Ministry of Public Health of Ukraine

Bogomolets National Medical University

Department of microbiology, virology and immunology

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STUDY GUIDE

OF THE PRACTICAL CLASSES COURSE

Part III

Specialties:

221 "Dentistry"

222 "Medicine"

225 "Medical Psychology"

226 "Pharmacy, industrial pharmacy"

228 "Pediatrics"

Kyiv – 2020

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Study guide of the practical classes was approved by cycle methodical commission on hygienic disciplines of the Bogomolets National Medical University on September 23, 2020.

CONTENTS:

Class №16

Topic: "Morphology and ultrastructure of viruses. Cultivation of viruses"

Actuality:

Viruses are the etiologic cause of a large group of infectious diseases that are characterized by various clinical manifestations and course, are highly contagious and can cause epidemics and pandemics. Today Virology develops intensively as one of biomedical sciences, although recently it has a narrow branch of science.

The importance of Virology is stipulanted for the leading role of viruses in human infectious diseases. Their impotance increases due to decreasing morbidity of bacterial, fungal and protozoal infections with limited ways of specific chemotherapy. Secondly, models of viruses as the simplest organized form of life uses for solving many fundamental questions of biology and molecular genetics (e.g., the doctrine of nirones, splicing, oncogenes). In modern science one of the most important contributions of Virology is the discovery of reverse transcriptase, the basis of genetic engineering.

The knowledge of the topic is necessary for understanding the pathogenesis of viral infections are studied at clinical departments. In physician's practice this is an important moment for the clinical and morphological differentiation of viral and other infections. At the practical lesson the students are able to master the methods of cultivation in cell culture, cultivation with virus-containing cell material. This is necessary for formation of the idea about methods of diagnose viral infections.

Specific objectives:

- To interpret the morphology and ultrastructure of viruses.
- To analyze the features of the interaction of viruses with living systems.
- To evaluate the results of the multiplication of viruses in living systems
- To analyze the methods of viral cultivation in the laboratory.

Basic knowledge, skills, experience needed for study of topic (interdisciplinary integration)

Names of the previous disciplines	Skills
Human anatomy	To analyze information about the structure of the human body and its organs and systems
Histology, cytology, embryology	To interpret the microscopic and submicroscopic structure of cells
Medical and Biological Physics	To interpret common physical and biophysical laws that underlie the biological processes
Medical biology	To explain the laws of biological processes with molecular-biology and cellular levels.
Medicinal chemistry	To interpret common physical and chemical laws that underlie cell development.

Enumeration of basic terms, parameters, characteristics that student has to learn for the lesson:

Term	Definitions
Virus	Virus is a of non-cellular form of organisms that is characterized by small size, lack of protein synthesis and energosynthesis, as well as obligate intracellular parasitism. Viruses exist in two qualitatively different forms: overcellular – virion and intracellular - virus.
Nucleocapsid	The protein sheath (capsid) that surrounds a single molecule of nucleic acid (DNA or RNA) and forms together the nucleocapsid. Capsid is composed of protein subunits, which are called capsomere.
Supercapsid	Supercapsid (peplos) is the external envelope of complex viruses that contain lipids, are penetrated glycoproteins as specific viral proteins. Peplomer is a structural subunit of supercapsid.
Productive infection	Type of interaction between virus and cells when the virus functions autonomously within the cell and its reproduction is independent of the reproduction of the cell's genetic apparatus. In this new generation the viruses are formed.
Abortive type of interaction between virus and cells	Abortive type of interaction between virus and cell take place when the reproduction of viruses is blocked at one stage, but infectious virions are not formed.
Integrative type of interaction between a virus and cells	Integrativny type of interaction between a virus and a cells occurs when the oncogenic RNA- and DNA-genomic viruses interact with the cell, and the nucleic acid integrates into the cellular chromosome and exists a provirus. As a result transformation of the hereditary characteristics of cells can occur. This process of combination of viral nucleic acid and the host`s cell chromosome is named virogeniya.
Defective viruses	Defective virus - is viruses lost part of its genome in the process of reproduction. They are divided into 4 groups: defective interfering particles, the integrated genomes of viruses, satellites and pseudovirions.

Theoretical questions for the lesson:

- The modern definition of viruses.
- Modern views of the viruses's nature and origin. Place of viruses in the system of all living.
- The history of discovery and the main stages of Virology. The contribution of scientists of our country.
- Classification of viruses. The main features of human and animal viruses.
- Morphology and ultrastructure of viruses. Symmetry types of viruses. Chemical composition, functuion of compound parts of viruses.

