

At the level of the body, viral infections are divided into 2 groups:

1) focal - the virus reproduces in cells locally at the site of the entrance gate;
2) 2) generalized - the virus, after local reproduction, hematogenously or lymphogenously spreads to various organs and tissues and forms secondary foci of infection.

Examples of focal infection - ARVI and AII, generalized - poliomyelitis, measles, smallpox

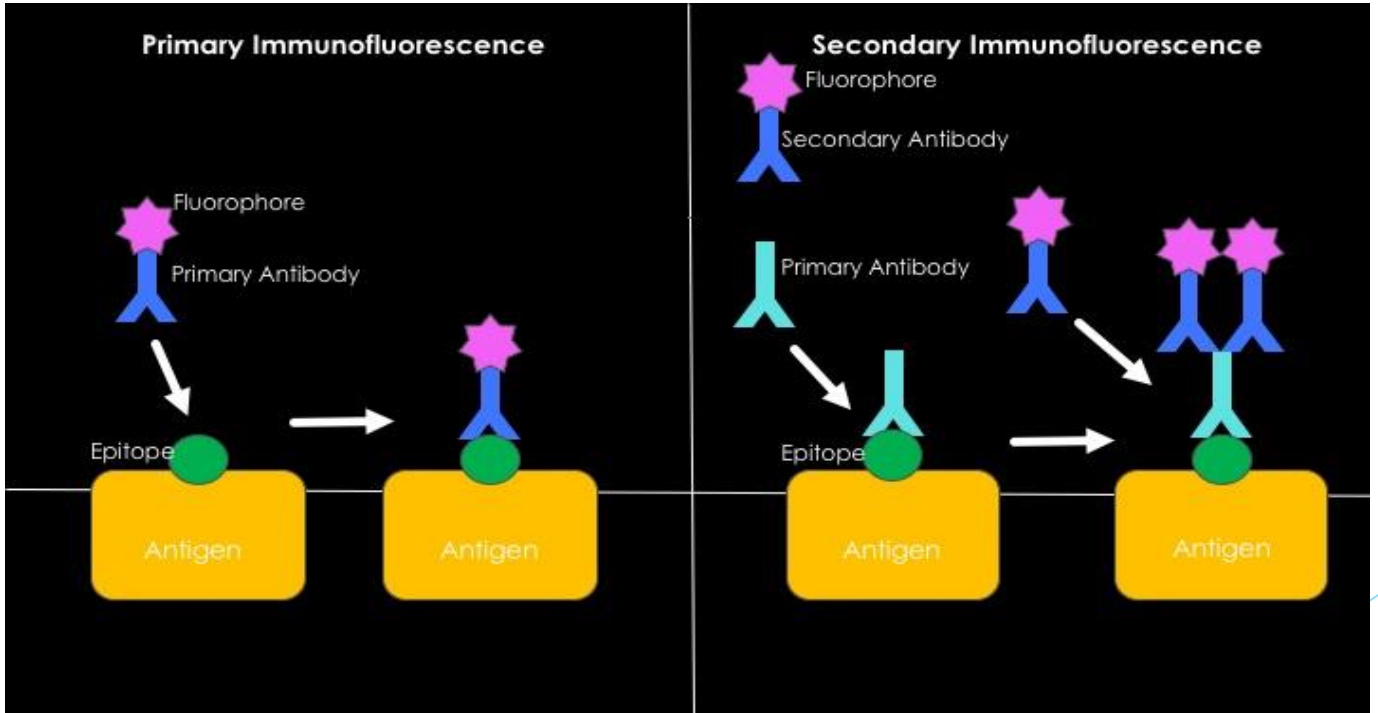

Focal infection
acute
persistent
Manifesto
Inapparant
Latent
Chronic
Generalized infection
acute
persistent
Manifesto
Inapparant
Latent
Chronic
slow

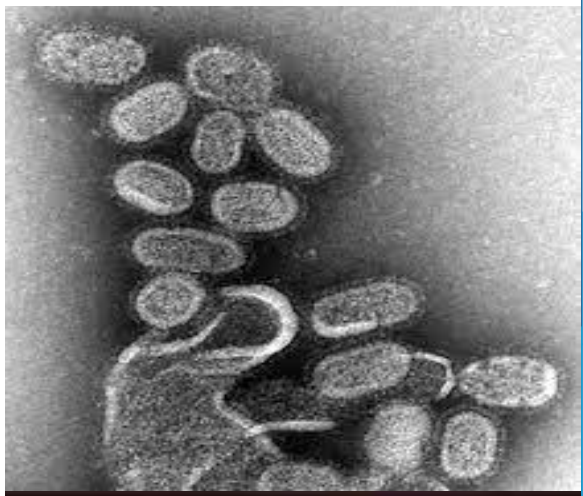
PRINCIPLES OF LABORATORY DIAGNOSIS OF VIRAL INFECTIONS

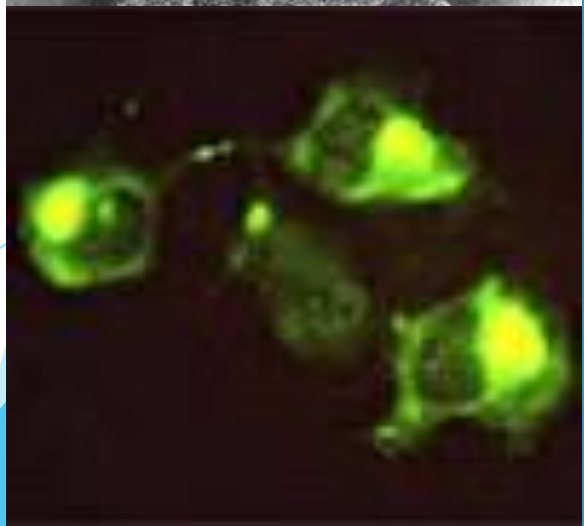
- The laboratory diagnosis of viral infections is based on 4 groups of methods:
- Group 1 - detection of the pathogen or its components directly in the clinical material taken from the patient, and receiving a response after a few hours (quick; express diagnostics).
- group 2 — virus isolation from clinical material, its indication and identification (virological diagnostic method).
- Group 3 — detection in biological fluids of patients (blood, cerebrospinal fluid) of antibodies to antigenic determinants of viruses (serological diagnosis of viral infections). In most cases, serological diagnosis requires paired sera taken in the acute phase of the disease and after 2–4 weeks.
- group 4 — detection of virus-specific fragments of the virus genome in biological material from the patient and cell culture (molecular biological methods of indication, identification of viruses).

Methods of express diagnostics

- Used for adenovirus, rhinovirus, herpesvirus (CMV, varicella) infections, influenza, RS infection, hepatitis, rotavirus infection and HIV
- The material for the study is: discharge of the nasopharynx, conjunctiva, blood, urine, feces, saliva, vesicle contents, biopsy material
- Apply: RIF, molecular hybridization (MG), EM, ELISA, RIA, PCR, IEM, IB, microscopy of smears of prints







Virological study

- The material for virological research in diseases accompanied by diarrhea or other gastrointestinal disorders suggesting a viral etiology (hepatitis A, rotavirus and enterovirus infections) are fresh portions of feces.
- In diseases of the respiratory system (influenza, parainfluenza, RS infection, adenovirus infection, etc.), the material for research is best obtained by aspiration of mucus, washings. Smears from the nasopharynx are less informative.
- In the presence of a vesicular rash (herpetic infection), the material for the study is a liquid aspirated by a needle from the vesicles. With petechial and maculopapular rash - both samples of mucus from the nasopharynx and feces.
- If neuroviral infections (poliomyelitis, arbovirus infections) are suspected, mucus from the nasopharynx, feces and cerebrospinal fluid should be taken for virological examination.
- Saliva is the material used to diagnose mumps and rabies.
- If cytomegalo- and papovavirus infections are suspected, the material may be urine.
- An attempt to isolate the virus from the blood can be made if infections are suspected caused by some arboviruses, herpes viruses, HIV.
- A brain biopsy may be performed in the diagnosis of herpetic encephalitis, SSPE, progressive rubella panencephalitis, Creutzfeldt-Jakob disease,

Isolation of the virus from clinical material is carried out by its inoculation into cell culture, chicken embryos or infection of laboratory animals with it.

- Indication of viruses in cell culture is carried out according to the CPD, RIF, RGA, RGA. Many enteroviruses cause early CPP (after a few hours). Cytomegaloviruses, adenoviruses, rubella virus cause CPP after a few weeks, and sometimes it is necessary to resort to obtaining a subculture. The presence of syncytium indicates the presence of viruses such as respiratory syncytial virus, measles, mumps, herpesviruses.
- Identification of viruses isolated in these systems is carried out using serological methods. Serological tests such as RTGA, RN, RTGA are used only for viral infections. RSK, RPHA, ELISA, RIA, RIF, RP, etc. are used to diagnose both viral infections and infections caused by other pathogens.
- Currently, methods of molecular diagnostics are widely used: MG, PCR.

