MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE
TARAS SHEVCHENKO NATIONAL UNIVERSITY OF KYIV

MICROBIOLOGY, IMMUNOLOGY, VIROLOGY

Laboratory Notebook

Part 2: Virology

For students specialty “Medicine”
of ESC “Institute of Biology and Medicine”

Видавничо-поліграфічний центр "Київський університет"
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Class № 1. **Date ________________**

TOPIC: GENERAL CHARACTERISTICS OF VIRUSES AND VIRUS STRUCTURE

List of questions to study:

1. The modern definition of viruses.
2. Place of viruses in the system of all living.
3. Morphology and ultrastructure of viruses.
4. Symmetry types of viruses. Chemical composition, functuion of compound parts of viruses.
5. Transmission electron microscopy

Theory

Viruses are infectious agents that reproduce only within intact living cells. They are so small and simple in structure, and so limited in almost all activity, that they challenge our definitions of life and of living organisms. The smallest viruses are comparable in size to a large molecule. Structurally, they are not true cells but subunits, containing only an essential nucleic acid wrapped in a protein coat.

Viruses come in an amazing variety of shapes and sizes. They are very small and are measured in nanometers, which is one-billionth of a meter.

Viruses can range in the size between 20 to 750 nm, which is 45,000 times smaller than the width of a human hair. The majority of viruses cannot be seen with a light microscope because the resolution of a light microscope is limited to about 200nm, so an electron microscope is required to view most viruses.

The basic structure of a virus is made up of a genetic information molecule and a protein layer that protects that information molecule. The arrangement of the protein layer and the genetic information comes in a variety of presentations. The core of the virus is made up of nucleic acids, which then make up the genetic information in the form of RNA or DNA. The protein layer that surrounds and protects the nucleic acids is called the **capsid.**

Viral capsid play several functions:

- Protect viral nucleic acid
- Interact with the nucleic acid for packaging
- Interact with vector for specific transmission
- Interact with host receptors for entry to cell and to release of nucleic acid.

Each capsid is constructed from identical subunits called **capsomers** made of protein. The capsid together with the nucleic acid is called **nucleoscapsid**. When a single virus is in its complete form and has reached full infectivity outside of the cell, it is known as a **virion**. Shape is a defining characteristic of viruses. A virus structure can be one of the following: **icosahedral, enveloped, complex and helical**.

Icosahedral viruses appear spherical in shape, but a closer look actually reveals they are icosahedral. The icosahedron is made up of equilateral triangles fused together in a spherical shape. This is the most optimal way of forming a closed shell using identical protein sub-units. The genetic material is fully enclosed inside of the capsid. Viruses with icosahedral structures are released into the environment when the cell dies, breaks down and lyses, thus releasing the virions. Examples of viruses with an icosahedral structure are the **poliovirus, rhinovirus, and adenovirus.**

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Viruses with **helical** structure has a capsid with a central cavity or hollow tube that is made by proteins arranged in a circular fashion, creating a disc like shape. The disc shapes are attached helically (like a toy slinky) creating a tube with room for the nucleic acid in the middle. All filamentous viruses are helical in shape. They are usually 15-19nm wide and range in length from 300 to 500nm depending on the genome size. An example of a virus with a helical symmetry is the **tobacco mosaic virus**.

Envelope viruses have a conventional icosahedral or helical structure that is surrounded by a lipid bilayer membrane, meaning the virus is encased or enveloped. The envelope of the virus is formed when the virus is exiting the cell via budding, and the infectivity of these viruses is mostly dependent on the envelope. The most wellknown examples of enveloped viruses are the influenza virus, Hepatitis C and HIV.

Complex viruses have a combination of icosahedral and helical shape and may have a complex outer wall or head-tail morphology. The head-tail morphology structure is unique to viruses that only infect bacteria and are known as bacteriophages. The head of the virus has an icosahedral shape with a helical shaped tail. The bacteriophage uses its tail to attach to the bacterium, creates a hole in the cell wall, and then inserts its DNA into the cell using the tail as a channel. The Poxvirus is one of the largest viruses in size and has a complex structure with a unique outer wall and capsid. One of the most famous types of poxviruses is the variola virus which causes smallpox.

Study of viral morphology. Electron Microscopy

Electron microscopy is a powerful tool for the study of viral morphology and size. Transmission electron microscopy (TEM) is based on the same principles as light microscopy, but it uses a beam of electrons rather than light to “illuminate” the specimen. Electron microscopes are usually very large and use a hot wire, called a filament, to produce a stream of electrons that are focused into a beam by magnets.

Much the same way as light-microscopes rely on glass slides that are transparent to light, you must use slides that are transparent to electrons. Electron microscope slides, which are called grids, are typically made of thin copper or nickel mesh and can be coated with carbon and heat-resistant plastics such as formvar (Fig. 1.1), depending on the sample to be examined. Biological samples and the carbon/plastic coating are transparent to electrons, yet they are extremely fragile, so the metal mesh provides stability and support. For liquid phage samples, such as lysates, 400-mesh copper grids coated with carbon and formvar are appropriate (“400-mesh” means that there are 400 holes in the copper foil of the grid).

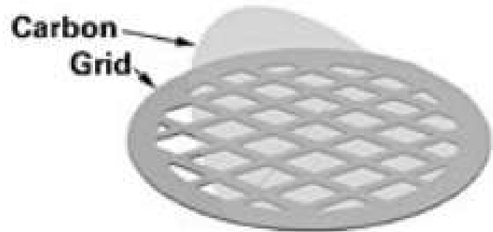

Fig. 1.1. Grids coated with formvar films for Transmission electron microscopy (TEM)

Electron microscope is of limited availability in most diagnostic laboratories. Direct electron microscopy requires specimens containing high titers (10^7 per ml) of viral particles. The major diagnostic application of electron microscopy is for the detection of certain nonculturable viruses, particularly those that cause gastroenteritis (e.g., caliciviruses, astroviruses, and rotaviruses).

PRACTICAL WORK

Protocol 1: Preparation of Formvar Film-coated Grids

Background

Electron microscope grids require that a hydrophilic plastic support film is applied to them to allow the attachment of viral particles. Many support films have been used with success, and the user should select a suitable support film for the purpose required. Support material that has been used includes Collodion, Formvar, Pioloform.

Objective: To prepare Formvar Film-coated Grids for Transmission electron microscopy.

Materials: 1% formvar solution in chloroform, plate under bowl to catch runoff water, distilled water, wide container for holding water, microscope slides, petri dishes, filter paper, grids, razor blade, fine pointed tweezers

Procedure:

1. Prepare 0,2 % formvar in chloroform. Filter solution to remove impurities and keep dry.
2. Put formvar into film caster or container to use with thistle tube.
3. Clean glass slides using distilled water and lens paper. Do not "over-clean" or films will not release from slides.
4. Fill slide chamber 3-4 cm deep with formvar. Keep covered as much as possible to minimize evaporation of chloroform.
5. Put slide in chamber and let sit 2-3 min.
6. Drain formvar from the chamber in a slow but steady stream.
7. Let the slide sit in covered chamber for 2-3 minutes to dry. This gradual drying results in more even, thinner film.
8. Remove slide from chamber and touch bottom to filter paper to remove any liquid formvar and complete drying.
9. Scrape edges of slide and ~½ cm from bottom of slide with a razor blade to loosen film.
10. Fill container with clean water and sweep surface with glass rod or lens paper to remove dust (Fig.1.2).
11. Breath moist air onto slide and slowly lower slide into the water at ~45° angle to float off film. Have tweezers ready in case film needs a little help freeing from slide. Films should be silver in color. Gold is usually too thick and gray is too thin.
12. Place grids onto the film with shiny or dull side down. Be consistent so that you always know which side has the film. Grids can be pressed down gently with round tipped glass rod if desired to ensure good contact.
13. Lower a clean glass over the grids at ~45° angle and remove film plus grids with a steady but quick down and turn motion. (Note: parafilm or pieces of filter paper can also be used to pick up grids. Glass slides are cleaner and outgas less when put into carbon coater.)
14. Remove excess water from the slide with filter paper and place in filter paper lined Petri dish to dry.

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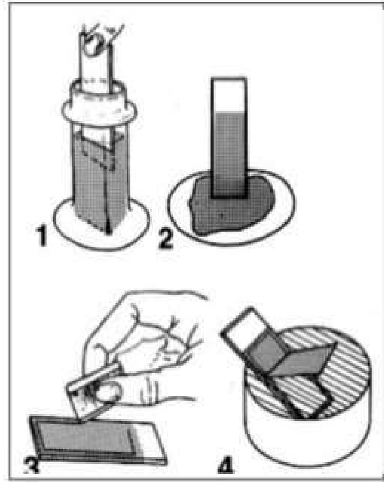

1) Dip slides in formvar
2) Drain excess formvar from slide
3) Score along edges
4) Immerse in water to release film

Fig. 1.2. Preparation of Formvar film

Protocol 2: Preparation of sample for TEM

Mounting Phage Samples for TEM and Staining with Uranyl Acetate

Background: The classic processing of biological specimens observed in a TEM needs fixation, dehydration, and a selective "staining" of investigated objects. "Staining", a means of receiving coloured images, cannot be effectively used in conjunction with an electron microscope. Instead, the enhancement of structures for TEM observation is effected, usually by impregnation with heavy metal salts of plumb and uranium.

Objective: To prepare your phage sample for viewing with a transmission electron microscope

Materials:

High-titer phage lysate, phage buffer, 200–400 mesh formvar-coated copper grids, filter paper, sterile, filtered water, 1 % uranyl acetate (filtered immediately before use).

Procedure:

1. Prepare your work area:p ut on a fresh pair of gloves and cover the designated work area with bench paper or a large Kimwipe to create a clean work surface.
2. Using a micropipettor, place 5 μl of onto the grid without touching the tip to the grid itself.
3. Allow the lysate to sit on the grid for 2 minutes. During this time the phage will settle and adsorb onto the grid.
4. Rinse the grid once using the following method:
   a) Using the forceps, turn the grid so it is at a 45° angle.
   b) Carefully pipette 60 μl of sterile water across the dark-and-shiny face of the grid, allowing it to drip off the other side into a Petri dish. It will take about six big drops.
   c) Rotate the grid back so that the dark-and-shiny side is facing up again. If necessary, wick away any excess water by placing a fresh wedge of filter paper against one edge of the grid.
5. Add 5 μl of 1 % uranyl acetate to the grid.
6. Important: Uranyl acetate is a very toxic compound. You should wear gloves throughout this procedure.
7. Immediately begin to wick off excess stain by using a wedge of filter paper. UA staining occurs by leaving a very thin layer of stain dried across the entire grid.
8. Place your grid in the designated grid box for storage. Be sure to accurately record the location of your grid in the box.
9. Transport your samples to your EM facility for imaging.
10. Observe your phage.
11. Calculate the capsid diameter and tail length relative to the size bar:
    A) Using a ruler, measure the widest point (edge-to-edge, not vertex-to-vertex) of the capsid and the length of the tail (excluding the capsid and any visible tail fibers). If possible, measure multiple phage heads and tails and average their respective values.
    B) Measure the length of the size bar with the ruler.
    C) Using the known and relative lengths of the size bar, calculate the length of the capsid and tail.

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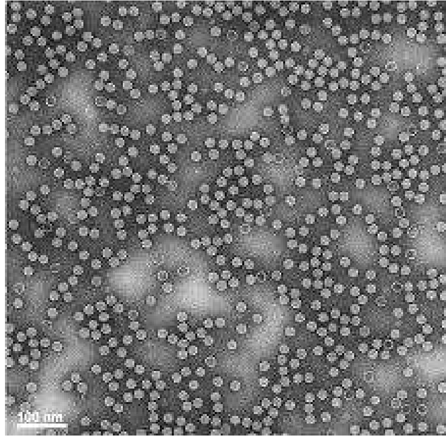
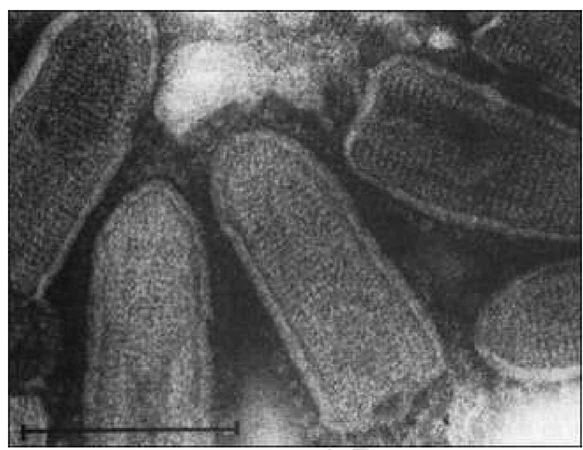
Example of culculation:
The phage on the right has an average capsid diameter of 1.6 cm (16 mm) and a tail length of 4.8 cm (48 mm).
The 100 nm size bar is measured to be 2.4 cm long.
To find the scaled (actual) size of the tail, use the simple ratio below to solve for the unknown scaled size:

$scaled\ size\ bar\ measured\ size\ bar = unknown\ scaled\ size$
$measured\ size$ 100 nm 24 mm =($unknown\ scaled\ tail\ size$)
48 mm $unknown\ scaled\ tail\ size$ = (100 nm)(48 mm) 24 mm
$unknown\ scaled\ tail\ size$ = 200 nm

Independent work

1. Calculate the length of the capsids (0,2 points)





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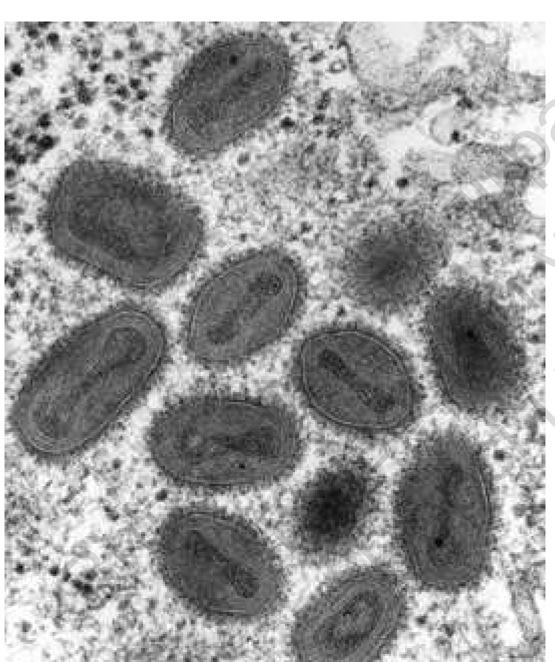

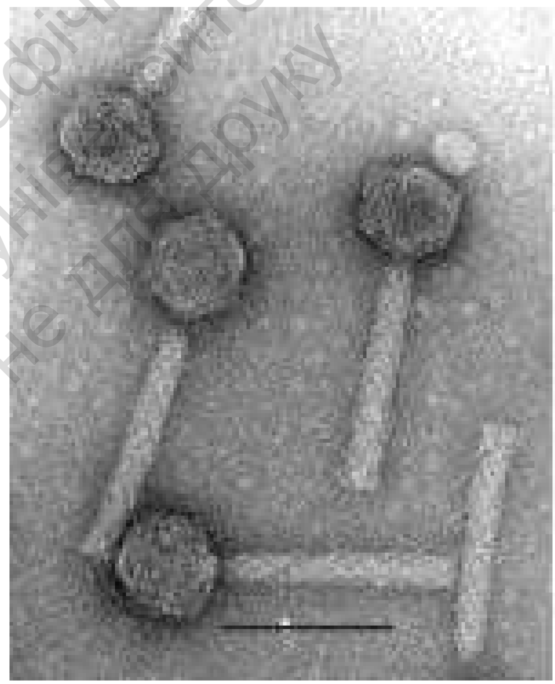

The bar is 100 nm

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2. Describe the basic structures of the viruses (0,1 points)

	Designation ____________ Characteristics____________ ____________ ____________ Examples ____________ ____________
	Designation ____________ Characteristics____________ ____________ ____________ Examples ____________ ____________
	Designation ____________ Characteristics____________ ____________ ____________ Examples ____________ ____________
	Designation ____________ Characteristics____________ ____________ ____________ Examples ____________ ____________

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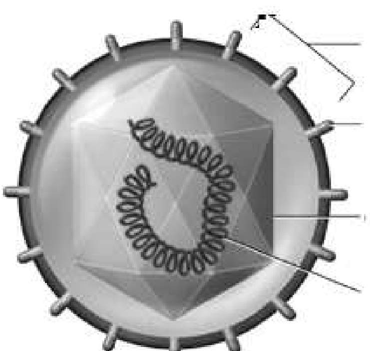
3. Specify the structural parts of the virion (0,1 points)



1. Envelope
2. Capsid
3. Nucleic acid
4. Spike

4. State living characteristics of viruses: (0,1 points)

____
____
____
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State nonliving characteristics of viruses:

____
____
____
____

Questions for self-control

1. List the properties that define a virus.
2. What is a virion?
3. Describe the general structure of viruses
4. Describe the four morphological classes of viruses
5. Compare the size of viruses to that of bacteria.
6. State why viruses can't replicate on environmental surfaces or in synthetic laboratory medium.
7. What is the function of virus coat ?

Score _________ Tutor signature__________________________

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Class № 2. **Date: ___________________**

TOPIC: VIRUS ISOLATION, CULTIVATION AND IDENTIFICATION: INTRODUCTION TO LABORATORY ANIMALS, CELL CULTURES, AND EMBRYONATED CHICKEN EGGS

List of questions to study:

1. To analyze features of virus interaction in living systems.
2. To evaluate the results of viruses multiplication in living systems.
3. To analyze laboratory methods of viruses cultivation using laboratory animals, cell cultures, and embryonated chicken eggs.

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Table 2.1. Basic terms that a student should learn during preparing to the lesson:

Term	Meaning
Infectivity	Ability of a pathogen to induce infection (disease) of a susceptible cell/organism
Infection	A process (an action) of infecting or the state of being infected (diseased)
Inoculation	Artificial and intentional infection of a susceptible cell/organism with a pathogen in the laboratory
Bioprobe / bioassay	A procedure to measure the presence or concentration of biological molecules, microorganisms, viruses, etc. In virology, test biological objects are inoculated with a virus for indication of virus presence and/or aggressiveness
Virus isolation	Selective 'extraction' of a given virus from a sample, diseased organism or environment
Virus indication	Confirmation of virus presence (or absence) using a living system
Virus identification	Determination of the nature (taxonomy) of the virus present in a diseased organism, sample or environment. Proven and/or highly specific diagnostic techniques are used (including microscopy, ELISA, PCR, etc.)
Virus cultivation	Accumulation of a known virus using an established specific living system (cell culture, embryonated chicken egg, etc.) susceptible to this virus
Model system	A well-characterized system with stable properties used in research for characterization of new object with unknown properties. Examples include specific cell cultures and laboratory animals, embryonated chicken eggs, etc.
Cell culture	A process of maintaining and/or growing cells under carefully controlled in vitro conditions outside of a living organism
Passage	A process of transfer of some cells from the culture to a fresh media in a different flask. This is periodically done for 'refreshing', maintaining/accumulation of cell culture
Cytopathic effect (CPE)	Morphological changes of cells resulting from virus reproduction and visible under the microscope. Some types of CPE are highly specific to viruses

Term	Meaning
Inclusion (or inclusion body)	Insoluble abnormal aggregates of virus particles or viral components in the cell cytoplasm or nucleus, and visible under the microscope with a special staining. Some types of inclusions are highly specific to viruses
Plaque	A limited area of adsorbed cell culture damaged by the virus. Visible with a naked eye. One virus particle forms a single plaque allowing plaque use for virus quantification
Embryonated chicken egg	Fertilized chicken egg used for research

Theory

1. How to cultivate viruses?

As viruses are not cells, they cannot grow in size or divide (reproduce themselves). Consequently, viruses ***cannot grow in nutrient medium – contrary to bacteria***!!!