- Reproduction of viruses. Stages and types of interaction between viruses and sensitive cells.
- Bacteriophage, the history of the study. The structure, classification of phages in morphology. Methods of qualitative and quantitative determination of bacteriophages. The practical use of bacteriophages.
- Forms of interaction between bacteriophages and bacterial cell. Virulent and moderate phages. Characteristics of productive interaction. Lysogenicity and phage conversion

Practical tasks performed in the classroom:

- To study the morphological and structural features of viruses, their interaction with cells.
- To describe the methods of viral cultivation.
- To investigate the cellular cultures, their using in virology, as well as culture media for growing cells.
- To infect the cellular cultures by virus-containing material. Methods of preparing of material for virological investigation.

Content of the topic:

At the practical lesson the students become study the methods of material collection, processing and transportation of material for virological investigation, preparation of material for the infection of living systems, study the samples of culture media, solutions and serums used for cell cultures, study the different types of cell cultures which are used in virology with demonstration; compare the methods of viral cultivation in different sensitive systems (cell cultures, chick embryos, sensitive laboratory animals); infect the cell culture with virus-containing material, infect the chick embryos and laboratory animals. The student writes down the results of completed tasks and teacher signs it.

Recommendations for the registration of protocol

Classification of cell cultures and tissues that used in virology

1. Tissue or organ culture			
Embryonic tissue organ or part of it that are supported in vitro and retain the cell differentiation and their functions			
2. Cell cultures			
Descriptions	**Primary trypsinized cultures**	**Transplanted cell cultures**	**Diploid cell cultures**
Morphology of cells compared with the original tissue	Don’t differ	Differ	Differ
Set of chromosomes	Diploid	Heteroploid	Diploid
Lifetime	Limited by 1-3 passages	Unlimited by the amount of passages	Limited by 20-100 passages
Growth in suspension	Impossible	Possible	Impossible

Signs of malignancy	Absent	Always present	Absent
Period of generation	3-7 days	2/3-1 days	1-15 days
Contact inhibition by growning on glass	Present	Absent	Present
Examples	1. Culture of monkey kidney cells 2. Fibroblast cell culture of human embryos 3. Cell culture of chicken embryo fibroblasts	1.HeLa (carcinoma of the cervix) 2. KB (oral humah carcinoma) 3.HEp-2 (human larynx carcinoma) 4. Vero (green monkey kidney)	Fibroblast cell lines of human embryo (WI-38, MRC-5, MRC-9, IMR-90), cows, pigs, sheeps

Classification of culture media for cell cultures

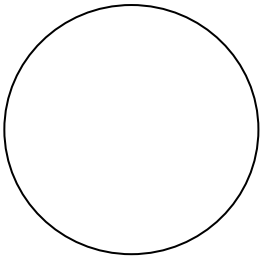
Natural	**Enzymatic hydrolysates**	**Synthetic**
Enders medium: 85-90% cow amniotic fluid, 5-10% of cow embryo extract, 5% horse serum	Aminopeptide Hemohidrolizate lactalbumin hydrolyzate	**1.** Balanced saline solutions: Earle"s solution, Hank"s solution, Dulbekko and Vogt solution **2.** Supported medium: 199 medium, Igl medium, IMMD (Igl medium modified by Dulbekko), MMI (Miller-Igl medium)

Used in the virology media for cell culture are divided into two main categories - **growth and support.**

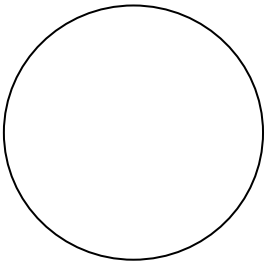
Growth media (GM), due to high content of serum are favourable for rapid cell growth. After the formation of a monolayer and before inoculation of virus growth the medium is removed and replaced with support medium. For preparing of the growth medium bovine serum (BS) or embrio calf serum (ECS) and antibiotics (penicillin and streptomycin) are added to the culture medium (e.g. IMMD), 5-10%.

Support media (SM) with low serum content can save the cell culture in the state of persistent slow metabolism during viral replication.

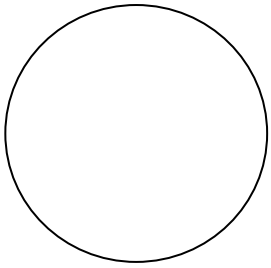
Task 1. To study different types of cell cultures used in virology with the demonstration preparations, paint in the protocol.



Primary trypsinized



Transplanted



Diploid

Task 2. To study technique of infecting of cell cultures by virus-containing material.