Basic principles of virus cultivation

- in laboratory animals
- in chicken embryos
- In cell (tissue) cultures

Cultivation of viruses in laboratory animals

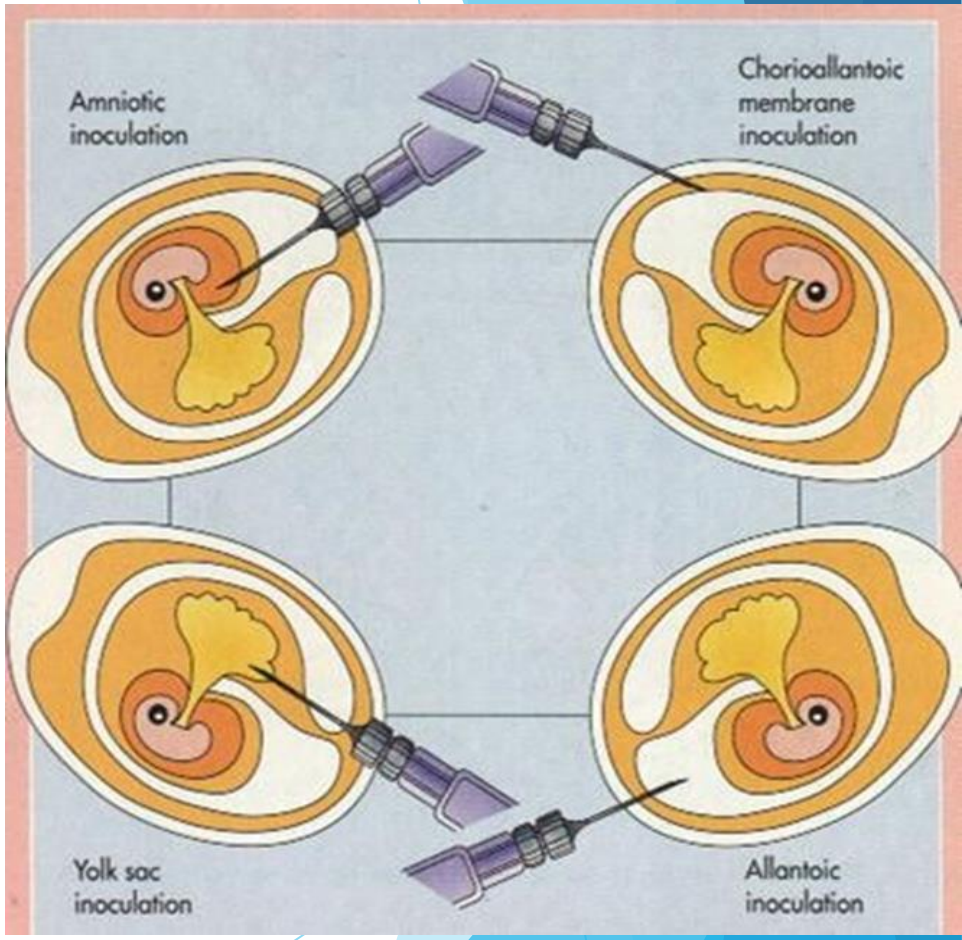
- In virological studies, newborn laboratory animals (white mice, rats, rabbits, monkeys, etc.)
- When infecting laboratory animals by various methods (subcutaneously, intramuscularly, intravenously, intranasally, intraperitoneally, etc.), the tropism of viruses must be taken into account.
- The use of laboratory animals is currently very limited due to species immunity of animals to human viruses, contamination of animals with foreign microbes, as well as economic and ethical considerations.

Cultivation of viruses in chicken embryos

- Chicken embryos are a favorable model for the cultivation of viruses due to the possibility of accumulating a large number of viruses in them, sterility and available techniques for working with them, etc.
- Typically 6-12 day old developing chick embryos (ECEs) are used.
- However, contamination of chicken embryos with a latent viral or bacterial infection is possible.

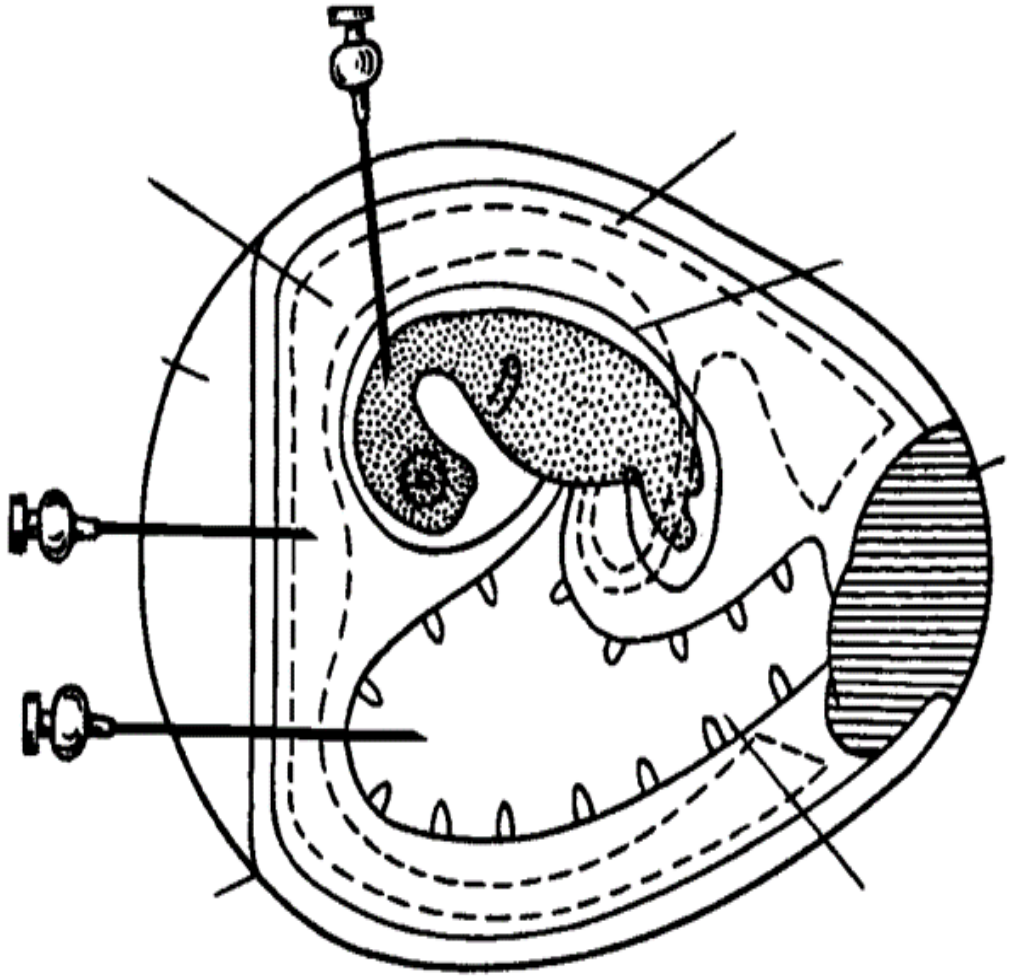
Infection of chick embryos

- Choose fertilized eggs aged in the refrigerator for no more than 10 days with a non-pigmented and clean shell (cannot be washed). The viability of the embryo is determined by candling; the living embryo is mobile, the pulsation of the heart is visible.
- Chicken embryos are infected under aseptic conditions. Before infection, the shell of the embryos is treated with 70% ethyl alcohol, wiped with iodine, and sometimes also flambéed.
- The choice of method of infection is determined by the tropism of the virus. Most often, infection is used in the allantoic cavity and on the chorionallantoic membrane, less often - in the amniotic cavity and in the yolk sac.



Infection of chick embryos

- In the shell above the border (previously outlined with a pencil) of the air chamber, a hole is made with a diameter of about 1 mm. The infecting liquid is injected in a volume of 0.1-0.2 ml, by inserting a needle to a depth of no more than 2-3.
- After the injection of the virus-containing material, the needle is removed and the hole in the shell is closed with a drop of molten paraffin.
- The infected embryo is opened after 48-72 hours of incubation, during the period of maximum accumulation of viruses.



Opening of infected embryos

- Before opening, the shell is treated with iodized alcohol. The shell is cut above the marked boundaries above the air chamber with sterile tools. In this case, the egg is held at a certain angle so that the shell does not fall inside.
- After the shell is removed, the CAO is examined by lifting it with tweezers in order to establish pathoanatomical changes in it (hemorrhages, whitish spots). The part of the CAO, on which the virus-containing material was applied, usually has the most pronounced changes.

Methods for the detection of viruses in an infected chick embryo

An indicator of infection of the embryo with a virus can be:

- Embryo death
- The appearance on the chorioallantoic membrane (CAO) of necrotic areas, nodules (pockmarks).
- Hemagglutination reaction with amniotic and allantoic fluid

Study of the infected CAO

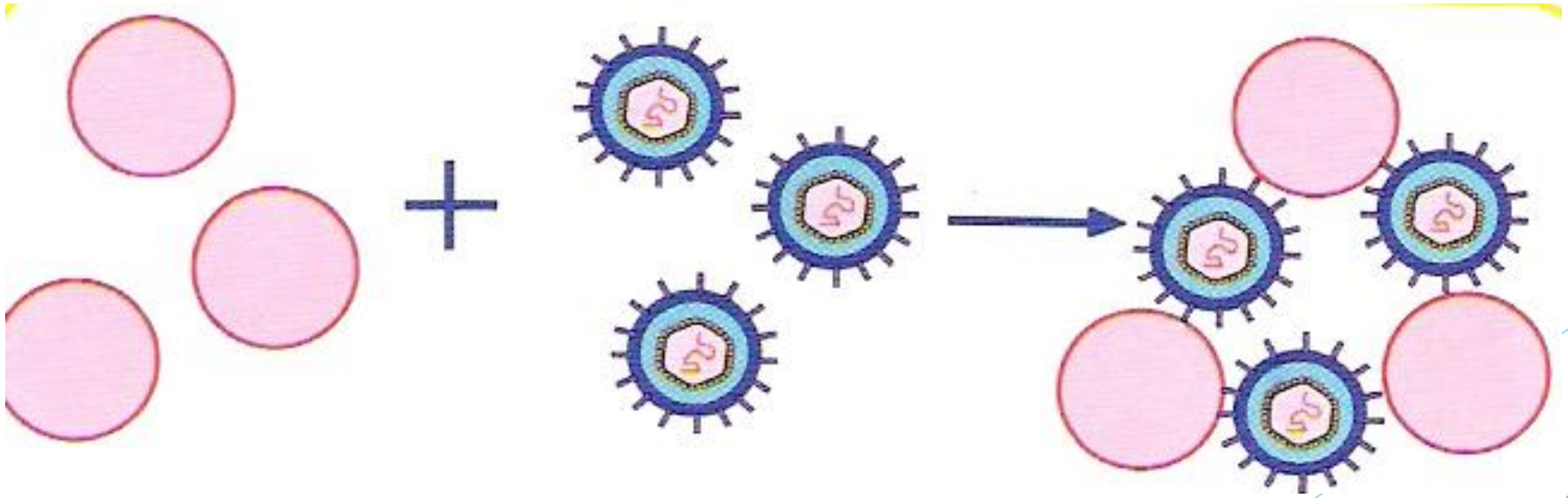
- For a more thorough examination, the CAO is lifted with tweezers and cut off with scissors.
- To examine and take the entire CAO, the embryo, yolk sac and protein are removed, and the chorioallantoic membrane is peeled off from the inner surface of the shell, removed and transferred to a sterile Petri dish with saline. The shell is rinsed, and then straightened with two tweezers so that it lies in one layer and can be examined over the entire surface.
- In order for pathological changes in the membrane to be seen more clearly, a sheet of black paper is placed under the Petri dish.