Hence, if you need to accumulate quantities of virus material (for research, vaccine making, etc.), you have to select a living cellular system capable to support the reproduction of ***infectious*** virus.

The primary purpose of virus cultivation is:

1. To isolate and identify viruses in clinical samples.
2. To do research on viral structure, replication, genetics and effects on host cell.
3. To prepare viruses for vaccine production.

Several different systems are used for virus isolation and cultivation. These include ***cell cultures***, ***embryonated chicken eggs*** (or chicken embryos), and ***laboratory animals***. Same approach is used for virus indication (presence) in pathological material.

The availability of suitable systems (such as the right cell line to be able to grow the suspected virus) is ***always a problem*** if you deal with the unknown virus. Not all viruses grow in the same system and not all viruses grow in cell culture, thus the need for a variety of options.

2. Laboratory animals

Logically, your first choice would be an animal susceptible to a given virus (for instance, a rabbit for myxomatosis, a chicken for avian flu, a cow for foot-and-mouth disease, etc.). However, you should remember that animals are risky (can be aggressive, can differ significantly between themselves) and also expensive. Another issue to consider is research ethics requiring strong evidences supporting the necessity of the animals for the experiments. Below are several important aspects to consider with laboratory animals used for virus cultivation:

- Animals are used for viruses that ***cannot be cultivated*** in embryonated eggs and tissue cultures.
- The selected animals should be ***healthy*** and free from any communicable diseases.
- Depending on the virus-animal system, the viruses can be inoculated by different routes (intraveinal, intraperitoneal, intranasal, subcutaneous, intracerebral, etc.).
- After inoculation, virus multiplies in host and ***develops disease***. The animals are observed for ***symptoms*** of disease and/or death.
- Then the virus is isolated and purified from the tissue(s) of these animals.

Benefits and drawbacks of animals' use in virology research

Advantages:

- diagnosis, pathogenesis and clinical symptoms are determined ***at the level of whole organism***,
- used for the study of immune responses (production of antibodies, etc.), epidemiology (routes of transmission) and oncogenesis,

- primary isolation of certain viruses which cannot be isolated otherwise,
- mice and rats provide reliable models for studying viral replication.

Disadvantages:

- expensive (animals, trained staff, safe facilities),
- difficulties in maintenance of animals (including risk of injuries and disease transmission),
- difficulty in choosing of animals for particular (especially unknown) virus,
- some human viruses cannot be grown in animals or do not cause disease,
- animal testing ***doesn't necessary translate to humans***,
- issues related to animal welfare (bioethics) as discussed above.

In other words, you shouldn't use laboratory animals if you can avoid it. Hence, the two most commonly used and preferred methods are ***cell cultures*** and ***embryonated chicken eggs***. These are less expensive, more stable, controlled and safe options successfully adapted for cultivation of a wide range of viruses.

3. Cell cultures

A cell culture is one of the preferred artificial living systems used in virology for:

1) Virus indication
2) Primary isolation of viruses from pathological material
3) Virus identification
4) Virus accumulation and/or maintaining for research, vaccine making, etc.
5) Studying mechanisms/activity of candidate drugs for antiviral therapy, etc.

The isolation of viruses in cell cultures is increasingly becoming the gold standard for virus diagnosis. Cell cultures represent a powerful, stable and genetically uniform tool for virology research. Several thousands of different established cell lines are known and at least 3,000 are deposited at American Type Culture Collection (ATCC) – the largest general culture collection in the world – allowing wide choice for nearly every occasion.

Benefits and drawbacks of cell cultures' use in virology research

Advantages:

- more standardized conditions thanks to genetic uniformity and controlled *in vitro* environment,
- wide choice of cell lines suiting most viruses/aims,
- fast results,
- much safer and 'cleaner' option comparing to laboratory animals,
- many steps (cell cultivation, etc.) can be automatized,
- lower cost.

Disadvantages:

- absence of cell cultures for some viruses,
- decrease of cell cultures' susceptibility to viruses with time,
- frequent nonspecific reactions of cell cultures on virus inoculation,
- requires facilities and trained technicians,
- cell cultures' reaction on virus inoculation and antiviral therapy ***differs significantly from the reaction of the host organism***.

There are many different types of cell cultures but most of them fall into one of the three categories shown in a table below.

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Table 2.2. Major types of cell cultures and their descriptive properties

Cell cultures			
Properties	**Primary trypsinized cultures**	**Diploid cell cultures**	**Transplanted cell cultures**
Morphology of culture cells comparing to the original tissue	Don't differ	Differ	Differ
Set of chromosomes	Diploid	Diploid	Heteroploid
Lifetime	Limited by 1-3 passages	Limited by 20-100 passages	Unlimited by the amount of passages
Growth in suspension	Impossible	Impossible	Possible
Signs of malignancy	Absent	Absent	Always present
Period of generation	3-7 days	1-15 days	2/3 - 1 day
Contact inhibition by growing on glass	Present	Present	Absent
Examples	1. Culture of monkey kidney cells 2. Fibroblast cell culture of human embryos 3. Cell culture of chicken embryo fibroblasts	Fibroblast cell lines of human embryo (WI-38, MRC-5, MRC-9, IMR-90), cows, pigs, sheep	1.HeLa (cells of carcinoma of the cervix) 2. KB (oral humah carcinoma cells) 3.HEp-2 (human larynx carcinoma cells) 4. Vero (green monkey kidney cells)

The following table details use of specific cell lines for isolation, identification and/or cultivation of some common viruses.

Table 2.3. Susceptibility of certain cell lines to viruses

Virus	Cell line
Herpes simplex virus (HSV)	Vero, Hep-2, human diploid (HEK and HEL), human amnion
Varicella zoster virus (VZV)	human diploid (HEL, HEK)
Cytomegalovirus (CMV)	human diploid fibroblasts
Adenovirus	Hep2, HEK
Poliovirus	MK, BGM, LLC-MK2, human diploid, Vero, Hep-2, Rhadomyosarcoma
Coxsackie B virus	MK, BGM, LLC-MK2, Vero, Hep -2
Echo viruses	K, BGM, LLC-MK2, human diploid, Rd
Influenza A virus	MK, LLC-MK2, MDCK
Influenza B virus	MK, LLC-MK2, MDCK
Parainfluenza virus	MK, LLC-MK2
Mumps virus	MK, LLC-MK2, HEK, Vero
Respiratory syncytial virus (RSV)	Hep-2, Vero
Rhinovirus	human diploid (HEK, HEL)
Measles virus	MK, HEK
Rubella virus	Vero, RK13

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Upon virus inoculation, three major types of reaction of cell cultures are distinguished:

1) Virus-induced cytopathic effect (CPE)
2) Cell proliferation (rapid division often seen with oncogenic viruses)
3) No reaction

CPE is most common and has diagnostic importance for many groups of viruses. Major types of CPE and inclusions are shown in the following tables.

Table 2.4. Types of cytopathic effect with typical examples of viruses

Cytopathic effect	Viruses	Morphology
Total destruction Total destruction of the cell layer, the most severe type of CPE	Enteroviruses (Poliovirus, Coxsackie, ECHO)	
Subtotal (partial) destruction	Some togaviruses, picornaviruses, and paramyxoviruses	
Focal degeneration Localized damage of the cell layer due to direct cell-to-cell transfer of the virus rather than diffusion through the extracellular medium	Smallpox virus Influenza virus	
Swelling and clumping Cells swell significantly and clump together in clusters (‘bunch of grapes’)	Adenoviruses	
Foamy degeneration Also known as vacuolization	Some retroviruses, paramyxoviruses, and flaviviruses	

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Cytopathic effect	Viruses	Morphology
Syncytium/symplast formation The plasma membranes of four or more cells fuse and produce an enlarged cell with at least four nuclei (polykaryon)	Measles virus Mumps virus Parainfluenza virus RS virus Herpes simplex virus	
Inclusion formation Virus-induced intracellular structures. May be single or multiple, small or large, and round or irregularly shaped, etc.	Many if not all Diagnostic importance: Rabies virus Smallpox virus Herpesviruses, etc.	

Table 2.5. Typical diagnostically important viral inclusion bodies

Type	Name and typical virus	Scheme	Picture
Intracytoplasmic	Guarnieri bodies (Smallpox virus (variola)) ***Big perinuclear 'virus plants' inside living cells***		
	Paschen bodies (Smallpox virus (variola)) ***Small numerous scattered virus particles often seen when the cells rupture***		

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Type	Name and typical virus	Scheme	Picture
	Negri bodies (Rabies virus)		
Intranuclear	Cowdry bodies (herpesviruses)		

4. Embryonated chicken eggs

Embryonated chicken eggs were first used for virus cultivation by Good Pasture back in 1931. Today, this model system is widely employed for isolation, cultivation and accumulation of many different (but not all!) human and animal (mostly avian) viruses. Chicken embryos lack proper immune system and also have large volumes of water in their different parts. Hence, they became a valuable 'tool' for mass production of vaccines for such viruses as flu, measles, coronaviruses, etc. (see figure below).

Benefits and drawbacks of embryonated chicken eggs' use in virology research

Advantages:

- gold standard for isolation and cultivation of some viruses,
- ideal substrate for the viral growth and replication,
- ***extremely cost-effective***, easy maintenance,
- the embryonated eggs are ***readily available***,
- wide range of tissues and fluids supporting different viruses,
- free from contaminating bacteria and many latent viruses (***naturally sterile***),
- specific and non-specific factors of defense (immunity, etc.) is not an issue,
- widely used for some ***vaccine production***.

Disadvantages:

- not many viruses can be cultivated,
- the site of inoculation varies for different viruses,
- reaction on virus inoculation ***differs significantly from the reaction of the host organism***.

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As shown on the following figure, the embryonated chicken egg has several parts:

- embryo,
- amniotic cavity (or sac) containing amniotic fluid – serves for cushioning and buoyancy,
- air sac – serves as a source of air and gas exchanger, and for cushioning,
- yolk sac – serves for feeding the embryo,
- albumin – serves as a source of water for the embryo and for non-specific defense,
- allantoic cavity containing allantoic fluid – serves for excretion.

Every cavity/sac is surrounded by a separate membrane, and the whole egg is protected by the shell with the underlying shell membrane.

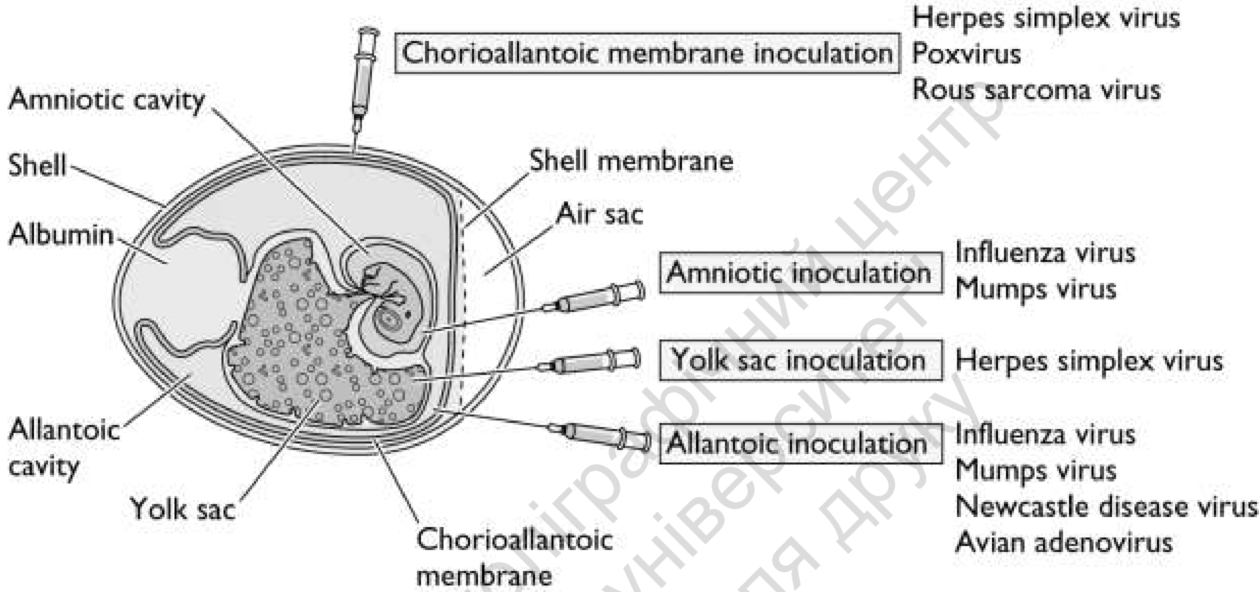

Figure 2.1. Anatomy of embryonated chicken egg and 4 major routes of its inoculation with viruses (adapted from Fenner et al., 1974)

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In the view of the anatomy of the chicken egg and virus specificity, several routes of virus inoculation were developed for ***different viruses and purposes*** (see figure and examples above). These include:

- chorioallantoic membrane (CAM) inoculation,
- amniotic sac inoculation,
- yolk sac inoculation,
- allantoic sac inoculation.

Chorioallantoic membrane (CAM) inoculation is mainly used for ***primary isolation*** of viruses infecting epithelial cells (poxviruses, herpesviruses, etc.). After inoculation and incubation, visible lesions called pocks are observed forming grey white area in transparent CAM. This method is suitable for plaque studies (i.e. for ***quantification of virus*** by calculating number of pocks/plaques).

Amniotic sac inoculation is mainly done for ***primary isolation*** of influenza virus and the mumps virus. Yolk sac inoculation is a simple method for ***growth and multiplication of viruses*** (e.g., herpesviruses). Allantoic sac inoculation is used for ***primary isolation*** of many avian viruses, as well as a standardized technique for ***production of vaccines*** of influenza virus, measles, yellow fever, rabies, etc.

Another thing to consider is the age of embryonated chicken eggs used for virus inoculation – it varies for different viruses. The lifespan of chicken embryo is about 20 days in total. ***For virus inoculation, 5-12 days old' embryonated chicken eggs are used*** (see table below).

Table 2.6. Identification of virus replication in embryonated chicken eggs

Age of the egg at inoculation	Part of embryo	Virus	Signs of virus multiplication
7-12	Yolk sac	Herpes simplex virus	Death
12	Chorioallantoic membrane (CAM)	Herpes simplex virus Poxviruses Rous sarcoma virus	Plaques
10	Allantoic sac	Influenza virus	Growth retardation and capillary damage. Hemagglutination
7		Mumps virus	Death
10	Amniotic sac	Influenza virus	Hemagglutination
7		Mumps virus	Death

Before virus inoculation, it's crucial to establish the position of the air sac of an embryo. For this purpose we use the light source in a dark chamber. This piece of equipment is called egg tester or ovoscope, or candling device, and the process is called candling.

At this step it is important to discard bad eggs, i.e. eggs which are physically damaged, have no embryo (unfertilized) or dead embryo.

After these preparatory steps the eggs can be inoculated. Viral growth and replication in egg embryo can be detected visually, and using hemagglutination test and other methods (see the table below and short explanation afterwards).

Typical examples of the results of virus reproduction in embryonated chicken eggs include thickening of and pocks on CAM (smallpox virus), hemorrhagic symptoms (blood vessels' damage) (influenza virus), retardation, hemorrhagic and death of chicken embryo (bluetongue virus), and others.

You should bear in mind that there is no universal model system which can be used for any virus or any research purpose. Choosing the correct model system is the first and most crucial step in virus research and clinics.

When dealing with ***virus isolation*** and ***cultivation***, please remember:

- there is no universal cell line or laboratory animal supporting reproduction of every virus,
- for isolation and cultivation of many viruses, there are established cell cultures and laboratory animals, and
- embryonated chicken eggs and cell cultures remain the option of choice.

5. Diagnostics of viral infections

Laboratory animals, cell cultures, and embryonated chicken eggs are most common examples of model systems extensively used in medical and veterinary virology for isolation, cultivation and identification of many viruses.

Certainly, this requires specific equipment and facilities in addition to trained staff. Therefore, these model systems are mostly used either in research (novel diseases, routes of their transmission, visual symptoms and pathologies, virus maintaining or accumulation for research use, etc.) or in production (drug clinical trials, vaccine making, etc.).

If it comes to diagnostics only, there are much faster, cheaper and reliable techniques based on virus biochemistry, serology or genetics. Three most common methods are:

1) Hemagglutination test
2) Enzyme-linked immunosorbent assay (ELISA)
3) Polymerase chain reaction (PCR)

Hemagglutination test is based on ability of some (but not many) viruses to nonspecifically bind red blood cells of certain laboratory animals. Hence, this reaction may be used for virus indication

in pathological material or ***sample of cell culture/embryonated chicken egg***. Today, hemagglutination test is commonly used for routine and efficient diagnostics of flu viruses and other related viruses. The disadvantages include small number of viruses which can be diagnosed coupled with low sensitivity.

Enzyme-linked immunosorbent assay (ELISA) is a classic and sensitive serological test based on antigen-antibody reaction. It is massively used for indication of huge variety of viruses (using specific antibodies raised in laboratory animals) and remains a 'gold standard' in virus diagnostics. In addition, ELISA is a unique tool for ***evaluating the profile of immune system reaction on virus infection***. In such case, virus proteins are used for detection of virus-specific antibodies in human blood.

Polymerase chain reaction (PCR) is another modern test widely used now for diagnostics of different viruses. It is based on complementarity of nucleotides, and uses specific oligoprobes for virus indication. PCR is by far the most sensitive test available. However, its use in clinics is limited to viruses with known (sequenced) genome.

ELISA- and PCR-based diagnostics is well-automatized in modern labs eliminating labor costs and human mistakes.

PRACTICAL WORK

Practical tasks performed during the class:

1. To perform virus inoculation and identify virus reproduction in the embryonated chicken eggs.
2. To identify virus reproduction in cell cultures by cytopathic effect (CPE).

Protocol 1: Virus inoculation and dissection of embryonated chicken eggs, and collection of virus-reach allantoic fluid

Background

Embryonated chicken eggs are a widely used model for indication and accumulation of influenza and influenza-like viruses. At the practical class the students learn the methods of virus isolation, cultivation and identification using embryonated chicken eggs; master virus inoculation of embryonated chicken eggs into the allantoic sac for virus indication in pathological material with subsequent study of pathology and collection of allantoic fluid for hemagglutination test. The students write down the results of completed tasks and teacher signs it.

Objective: To inoculate the embryonated chicken eggs with influenza virus into allantoic sac, study induced pathology and collect allantoic fluid for hemagglutination test

Materials: Influenza virus isolate (mock inoculation solution), embryonated chicken eggs, egg racks, candling device, pencils, cotton wool, 70% alcohol, 5% iodine, egg shell punch, scissors, pincers, syringes, glass pipettes with a hand pump, Petri dishes, stationery tape ('Scotch'), gloves, medical masks, discard tray.

Procedure (performed using gloves and medical masks):

1. Using candling device (ovoscope), do the following:
   1.1 Examine the structure of embryonated chicken egg (8-11 days old) for vitality signs:
      a) embryo shape and active mobility,
      b) presence of developed blood vessels' pattern,
      c) absence of physical damage (cracks, etc.).
   1.2 Mark the air sac border using a pencil.
2. Set embryo on the egg rack and treat shell (from the side of the air sac) as follows:
      d) 70% alcohol,
      e) 5% iodine.
3. Inoculate the embryonated chicken egg into the allantoic sac as follows:

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f) Swab the egg shell punch with 70% alcohol. Place used cotton wool in discard tray.
g) Pierce a hole in the end of the egg at the marked inoculation site.
h) Attach needle to a sterile 1 mL syringe.
i) Draw inoculum into 1 mL syringe.
j) Keeping the needle and syringe vertical, place the needle through the hole into the egg shell approximately 16 mm deep to reach the allantoic sac.
k) Inject 0.1 mL of inoculum into the egg.
l) Withdraw the needle from the egg.
m) Seal the hole in the shell with stationery tape or melted wax.
n) Properly discard used needles and syringes.
o) Place the inoculated eggs into an incubator.