Questions for self-control

- What signs are basic of modern classification of viruses?
- The property of all living is the ability to reproduce. Viruses have one type of reproduction. How is it called? What is its essence?
- What special features of reproductions of RNA and DNA - genomic viruses? What is the role of reverse transcriptase in retroviruses reproduction?
- Viruses are very persistent in the environment, to the action of sterilizing and disinfecting agents. What features of virus's structure form this property and play favorable role in the development of viral epidemics and pandemics?
- Laboratory animals are primary models for the virus's cultivation were. Name the benefits and limitations of their using.
- Viruses can be obtained in large quantities relatively easy with one of the methods of cultivation. Which method of virus's cultivation is it? Why it can"t be considered universal?
- Viruses are specific parasites of human cells, animals and plants. How is this feature used for cultivation of viruses in vitro?
- What is the difference between moderate and virulent bacteriophages? What is their practical using?

Class №17

Topic: “The cultivation of viruses. Indication of viral reproduction”

Actuality:

In most cases the diagnosis of viral infections is based on the isolation of virus from infectious material and subsequent identify. The correct collecting of material for research, timely delivery to the laboratory with correct preservtion conditions, correct choice of methods of viruses’s cultivation and identification enable for timely diagnosis of viral diseases.

The purpose of this lesson is to study indication of viral reproduction. To achieve this purpose, it is necessary to be able to identify viruses in different sensory systems (cell cultures, chick embryos, sensitive laboratory animals); distinguish different types of CPA in cell culture, to know the method of making plaques formation test under agar and bentonite layer; perform consideration "color test" which is maked for indication of viruses in cell culture; account haemagglutination test and reaction of gemadsorbtion.

In practice the students may learn different methods of indication of viruses, learn the received results interpretation.

Specific objectives:

- To interpret morphology and ultrastructure of viruses.
- To analyze features of virus interaction in living systems.
- To evaluate the results of viruses’ multiplication in living systems.
- To analyze laboratory methods of viruses’s cultivation in the.

Basic knowledge, skills, experiences needed for study of topic (interdisciplinary integration). See a class №16.

A list of key terms, parameters, characteristics that student should learn for the class:

Term	Significance
Cytopathic effect of the virus (CPE)	CPE are visible under the microscope, morphological changes of cells resulting in intracellular virus reproduction.
Inclusion	Inclusios are accumulation of virions or their individual components in the cell cytoplasm or nucleus that are revealed under the microscope with a special coloring. Variola virus forms cytoplasmic inclusion - Guarnieri bodies. Herpes viruses and adenoviruses form intranuclear inclusions.
Plaques	Plaques are limited areas of damaged by virus’s cells that were cultivated under nutrient agar medium at bentonite. They are visible as bright spots among stained living cells. One virion forms a single plaque.
Hemagglutination test	Hemagglutination test is based on the ability of some viruses to cause agglutination of erythrocytes by viral glycoprotein spikes - hemagglutinins.

Reaction of hemadsorbtion	Reaction of hemadsorbtion - the ability of infected by viruses culture cells to adsorb red blood cells on the surface.
Color reaction	Color reaction is appraised by color change indicator, which is in the nutrient culture medium. If viruses do not multiply in cell culture, the living cells secrete acid metabolism products and change pH of medium and therefore the color of indicator change. Normal metabolism of cells is disturbed during viral reproduction, as a result the cells die, but medium preserves its original color.

Theoretical questions for the lesson:

- Methods of the study of viruses and their estimation.
- Features of the collection, processing and transportation of material for virological research.
- Methods of preparing virus-containing material and techniques of infection sensitive to virus infective systems.
- Methods of viruses' cultivation and their estimation.
- Viral hemagglutination test (HT) and hemadsorption (HAT) test. The mechanism, practical importance, using, diagnostic significance.
- Cytopathic action of viruses (CPA). CPA classification of the viruses.

Practical tasks performed in the classroom:

- To make hemagglutination test to detect the virus in alantoic fluid of chicken embryo, to determine the titer of the virus.
- To identify virus reproduction in cell cultures by cytopathic effect (CPE), form the plaques, "color" reaction, reaction of hemadsorbtion.
- To examine different types of CPA of the virus.

Content of the topic:

At the practical class the students learn the methods of virus detection in different sensitive systems (cell cultures, chick embryos, sensitive laboratory animals); learn to detect the virus in cell culture by cytopathic action, different types of CPA in cell culture; study methodology of plaque reaction under the agar and bentonite covers; studying viral plaques in cell culture under agar and bentonite covers; perform accounting "color test" maked to indicate viruses in cell culture; perform account of hemadsorbtion test for virus indication in cell culture; perform account of hemagglutination test for indication of viruses in chorion allantois fluid of chicken embryo. The students write down the results of completed tasks and teacher signs it.