Obtaining amniotic and allantoic fluid

- The allantoic fluid is sucked off with a pipette, which is used to pierce the shell membrane and CAO. They put bacteriological control of the virus-containing material by inoculation on the BCH, or sugar broth. Detection of the virus in the material is carried out using the hemagglutination test (HHA) and stored frozen at -4C
- Taking amniotic fluid is carried out after removal of allantoic fluid. To do this, the pipette is introduced into the amnion between the head and body of the embryo and sucked off with a Pasteur pipette.

Hemagglutination reactions with amniotic and allantoic fluid

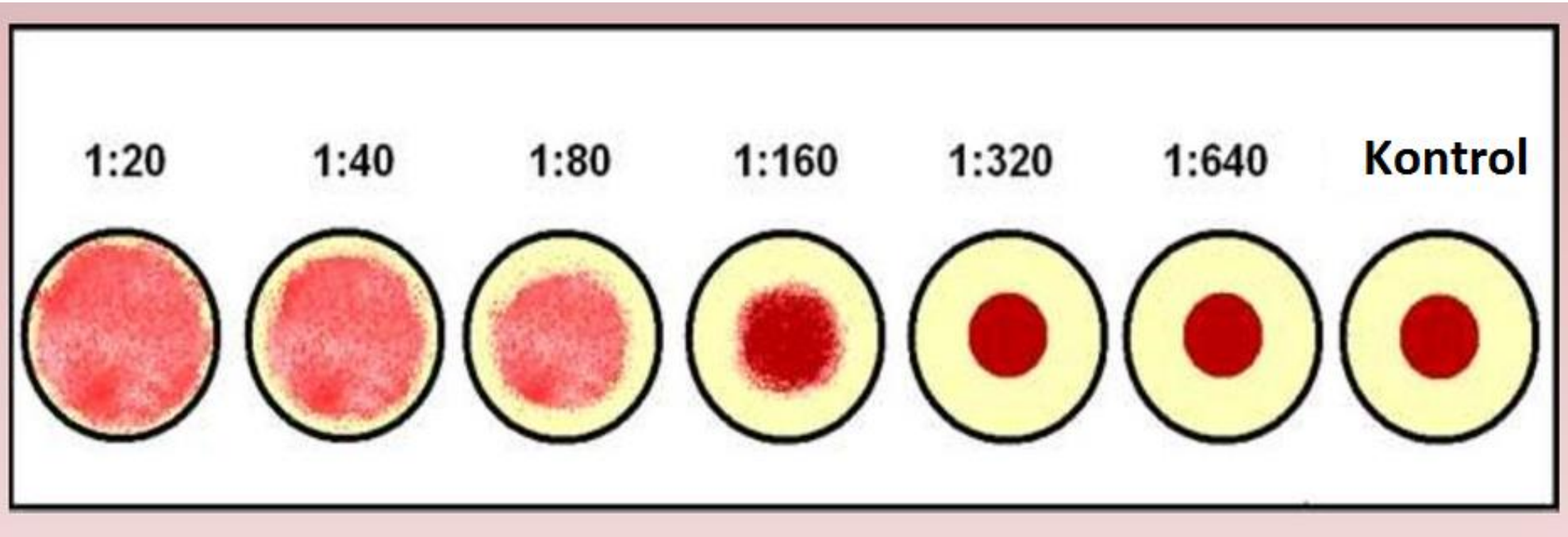
- Detection of the virus in the allantoic and amniotic fluids of an infected embryo is carried out by setting up a hemagglutination reaction.
- The reaction is based on the ability of the antigens of some viruses (hemagglutinins) to agglutinate (glue) animal erythrocytes and is used in the indication of viruses



Haemagglutination Reaction Technique

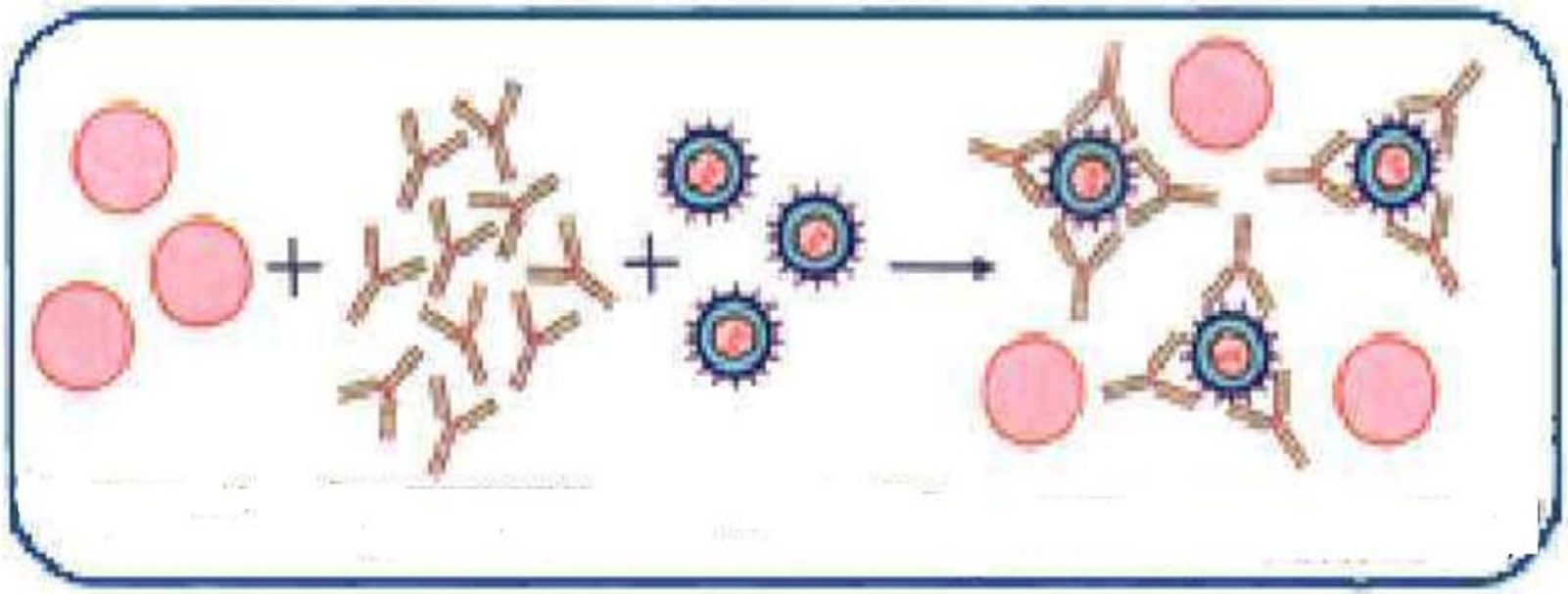
- After opening, the amniotic and allantoic fluid is poured into test tubes or holes of a plexiglass plate in a volume of 0.5 ml (0.5 ml of the same fluid of an uninfected embryo is taken for control). Then, 0.2 ml of a 1% suspension of washed chicken erythrocytes is added and kept at room temperature.
- The reaction results are taken into account 40 minutes after erythrocyte sedimentation;
- (++++) pronounced hemagglutination — a thin film of stuck together erythrocytes at the bottom of the tube;
- (+++) – the presence of gaps in the film;
- (++) - the presence of a film of adherent erythrocytes with scalloped edges;
- (+) - flocculent sediment of erythrocytes, surrounded by a zone of lumps of agglutinated erythrocytes;
- - sharply defined erythrocyte sediment indistinguishable from control
- The presence of hemagglutination in experimental tubes, while its absence in control tubes, indicates the content of the virus in the test liquid.

Haemagglutination Reaction Technique

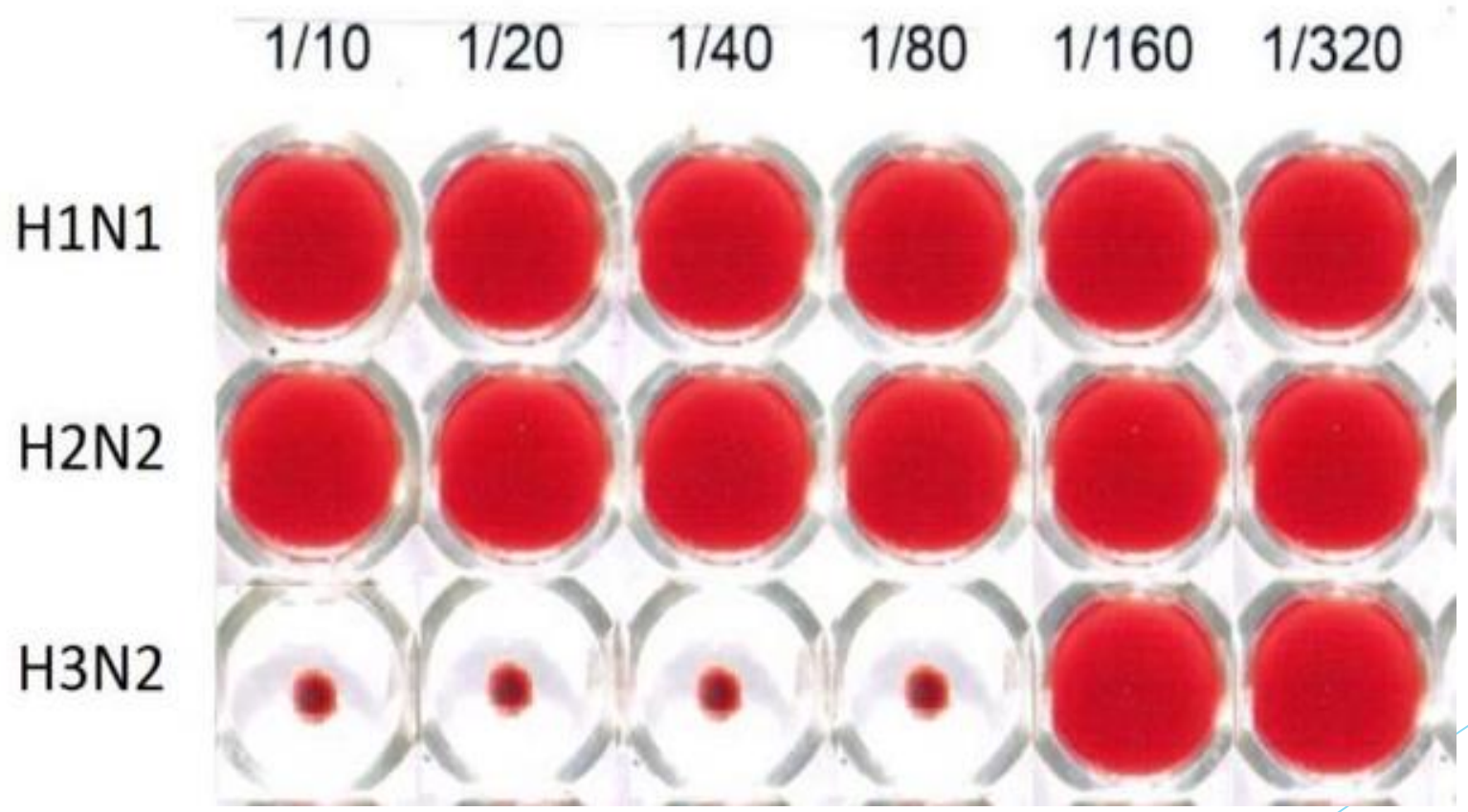


Hemagglutination inhibition reaction

- Used in the identification of certain viruses (influenza, measles, tick-borne encephalitis, etc.)
- To determine the type of virus in the test material, serum containing antibodies to a specific type of virus is added to it.
- In the presence of a virus in the test material, antibodies complementary to it inactivate the virus and hemagglutination of erythrocytes does not occur