4. Collect allantoic fluid for hemagglutination test as follows:
   p) Repeat the step 1.1 to confirm the air sac position.
   q) If it's unchanged, remove the stationery tape and disinfect the egg using 70% alcohol and 5% iodine. If inoculation site (previously made egg shell hole) cannot be used again, repeat the steps 1.2, 2, and 3 (a), 3 (b).
   r) Insert the glass pipette through the hole into the egg shell to reach the allantoic sac.
   s) Collect allantoic fluid using glass pipette with a hand pump (typically 2-10 mL) into a sterile tube with a lid, taking care not to allow the fluid into the pump and not to damage the egg membranes, etc. ***Collected allantoic fluid will later be used for hemagglutination test***.

5. After collection of allantoic fluid, examine pathologies of virus-inoculated embryonated chicken eggs as follows:
   t) Flame scissors and pincers.
   u) Using scissors and pincers, carefully remove the egg shell located above the air sac (remove the upper 'hollow' part of the egg shell).
   v) Carefully cut and fold the chorioallantoic membrane visible in the cutout.
   w) Position the egg over the bottom of Petri dish.
   x) Empty the egg into the Petri dish taking care not to damage the embryo and other parts of the egg.
   y) Examine the egg content for pathologies (anatomic abnormalities), i.e. blood vessel damage, pocks, etc.
   z) Record your findings and make a sketch in your workbook below.

Results of examination of virus-inoculated embryonated chicken egg:

6. The following pathologies have been found:___________________________

____

7. Make a sketch of the de-embryonated chicken egg and indicate its parts:

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Practical importance: The students become familiar with virus isolation, indication and cultivation using embryonated chicken eggs for virus diagnostics, research and vaccine production.

Protocol 2: Indication of virus reproduction in cell cultures (SELF-WORK)

Background

Upon virus inoculation, three major types of reaction of cell cultures are distinguished. Virus-induced cytopathic effect (CPE) is most common and has diagnostic importance for many groups of viruses. At the practical class the students learn the methods of virus isolation, cultivation and identification using cell cultures; learn how to detect the virus in cell culture by its cytopathic effect. The students write down the results of completed tasks and teacher signs it.

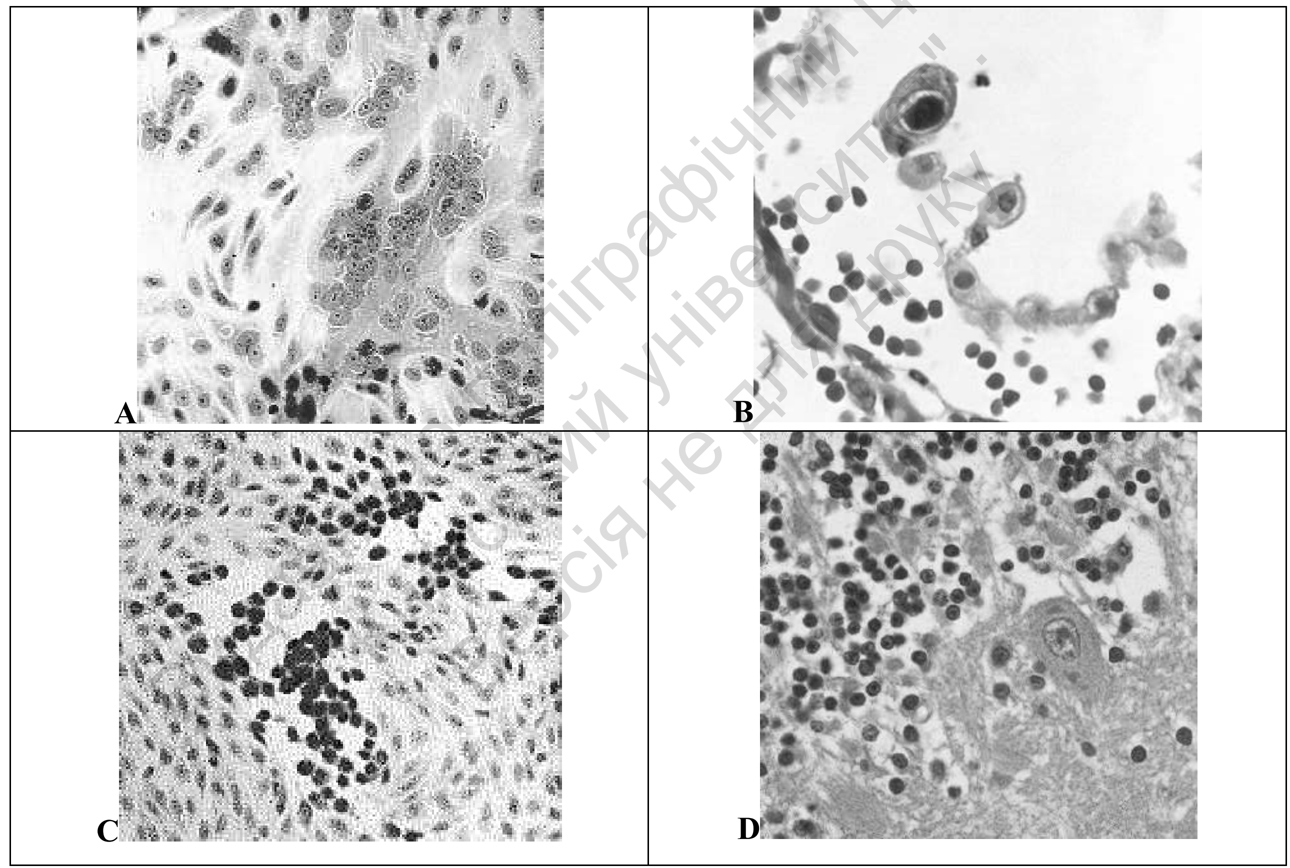
Objective: To identify virus reproduction in cell cultures by cytopathic effect (CPE)

Materials: Handouts with cell lines demonstrating with typical CPEs specific for different viruses (poxviruses, adenoviruses, herpesviruses, etc.).

Procedure:

1. Examine the handouts.
2. Record your findings in your workbook below.

Handouts of virus-induced CPE in the cell culture:



The following pathologies have been found:

A ____ typical for the following virus(es)
____

B ____ typical for the following virus(es)
____

C ____ typical for the following virus(es)
____

D ____ typical for the following virus(es)
____

Practical importance: The students become familiar with virus indication in cell culture by virus-specific cytopathic effect (CPE) for subsequent virus identification.

Independent work

1. A sample from a patient with severe fever and hemorrhagic symptoms on skin was delivered to your lab. Explain your actions for: 1) your own safety; 2) virus indication in this sample. (0,2 points)

____

____

____

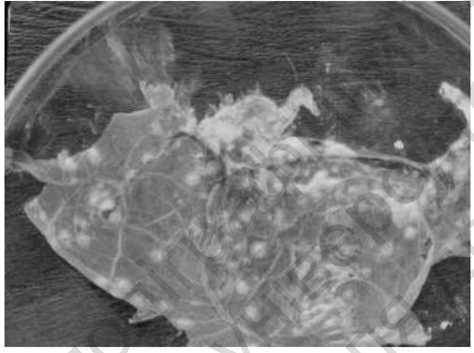
2. You're clinical trial manager organizing testing for candidate generic drug relieving the symptoms of acute respiratory infection. WHICH model system would you use and WHY? (0,2 points)

____

____

____

3. After dissecting embryonated chicken egg previously inoculated with unknown virus, you've seen the following:



Describe the symptoms, indicate possible virus(es). (0,1 points)

____

Questions for self-control

1) Viruses are specific parasites of human cells, animals and plants. How this feature is used for virus cultivation *in vitro*?
2) Laboratory animals are primary models for virus cultivation. Name the benefits and limitations of their use.
3) What is cytopathic effect and how can it be used for virus indication?
4) Viruses can be obtained in large quantities relatively easy and inexpensive with one of the methods of cultivation. Which method of virus cultivation is it? Why it cannot be considered universal?
5) Compare the benefits of laboratory animals, cell cultures and embryonated chicken eggs, and then choose a cost-efficient system suiting MOST of viruses. Argue your option.

Situational task. In 48 hours after inoculation of embryonated chicken egg with nasopharyngeal swab from a patient with possible flu, hemorrhagic symptoms were discovered on chorioallantoic membrane. The embryo died. 1) Describe optimal way of virus indication in the allantoic fluid. 2) Describe other methods to confirm your diagnosis.

Situational task. A patient was bitten by a stray dog in a shin. The dog was caught afterwards. A sample of its spinal fluid was delivered to your laboratory for rabies diagnostics. 1) Describe optimal way of virus indication in the sample. 2) Describe other methods to confirm your diagnosis. 3) What would be you actions towards the patient in case of positive results for rabies?

Score _________ Tutor signature ____________________________

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Class № 3. **Date ________________**

TOPIC: LABORATORY DIAGNOSTICS OF DISEASES CAUSED BY ORTHO- AND PARAMYXOVIRUSES

List of questions to study:

- General characteristics and classification of Orthomyxoviruses. Classification of human influenza viruses.
- General characteristics of influenza viruses: genome structure and characteristics, chemical composition and antigenic structure. Antigenic diversity (shift and drift) and its consequences.
- Resistance and sensitivity of influenza viruses to physical and chemical factors.
- Methods of cultivation Orthomyxoviruses.
- Origin of influenza and mechanism of its transmission.
- Pathogenesis of influenza. Role of virus persistence for preservation of important epidemic strains in humans and animals.
- Features of laboratory diagnostics of influenza.
- Specific prevention and treatment of influenza.
- Paramyxoviruses. Taxonomy and characteristics of the family. Differentiation with orthomyxoviruses. Parainfluenzaviruses, properties, importance for human pathology. Pathogenesis, immunity, diagnostics. Mumps virus, properties, pathogenesis, immunity, specific prophylaxis. Morbilivirus, morphology, properties, pathogenesis, immunity, specific prophylaxis. HRSV, properties, importance for human pathology.

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Theory

Basic terms that a student should learn during preparing to the lesson:

Term	Meaning
Viral glycoproteins	Viral glycoproteins are structural surface proteins of the outer shells of complex viruses that consist of the outer (hydrophilic) part at the ends of the aminogroup (N-end), and immersed hydrophobic part in the lipid layer that contained at the end of hydroxyl group (C-end).
	Viral glycoproteins are specific antigens. The main function of viral glycoproteins are interaction with specific receptors on the cell surface, i.e., specific adsorption of virus to cells. Another feature is its participation in the viral fusion with cell membranes, leading to virus penetration into the cells and strip down (release of genomes).
Antigenic shift	Antigenic shift is such variability of influenza virus which leads to the emergence of strains with new surface glycoproteins and leads to appearance of radical update of antigens.
Antigenic drift	Antigenic drift is a partial change of hemagglutinin when one or more aminoacids change due to point mutations. It leads to formation of the strains with slightly updated antigenic properties.
Viral population	Viral population is a particular type of virus originating from a single viral part and reproducing in natural or experimental sensitive systems, forming unlimited number of generations in it.
Adaptation of the virus	Adaptation of the virus is the virus's ability to multiply rapidly in cell culture of new host or when cultivation conditions change.

Virological surveillance

Collection, storage and transport of specimens

Clinical specimens that are to be tested for influenza viruses can be collected either as part of routine patient care (through sentinel surveillance) or during outbreak investigations.

The successful isolation of an influenza virus depends upon the prompt collection of high-quality specimens, the rapid transportation of specimens to the testing laboratory, and appropriate transport and storage conditions prior to testing. Ideally, respiratory specimens and acute-phase serological specimens should be collected within 3 days of the onset of clinical symptoms.

Virus isolation in cell culture

The isolation of viruses in cell cultures is increasingly becoming the gold standard for virus diagnostics. However a laboratory must maintain several cell lines to allow for the detection of a variety of respiratory pathogens. Since standard virus-isolation procedures take several days before results are available they are usually of limited use in clinical settings for the prompt diagnostics of influenza.

Virus isolation in embryonated chicken eggs

In recent years, the use of cell cultures has surpassed the use of embryonated eggs to isolate and culture influenza viruses. However, only viruses grown in embryonated eggs can be used as seed viruses for the production of the majority of influenza vaccines. For this reason, laboratories that have the capability to isolate influenza viruses in eggs are encouraged to maintain this capacity.

Hemagglutination test and Hemagglutination Inhibition test

Certain viruses have a protein on their surface that interacts with red blood cells (RBC) and is able to attach to them. This property is called hemagglutination and the surface protein of the virus is hemagglutinin. The inhibition or blocking of this activity is the basis of the hemagglutination inhibition (HI) test. The most well-known virus with this property is the influenza virus. Like the virus neutralization (VN) test, the patient's serum sample is incubated with the virus of interest but instead of growing the virus in cells, red blood cells are added to the virus-serum mix. If antibodies are present, the hemagglutination activity will be blocked; if no antibodies are present the virus will agglutinate (bind together). In this case the red blood cells are the indicator (Fig 3.1).

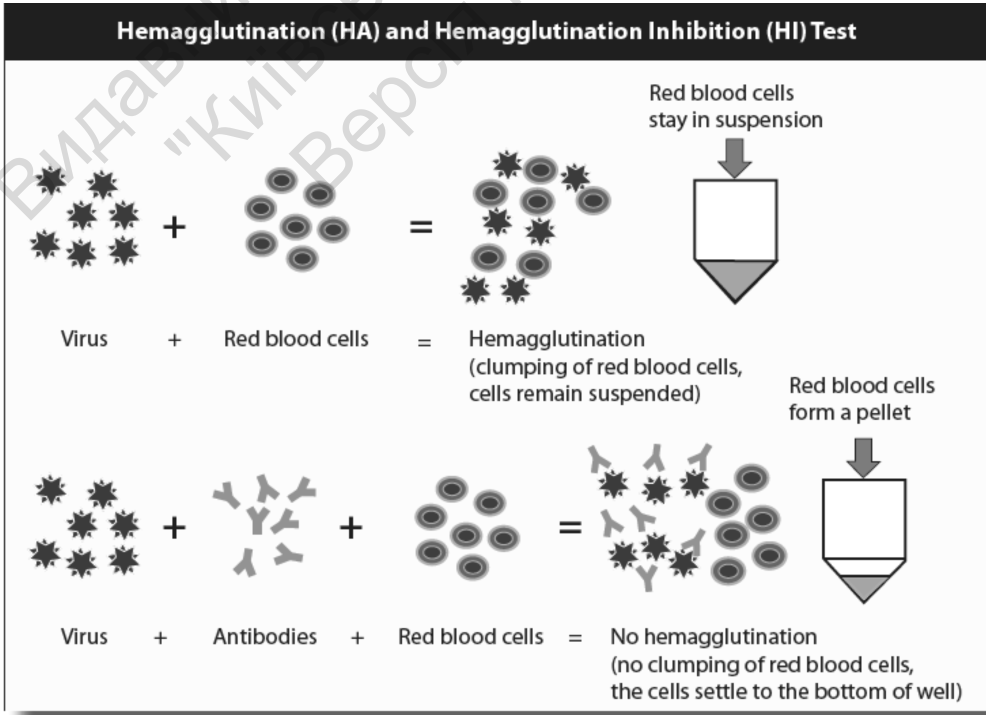

Fig. 3.1. Scheme of hemagglutination test (HA) and hemagglutination inhibition test (HIT)

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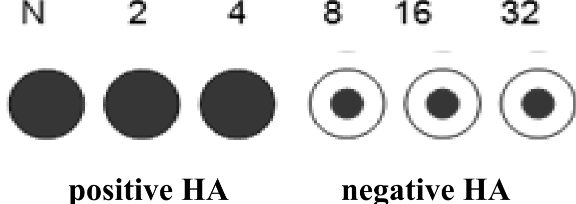
Influenza viruses bind to red blood cells using the hemagglutinin causing the formation of a lattice.

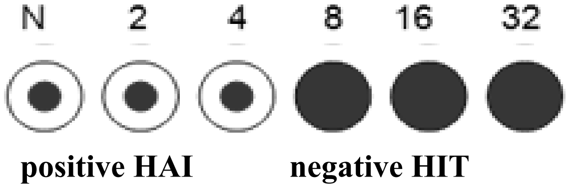
HA: two-fold serial dilutions of a virus are prepared, mixed with red blood cells, and added to the wells of a plastic plate. The red blood cells that are attached to virus particles form a lattice that coats the well. The red blood cells that are not bound by virus sink to the bottom of a well and form a button.



positive HA **negative HA**

The basis of the HIT assay is that antibodies to influenza virus will prevent attachment of the virus to red blood cells.

By adding specific antibodies to the virus it is possible to block this interaction and detect the virus. If antibodies to the virus are specific, hemagglutination will not be observed.



positive HAI **negative HIT**

Identification of the hemagglutinin subtype of viral isolates by hemagglutination inhibition testing

The hemagglutination inhibition test (HIT) is an extremely reliable assay for typing, subtyping and further determining the antigenic characteristics of influenza viral isolates provided that the reference antisera used contain antibodies to currently circulating viruses. The antisera used are based on antigen preparations derived from either the wildtype strain or a high-growth reassortant made using the wild-type strain or an antigenically equivalent strain hemagglutination that occur naturally in sera; the need to standardize reference and test antigens each time a test is performed; and the need for specialized expertise in reading the results of the test. Nevertheless, the HIT test remains the assay of choice for global influenza surveillance and for determining the antigenic characteristics of influenza viral isolates.

Serological diagnostics of influenza by hemagglutination inhibition testing

Diagnosing influenza by virus isolation in cell culture definitively identifies the infecting strain and is usually more rapid than serological diagnostics. However, serological diagnostics is an important approach when clinical specimens are unobtainable or when a laboratory does not have the resources required for virus isolation. Serological methods such as the HIT test are essential for many epidemiological and immunological studies and for evaluation of the antibody response following vaccination. Serological methods are also very useful in situations where identification of the virus is not feasible. Demonstration of an acute influenza infection using serology requires a significant increase in antibody titres (i.e. 4-fold or greater) between acute-phase and convalescent-phase serum samples. The demonstration of such a significant increase may establish the diagnostics of a recent infection even when attempts to detect the virus are negative.

Virus identification by immunofluorescence antibody staining

Immunofluorescence antibody (IFA) staining of virus-infected cells in original clinical specimens and field isolates is a rapid and sensitive method for diagnosing respiratory and other viral infections. During recent years, monoclonal antibodies against several clinically important respiratory viruses have become commercially available and have been thoroughly evaluated in many laboratories. It is preferable for IFA staining to be performed on isolates rather than original clinical specimens as this allows any virus that is present to first be amplified, and if required used in

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other studies. However, where rapid diagnostics is needed, this procedure is often carried out on clinical specimens. Because commercially available rapid tests for diagnosing influenza infection differ with regard to the type of specimen required, as well as their complexity, specificity and sensitivity, WHO recommends that such assays should be used in conjunction with other laboratory tests.

Molecular identification of influenza isolates

The direct molecular identification of influenza isolates is a rapid and powerful technique. The reverse-transcription polymerase chain reaction (RT-PCR) allows template viral RNA to be reverse transcribed producing complementary DNA (cDNA) which can then be amplified and detected. This method can be used directly on clinical samples and the rapid nature of the results can greatly facilitate investigation of outbreaks of respiratory illness. For example, genetic analysis of influenza virus genes (especially the HA and NA genes) can be used to identify an unknown influenza virus when the antigenic characteristics cannot be defined. Genetic analyses also can be used to monitor the evolution of influenza viruses and to determine the degree of relatedness between viruses from different geographical areas and those collected at different times of the year.

Rapid influenza diagnostic tests (RIDTs) are immunoassays that can identify the presence of influenza A and B viral nucleoprotein antigens in respiratory specimens, and display the result in a qualitative way (positive vs. negative).