Recommendations for the registration of protocol.

Identification of the cytopathic action of viruses in cell culture.

Cytopathic effect is degenerative cell changes that occur as a result of a virus reproduction in the cells.

Types of cytopathic effect		Viruses that cause cytopathic effect
Complete degeneration		Poliovirus Coxsackie viruses ECHO viruses
Partial degeneration	**Type of grapes form**	Adenoviruses
	Type of focal destruction	Smallpox virus Influenza virus
	Type of symplast generating	Measles virus Mumps virus Parainfluenza virus RS-virus Herpes simplex virus
Proliferation		Oncogenice viruses

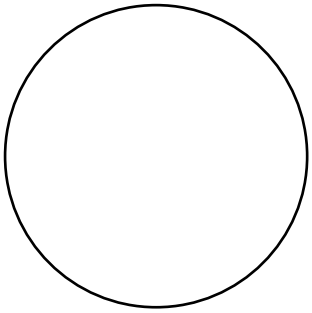
Appearance of intranuclear inclusions is detected by staining by Romanovsky-Giemsa, with fluorescent microscopy, electron microscopy.

Plaque reacts under the agar and bentonite

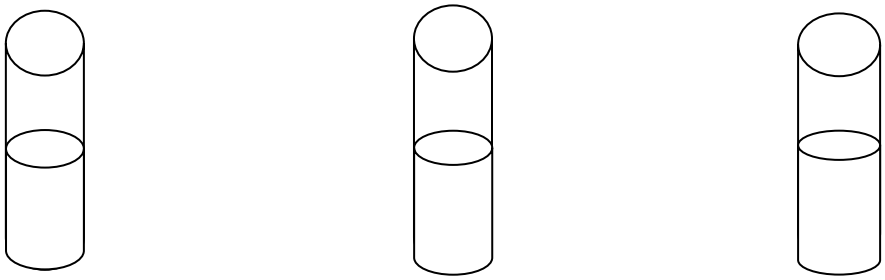
Hemagglutination test.

Positive ***Negative***

Reaction of haemadsorbtion.



"Color" test



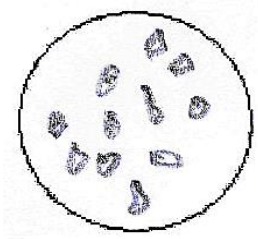
Control *Positive* *Negative*

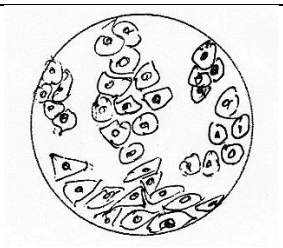
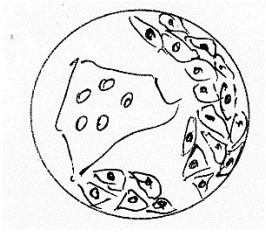
"Color" test. As a result of vital cell activity acid products accumulate in nutrient medium (NM), which change the nutrient medium pH (yellow). The cell metabolism inhibites and pH does not change when cell culture is infected by cytopathogenic viruses (the medium remains red).

Identification of virus replication in chicken embryos

Part of embrio	**Viruses**	**Features of multiplication**
Yolk-sac	Herpes simplex viruses	Death
Chorion	Herpes simplex viruses Poxviruses Rous sarcoma virus	Plaques
Alantoyis	Influenza virus	Retard of growing and capillarotoxicosis. Hemagglutination.
	Mumps virus	Death
Amnion	Influenza virus	Hemagglutination
	Mumps virus	Death

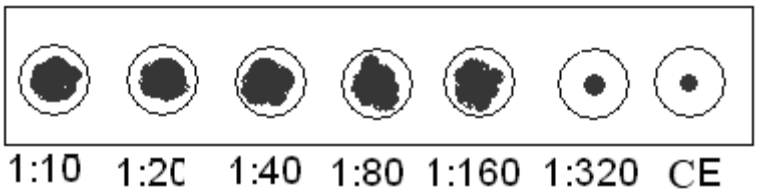
Task 1. To **s**tudy different types of virus CPE with demonstration preparations.

Type of CPE	**Viruses that cause CPE**	**Type CPE**
Complete degeneration	Polioviruses, Coxsackie viruses, ECHO viruses	 Complete degeneration

Type as grapes-like clusters	Adenoviruses	 CPE type as grapes form
Type of symplast generating (giant cells or syncytia)	Measles virus, mumps virus, parainfluenza virus, RS virus, herpes virus.	 CPE as symplast generating

Task 2. To make account of hemagglutination test for revealing influenza viruses in chorion-alantoyis fluid.