Haemagglutination Inhibition Technique



Cultivation of viruses in cell (tissue) culture

- Cell (tissue) culture consists of individual cells of an organ or tissue that are able to reproduce their vital functions in nutrient media.
- Cells obtained from various organs and tissues of humans, animals, birds, and other biological objects are propagated outside the body on artificial nutrient media.

Getting cell cultures

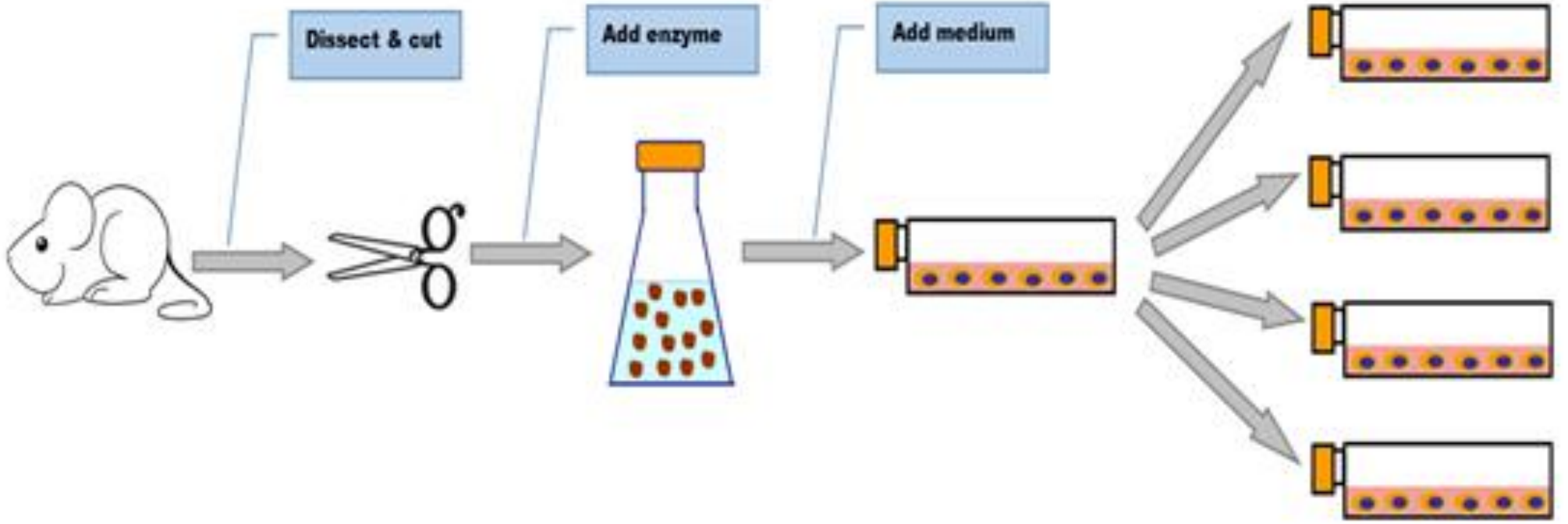


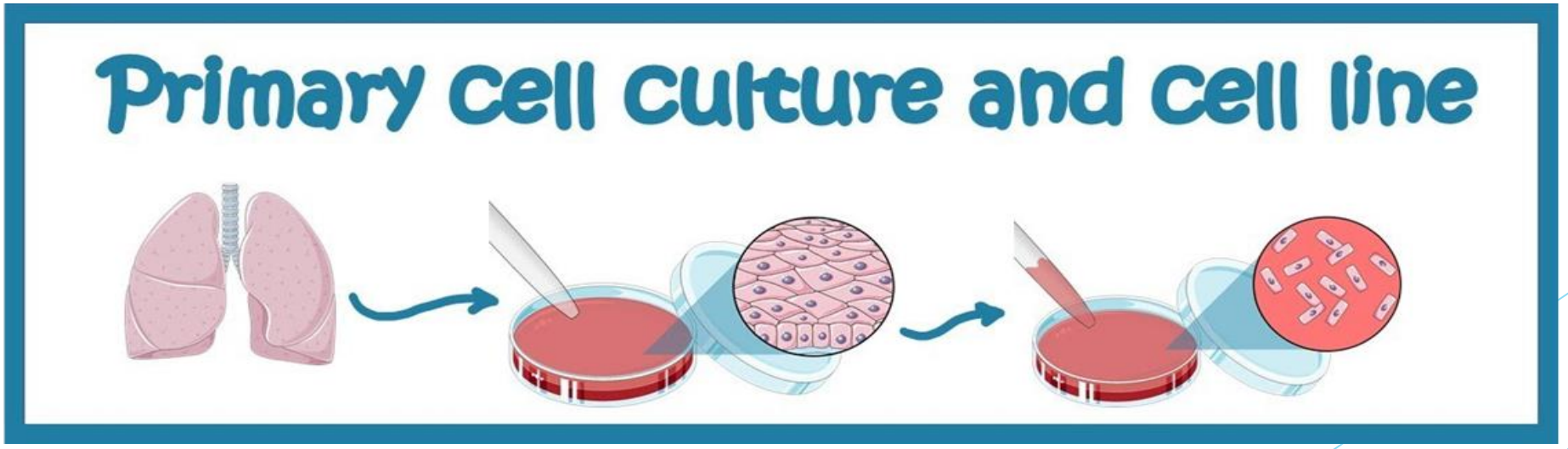
Fig1： the flow chart for primary cell culture

Cultivation of viruses in cell (tissue) culture

- Cell (tissue) cultures:
  - single
  - layer
  - suspension
- organ Single layer cell cultures
  - Primary cell cultures
  - Continuous (stable) cell cultures
  - Semi-permanent cell cultures

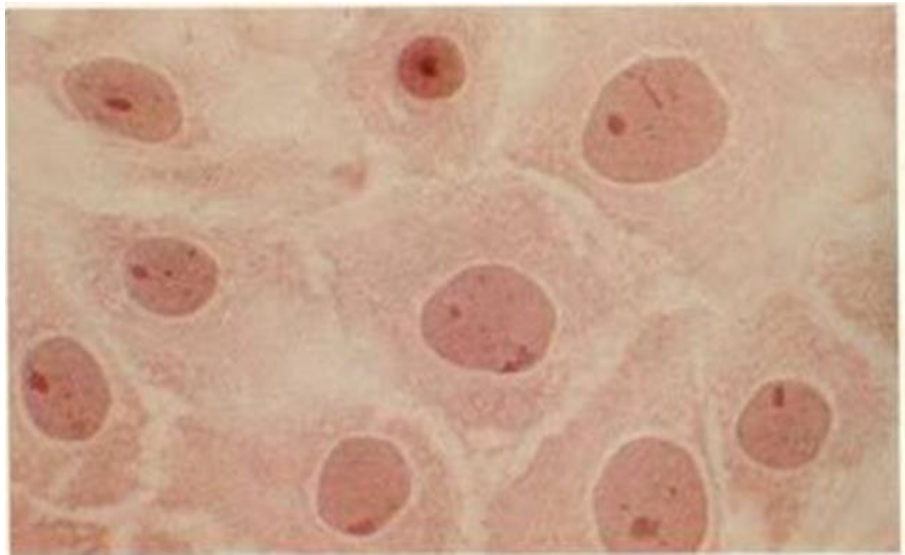
Primary cell cultures

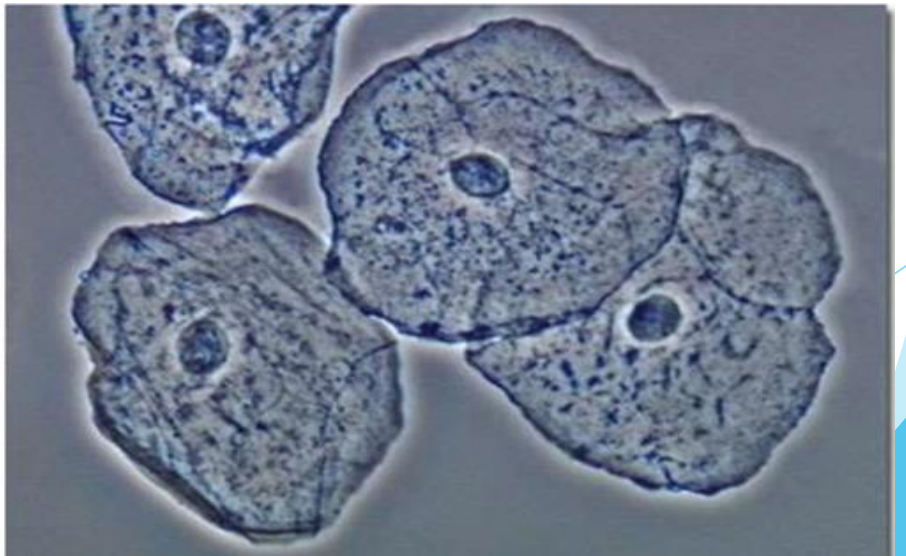
- Primary cell cultures are obtained by treating pieces of animal or human tissue with proteolytic enzymes.
- Cells formed by disintegration settle, attach and spread out on the surface of glass or plastic.
- Once the primary culture has reached a monolayer state, it can be subcultured or subcultured into a second culture vessel with trypsin or Versen's solution. Primary cell cultures are able to multiply a limited number of times, and therefore withstand no more than 5-10 passages.