Table 3.1. Influenza Virus Testing Methods

Method	Acceptable Specimens	Test Time
Viral cell culture (conventional)	nasopharyngeal (NP) swab, throat swab, NP or bronchial wash, nasal or endotracheal aspirate, sputum	3-10 days
Rapid cell culture (shell vials; cell mixtures)	As above	1-3 days
Immunofluorescence, Direct (DFA) or Indirect (IFA) Antibody Staining	NP swab or wash, bronchial wash, nasal or endotracheal aspirate	1-4 hours
RT-PCR (single-plex and multiplex; real-time and other RNA-based) and other molecular assays	NP swab, throat swab, NP or bronchial wash, nasal or endotracheal aspirate, sputum	Varied (Generally 1-6 hours)
Rapid Influenza Diagnostic Tests (antigen)	NP swab, (throat swab), nasal wash, nasal aspirate	<30 min

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PRACTICAL WORK

Practical tasks performed in the classroom:

1. To learn the scheme of virological diagnostics of influenza.
2. To take into account Hemagglutination test to study and determine virus titer. To make a conclusion.
3. To learn diagnostic, prophylactic and therapeutic drugs used for influenza treatment.

Protocol 1: Determination of the titer of influenza virus by the hemagglutination reaction

Background

In preparing the scheme for laboratory diagnostics, the students use the self-training knowledge and practical class knowledge, perform accounting of reactions (determine the presence of virus in the Hemagglutination test; take in account Hemagglutination Inhibition test performed for serological diagnostics of influenza), learn the drugs used for laboratory diagnostics and prevention of influenza: influenza diagnostics, diagnostic sera, vaccines: live, inactivated (of whole virions), chemical (Split, subunit vaccines, normal human immunoglobulin, various types of interferon and write to the protocol.

The student writes down the results of completed tasks and teacher signs it.

Materials: viral isolates, red blood cells (RBCs; chicken or guinea-pig), physiological saline (0.85% NaCl); microtitre plate.

Procedures:

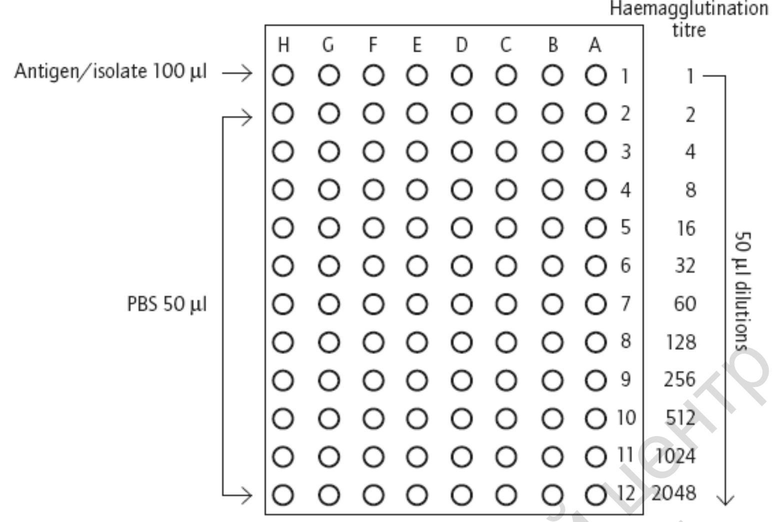

Fig. 3.2. Schematic outline of hemagglutination titration of viral isolates

Titration to determine HA titre of a suspension of virus

1. Choose the appropriate type of 96-well microtitre plate and label plate oriented as shown in FIGURE 2.

2. Add 50 мl physiological saline to wells 2 to 12 of each row (i.e. A2–A12; B2–B12; etc. up to H2–H12).

3. Add 100 мl of each viral isolate to the **first wells** of rows A–F (i.e. A1–F1).

4. Prepare an RBC control in row H (well H1) by adding 50 мl physiological saline.

5. Prepare C + control in row G (well G1) by adding 50 мl standard viral isolate.

6. Make serial 2-fold dilutions by transferring 50 мl **from the first to successive wells** of each row (i.e. A1 to A2; A2 to A3; etc. up to A11 to A12). Discard the final 50 мl.

7. Add 50 мl of standardized RBCs to each well.

8. Mix using a laboratory shaker for 10 seconds or by manually agitating the plates thoroughly.

9. Cover and incubate the plates at room temperature. Check the RBC control for complete settling of the cells.

10. Record and interpret the results.

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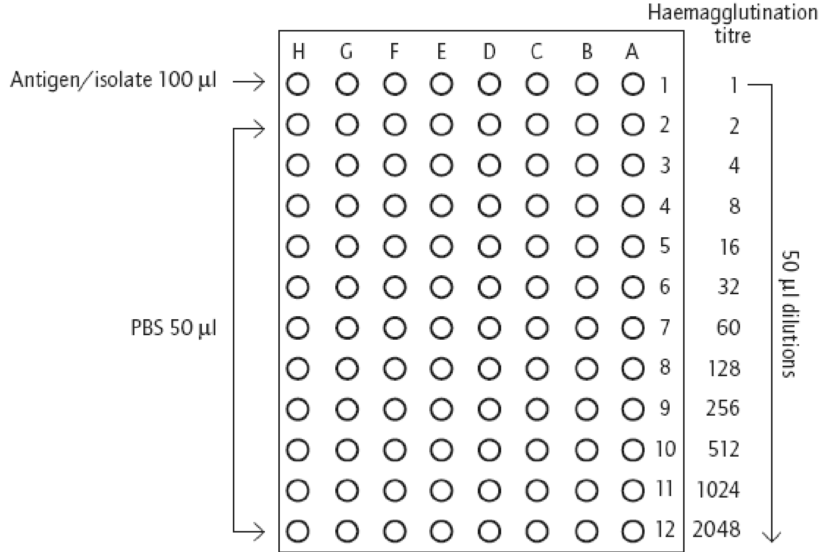
Results



Interpretation of results

Single cells roll down the sides of the V-bottom well and settle as a sharp button. Agglutinated cells do not roll down the sides of the well to form a button. Instead, they settle as a diffuse film.

Negative HA result = a sharp button

Positive HA result = a diffuse film

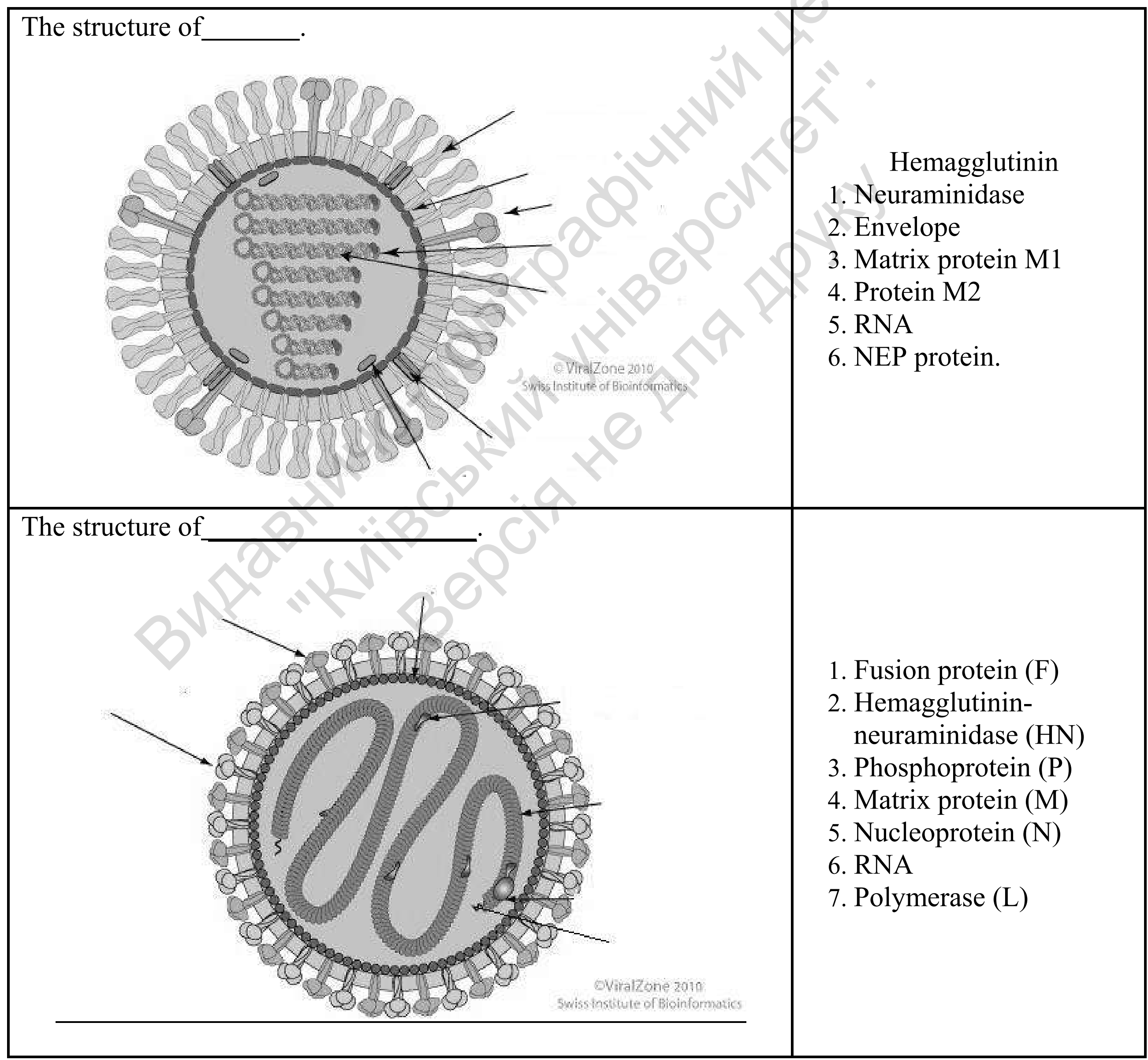
Red blood cell control = a sharp button

The hemagglutination titration ***end-point*** is defined as the highest dilution of virus that still causes complete hemagglutination. The hemagglutination ***titer*** (or ***titre***) is the reciprocal of this dilution. For example, if a virus causes complete hemagglutination up to a 1:256 dilution then the HA titre of the virus stock is 256.

One ***unit*** of hemagglutination is contained in the end-point dilution of the HA titration. A hemagglutination unit is defined as the amount of antigen (virus) needed to agglutinate an equal volume of a standardized RBC suspension.

Independent work

1. Write in virus family name, indicate respective structural elements of virion (0,2 points)



The structure of_______.	Hemagglutinin 1. Neuraminidase 2. Envelope 3. Matrix protein M1 4. Protein M2 5. RNA 6. NEP protein.
The structure of__________________. ______________________________	1. Fusion protein (F) 2. Hemagglutinin-neuraminidase (HN) 3. Phosphoprotein (P) 4. Matrix protein (M) 5. Nucleoprotein (N) 6. RNA 7. Polymerase (L)

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2. Name taxonomy of the viruses (0,1 points)

	Influenza virus	Parainfluenza virus	Measles virus	Mumps virus	Respiratory-syncytial virus
Family					
Genus					

3. Fill in the table (0,1 points)

	Influenza virus	Parainfluenza virus	Measles virus	Mumps virus	Respiratory-syncytial virus
Genome					
Shape					
Diagnistics					
Antigenic stability					

4. Prevention of ortho – and paramyxoviruses (0,1 points)

Virus	Prophylaxis	Treatment
Influenza		
Parainfluenza		
Measles virus		
Mumps virus		
Respiratory-syncytial virus		

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Questions for self-control

1. Orthomyxoviridae family. History of discovery, biological properties, classification.
2. Methods of laboratory diagnostics of influenza and its estimation.
3. Antigenic structure and types of antigenic variability of influenza virus. Modern hypotheses explaining antigenic variability of orthomyxoviruses.
4. Pathogenesis and immunity during influenza. The role of specific and nonspecific mechanisms of immunity to influenza.
5. The problem of specific prophylaxis and therapy of influenza. Preparation their evaluation.

6. Paramyxoviridae family, the general characteristics. Parainfluenza viruses, their biological properties. Role in the development of human pathology. Laboratory diagnostics of parainfluenza infections.
7. Measles virus biological properties and cultivation. Pathogenesis of infection. Laboratory diagnostics and specific prophylaxis.
8. Mumps (epidemic parotitis) virus. Pathogenesis of infection. Laboratory diagnostics and specific prophylaxis of mumps.
9. Paramyxoviridae family. General characteristics. Respiratory syncytial virus. Biological properties, role in the development of human pathology.
10. Methods of diagnosing of diseases caused by RS-virus.

Score _________ Tutor signature__________________________

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Class № 4. **Date ________________**

TOPIC: PICORNAVIRUSES. LABORATORY DIAGNOSIS OF ENTEROVIRUSES INFECTIONS

List of questions to study:

- General characteristics of the family Picornaviridae, classification.
- Structure and chemical composition of enteroviruses.
- Sensitivity of enteroviruses to physical and chemical factors.
- Antigenic structure of enteroviruses.
- Cultivation and features of reproduction in sensitive cells.
- Pathogenesis, clinical manifestations and immunogenesis of poliomyelitis, Coxsackie virus and ECHO-virus infection.
- Principles and methods of laboratory diagnosis of enteroviruses infections.
- Principles of specific prevention of enteroviral infections. Comparison of live and inactivated poliovaccines.

Theory

Poliovirus

Diseases: Paralytic poliomyelitis and aseptic meningitis.

Characteristics: Naked nucleocapsid with single-stranded, positive-polarity RNA. No virion polymerase. There are three serotypes.

Transmission: Fecal-oral route.

Pathogenesis: The virus replicates in the pharynx and the gastrointestinal tract. It can spread to the local lymph nodes and then through the bloodstream to the central nervous system.

Most infections are asymptomatic or very mild. Aseptic meningitis is more frequent than paralytic polio.

Paralysis is the result of death of motor neurons, especially anterior horn cells in the spinal cord. Pathogenesis of postpolio syndrome is unknown.

Laboratory Diagnosis: Recovery of the virus from spinal fluid indicates infection of the central nervous system. Isolation of the virus from stools indicates infection but not necessarily disease.

It can be found in the gastrointestinal tract of asymptomatic carriers. The virus can be detected in cell culture by CPE and identified by neutralization with type-specific antiserum.

A significant rise in antibody titer in convalescent-phase serum is also diagnostic.

Poliovirus can be detected in specimens from the throat, and feces (stool), and occasionally cerebrospinal fluid (CSF), by isolating the virus in cell culture or by detecting the virus by polymerase chain reaction (PCR).

CDC laboratories conduct testing for poliovirus including:

- ✓ Cell culturing
- ✓ Intratypic differentiation
- ✓ Genome sequencing
- ✓ Serology
- ✓ Virus Isolation

Virus isolation in cell culture is the most sensitive method to diagnose poliovirus infection. Poliovirus is most likely to be isolated from stool specimens. It may also be isolated from pharyngeal swabs. Isolation is less likely from blood or CSF.

To increase the probability of isolating poliovirus, collect at least **two stool specimens 24 hours apart from patients** with suspected poliomyelitis. These should be collected as early in the course of disease as possible (ideally within 14 days after onset).

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Real-time reverse transcription PCR is used to differentiate possible wild strains from vaccine-like strains (“intratypic differentiation”), using virus isolated in culture as the starting material.

Partial genome sequencing is used to confirm the poliovirus genotype and determine its likely geographic origin.

Serologic testing

Serology may be helpful in supporting the diagnosis of paralytic poliomyelitis, particularly if a patient is known or suspected to not be vaccinated. An acute serum specimen should be obtained as early in the course of disease as possible, and a convalescent specimen should be obtained at least three weeks later.

CSF analysis

Detection of poliovirus in CSF is uncommon. CSF usually contains an increased number of leukocytes [from 10 to 200 cells/mm^3 (primarily lymphocytes)] and a mildly elevated protein (from 40 to 50 mg/dL). These findings are nonspecific and may result from a variety of infectious and noninfectious conditions.

Treatment: No antiviral therapy is available.

Prevention: Disease can be prevented by both the inactivated (Salk) vaccine and the attenuated (Sabin) vaccine; both induce humoral antibody that neutralizes the virus in the bloodstream. The oral Sabin vaccine is used for routine childhood immunizations, because it (1) induces IgA immunity in the gut, thereby interfering with transmission; (2) induces immunity of longer duration; and (3) is administered orally.

Current practice in the United States is to give two immunizations of the inacti- vated vaccine followed by the live, attenuated vaccine.

The inactivated vaccine induces antibodies, which can prevent virulent revertants in the live vaccine from causing paralytic poliomyelitis.

Immune globulins are available but rarely used.

Coxsackieviruses

Diseases: Aseptic meningitis, herpangina, pleurodynia, myocarditis, and pericarditis are the most important diseases.

Characteristics: Naked nucleocapsid with single-stranded, positive-polarity RNA. No virion polymerase. Group A and B viruses are defined by their different pathogenicity in mice. There are multiple serotypes in each group.

Transmission: Fecal-oral route.

Pathogenesis: The initial site of infection is the oropharynx, but the main site is the gastrointestinal tract. The virus spreads through the bloodstream to various organs.

Laboratory Diagnosis: The virus can be detected by CPE in cell culture and identified by neutralization.

A significant rise in antibody titer in convalescent-phase serum is diagnostic.

Treatment: No antiviral therapy is available.

Prevention: No vaccine is available.

PRACTICAL WORK

Background

In practice the students study the biological properties of Picornaviridae family, Enterovirus genera and some of its representatives (poliovirus, Coxsackie viruses and ECHO viruses). The students make the scheme of diagnosis of enterovirus diseases. Students lean the cytopathic effect of polioviruses in cell cultures at the demonstrational preparations.

To identify the unchanged cells culture (control) on the demonstration preparations and cytopathic effect of poliovirus as type of complete degeneration.

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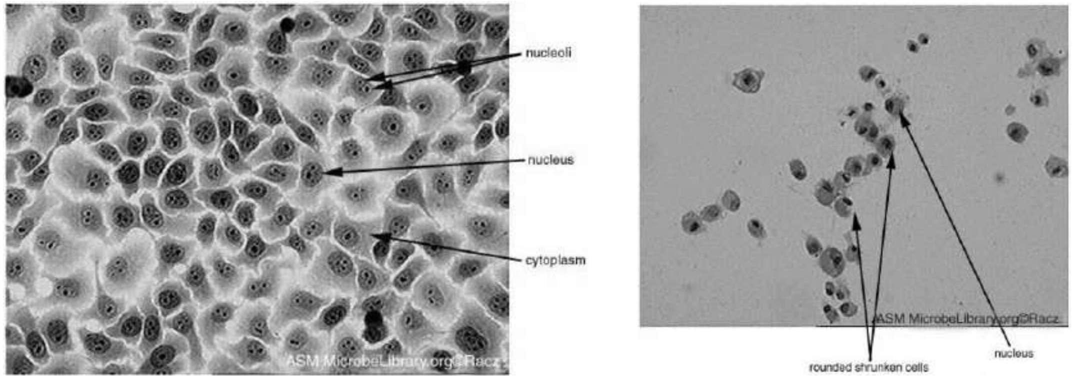

healthy cells culture **cytopathic effect of poliovirus**

Protocol 1: Neutralization test for identification of polioviruses

Materials: sterile 96-well flat bottomed dell culture microtitre plates with lids; sterile, non-toxic plate sealers (if no CO_2 incubator will be used); 5 ml sterile tubes for dilution; 1 ml and 2 ml disposable plastic pipettes; sterile 50 µl droppers or pipettes with aerosol-resistant tips (ARTs); flask of healthy cells of the type in which the virus was confirmed (usually L20B); polio antiserum pools; maintenance medium

Procedures:

Label the edge of the microtitre plate as indicated in Figure (for two unknown virus isolates).