HA is agglutination of erythrocytes under the action of viruses.



Dilutions of virus-containing material

***CE- control of erythrocytes.**

Questions for self-control

- Support medium turbidited after infection of cell cultures and incubation in the thermostat. What does it mean?
- What are the biological features of the virus reproduction in the chicken embryos?
- When we can use hemagglutination test (HA) for virus indication? How is it performing?
- What are the signs of virus reproduction in laboratory animals?
- Large multinuclear cells can be revealed during cultivating of measles virus in cell culture. How can such changes be called in infected cells? What other types of CPE can be formed in cell culture?
- Can viruses multiply in cell culture without the appearance of CPE? If so, how to detect this type of reproduction?

Class №18

Topic: “Serological reactions in virology”

Actuality:

Serological identification of viral antigens with standard diagnostic sera and serological diagnosis of viral diseases base on the detection of antibodies in the sera with standard antigens as diagnosticums. It is the main direction of research in virological and immunological laboratories. The knowledge of the serology and understanding the principles and features of performing serological tests are the necessary for effective laboratory diagnosis of viral diseases and, consequently, the effective for its treatment. These data demonstrate the actuality of the topic at this practical class and direct to form positive motivation for stidy.

Specific objectives:

- To learn the laws of immunity to viral infections.
- To learn the methods of serological tests used in virology.

Basic knowledge, skills, experiences needed for study of topic (interdisciplinary integration). See a class №16.

A list of key terms, parameters, characteristics that student should learn for the class:

Term	Significance
Complement	Complement is a complex of serum proteins activated by antigen-antibody complex and other factors, and release of membrane-attack enzymes. It provides nonspecific protection against heterogenous agents with cellular origin.
Diagnostic sera	Diagnostic sera are blood products of animals (rabbits, sheeps, horses, etc.) conteining high levels of specific antibodies.
Monoclonal antibodies	Monoclonal antibodies are the preparations of antibodies highly specific to one of antigenic determinants. They are derived from one clone of cells-producers in vitro.
Diagnosticum	Diagnosticum is the diagnostic preparation containing antigenes.
Hemolytic serum	Hemolytic serum is a serum containing antibodies as hemolysins. It is obtained by 3-4 single intravenous immunization of rabbits by 50% suspension of sheep erythrocytes and others and by next inactivation at 56 º C later.

Theoretical questions for the lesson:

- Features of serological tests used in virology
- Method of paired sera.
- Features of viral diagnosticums.
- Complement fixation test reaction and feature of its using in virology.

- Reactions used in virology exclusively - the Inhibition hemagglutination test and hemadsorption inhibition test, virus neutralizatoin test.

Practical tasks performed in the classroom:

- To perfom the reaction of hemagglutination inhibition test for serological diagnosis of viral infections, evaluate the result of reactions and make conclusions.
- To learn the scheme of reaction and make account virus neitralization test for serological identification of the virus.
- To learn the scheme and make account of complement fixation test (CFT) for serological diagnosis of viral diseases. To substantiate a conclusion.
- To evaluate the result of Indirect hemagglutination test for serological diagnosis of viral infection.

Content of the topic:

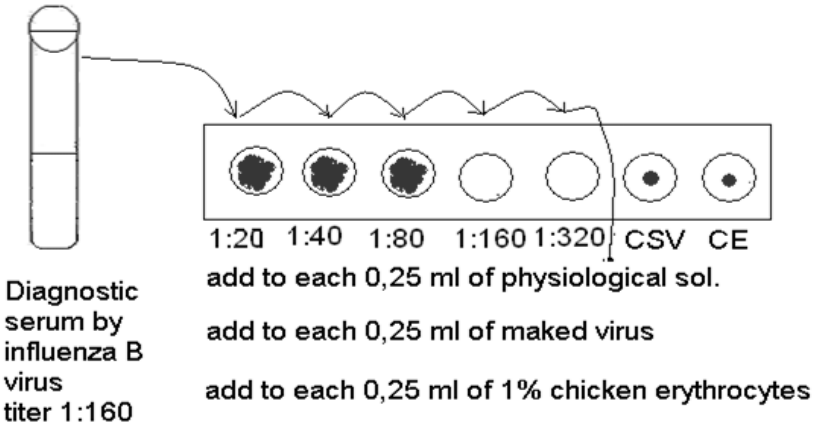
At practical class the students learn laws of immunity of viral infections, the role of humoral and cellular mechanisms take part in the formation of immunity and antigenic structure of viruses, the methodology of perfoming and principles of accounting the serological tests used in virology: hemagglutination test, hemagglutination inhibition test, complement fixation test, virus neutralization tests. They perform accounting hemagglutination inhibition test used for serological identification and diagnosis of viral infections. They take into account the complement fixation test - to detect antiviral antibodies, virus neutralization test - to identify viruses. The students write down the results of completed tasks and teacher signs it.