Primary cell cultures

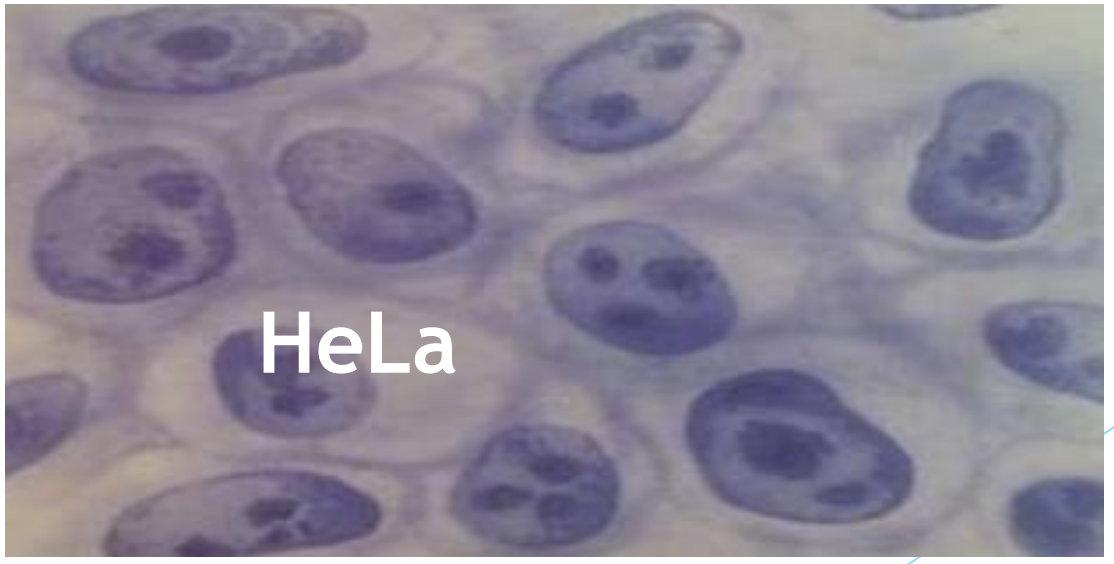
- Primary cell cultures are obtained from human or animal embryonic tissue, since it is embryonic cells that have a high potential for growth and reproduction.
- Cell cultures often contain a mixture of several tissue types, eg skin, bone, muscle.
- According to this principle, cultures of human embryonic fibroblasts (HEF) and chicken embryonic fibroblasts (FEK), human kidney cells (HEK), etc. are made. When obtaining such cultures, human embryo tissues (after abortions) or 8-12-day-old chicken embryos are used.
- Cell cultivation is carried out in glass or plastic dishes with strict observance of asepsis rules.





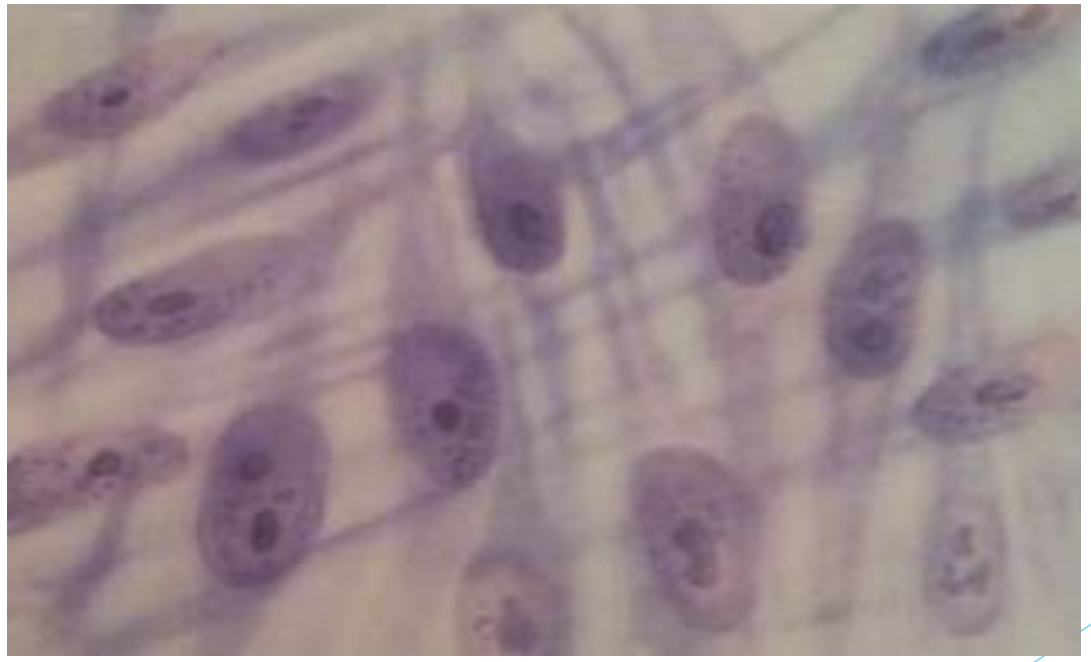
Continuous cell cultures

- Transplanted (stable) cell cultures are capable of multiplying indefinitely for a long time (tens of years), i.e. withstand multiple passages
- They are obtained mainly from tumor or embryonic tissues with a high potential for growth.
- The following lines of transplanted cells have been obtained and most widely used in virological practice: (A-0, A-1, FL) - from human amnion cell culture, HeLa - from cervical carcinoma; Hep-2 - from carcinoma of the larynx; Detroit-6 - from lung cancer metastasis to the bone marrow; RD - from human rhabdomyosarcoma



Diploid (semi-transplantable) cell line

- A diploid cell line is a cell line in which more than 75% of the cells have the karyotype of normal cells of the original species.
- Many of these cultures are able to maintain a diploid set of chromosomes even after 50-80 or more passages.
- To obtain a diploid cell line, fibroblasts isolated from human and animal embryonic tissue are used.

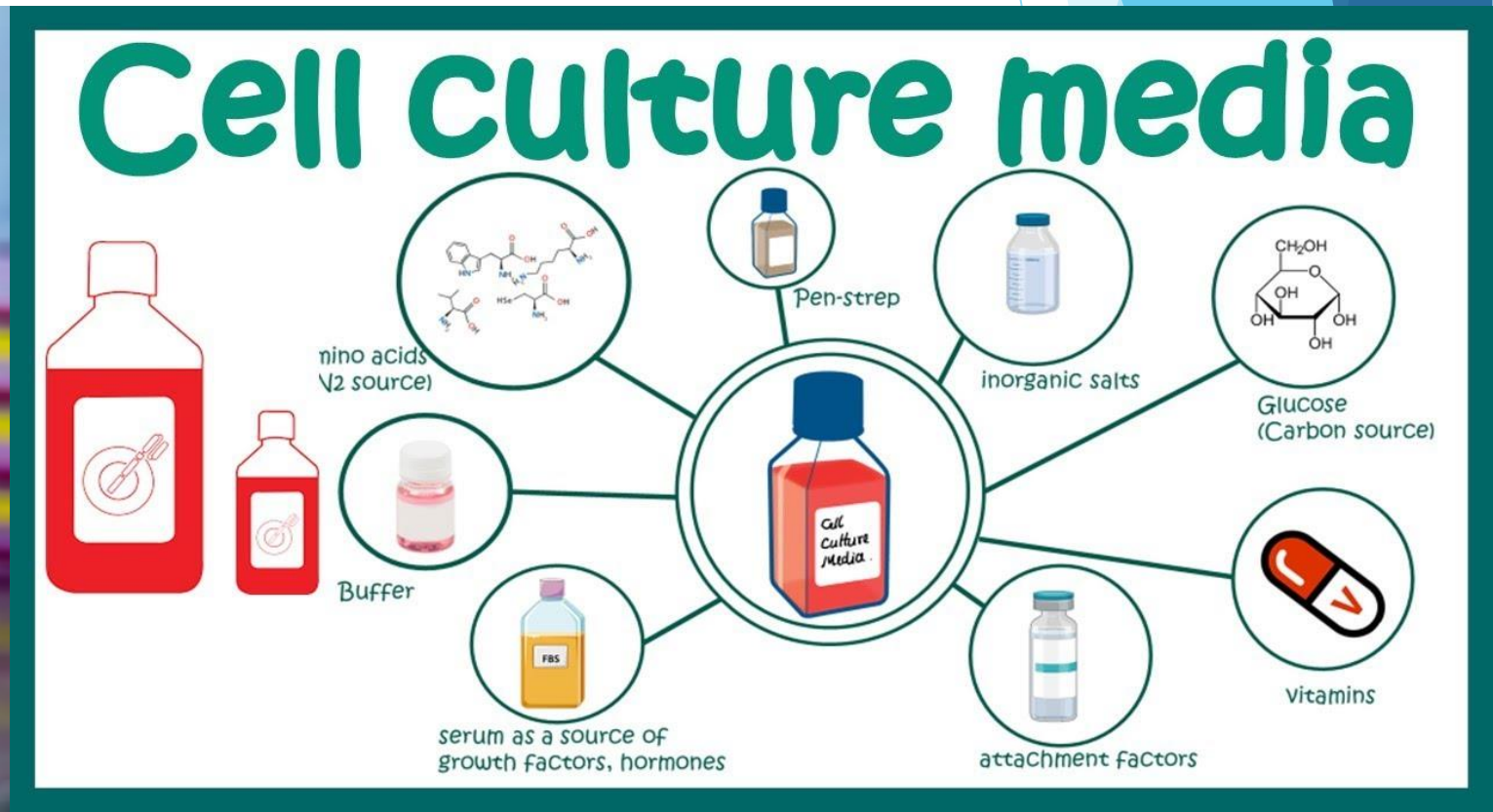


Culture media used for growing cell cultures

- The media contain a complete set of amino acids, vitamins and growth factors.
- Along with dry media and ready-made components, ready-made liquid media are produced (199, Needle, lactalbumin hydrolyzate, dry media and concentrates)
- Environments are divided into growth and support. For growing cell cultures, growth media enriched with human and animal sera (e.g. bovine serum, fetal calf serum, etc.) are used. The serum content in the medium can be 2 - 30%

Nutrient media used for growing cell cultures

Phenol red is added to the medium, which becomes yellow-orange in an acidic medium, and raspberry (dark red) in an alkaline medium.