1. Distribute 50 µl of each of the four antiserum pools in columns 1 to 8, rows A to D, using a different dropper/pipette tip for each pool.
2. Add 50 µl medium to virus control wells, A9 to D10.
3. Add 50 µl medium to back titration wells E1 to H10.
4. Add 100 µl medium to cell control wells G11 to H12 and cover plate.
5. Label dilution tubes 10-1to 10-7, marking each set with specimen number (Fig. 4.1).

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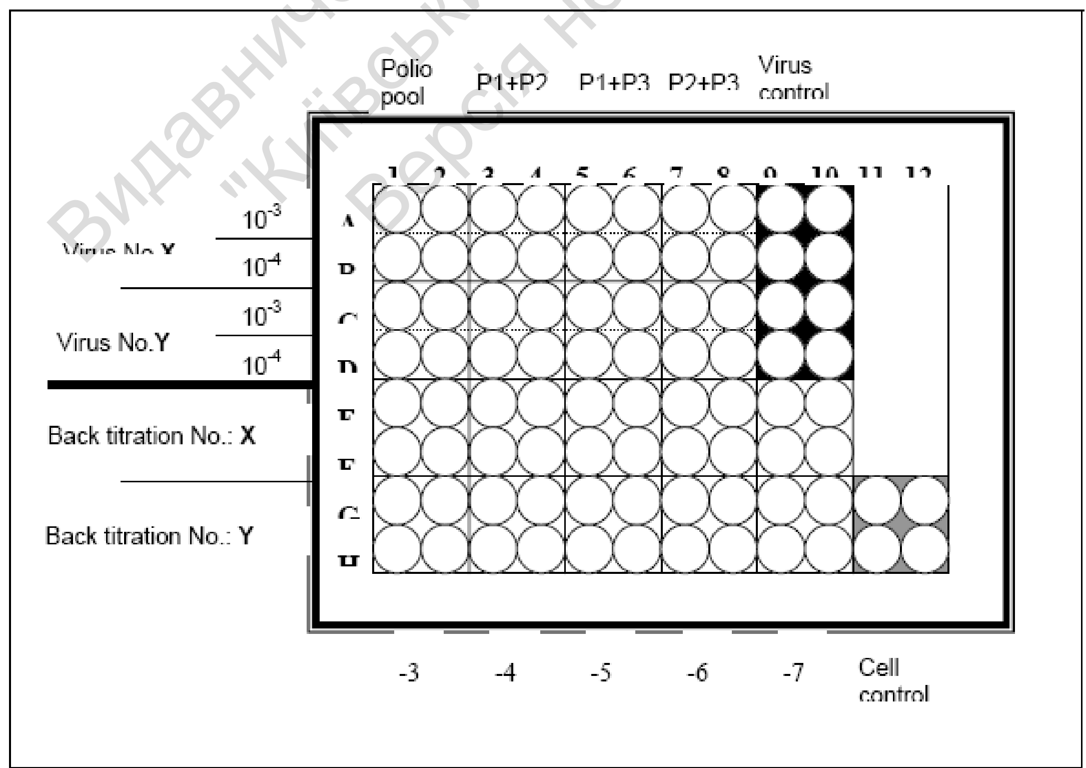

Figure 4.1. Plate set-up for poliovirus identification test

6. Dispense 0.9 ml medium to tubes 1–2 and 5–7, and 1.8 ml to tubes 3 and 4.
7. Add 0.1 ml virus to first tube (=10-1 dilution) using sterile pipette or pipette with ART tip.
8. Take another pipette/pipette tip, mix thoroughly but gently to avoid aerosols.
9. Transfer 0.1 ml to the second tube and discard pipette/pipette tip.
10. Repeat dilution steps, transferring 0.2 ml to tubes 3 and 4 (Figure1).
11. Add virus to the back titration wells of the microplate beginning at the 10-7dilution in columns 9 and 10, rows E and F.
12. The same dropper/pipette ART tip may be used for one isolate, working from highest to lowest dilution, 10-7 to 10-3.
13. Add 50 μl virus to the test wells as indicated: 10-3 dilution of isolate 1 to wells A1-A10, 10-4 dilution to wells B1–B10 etc.
14. Repeat the last two steps for the second isolate in rows G and H for the back titration, in wells C1–C10 for the 10-3 dilution of isolate 2, and in wells D1–D10 for the 10-4 dilution of isolate 2.
15. Cover the plate with the lid and incubate between one and three hours at 36°C.
16. Distribute 100 μl of cell suspension into test and control wells.
17. Examine and record daily, using an inverted microscope, for development of CPE.
18. Continue observation and recording until 24 hours after the virus control wells show 100% CPE (usually 3–5 days).

Results

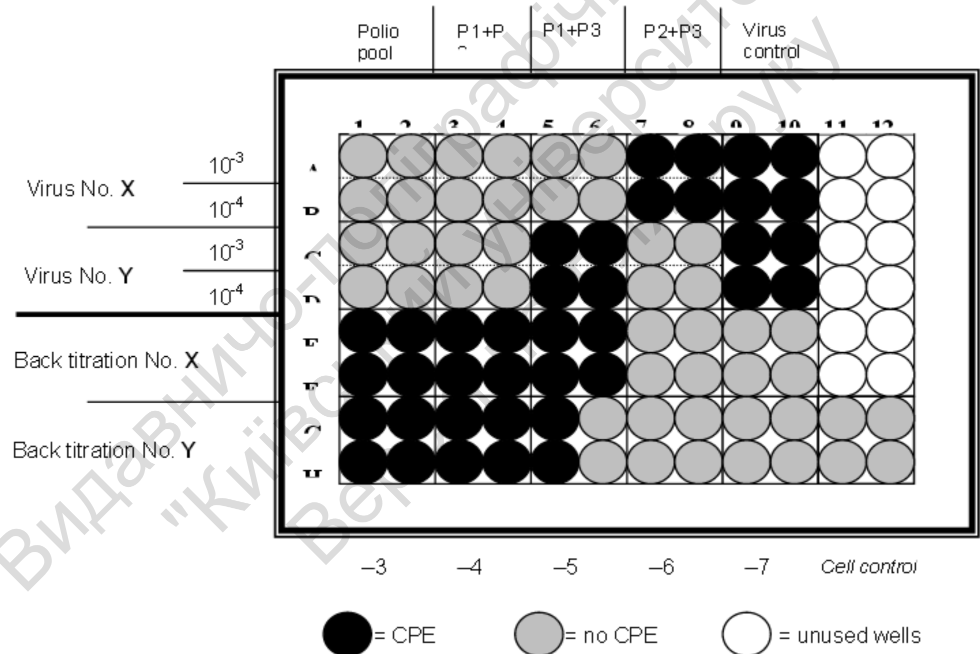

Fig 4.2. Identification of poliovirus isolates using the microtechnique

Interpretation of results

Cell control wells should have a complete monolayer of cells. Virus control wells should show complete CPE. The back titration should confirm that the amount of virus used in the test for one of the dilutions was within the range 32–320 CCID50 . The test result should be read from the dilution that corresponds to the correct virus titre for the test. If the virus titre in the test is not within the acceptable range, the test should be repeated with the dilutions adjusted (either higher or lower, as appropriate) to contain the correct amount of virus. The antiserum pools that prevent the development of CPE indicate the identity of the virus isolate or mixture of viruses. Failure of a virus to replicate in the presence of a pool of antisera is due to the neutralization of infectivity by one of the antisera present in the pool. Figure shows the typical lay-out of a test plate with interpretation of results.

Fill in the table. Interpretation of virus neutralization patterns in the poliovirus identification test

Pool P1+P2+P3	Pool P1+P2	Pool P1+P3	Pool P2+P3	Virus identification

\+ = CPE 0 = no CPE

Independent work

1**. Write in virus family name, indicate respective structural elements of virion** (0,1 points)

The structure of_________________virus.

1. Capsid
2. VPg
3. RNA

2. Name taxonomy of the viruses (0,1 points)

	Poliovirus	Coxsackie virus	ECHO virus	Foot-and-mouth disease virus
Family				
Genus				

3. Fill in the table (0,1 points)

	Poliovirus	Coxsackie virus	ECHO virus	Foot-and-mouth disease virus
Genome				
Shape				
Size of virion				
Type of symmetry				

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4. **Diagnosis** (0,1 points)

Virus	Antigen Detection	Detection of virus-specific Antibodies	Molecular Detection Methods
Poliovirus			
Coxsackie virus			
ECHO virus			
Foot-and-mouth disease virus			

5. **Prevention of Picornaviruses** (0,1 points)

Virus	Prophylaxis	Treatment
Poliovirus		
Coxsackie virus		
ECHO virus		

Questions for self-control

1. What type of nucleic acid does Picornaviridae have?
2. What structure do picornaviruses have?
3. What are the defining features of Enterovirus genus?
4. Which viruses do belong to the Enterovirus genus?
5. What is the antigenic structure of enteroviruses?
6. Which cultivation methods may be used for enteroviruses?
7. What are the stages of interaction between enteroviruses and sensitive cells?
8. How many serological types of polio viruses are there?
9. What is the pathogenesis of poliomyelitis?
10. What are the methods for laboratory diagnostics of poliomyelitis?
11. Which vaccines can be used for prevention of poliomyelitis? What are the positive properties and negative properties of live and inactivated vaccines?
12. What are the criteria for differentiation between Coxsackie A viruses and Coxsackie B viruses?
13. Rotaviruses: characteristics, role in pathology.

Score _________ Tutor signature___________________________

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Class № 5. **Date ____________**

TOPIC: RETROVIRUSES. HIV. LABORATORY DIAGNOSIS OF HIV INFECTION HUMAN IMMUNODEFICIENCY VIRUS

List of questions to study:

1. General characteristic and classification of oncogenic viruses.
2. Virus-genetic tumor formation theory by L.A. Zilber. Mechanisms of viral carcinogenesis.
3. Morphology and chemical composition of human immunodeficiency virus. Types of HIV.
4. Origin and evolution of HIV. Peculiarities of the genome.
5. Cultivation of HIV, stages of interaction between viruses and susceptible cells.
6. Target cells for HIV in humans, characteristics of the viral surface receptors.
7. The mechanism of immunodeficiency. AIDS-associated pathologies (opportunistic infections and tumors).
8. Methods of laboratory diagnosis of AIDS (immunological, genetic).
9. Prospects for specific prevention and therapy of HIV infection.

Theory

Disease: Acquired immunodeficiency syndrome (AIDS).

Characteristics: Enveloped virus with two copies (diploid) of a single-stranded, positive-polarity RNA genome.

RNA-dependent DNA polymerase (reverse transcriptase) makes a DNA copy of the genome, which integrates into host cell DNA. Precursor polypeptides must be cleaved by virus-encoded protease to produce functional viral proteins.

The *tat*[1] gene encodes a protein that activates viral transcription. It is a type D retrovirus (lentivirus). Antigenicity of the gpl20 protein changes rapidly; therefore, there are manyserotypes.

Transmission: Transfer of body fluids, eg, blood and semen. Also transplacental and perinatal transmission.

Pathogenesis: Two receptors are required for HIV to enter cells. One receptor is CD4 protein found primarily on helper T cells. HIV infects and kills helper T cells, which predisposes to opportunistic infections. Other cells bearing CD4 proteins on the surface, e.g., astrocytes, are infected also.

The other receptor for HIV is a chemokine receptor such as CCR5. The NEF protein is an important virulence factor. It reduces class I MHC protein synthesis, thereby reducing the ability of cytotoxic T cells to kill HIV-infected cells. Cytotoxic T cells are the main host defense against HIV.

Laboratory Diagnosis: Virus can be isolated from blood or semen, but this procedure is not routinely available. Diagnosis is 'usually made by detecting antibody with ELISA as screening test and Western blot as confirmatory test.

Determine the "viral load", ie, the amount of HIV in the plasma, using PCR-based assays. PCR-based assays can also detect viral RNA in infected cells, which is useful to detect early infections.

Treatment: Azidothymidine (AZT), 3TC, d4T, ddl, and ddCinhibit HIV replication by inhibiting reverse transcriptase. Protease inhibitors, eg, indinavir, prevent cleavage of precursor polypeptides. Highly active retroviral therapy (HAART) consists of two nucleoside inhibitors and one protease inhibitor. Non-nucleoside inhibitors such as nevirapine are also useful.

Clinical improvement occurs, but the virus persists. Treatment of the opportunistic infection depends on the organism.

Prevention: Screening of blood prior to transfusion for the presence of antibody. "Safe sex," including the use of condoms.

AZT with or without a protease inhibitor should be given to HIV-infected mothers and their newborns. AZT, 3TC, and a protease inhibitor should be given after a needle-stick injury. There is novaccine.

Practical work

On the practice, students are introduced to the basic of classification and biological properties of retroviruses, morphological, physico-chemical properties, ultrastructure and antigenic structure of HIV, laboratory diagnostics and specific prevention and treatment of HIV infection prospects. Students examine and analyze the scheme of polymerase chain reaction (PCR) with the purpose of the laboratory diagnosis of HIV/AIDS as well as its modification, which provides a quantitative determination of HIV RNA in blood of patients. Learn principles of western blot. Assess the results of laboratory diagnosis of HIV-infection by ELISA.

During composing the scheme of the laboratory diagnosis of HIV infection and AIDS, students use the knowledge acquired during self-training and in the process of consideration of the topic in class. In addition, students are acquainted with drugs used for laboratory diagnosis of HIV-infection. Completed protocols are signed by the teacher.

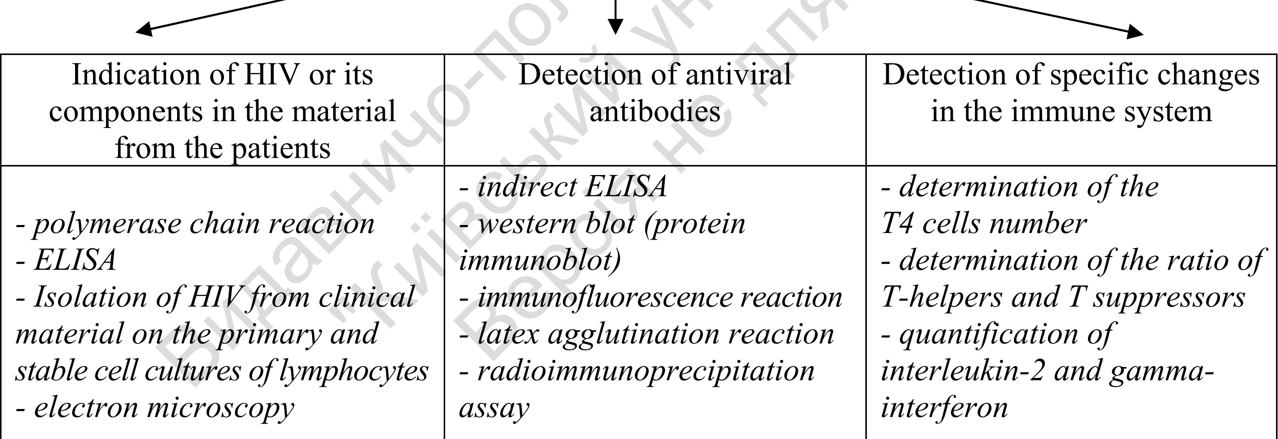
Recommendations for design of the protocol

- Students learn and enter the classification of retroviruses into the protocol
- Students learn and enter the scheme of the laboratory diagnosis of HIV/AIDS into the protocol
- Students assess the results of laboratory diagnosis of HIV-infection by ELISA
- Students characterize and add to the protocol the main drugs used for treatment of HIV/AID

Protocol 1:

1. To write the scheme of the laboratory diagnosis of HIV / AIDS in the protocol.

Scheme of the laboratory diagnosis of HIV/AIDS



Indication of HIV or its components in the material from the patients	Detection of antiviral antibodies	Detection of specific changes in the immune system
- polymerase chain reaction *- ELISA* *- Isolation of HIV from clinical material on the primary and stable cell cultures of lymphocytes* *- electron microscopy*	*- indirect ELISA* *- western blot (protein immunoblot)* *- immunofluorescence reaction* *- latex agglutination reaction* *- radioimmunoprecipitation assay*	*- determination of the T4 cells number* *- determination of the ratio of T-helpers and T suppressors* *- quantification of interleukin-2 and gamma-interferon*

2. To assess the results of laboratory diagnosis of HIV-infection by ELISA.

Conclusions: B1, E3, G2 – seropositive samples.

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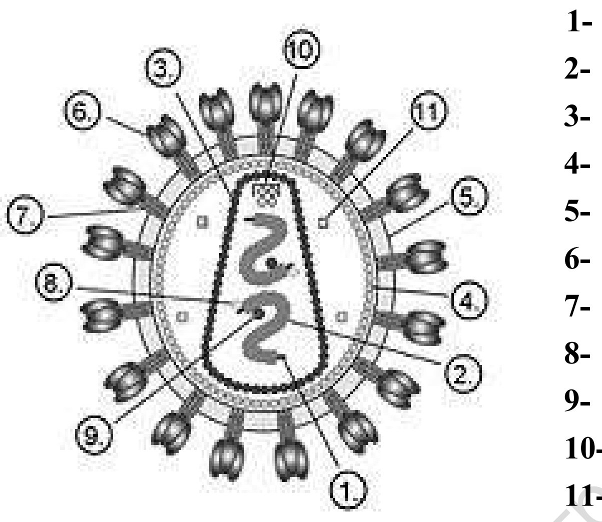
Protocol 2:

1. To read and add to the protocol the main drugs used for treatment of HIV/AIDS.
2. Nucleoside analog of reverse transcription inhibitors (zidovudine, lamivudine, stavudine etc.).
3. Non-nucleoside reverse transcriptase inhibitors (efavirenz, nevirapine, delavirdine etc.).
4. Protease inhibitors (indinavir, ritonavir, ABT-378).

Independent work

1. Write components of structure of HIV. (0,1 points)



1-
2-
3-
4-
5-
6-
7-
8-
9-
10-
11-

2. Name morphological properties of HIV (0.2 points)

	HIV
Genome	
Shape	
Size of virion	
Site of synthesis of ribonucleoprotein	
Type of symmetry	

3. Fill in the table (0.2 points)

Medicine	Mechanism of action
Abacavir (ABC)	
Didanosine (ddI)	
Lamivudine (3TC)	
Stavudine (d4T)	
Zidovudine (ZDV or AZT)	
Abacavir (ABC)	
Efavirenz (EFV or EFZ)	
Nevirapine (NVP)	
Indinavir (IDV)	
Lopinavir+ritonavir (LPV/r)	
Nelfinavir (NFV)	
Enfuvirtide	

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Questions for the self-control

1. What is the structure of human immunodeficiency virus?
2. What are the enzymes HIV has?
3. Which cell receptor interacts with HIV?
4. What physical factors are harmful to HIV?
5. What are the violations of cellular immunity observed in patients with AIDS?
6. What are the mechanisms of HIV transmission?
7. In which biological fluids antibodies to HIV can.

Score _________ Tutor signature__________________________

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Class № 6. **Date ________________**

TOPIC: VIRAL HEPATITIS

List of questions to study:

1. Etiologic agents of viral hepatitis, their properties and classification.
2. Viral hepatitis A.
3. Hepatitis B virus structure specifics.
4. Viral hepatitis C.
5. Viral hepatitis D.
6. Viral hepatitis E.

Theory

Hepatitis is a term that describes inflammation of the liver, and can be caused by a virus or other non-viral causes. Inflammation is a natural reaction of the body to injury and often causes swelling and tenderness. The condition can be self-limiting or can progress to fibrosis (scarring), cirrhosis or liver cancer.

Hepatitis viruses are the most common cause of hepatitis in the world but other infections, toxic substances (e.g. alcohol, certain drugs), and autoimmune diseases can also cause hepatitis.

Viral hepatitis are a group of distinct diseases that affect the liver. Each have different hepatitis symptoms and treatments. Some causes of hepatitis include recreational drugs and prescription medications. Laboratory tests can determine hepatitis types.