Recommendations for the registration of protocol

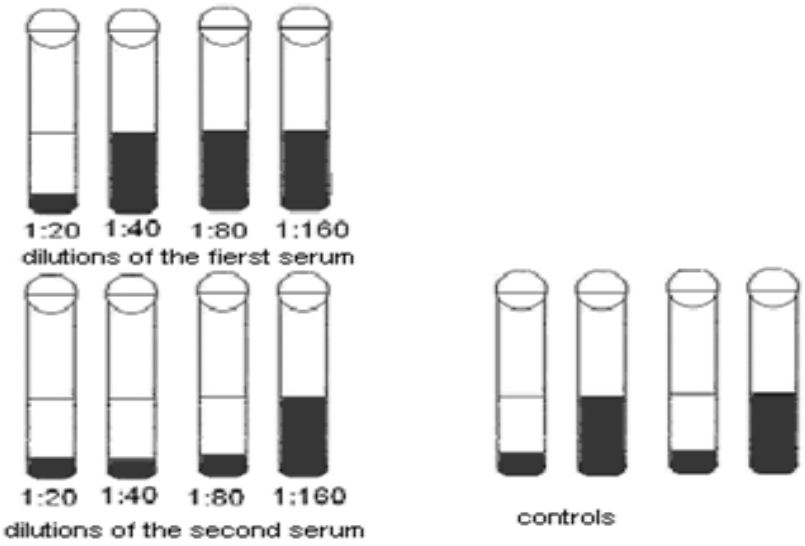
Features of serological diagnosis of viral diseases

1. Antibodies are detected in paired sera, the first serum was taken at the initial stage of the disease, the second - after 2 weeks.
2. Sera are eliminated from viral inhibitors.
3. The reaction is considered positive if the titer of antibodies in the second serum increases in 4 or more times.

Scheme hemagglutination inhibition test (HIT) for serological identification of influenza viruses