Culture glassware

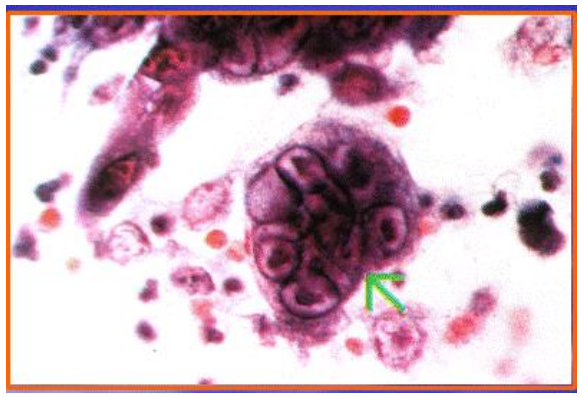
Culture glassware

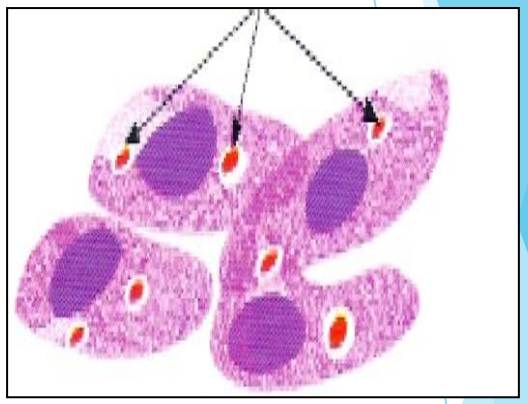
Virus detection methods in cell culture

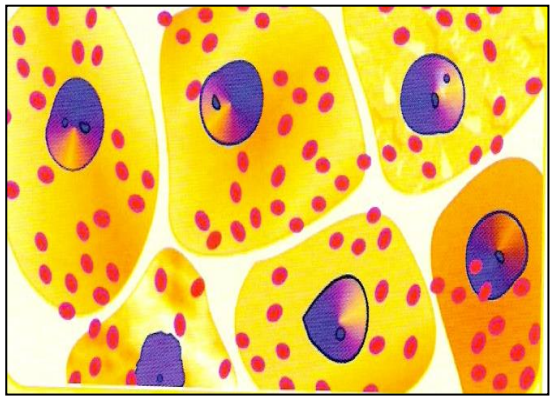
- Reproduction of viruses in cell culture is not always accompanied by a visible effect
- The reproduction of viruses in a cell culture infected with virus-containing material can be judged on the basis of the phenomena

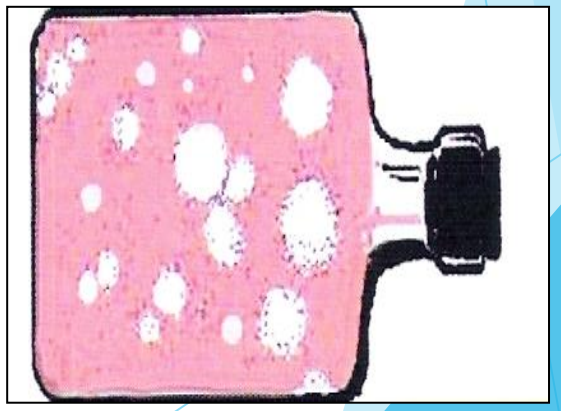
Virus detection methods in cell culture

Cytopathogenic effect (CPE), intracellular inclusions (bodies), hemadsorption phenomenon, "negative colonies", "color test"





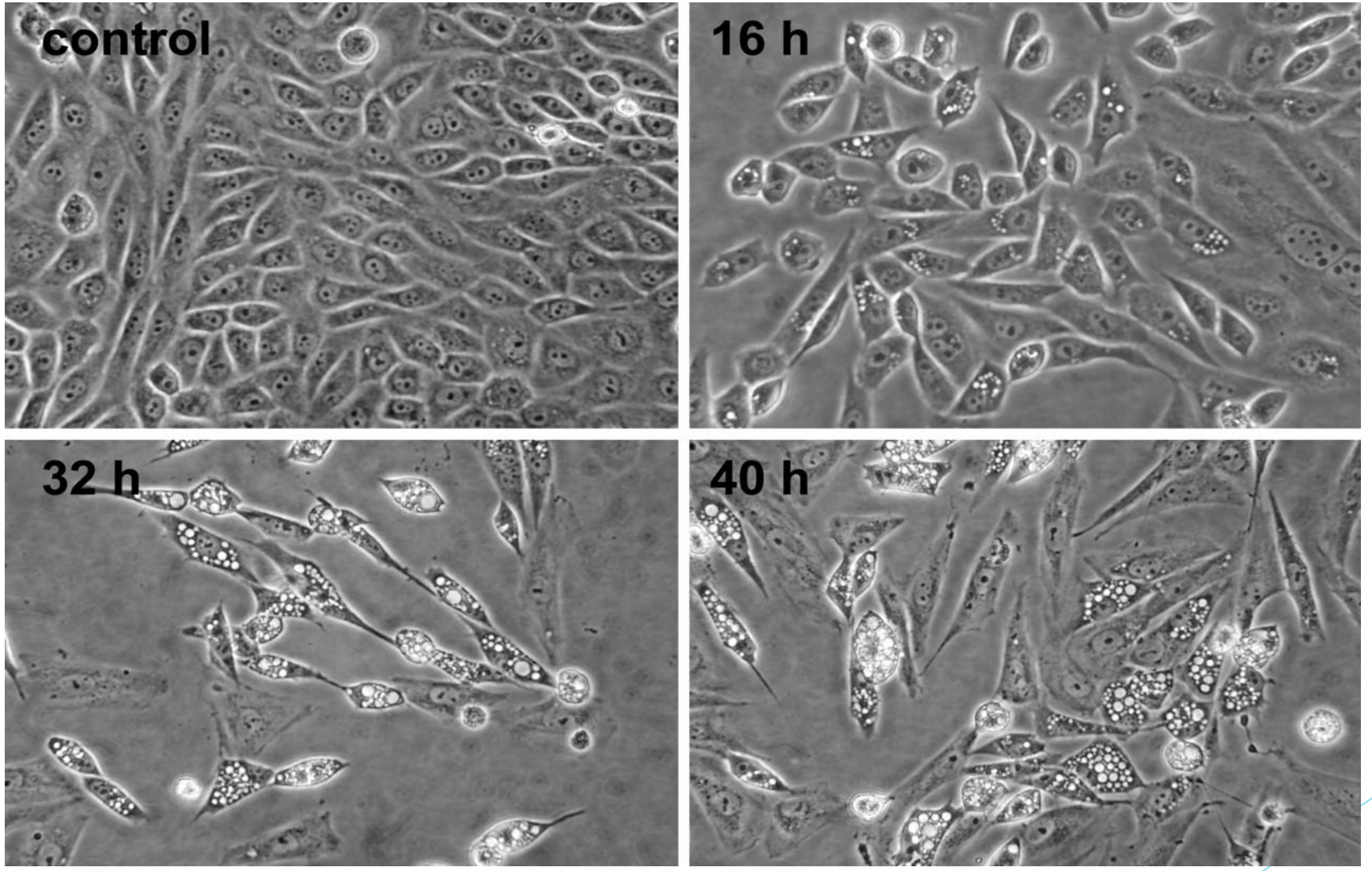




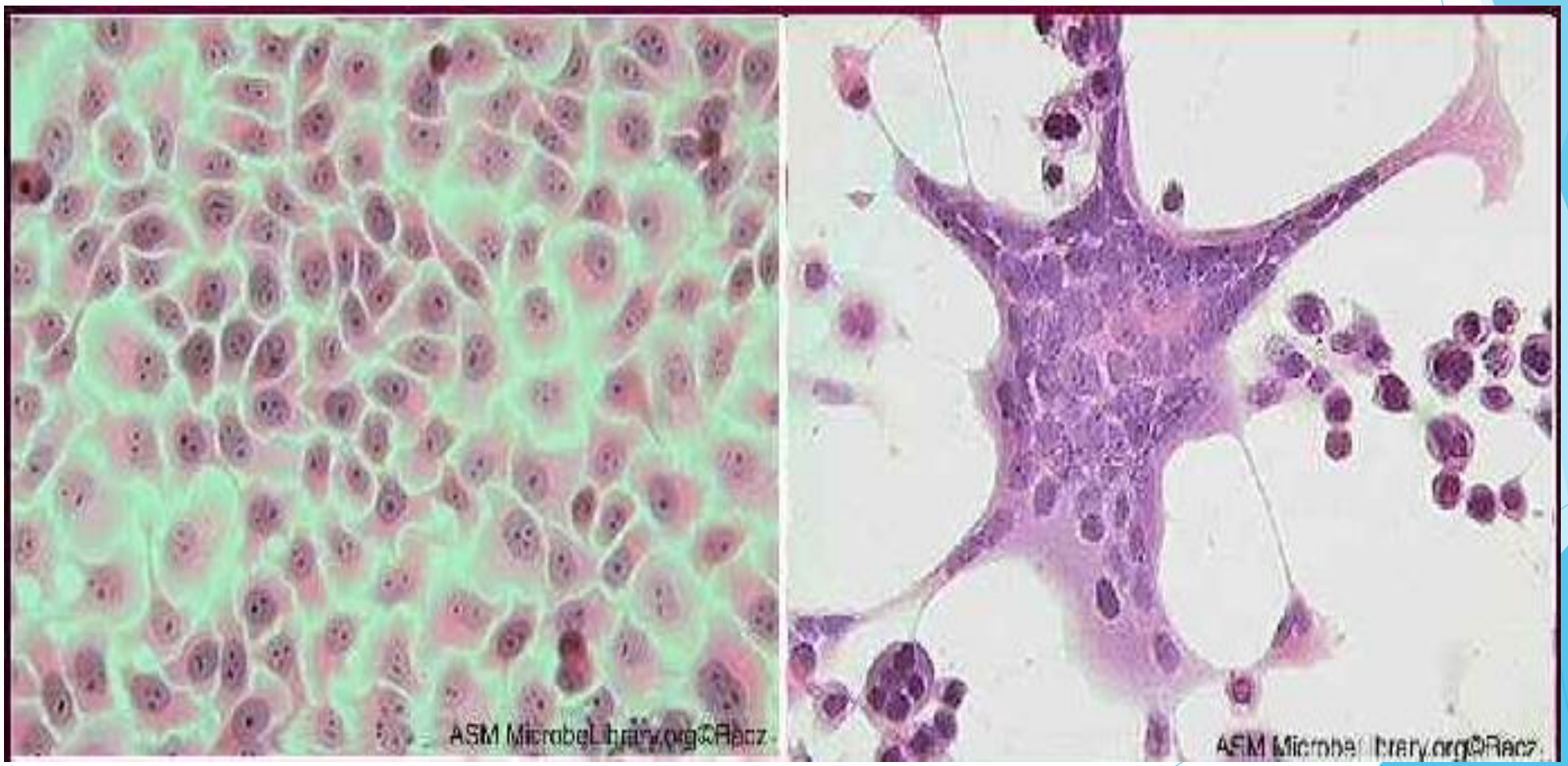
Cytopathogenic effect (CPA)