There are 5 main hepatitis viruses, referred to as types A, B, C, D and E (table 6.1). These 5 types are of greatest concern because of the burden of illness and death they cause and the potential for outbreaks and epidemic spread. In particular, types B and C lead to chronic disease in hundreds of millions of people and, together, are the most common cause of liver cirrhosis and cancer.

Recent studies of the so-called hepatitis F virus allowed affirmed its heterogeneity, so term HFV is no longer in use. Participation of recently described viruses TTV and SEN, and some animal viruses (Peking ducks, Canadian forestry marmot et al.) in human pathology and the possible degree of organ damage is under debate.

Hepatitis G virus (also known as GB virus) is known to infect humans, but is not known to cause human disease.

Hepatitis A and E are typically caused by ingestion of contaminated food or water. Hepatitis B, C and D usually occur as a result of parenteral contact with infected body fluids. Common modes of transmission for these viruses include receipt of contaminated blood or blood products, invasive medical procedures using contaminated equipment and for hepatitis B transmission from mother to baby at birth, from family member to child, and also by sexual contact.

Hepatitis A is always an acute, short-term disease, while hepatitis B, C, and D are most likely to become ongoing and chronic. Hepatitis E is usually acute but can be particularly dangerous in pregnant women.

While all hepatitis viruses cause liver disease, they vary in important ways.

Hepatitis A virus (HAV) is a species of virus in the order *Picornavirales* in the family *Picornaviridae* and is the type species of the genus *Hepatovirus*. HAV is not enveloped and contains a single-stranded RNA packaged in a protein shell

HAV is present in the faeces of infected persons and is most often transmitted through consumption of contaminated water or food. Certain sex practices can also spread HAV. Infections

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are in many cases mild, with most people making a full recovery and remaining immune from further HAV infections. However, HAV infections can also be severe and life threatening. Most people in areas of the world with poor sanitation have been infected with this virus. Safe and effective vaccines are available to prevent HAV.

Hepatitis B virus (HBV) is a partially double-stranded DNA virus, a species of the genus *Orthohepadnavirus* and a member of the *Hepadnaviridae* family of viruses. HBV is transmitted through exposure to infective blood, semen, and other body fluids. The virus particle, called Dane particle (virion), consists of an outer lipid envelope and an icosahedral nucleocapsid core composed of protein. The nucleocapsid encloses the viral DNA and a DNA polymerase that has reverse transcriptase activity similar to retroviruses

HBV can be transmitted from infected mothers to infants at the time of birth or from family member to infant in early childhood. Transmission may also occur through transfusions of HBV-contaminated blood and blood products, contaminated injections during medical procedures, and through injection drug use. HBV also poses a risk to healthcare workers who sustain accidental needle stick injuries while caring for infected-HBV patients. Safe and effective vaccines are available to prevent HBV.

Hepatitis C virus (HCV) is a small, enveloped, positive-sense single-stranded RNA virus of the family *Flaviviridae*. The hepatitis C virus particle consists of a lipid membrane envelope that is 55 to 65 nm in diameter. Two viral envelope glycoproteins, E1 and E2, are embedded in the lipid envelope. They take part in viral attachment and entry into the cell. Within the envelope is an icosahedral core that is 33 to 40 nm in diameter. Inside the core is the RNA material of the virus.

HCV is mostly transmitted through exposure to infective blood. This may happen through transfusions of HCV-contaminated blood and blood products, contaminated injections during medical procedures, and through injection drug use. Sexual transmission is also possible, but is much less common. There is no vaccine for HCV.

Hepatitis D virus (HDV) is a small spherical enveloped virusoid. The HDV is a small, spherical virus with a 36 nm diameter. It has an outer coat containing three kinds of HBV envelope protein - large, medium, and small hepatitis B surface antigens - and host lipids surrounding an inner nucleocapsid. The nucleocapsid contains single-stranded, circular RNA of 1679 nucleotides and about 200 molecules of hepatitis D antigen (HDAg) for each genome.

HDV is considered to be a subviral satellite because it can propagate only in the presence of the hepatitis B virus. Transmission of HDV can occur either via simultaneous infection with HBV (coinfection) or superimposed on chronic hepatitis B or hepatitis B carrier state (superinfection).

HDV infections occur only in those who are infected with HBV. The dual infection of HDV and HBV can result in a more serious disease and worse outcome. Hepatitis B vaccines provide protection from HDV infection.

Hepatitis E virus (HEV) is a positive-sense, single-stranded, nonenveloped, RNA icosahedral virus.The virus has since been classified into the genus *Orthohepevirus*, and has been reassigned into the *Hepeviridae* family.

HEV is mostly transmitted through consumption of contaminated water or food. HEV is a common cause of hepatitis outbreaks in developing parts of the world and is increasingly recognized as an important cause of disease in developed countries. Clinically, it is comparable to hepatitis A, but in pregnant women, the disease is more often severe and is associated with a clinical syndrome called fulminant liver failure. Pregnant women, especially those in the third trimester, have a higher rate of death from the disease of around 20%. Safe and effective vaccines to prevent HEV infection have been developed but are not widely available.

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Table 6.1. Characteristics of hepatitis viruses

Characteristics of viruses	HAV	HBV	HCV	HDV	HEV	HGV
Type of nucleic acid	ssRNA(+)	dsDNA, circular, incomplete	ssRNA(+)	ssRNA, defective virus	ssRNA(+)	ssRNA(+)
Systematic position	*Picornaviridae*	*Hepadnavirid ae*	*Flaviviridae*	*Deltavirus*	*Hepeviridae*	*Flaviviridae*
Virion size (nm)	27	40	80	36	32–34	60
Structure	Simple	Complex	Complex	Complex	Simple	Complex
Cultivation in the cell culture	Hepatoma-cells	Hepatoma-cells	Huh7.5.1	Hepatoma-cells	Modified hepatoma-cells	?
Pathogenicity for animals	Chimpan-zees, marmozets	Chimpanzees	Chimpan-zees	Chimpan-zees	Chimpanzees	?
Replication in hepatocytes	Cytoplasm	Nucleus	Cytoplasm	Nucleus	Cytoplasm	?
Antigenic variants	Viral specific antigen	HBsAg, HBcAg, HBeAg, HBxAg	Few subtypes	Two forms: small, big	Hetero-geneous	Five phylogenetic groups
Oncogenicity	–	+	+	+	–	+
Association with other HV				HBV		HCV HBV
The mechanism of transmission	Fecal-oral	Parenteral, sexual	Parenteral, sexual	Parenteral	Fecal-oral	Parenteral
Factors of transmission	Water, food	Blood, sperm, exretion from vagina	Blood	Blood	Water, food	Blood
Groups of risk	Children	Doctors, blood recipients, drug users, sexual partners, children of HBV-positive mothers	Doctors, blood recipients, drug users, sexual partners, hemodialy sis patients	Patients with hepatitis B, doctors, recipients, drug users	Young people from Asia, Africa	Doctors, blood recipients, drug users, sexual partners, hemodialys is patients
Prevention	Inactivated and live vaccine	1. Plasma vaccine (from blood of HBsAg-carriers) 2. Geneticall y engineered 3. Recombin ant from poxvirus	Interferon Protease ihibitor Polymeras e inhibitor	Vaccination against HBV	Not developed	Not developed

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PRACTICAL WORK

In practice, students are introduced to modern methods of laboratory diagnosis of viral hepatitis; acquainted with rapid test for the detection of Hepatitis B surface antigen. Completed protocols are signed by the teacher.

Protocol 1:

Background

HBsAg rapid test is a direct binding test for the visual detection of hepatitis B surface antigen (HBsAg) in serum, plasma or whole blood. It is used as an aid in the diagnosis of hepatitis B infection. The test is based on the principle of sandwich immunoassay for determination of HBsAg in serum or whole blood. Monoclonal and polyclonal antibodies are employed to identify HBsAg specifically. This one step test is very sensitive and only takes 10 minutes. Test results can be read visually without any instrument.

Objective: To detect of hepatitis B surface antigen (HBsAg) in whole blood.

Materials: rapid test device for screening and diagnosis of Hepatitis B, Distilled water, Petri dishes, Filter paper.

Procedure:

1. This test strip contains a membrane strip, which is pre-coated with mouse monoclonal anti-HBs capture antibody on test band region. The mouse monoclonal anti-HBs-colloid gold conjugate and sample moves along the membrane chromatographically to the test region (T) and forms a visible line as the antibody-antigen-antibody gold particle complex forms. Both the Test Line and Control Line are not visible before applying any samples. The Control Line is used for procedural control. Control line should always appear if the test procedure is performed properly and the reagents of control line are working.
2. Sample collection: fingerstick or venipuncture.
3. Dispense 75 µl of whole blood.
4. Dispense 1 drop of buffer.
5. Read the result at 30 mins.

Note: For most positive samples, the line in the test region (T) can appear before 30 minutes. However, test reading and interpretation should only be performed after 30 minutes following sample deposit.

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Independent work

1. Name taxonomy of the viruses of hepatitis A, B, C, D, E, G (0,1 points)

Virus	**Hepatitis A**	**Hepatitis B**	**Hepatitis C**	**Hepatitis D**	**Hepatitis E**	**Hepatitis G**
Family						
Genus						

2. Draw the structure of hepatitis viruses (0,1 points)

	Hepatitis A virus ________________________ ________________________ ________________________ ________________________ ________________________ ________________________ ________________________

	Hepatitis B virus ________________________________ ________________________________ ________________________________ ________________________________ ________________________________ ________________________________ ________________________________
	Hepatitis C virus ________________________________ ________________________________ ________________________________ ________________________________ ________________________________ ________________________________ ________________________________ ________________________________ ________________________________

3. Fill in the table (0,2 points)

Virus	Source of infection	Route of transmission	Signs and symptoms
Hepatitis A			
Hepatitis B			
Hepatitis C			
Hepatitis D			
Hepatitis E			
Hepatitis G			

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4. Prevention of hepatitis (0,1 points)

Virus	Prophylaxis	Treatment
Hepatitis A		
Hepatitis B		
Hepatitis C		
Hepatitis D		
Hepatitis E		
Hepatitis G		

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Questions for the self control

1. Specify which hepatitis B virus antigens can be detected in the serum of patients with viral hepatitis B. Which hepatitis B virus antigens can be detected only in hepatocytes?
2. Which markers of acute hepatitis B can be detected in the blood of the patient?
3. Which markers of acute hepatitis A can be detected in the blood of the patient?
4. Which marker can be detected in blood after vaccination against hepatitis B?
5. What medications are used for specific prophylaxis of hepatitis B?
6. What is the minimum infectious dose of hepatitis B virus during parenteral enters?
7. Which hepatitis viruses can cause the development of primary hepatocellular carcinoma?
8. What are the reasons of high percentage of chronic cases and resistance to antiviral therapy during viral hepatitis C.
9. What measures are used for prophylaxis of hepatitis B?
10. Which viral hepatitis can be diagnosed in Ukraine? What methods of laboratory diagnosis should be used?
11. What viruses are causative agents of viral hepatitis TTV and Sen? Is registered in Ukraine cases of these diseases? Are there any cases of such diseases registered in Ukraine?

Score _________ Tutor signature____________________

Class № 7. **Date ______________**

TOPIC: BACTERIOPHAGES

List of questions to study:

6. General characteristics of bacteriophages.
7. Types of phage's lifecycles
8. Methods for bacteriophages' titration.
9. Use of bacteriophages in medical practice.

Theory

A **bacteriophage** is a virus that infects bacteria. Bacteriophages are among the most common and diverse entities in the biosphere. Bacteriophages are ubiquitous viruses, found wherever bacteria exist. It is estimated there are more than 10^{31} bacteriophages on the planet, more than every other organism on Earth, including bacteria. Like other types of viruses, bacteriophages vary a lot in their shape and genetic material. Phage genomes can consist of either DNA or RNA, and can contain as few as four genes or as many as several hundred. The capsid of a bacteriophage can be icosahedral, filamentous, or head-tail in shape. The head-tail structure seems to be unique to phages and is not found in eukaryotic viruses. Phages replicate within the bacterium following the injection of their genome into its cytoplasm. The steps that make up the infection process are called the **lifecycle of the phage**.

Some phages (**lytic phages**) can only reproduce via a **lytic lifecycle**, in which they burst and kill their host cells. Other phages (**temperate phages**) can alternate between a lytic lifecycle and a **lysogenic lifecycle**, in which they don't kill the host cell (and are instead copied along with the host DNA each time the cell divides). Many (but not all) temperate phages can integrate their genomes into their host bacterium's chromosome, together becoming a **lysogen** as the phage genome becomes a **prophage**. Several human pathogens, such as the diphtheria bacillus, are lysogenized by viruses whose DNA directs the synthesis of toxins that are harmful to the human host.

Bacteriophages can be effectively used to treat bacterial infections. Recent renewed interest in **phage therapy** is dictated by its advantages most importantly by their specificity against the bacterial targets. This prevents complications such as antibiotic-induced dysbiosis and secondary infections.

The limited host range of many phages makes them useful for distinguishing different strains within the same bacterial species. The great advantage of typing bacteria by phage in this way is that it will detect differences between strains that are identical by serological and other tests, so that precise surveys can be made of the distribution and spread of a given phage type of pathogen within a community. **Phage typing** for epidemiological purposes has been particularly successful in *Salmonella* infections, notably typhoid fever and in staphylococcal infections in hospital.

PRACTICAL WORK

Protocol 1: Double-Layer Agar technique (double agar overlay method)

Background

The procedure requires the use of a Double-Layer Agar (DLA) technique also known as double agar overlay method, in which the hard agar serves as a base layer (to form gel), and a mixture of few phage particles (diluted stock) and a very large number of host cells in a soft agar forms the upper overlay. When the plates are incubated, susceptible bacterial cells multiply rapidly and produce a lawn of confluent growth on the medium. When one phage particle adsorbs to a susceptible cell, penetrates the cell, replicates and release new phage particles which infect other bacteria in the vicinity of the initial host cell. The growth or spread of the new viruses is then restricted or limited to the neighboring cells by the gel. This cycle is repeated until large numbers of bacteria have been destroyed. The destroyed cells produce single circular areas called plaques in the

bacterial lawn, where there is no growth of bacteria because the phage progeny originating from single virus particles have multiplied sufficiently to kill bacteria over an easily visible area. Eventually the plaque becomes too large to be visible to our naked eye. Each plaque represents the lysis of a phage-infected bacterial culture and can be designated as a plaque-forming unit (PFU) and is used to quantitate the number of infective phage particles in the culture. Dyes that stain the living cells are frequently used to enhance the contrast between the plaques and the living cells. Therefore the dead cells in the plaque will appear as unstained against the colored background. Only viruses that have the ability to cause visible damage of cells can be assayed using this way.

Purpose:

To demonstrate the ability of bacteriophage to replicate inside a susceptible host cell and to determine the titer of a coliphage by the plaque assay method.

Materials:

Overnight broth culture of *Escherichia coli* strain B, coliphage T4, test tubes containing 4.5 ml LB broth, test tubes containing 2.5 ml soft LB agar (0.7%), plates of LB agar at room temperature Sterile 1.0- and 5.0-ml pipettes Pipette bulb or other pipetting device

Procedures:

1. Prepare enough LB plates for the experiment. Plates should be prewarmed at 37 ºC.
2. Prepare serial dilutions of filtrate, containing the phage of interest: 1:10, 1:100, 1:1000 etc. (Fig. 1).
3. Prepare top agar (0,7%) media. It must be maintained on waterbath at 45-50°C.
4. Prepare suspension of phage sensitive bacteria.
5. Mix 1ml of each phage dilution with 0,1 ml of bacteria and 2,5 ml of top agar and immediately pour onto LB plates.
6. Allow plates to cool until agar has set. Invert the plates (lid side down), and incubate 12-24 hours at 37°C.
7. Count plaques and record the results in the table below. Phage activity can be expressed as a titer (maximal dilution at which phages retain ability to lyse bacteria). More precisely phage activity can be calculated as the concentration of pfu (plaque forming units) in the ml of original material.

N = n (number of plaques) x dilution.

Example: on plate with phage dilution 10^{-5} 15 plaques were found. N = 15x105 = 1,5x106 pfu/ml of initial material.

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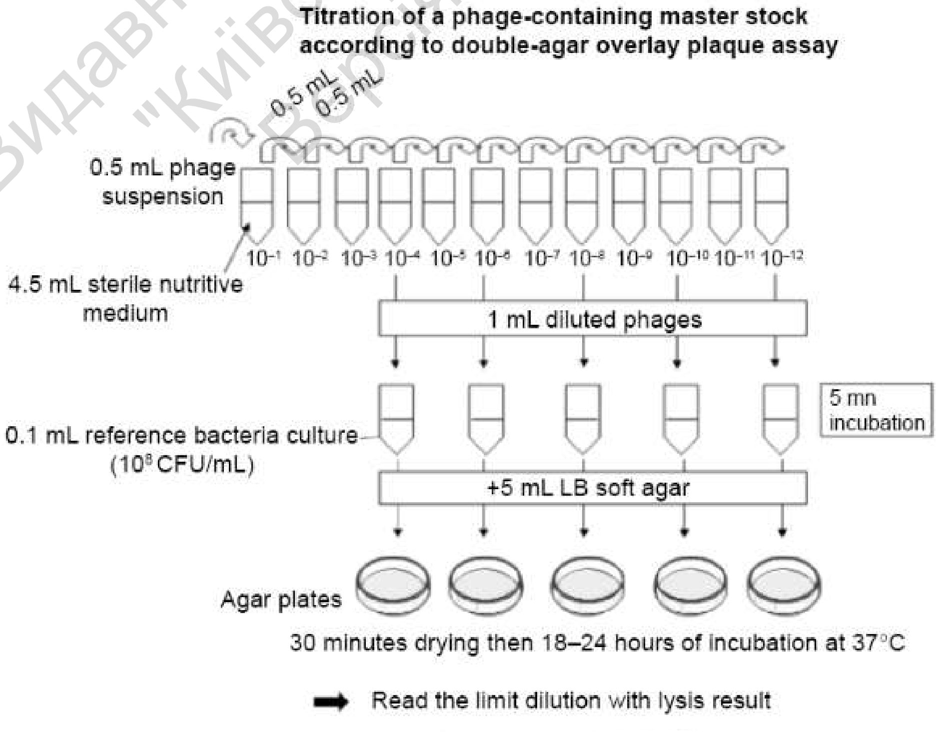


Fig. 7.1. Agar Overlay Technique

Results

№	Phage dilution	Plaques per plate

Protocol 2: Phage typing

Background

Bacteriophages are specific in their action. A particular phage may be very specific in that it will infect only a few strains of a certain bacterial species. On the other extreme, another phage may infect strains of two or more species of a particular genus. Susceptibility to lysis by a particular phage may be the only apparent phenotypic difference between two bacterial strains and may be the only means by which a strain causing an outbreak of disease can be recognized. This observation is the basis for phage-typing, a procedure for characterizing and detecting bacterial strains by their reaction (susceptibility or resistance) to various known strains of phages (fig. 2).

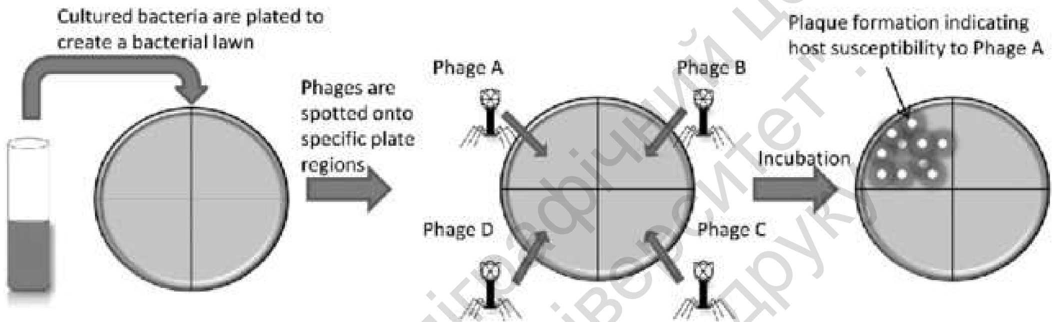


Fig. 7.2. Phage typing

The bacterial sample to be typed is plated together with a series of bacteriophages to create a bacterial lawn where clearings/plaque formation is indicative of bacteriophage replication and host susceptibility to the specific bacteriophage.