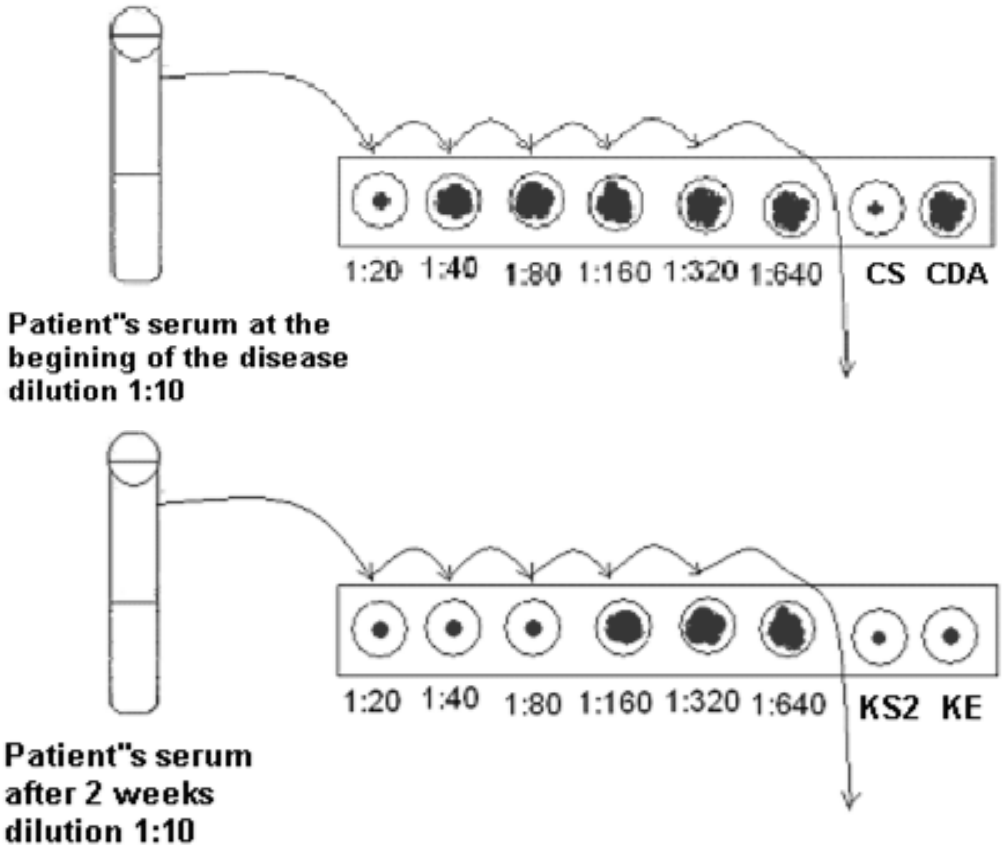


Scheme of CFT in viral infections



The content of tubes: 1-4 – dilutions of investigated serum (serum + antigen + complement), 5 - control of antigen to anticomplementation (antigen + complement + physiological solution + hemolytic system), 6 - control of antigen to hemotoxigenicity (antigen + physiological solution + hemolytic system), 7-8 antigen control with normal tissue to hemotoxigenicity and anticomplementation.

Scheme of hemagglutination inhibition test (HIT) for serological diagnosis of influenza



Questions for self-control

- What biological models can be used for virus neutralization test?
- What are the features of serological diagnosis of viral diseases?
- What is the purpose of collecting paired serum for serological diagnosis of viral infections?
- Features of performing complement fixation test in viral infections.
- What is hemolytic serum and how is it obtained.
- What is hemolytic system?
- What additional controls are to be performed in complement fixation test?

Class №19

Topic: “Orthomyxoviruses. Laboratory diagnosis of influenza”

Actuality:

Through the wide spreading and high levels of morbidity, influenza and acute respiratory infections continue to be the actual problem of Ukraine's health system. More than 13-20 million people suffer from these diseases every year that is over 90% of all registered infections.

The peculiarities of the structure and genetics of influenza viruses, their wide spread not only among people but also among animals, the ability of intense variability and as a result severe epidemics and pandemics determine scientific and practical importance of all problems related to influenza. Due to new environmental and socio-economic conditions, environmental pollution, global warming range of natural hosts of influenza agents and contacts between them are changing; the basis for the emergence of new types of influenza viruses form. There is real threat of the emergence of pandemic virus strain due to recombination of influenza viruses of human and birds. The consequences of such pandemic could be catastrophic on a global scale. All this testifies about actuality of the topic and directs to form of positive motivation for learning.

Specific objectives:

- Learn the biological properties of influenza viruses.
- Learn the techniques of virological and serological diagnosis of influenza.
- Analyze basic modern principles of the treatment and prevention of influenza.

Basic knowledge, practical skills, experiences needed for study of topic (interdisciplinary integration). See a class №16.

A list of key terms, parameters, characteristics that student should learn for the class:

Term	Significance
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 is changed.

Theoretical questions for the lesson:

- 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.
- 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 diagnosis of influenza.
- Specific prevention and treatment of influenza.

Practical tasks performed in the classroom:

- To learn the scheme of virological diagnosis of influenza. To take into account Henagglutination test to study and determine virus titer, as well as Hemagglutonation Inhibition test for serological identification of influenza viruses. To make a conclusion.
- To perform Hemagglutonation Inhibition test for serological diagnosis of influenza, take into account the results and make conclusion.
- To learn diagnostic, prophylactic and therapeutic drugs used for influenza treatment.

Content of the topic.

At practical class, the students learn morphological, physical and chemical properties, ultrastructure and antigenic structure of the family *Ortomyxoviridae*, types and mechanisms of antigenic variation.

In preparing the the scheme of laboratory diagnosis the students use the self-training knowledge and practical class knowledge, perform accounting of reactions (determine the presence of virus in the Hemagglutonation test; take into account of Hemagglutonation Inhibition test performed for serological identification of influenza viruses; take in account Hemagglutonation Inhibition test performed for serological diagnosis of