- In the process of reproduction in cell culture, some viruses have a cytopathogenic effect (CPE), that is, cell degeneration.
- CPD is manifested by vacuolization of the cell cytoplasm, destruction of mitochondria, rounding and death of cells.
- The nature of the CPE allows this phenomenon to be used for the indication and identification of viruses.
- CPP may differ for different types of virus

Cytopathogenic action

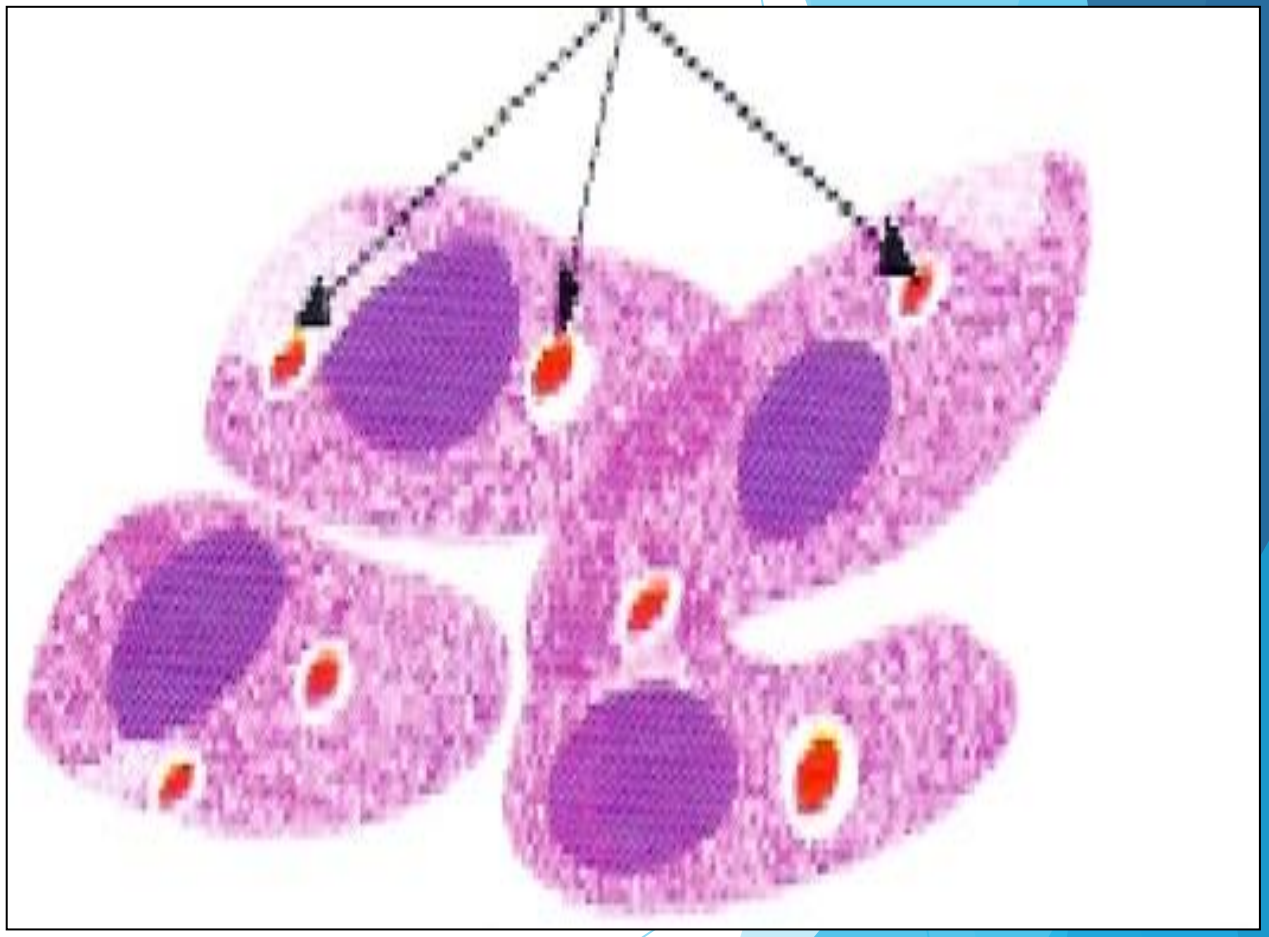


CPD herpes simplex virus



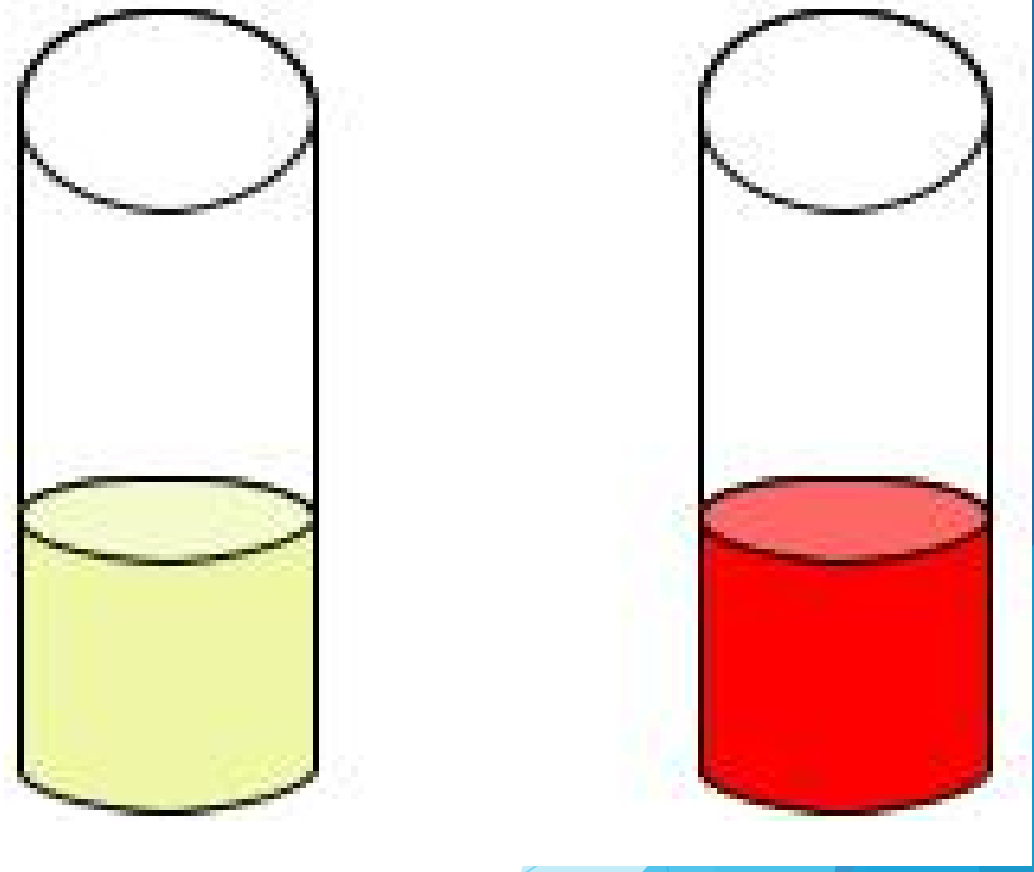
Intracellular inclusions

- Some viruses can be detected and identified by intracellular inclusions that form in the nucleus or cytoplasm of infected cells.
- Inclusions may differ in size (0.2-25 microns), shape (round or irregular) and number.
- They are clusters of viral particles and are detected by Giemsa staining or fluorochromes.



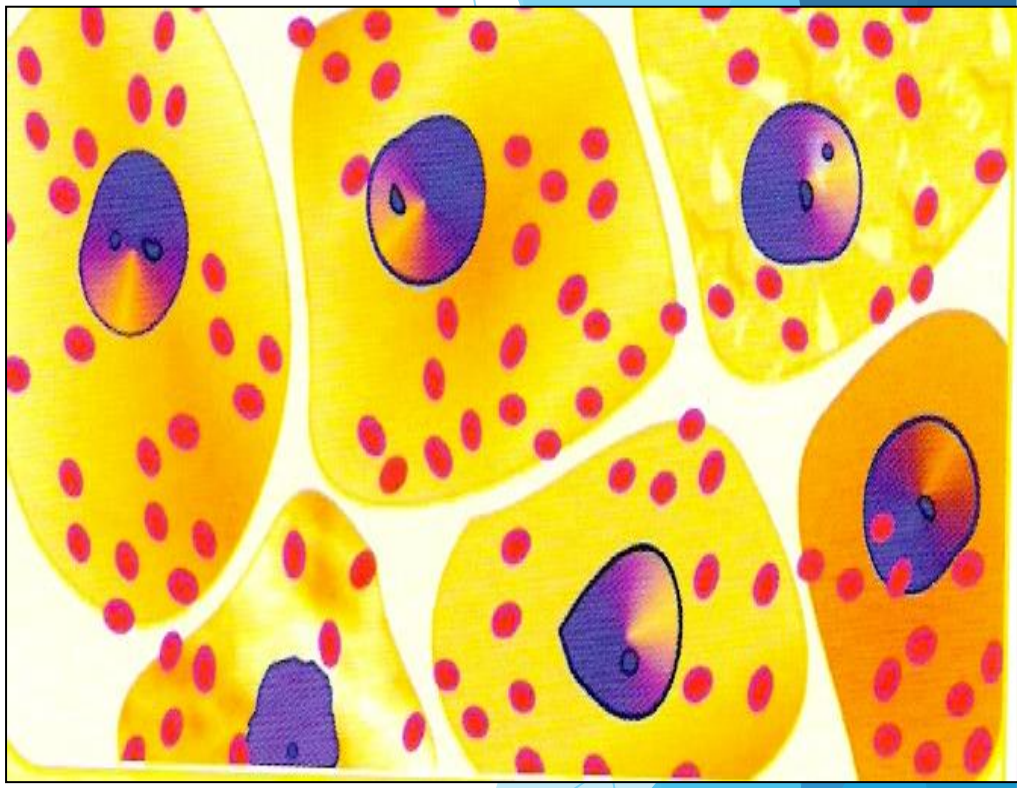
"Color test"

- The reproduction of viruses in cell culture can be judged by the "color reaction". To do this, use cell cultures growing on media containing indicators (eg, methyl red)
- When viruses reproduce in cell culture, their normal metabolism is disrupted (the cells die) and the medium retains its original color.
- If viruses do not multiply in cell culture, then living cells secrete acidic metabolites that change the pH and, accordingly, the color of the indicator in the medium.