The phage-typing procedure is sometimes employed in epidemiology to identify and trace the progress of an infectious agent. This has been a standard procedure for strains of *Staphylococcus aureus* involved in outbreaks of food poisoning or other infections. A species-specific phage is used routinely in the identification of *Bacillus anthracis*, the causative agent of anthrax. Thus, reproduction of anthrax in laboratory animals is not necessary to confirm identification of this organism.

Purpose:

To estimate sensitivity of tested bacterial culture to set of bacteriophages by spot-test method.

Materials:

Broth cultures of potential host strains, phage strains (each appropriately diluted), serological pipettes (5ml) and bulbs, micropipettes, appropriate micropipette tips, glass tubes and caps (13mm), soft LB agar (0.7%) , pre-poured agar (1,4%) plates, 37°C incubator, water bath.

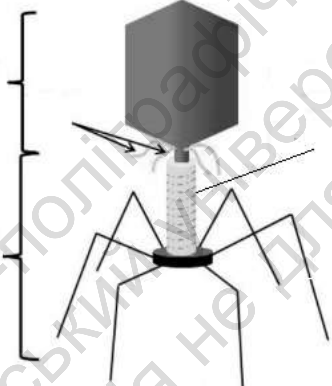
Procedures:

1. Label the agar plate clearly. Divide the plate with a marker and label each section.
2. Prepare the top agar: heat the soft LB agar in a microwave, interrupting and shaking periodically.
3. While the top agar is very warm, use a serological pipette to add ~4.5 mL of it to the sterile tube and place it in the water bath (at 45°C).
4. To the soft agar tube, add 100 μL of an overnight bacterial culture. Briefly vortex the tube to mix.

5. Pour the contents of the tube over the plate and swirl or shake until it is evenly spread across the surface. The soft agar begins to solidify after about 10 seconds, so do this carefully but quickly!
6. Allow the plates to cool and solidify (~10 minutes).
7. Using a micropipettor, transfer 10 μL of liquid phage sample onto the plate in the appropriate place. Try not to physically touch the pipette tip to the agar, as this will interfere with even lawn growth. Also, avoid making bubbles, as these will scatter the sample across the plate. Replace the tip for another sample.
8. Allow the liquid from the spots to absorb into the agar (generally 30 minutes).
9. Incubate inverted plates at 37°C for ~16 – 24 hours, or until a visible bacterial lawn grows.
10. After incubation, check spots for clearing. A positive spot test will appear as complete obliteration of the entire drop area, whereas a negative spot test will result in the bacterial lawn growing normally in the region of the spot. Typically, the concentration of phage allows plaques to “grow” together to form a bacterial death zone the size of the drop area. Positive spot tests are NOT a plaque; no morphologies are associated. Rather, they are many plaques that fused together and destroy all bacterium in their wake.

Independent work

1. Identify the structural components of bacteriopage T4 (0,1 points)



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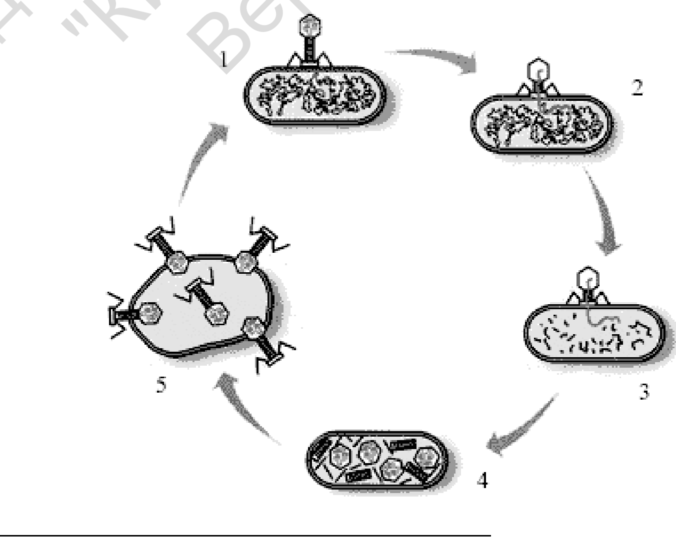
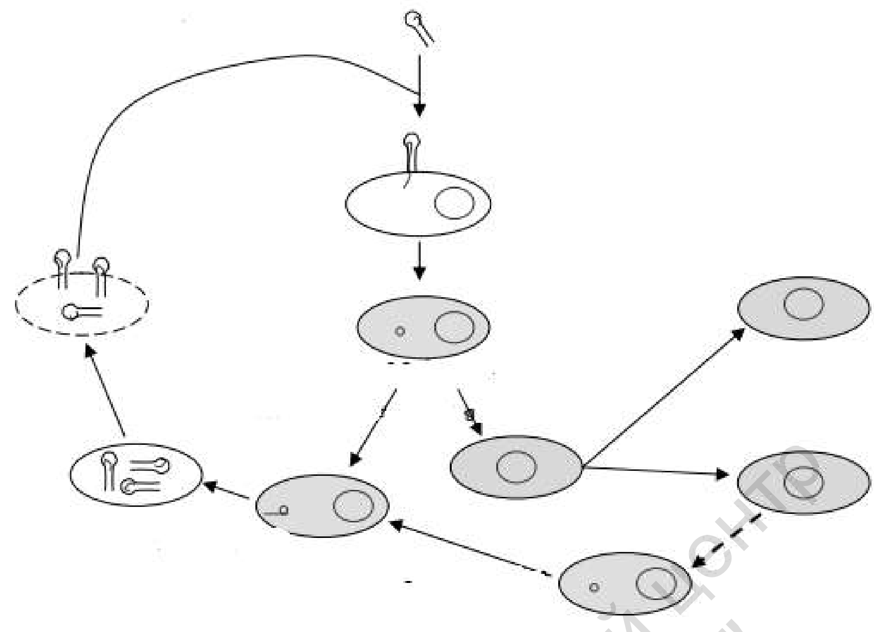
2. Describe the main steps in replicative cycle of virulent bacteriophages (0,2 points)



1. ____
2. ____
3. ____
4. ____
5. ____

3. Complete the schematic diagram of phage λ life cycle (0,2 points)



Questions for self-control

1. What is the definition of bacteriophage?
2. What is a virulent bacteriophage?
3. What is a lysogenic bacteriophage?
4. Why lysogenic bacteriophages are important in some diseases?
5. How bacteriophages can be determined in a sample?
6. What is the definition of plaque?
7. What is the function of the bacteriophage capsid?
8. What is the host specificity for a bacteriophage?
9. How bacteriophages can be applied in medicine?
10. In order to prevent postoperative complications in the abdominal cavity of the patient, 50 ml of polyvalent staphylococcal bacteriophage was administered. What is the mechanism of action of this drug?

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Class № 8. **Date ________________**

TOPIC: HERPESVIRUSES, ADENOVIRUSES. LABORATORY DIAGNOSIS OF HERPESVIRUS AND ADENOVIRUS INFECTIONS

List of questions to study:

1. The morphology, reproduction particularities and laboratory cultivation of Herpesviruses and Adenoviruses.
2. The pathogenesis, clinical symptoms and immunogenesis of Herpesviruses and Adenoviruses infection.
3. The principles and methods of laboratory diagnostics of Herpesviruses and Adenoviruses infection.
4. The principles of treatment and prophylaxis of adenoviruses and herpesviruses infections.

Theory

Herpesviruses

There are more than 100 known herpesviruses which are currently classified into three subfamilies called alpha, beta, and gamma. All but one of the known viruses infects vertebrates. Among these are nine distinct herpesviruses that infect humans. Human herpesviruses are best known as the cause of discomfort from recurring “cold sores”, lesions of the mucosa of the mouth and lips. Genital herpes lesions are a common sexually transmitted disease, and chickenpox is a common childhood disease that can be serious particularly when people are first exposed as adults. These three relatively mild but often annoying human diseases are caused, respectively, by herpes simplex virus types 1 and 2, and by varicella-zoster virus. However, under conditions of reduced immune response found in newborns, transplant recipients, and AIDS victims herpesviruses can cause serious and often fatal disease, including encephalitis, pneumonia, and hepatitis.

The Herpesviridae are grouped in three subfamilies denoted, and, based on biological and physical properties including cell tropism and genome organization. Some cancers are associated with infection by the herpesviruses Epstein–Barr virus and Kaposi’s sarcoma virus (human herpesvirus 8). These cancers are rare or localized to specific populations, and other factors probably contribute to their incidence.

All herpesviruses contain a double-stranded linear DNA genome, which is between 120 and 230 kb in length depending on the virus species. Herpesviruses are enveloped and approximately spherical, with a diameter of 100–300 nm. They possess a 100-nm icosahedral nucleocapsid (T = 16) that contains at least six proteins and the viral DNA. The nucleocapsid is surrounded by or embedded within a structure known as the tegument. The tegument is composed of about 20 different virus-encoded proteins and its thickness can vary, even within a single virion. Outside the tegument is the envelope containing a dozen or more virus glycoproteins. Many, perhaps all, of the glycoproteins are present in 600 or more spikes of which several different morphological types can be distinguished. One type of spike is 20 nm long and has a globule at its terminus.

One of the most intriguing characteristics of herpesviruses is their ability to establish latent infections. After an initial infection, viral DNA is harbored in a latent state in neurons, B or T lymphocytes, or other cell types. Latently infected individuals can remain without symptoms for months or years, or even their entire life. Reactivation of latent virus can lead to recurrent disease, such as repeated outbreaks of labial or genital herpes, or herpes zoster (“shingles”), a localized rash that can occur years after the initial chickenpox infection. People whose immune systems are impaired can suffer reactivation of cytomegalovirus or Kaposi’s sarcoma virus with dire results.

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Table 8.1. Human Herpesvirus (HHV) classification

Subfamily	Genus	Name	Pathophysiology
Alphaherpes virinae	Simplex-Virus	Herpes simplex virus-1 (HSV-1)	Oral herpes, as well as other herpes simplex infections
		Herpes simplex virus-2 (HSV-2)	Genital herpes, as well as other herpes simplex infections
	Varicellovirus	Varicella zoster virus (VZV)	Chickenpox and shingles
Betaherpesvi rinae	Cytomegalovirus	Cytomegalovirus (CMV)	Infectious mononucleosis-like syndrome, retinitis, etc.
	Roseolovirus	Roseolovirus, Herpes lymphotropic virus (HHV-6)	Sixth disease (roseola infantum or exanthem subitum)
		Roseolovirus (HHV-7)	
Gammaherp esvirinae	Lymphocryptovirus	Epstein-Barr virus (EBV), lymphocryptovirus	Infectious mononucleosis, Burkitt's lymphoma, CNS lymphoma in AIDS patients, post-transplant lymphoproliferative syndrome (PTLD), nasopharyngeal carcinoma, HIV-associated hairy leukoplakia
	Rhadinovirus	Kaposi's sarcoma-associated herpesvirus (KSHV)	Kaposi's sarcoma, primary effusion lymphoma, some types of multicentric Castleman's disease

The main properties of Herpesviruses

1. Icosahedral symmetry of nucleocapsid;
2. Large viruses 150-250 nm in diameter; enveloped with lipid bilayer membrane;
3. Linear DNA genomes encoding 100-200 genes; molecular weight 54-94 mllion daltons;
4. Capsid consists of 162 capsomeres: 150 hexons; 12 pentons on the tops;
5. The herpesvirus capsid is surrounded by an amorphous region called “tegument” that contains numerous viral proteins that function upon entry of the virion into the cell.
6. Most herpesviruses establish latent infections in their hosts after an initial primary infection. Latent herpesviruses may be reactivated regularly, as in cold sores, or only after many years, as in shingles.
7. Three subfamilies: alpha, beta, and gammaherpesvirus.
8. Nine human herpesviruses, including herpes simplex virus, varicella-zoster virus, Epstein–Barr virus, cytomegalovirus. Over 100 known herpesviruses from many animal species.

Herpes Simplex Virus Type 1

Diseases: Herpes labialis (fever blisters or cold sores), keratitis, encephalitis.

Characteristics: Enveloped virus with icosahedral nucleocapsid and linear double- stranded DNA. No virion polymerase. One serotype; cross-reaction with HSV-2 occurs. No herpes group-specific antigen.

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Transmission: By saliva or direct contact with virus from the vesicle.

Pathogenesis: Initial vesicular lesions occur in the mouth or on the face. The virus then travels up the axon and becomes latent in sensory (trigeminal) ganglia. Recurrences occur in skin innervated by affected sensory nerve and are induced by fever, sunlight, stress, etc.

Dissemination occurs in patients with depressed cell-mediated immunity.

Laboratory Diagnosis:

Virus causes cytopathic effect (CPE) in cell culture. It is identified by antibody neutralization or fluorescent-antibody test.

Tzanck smear of cells from the base of the vesicle reveals multinucleated giant cells with intranuclear inclusions. These giant cells are not specific for HSV-1; they are seen in the vesicular lesions caused by HSV-2 and varicella-zoster virus as well.

A rise in antibody titer can be used to diagnose a primary infection but not recurrences. Intranuclear inclusions seen in infected cells. HSV encephalitis can be diagnosed using a PCR assay to detect HSV-1 DNA in spinal fluid.

Treatment: Acyclovir for encephalitis and disseminated disease. Acyclovir has no etiect on the latent state of the virus. Trifluorothymidine for keratitis.

Primary infections and localized recurrences are self-limited. A variety of over- the-counter drying agents can be used to promote healing.

Prevention: Recurrences can be prevented by avoiding the specific inciting agent such as intense sunlight. Acyclovir can reduce recurrences.

No vaccine is available.

Herpes Simplex Virus Type 2

Diseases: Herpes genitalis, aseptic meningitis, and neonatal infection. Characteristics: Enveloped virus with icosahedral nucleocapsid and linear double- stranded DNA. No virion polymerase. One serotype; cross-reaction with HSV-1 occurs. No herpes group-specific antigen.

Transmission: Sexual contact in adults and during passage through the birth canal in neonates.

Pathogenesis: Initial vesicular lesions occur on genitals. The virus then travels up the axon and becomes latent in sensory (lumbar or sacral) ganglion cells. Recurrences may be induced by stress.

Laboratory Diagnosis: Virus causes CPE in cell culture. Identify by antibody neutralization or fluorescent-antibody test. Tzanck smear reveals multinucleated giant cells but is not specific for HSV-2. A rise in antibody titer can be used to diagnose a primary infection but not recurrences.

Treatment: Acyclovir is useful in the treatment of both primary and recurrent disease. It has no effect on the latent state.

Prevention: Primary disease can be prevented by protection from exposure to vesicular lesions. Recurrences can be reduced by the long-term use of oral acyclovir. There is no vaccine.

Varicella-Zoster Virus

Diseases: Varicella (chickenpox) in children and zoster (shingles) in adults. **Characteristics:** Enveloped virus with icosahedral nucleocapsid and linear double- stranded DNA. No virion polymerase. One serotype.

Transmission: Varicella is transmitted primarily by respiratory droplets. Zoster is not transmitted; it is caused by a reactivation of latentvirus.

Pathogenesis: Initial infection is in the respiratory tract. It spreads via the blood to the internal organs such as the liver and then to the skin.

After the acute epispde of varicella, the virus remains latent in the sensory ganglia and can reactivate to cause zoster years later, especially in older and im- munocompromised individuals.

Laboratory Diagnosis: Virus causes CPE in cell culture and can be identified by fluorescent-antibody test.

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Multinucleated giant cells seen in smears from the base of the vesicle. Intranuclear inclusions seen in infected cells.

A 4-fold rise in antibody titer in convalescent-phase serum is diagnostic

Treatment: No antiviral therapy is indicated for varicella or zoster in the immunocompetent patient. In the immunocompromised patient, acyclovir can prevent dissemination.

Prevention: Vaccine contains live, attenuated virus. Immunocompromised patients exposed to the virus should receive passive immunization with varicella-zoster immune globulin (VZIG) and acyclovir to prevent disseminated disease.

Cytomegalovirus

Diseases: Cytomegalic inclusion body disease in infants. Mononucleosis in transfusion recipients. Pneumonia and hepatitis in immunocompromised patients.

Characteristics: Enveloped virus with icosahedral nucleocapsid and linear double- stranded DNA. No virion polymerase. One serotype.

Transmission: Virus is found in many human body fluids, including blood, saliva, semen, cervical mucus, breast milk, and urine.

It is transmitted via these fluids, across the placenta, or by organ transplantation.

Pathogenesis: Initial infection usually in the oropharynx. In fetal infections, the virus spreads to many organs, eg, central nervous system and kidneys.

In adults, lymphocytes are frequently involved. A latent state occurs in leukocytes. Disseminated infection in immunocompromised patients can result from either a primary infection or reactivation of a latent infection.

Laboratory Diagnosis: The virus causes CPE in cell culture and can be identified by fluorescent-antibody test. "Owl's eye" nuclear inclusions are seen. A 4-fold rise in antibody titer in convalescent-phase serum is diagnostic.

Treatment: Ganciclovir is beneficial in treating pneumonia and retinitis. Acyclovir is ineffective. Prevention: No vaccine is ayailable. Ganciclovir suppresses retinitis. Do not transfuse CMV antibody- positive blood into newborns or antibody-negative immunocompromised patients.

Epstein-Barr Virus

Characteristics: Enveloped virus with icosahedral nucleocapsid and linear double- stranded DNA. No virion polymerase. One serotype.

Transmission: Virus found in human oropharynx and B lymphocytes. It is transmitted primarily by saliva.

Pathogenesis: Infection begins in the pharyngeal epithelium, spreads to the cervical lymph nodes, then travels via the blood to the liver and spleen.

Laboratory Diagnosis: The virus is rarely isolated. Lymphocytosis, including atypical lymphocytes, occurs.

Heterophil antibody is typically positive (Monospot test). A significant rise in EBV-specific antibody to viral capsid antigen is diagnostic.

Treatment: No effective drug is available.

Prevention: There is no drug or vaccine.

Virological diagnostics for herpes infection

Early diagnostics: morphological examination of damaged tissues and isolation of virus. Scrapings and smears from rash elements are used as a material.

Smears are usually stained by Gimsa method or by hematoxylin-eosin. Giant cells formation and nuclear inclusion development are characteristic for herpes infection.

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Smears can be stained with fluorescent antibodies (FAT). Herpes antigens may be found in multinucleated, giant and unchanged cells. The method allows to detect herpes infection in brain, spinal cord and other tissues (liver) in lethal cases.

Virus **can be isolated** by 12-days chicken embryo inoculation. Material is applied on allantois membrane. Embryo is incubated for 48 hours at 35 C. Allantois membrane damages are observed. Giant and multinucleated cells with nuclear inclusions are revealed by microscopy.

Cell culture inoculation. Typical CPE includes multinucleated cells formation with nuclear inclusions and round cell degeneration;

Suckling mice inoculation. Mice are infected in brain or in abdominal cavity. The disease appears in 3-4 days and kills animals;

Rabbits inoculation. Rabbits are infected on scarified cornea or in brain: specific keratitis or lethal encephalitis develops respectively.

Identification of isolated viruses is performed by FAT or NT.

Virological diagnostics for chicken pox

Early diagnostics: microscopy of material from lesions, viral antigens, DNA detection or virus isolation in cell culture.

The best results are achieved by microscopy of material from fresh vesicular: multinucleated giant cells with nuclear inclusions are characteristic.

For rapid identigication FAT method is usually used. Specific antigen can be revealed extracellularly as bright grains or intracellular.