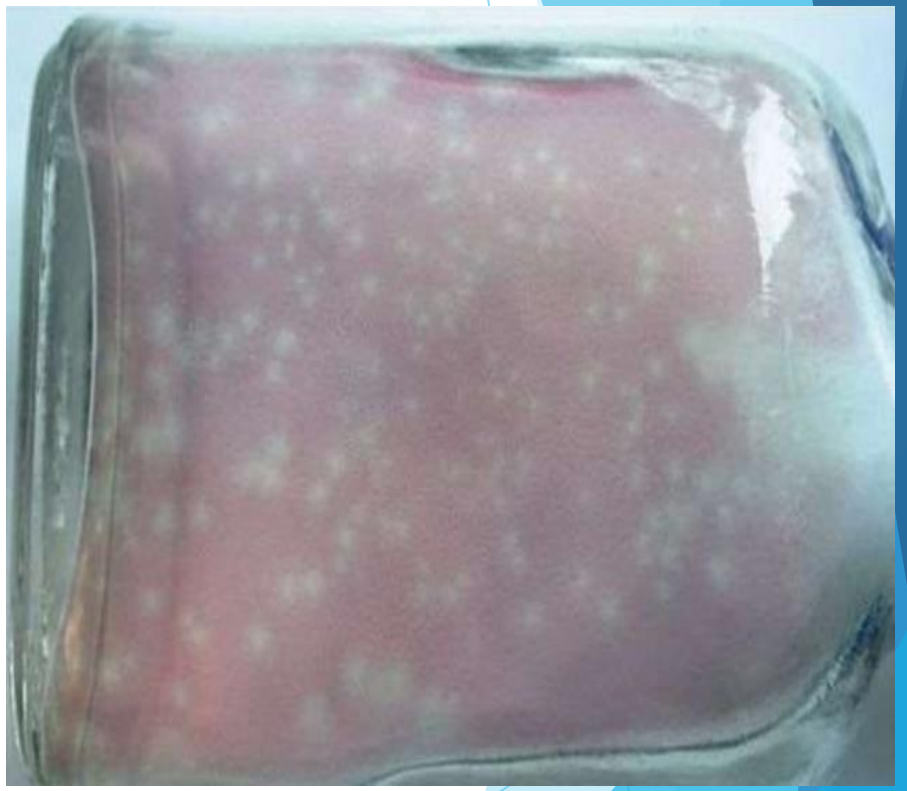
Hemadsorption phenomenon

- The hemadsorption phenomenon is another method used to indicate viruses in cell culture. The phenomenon is based on the ability of cell cultures infected with viruses to adsorb erythrocytes on their surface. For example, on the surface of paramyxo- and orthomyxoviruses there are hemagglutinins that promote hemadsorption.
- The mechanisms of hemadsorption and hemagglutination reactions are similar.



"Negative Colonies"

- Reproduction of some viruses in cell culture leads to the death of certain areas and the formation of "negative colonies", which is also used in the indication of viruses.
- The addition of agar to the nutrient medium limits the spread of viruses throughout the cell monolayer, as a result, foci of necrosis are limited from each other.
- Affected areas (dead cells) look like light spots against the background of a colored monolayer of living cells.

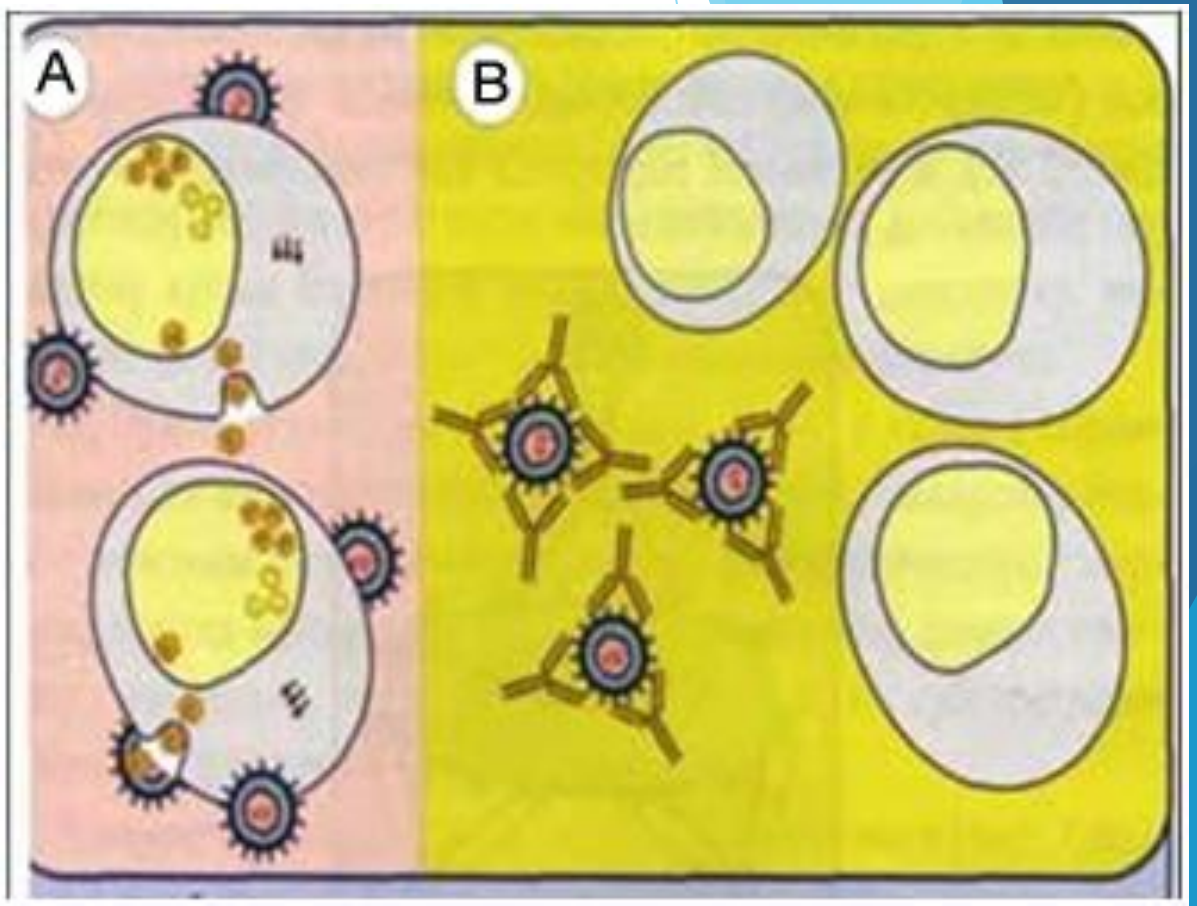


Interference phenomenon

- To detect viruses that do not give a distinct CPE in cell culture, the phenomenon of interference is used. The phenomenon of interference is a phenomenon when a cell infected with one virus becomes resistant to infection by another virus.
- For example, the rubella virus multiplies in a number of cell cultures without CPE and is detected by the phenomenon of interference when the primary cell culture is infected with other cytopathogenic viruses.
- As an inducer for superinfection, the vesicular stomatitis virus is used, the reproduction of which in cell culture is always accompanied by the development of CPD. Due to the multiplication of the rubella virus in cell culture, the multiplication of the vesicular stomatitis virus is not accompanied by visible CPE, which indicates the phenomenon of interference. If the rubella virus does not multiply in cell culture, then the reproduction of vesicular stomatitis in cell culture will be accompanied by visible CPP

Virus neutralization reaction

- The virus neutralization reaction (biological neutralization)-RBN is used in the identification of viruses.
- Under the action of neutralizing antibodies, viruses lose their ability to cause disease in laboratory animals, cause CPE in cell and tissue culture, and multiply in chicken embryos.



Serological diagnosis of viral infections

- Detection in biological fluids of patients (blood, cerebrospinal fluid) antibodies to antigenic determinants of viruses.
- A single serological test only in rare cases allows diagnosing a viral disease (for example, with HIV infection).
- in most cases, serological diagnosis requires paired sera taken during the acute phase of the disease and 2–4 weeks later.
- The detection of a four-fold or more increase in antibody titer is usually considered as a diagnostic sign of an acute viral infection.

Molecular biological methods of indication, identification of viruses

- Detection of virus-specific fragments of the virus genome in biological material from a patient and in cell culture.
- Molecular biological indication of viruses in biological material (dot blotting, in sinu hybridization, sandwich hybridization):
- - methods of molecular hybridization (microgen method) - are carried out in order to identify virus
- -specific fragments of the virus genome in the material; characterized by high sensitivity and specificity; used to detect cytomegaloviruses, herpes viruses, hepatitis viruses; – polymerase chain reaction (PCR) and quantitative PCR;
- - Western blotting - based on the detection of specific antibodies to the antigens of the virus circulating in the blood;
- - determination of virus-infected cells by flow cytometry - used for HIV infection, infectious mononucleosis, hepatitis C, cytomegalovirus infection.
- Using the PCR method, it is possible to carry out the indication of viruses in the material, identification and differentiation with related infectious agents, genotyping of virus isolates and cloning of fragments of their genome without the need for cultivation in cell culture.