Virus can be **isolated** in cell culture. Characteristic CPE – the development of giant multinuclear cells or round-cell degeneration. Eosinofilic nuclear inclusions are often observed. Identification of isolated viruses is performed by FAT or NT.

Retrospective diagnostics: specific antibodies are revealed in ELISA, CFT or NT in paired sera B) retrospective diagnostics. For serological diagnostics CFT or ELISA in paired sera are used.

Virological diagnostics for EBV infection

Heterophilic antibodies detection – natural antibodies (IgM), which agglutinate erythrocytes of unrelated species (sheep, bull, horse etc). This phenomenon is found in approximately 90% EBV patients. Heterophilic antibodies sometimes present in blood of healthy persons in low titer.

Paul-Bunnell test (Hanganutziu-Deicher reaction) – standard method for infection mononucleosis diagnostics. It is based on sheep erythrocytes hemagglutination by patient`s serum. Diagnostic titer is 1:128–1:256. Heterophilic antibodies are found 3–4 week of the disease. **Paul-Bunnell test** are positive in leucosis, viral hepatitis, CMV infection, Burkitte lymphoma, rheumatoid arthritis, serum sickness. The antibodies titer does not reflect the severity of the disease.

The monospot test is a rapid test for infectious mononucleosis due to Epstein–Barr virus (EBV). The test is sensitive for heterophile antibodies which agglutinate horse erythrocytes. Commercially-available test kits are 70–92% sensitive and 96–100% specific. It will generally not be positive during the 4-6 week incubation period before the onset of symptoms. It will also not generally be positive after active infection has subsided, even though the virus persists in the same cells in the body for the rest of the carrier's life.

Serological diagnostics. Tests for heterophilic antibodies are relatively not sensitive and if negative can not exclude EBV infection. In this case other serological tests are useful:

ELISA for IgM and IgG to EBV capsid antigen. Its concentration reaches maximum in 2 weeks and diminishes during 2–3 months. IgM to EBV capsid antigen testifies for recent infection, IgG – infection in the past.

ELISA for antibodies to early EBV antigens. Its concentration reaches maximum in 2 weeks of the disease.

ELISA for antibodies to nuclear EBV antigen. They appear approximately in 4 weeks of the disease and persist lifelong.

Table 8.2. Antibodies to EBV antigens

Antibodies		Period of the disease	Persistence	Specificity, %
Capsid antigens	IgM	Beginning	4-8 weeks lifelong	100
	IgG			100
Early antigens	Anti-R	3-5 weeks	3-6 months	70
	Anti-D	2 weeks – 4 month s	2 months – years	low
Nuclear antigen		3-4 weeks	lifelong	100

Medications for herpes virus treatment and prophylaxis:

1. modified nucleosides which inhibit the virus replication (interferon inductors, acyclovir);
2. gamma-globulins against the Varicella zoster virus;
3. immunomodulators (Levamisole).
4. vaccines:
   - inactivated vaccine of Herpes virus strains of I and II types;
   - alive attenuated vaccine against the human cytomegalovirus.

Adenoviruses

Adenoviruses were first isolated by Wallace Rowe in 1953 from tissue culture cell lines derived from tonsils and adenoid tissues of sick children. So, adenoviruses were named after adenoids, a glandlike collection of lymphoid tissue in the nasopharynx. Many human adenoviruses establish a long-term infection in this tissue and adenoviruses were first isolated from human adenoids

Adenoviruses are widespread viruses of mammals and birds, but a few of the known viruses are able to infect reptiles or frogs. The virions are a T = 25 icosahedron, 70–90 nm in diameter, with fibers 9 to 77 nm in length projecting from the 12 fivefold axes of the icosahedron (Figs. 2.1 and 2.12).

Icosahedral virions contain about a dozen proteins, of which 4 are present in the core. The major structural proteins are a hexon protein called II, three copies of which form a hexon, of which there are 240 in the virion, and a penton protein called III, five copies of which form a penton base, of which there are 12 in the virion. The genome of adenoviruses is a linear dsDNA of size 26 to 45 kb. Double-stranded linear DNA genome; encoding 30 genes.

A terminal protein that served as a primer during DNA replication is covalently attached to the 5¢ end of both strands.

Fifty-one human adenoviruses have been distinguished on the basis of serological reactivity – an adenovirus is considered distinct if it resists neutralization by antisera against the other known adenoviruses. All belong to the genus Mastadenovirus and have a genome size of 30–36 kb. The 51 viruses are simply numbered in order of their isolation and are often referred to as Ad1, Ad2, etc., or more formally as HAdV-1, etc., to distinguish them from adenoviruses that infect other species. The human viruses were originally divided into six subgroups on the basis of serological crossreactions in a hemagglutination-inhibition assay.

This grouping correlated with a number of other properties of the viruses as well, such as their ability to form tumors in rodents. These original subgroups are now considered to be different adenovirus species, human adenovirus A through F, with grouping relying on sequence identities where possible.

Because they replicate to high titer in cultured human cells, several human adenoviruses have been intensively studied, in particular Ad2, Ad5, and Ad12.

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The human adenoviruses replicate primarily in the upper respiratory tract or in the gastrointestinal tract. Some replicate well in both while others express a tropism for one or the other. Spread of the viruses is by a respiratory route or by an oral–fecal route. Many infections by adenoviruses appear to be asymptomatic or to result in only mild illness, but about 5% of acute respiratory disease in children under 5 years old is due to adenovirus infection. Some serotypes can also cause gastroenteritis, but the overall importance of these viruses as causative agents of gastroenteritis is not resolved. Ad1, 2, and 5 are the most common viruses found in human populations, and antibodies to these viruses are present in about one-half of all children. Ad7, and to some extent Ad3 and Ad4, are the adenoviruses most often associated with severe disease, and Ad7 accounts for about 20% of adenoviruses reported to the World Health Organization.

Adenoviruses also cause respiratory disease in adults and probably account for about 3% of such illnesses. The disease is usually mild, but Ad4 and Ad7 have caused epidemics of more serious respiratory illness in military recruits. Such epidemics of acute respiratory disease have resulted in the infection of 80% of the recruits in a unit and 20–40% of these have required hospitalization.

Adenoviruses are being developed as vectors to immunize people against other viruses and for gene therapy.

The main properties of Adenoviruses

1. Naked icosahedral capsid. Diameter 70–90 nm;
2. Capsid consists of 252 capsomeres: 240 hexons; 12 pentons with fibers associated with each penton. Knobbed fibers protruding from each of 12 vertices. Eleven proteins in virion;
3. Linear, double-stranded DNA, 30–36 kb; encoding 30 genes.
4. Fifty human serotypes in six subgroups (A through F). Other adenoviruses in cattle, mice, birds, etc.
5. Diseaes: respiratory syndromes including pneumonia, but not common colds. Eye and gastrointestinal infections.
6. Some adenoviruses can induce cancer in experimental animals but not in humans.
7. Adenoviruses are widely used as gene therapy and anticancer virus vectors.

Virological diagnostics for adenovirus infection

1. Nasopharyngeal and conjunctival washes and scrapings, feces, urine, biopsy and autopsy are used as a material.
2. Fast methods include viral antigens and DNA detection in the material: usually FAT or ELISA in situ are used.
3. Virus isolation:different epithelial cell lines (HEK, HELA, A-549) are used. Characteristic CPE includes: small cell degeneration with cell agglomeration (grape like); cell rounding; cytoplasmic and nuclear inclusions; cells death; Virus identification is performed by NT, FAT, CFT; PCR;EM and IEM.
4. Retrospective diagnostics (for epidemiological purposes) includes ELISA, HIT, CFT in paired sera.

PRACTICAL WORK

Protocol 1: Laboratory diagnostics of herpesvirus and adenovirus infection

Background

On the practical course the students are studying the Herpesviruses and Adenoviruses classification, its morphological structure, the cultivation, the particularity of reproduction; the role of Adenoviruses and Herpesviruses in the human pathology, the pathogenesis and immunogenesis of diseases; methods of laboratory diagnostics of Adenoviruses and Herpesviruses infection; principles of treatment, prophylaxis of Adenoviruses and Herpesviruses infection; are studying the formation of symplast caused by the Herpesviruses; are assessing the indirect hemagglutination reaction performed with the purpose of serological diagnostics of adenoviruses infections; are learning the medications used for the diagnostic and specific prophylaxis of Adenoviruses

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infections and Herpesviruses. The completed tasks students are noting to the protocol and afterwards present the protocol to the teacher for the signature obtaining.

Objective: To learn the cytopathic effect caused by herpesvisuses and adenoviruses. To sassess the indirect hemagglutination reaction performed with the purpose of serological diagnostics of adenoviruses infections (determine the virus titer).

Protocol 1: Describe the morphology of non-infected cells (control) and the cytopathic effect of human herpesvirus type 1 on demonstration microscopic slides (Fig. 8.1 and Fig. 8.2).

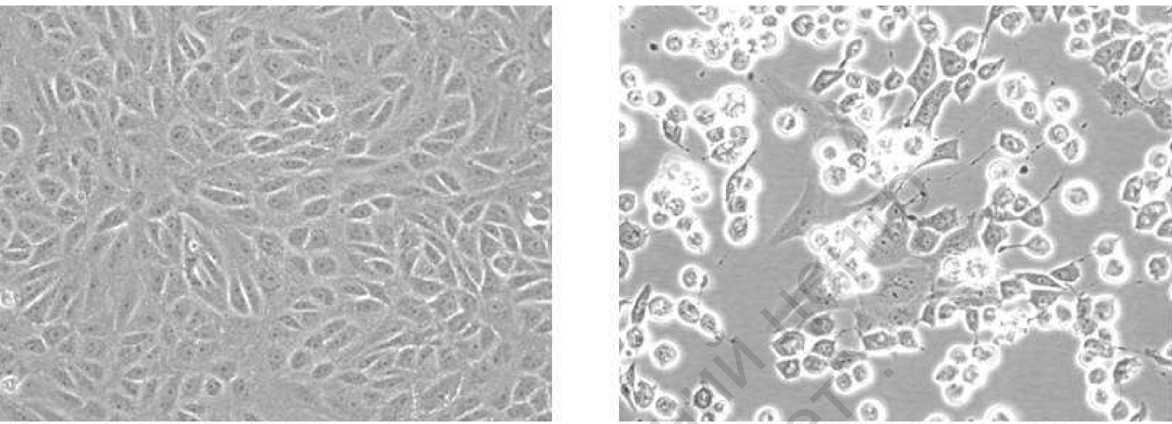

Fig. 8.1. Uninfected cells culture. Infected cell culture showing CPE

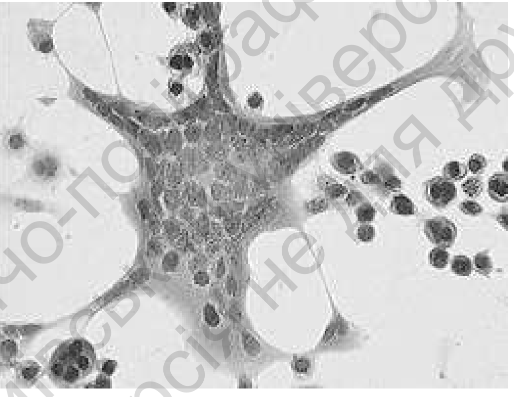

Fig 8.2. Infected cell culture showing CPE

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Describe the morphology of non-infected cells (control) and the cytopathic effect of human adenovirus type 1 on demonstration microscopic slides (Fig. 8.3).

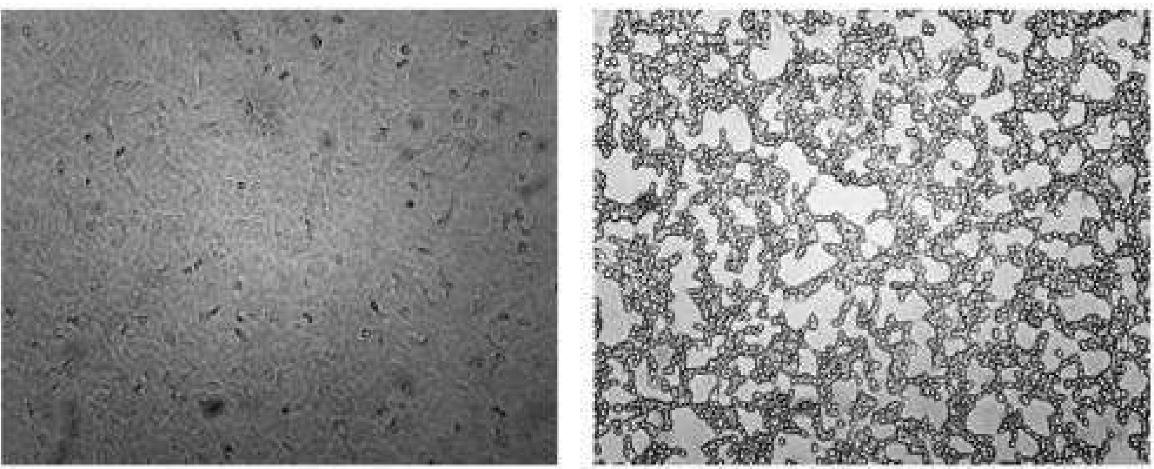

Fig. 8.3. Uninfected cells culture. Infected cell culture showing CPE

Protocol 2. To assess the results of passive hemagglutination reaction performed with the purpose of serological diagnostics of adenoviruses infections.

	1:20	1:40	1:80	1:160	1:320	C (ED)
Firs serum (w/d 1:10)						
Second serum (w/d 1:10)						

Comments: w/d – the working dilution
C (ED) – the control of erythrocytic diagnosticum
Please make the conclusion.
T=

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Independent work

1. Name taxonomy of the smallpox, herpes simplex, varicella-zoster, Epstein-Barr viruses (0,05 point).

	Smallpox virus	Herpes simplex virus	Varicella-zoster viruss	Epstein-Barr virus	Cytomegalo virus
Family					
Genus					

2. Fill in the table. Classification of human herpesviruses (0,15 point).

Species		Subfamily	Cytopathology	Site of latent infection
Official name	**Common name**			

Name morphological properties of the viruses.

	Herpes simplex virus	Varicella-zoster viruss	Epstein-Barr virus
Genome			
Shape			
Size of virion			
Site of synthesis of ribonucleoprotein			
Antigenic stability			

3. Fill in the table. Virological investigations of the chickenpox (varicella), zoster, herpes simplex, infectious mononucleosis (0,1 point).

	Specimens	Object for cultivation of the virus	Indication of the virus	Identification of the virus
Chickenpox (varicella)				
Herpes simplex				
Zoster				
Infectious mononucleosis				

a. Name taxonomy of the adenoviruses. (0,1 point)

	Adenovirus	Specimens
Family		
Genus		

Fill in the table. Virological investigations of the adenovirus infections.

	Specimens	Object for cultivation of the virus	Indication of the virus	Identification of the virus
Adenovirus infection				

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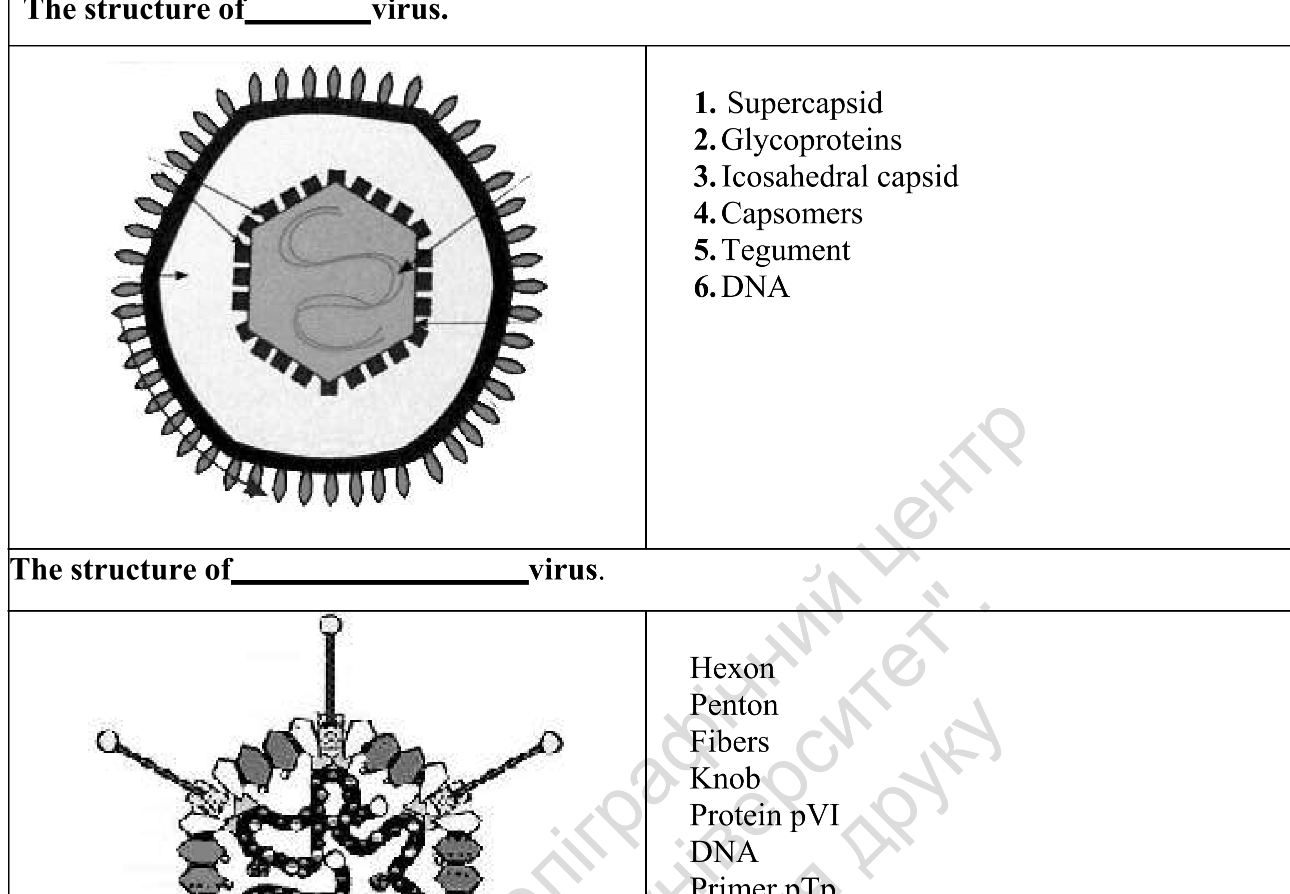
b. Write in virus family name, indicate respective structural elements of virion (0,1 point)

The structure of________virus.	
	**1.** Supercapsid **2.** Glycoproteins **3.** Icosahedral capsid **4.** Capsomers **5.** Tegument **6.** DNA
The structure of___________________virus.	
	Hexon Penton Fibers Knob Protein pVI DNA Primer pTp Protease p23

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Questions for the self-control:

1. The general characteristic of DNA viruses, its classification.
2. The morphology and particularity of reproduction of Herpesviruses and Adenoviruses.
3. Cultivation.
4. The pathogenesis, clinical symptoms and immunogenesis of Herpesviruses and Adenoviruses infection.
5. The principles and methods of laboratory diagnostics of Herpesviruses and Adenoviruses infection.
6. The principles of treatment and prophylaxis of adenoviruses and herpesviruses infections.
7. Herpesviridae family, biological properties, the value in the development of human pathology. Laboratory diagnosis of diseases. Genetic methods for diagnosis.
8. Adenoviridae family. Biological properties. Antigenic structure. Cultivation. Pathogenesis and laboratory diagnosis of infection caused by adenoviruses. Immunity. Specific prevention.

Score _________ Tutor signature___________________________

Навчальне видання

МІКРОБІОЛОГІЯ, ІМУНОЛОГІЯ, ВІРУСОЛОГІЯ

Робочий зошит

Частина 2: Вірусологія

(Англійською мовою)

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