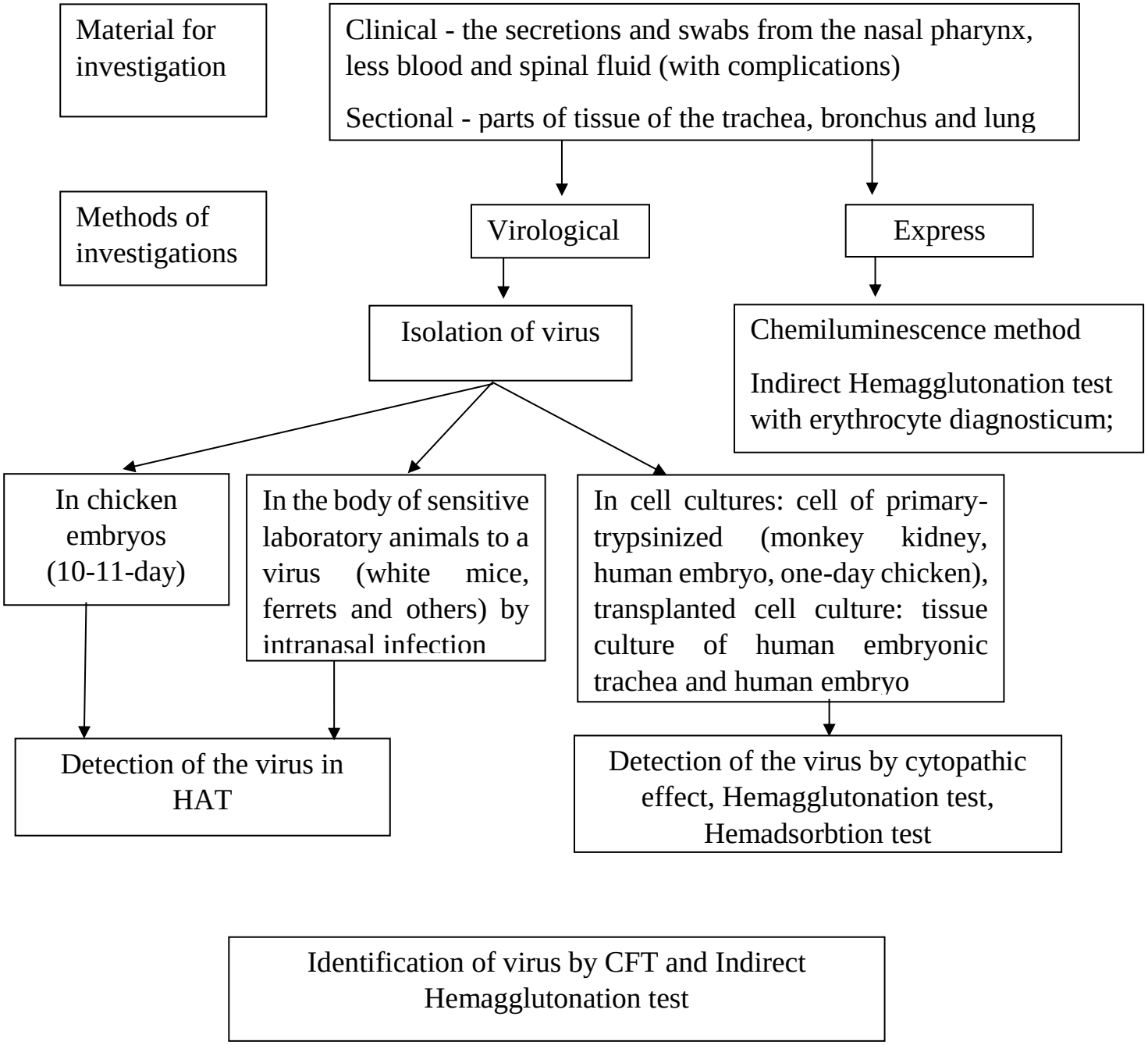
influenza), learn the drugs used for laboratory diagnosis and prevention of influenza: influenza diagnostics, diagnostic sera, vaccines: live, inactivated (of whole virions), chemical (split vaccines, subunit vaccines), normal human immunoglobulin, various types of interferon and write to the protocol.

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

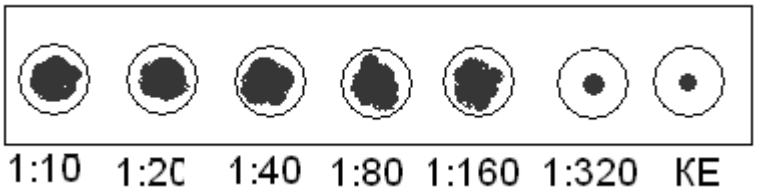
Recommendations for the registration of protocol.

Task 1. To lean the scheme of laboratory diagnosis of influenza.

Scheme of laboratory diagnosis of influenza



Task №2. To use the Indirect Hemagglutonation test to detect the virus with ability of hemagglutination.



Task 3. To use the Indirect Hemagglutonation test for serological identification and serological diagnosis of influenza (See Practical class №18).

Task 4. Learn the preparations for specific prevention of influenza.

1. Live allantois dry vaccine of Influenza virus for intranasal introduction. (vaccines of the 1 generation) (Russia).
2. Purified inactivated split influenza vaccine of "Vaxigryp" (France).
3. Inactivated subunit vaccine "Influvak" (Netherlands).

Questions for self-control.

- What family do influenza viruses belong to?
- What is the morphology of influenza virus?
- What is the strategy of the genome of influenza virus?
- What proteins determine the strain specificity of influenza virus?
- What is the mechanism of antigenic shift of influenza virus?
- What biological systems use for detection of influenza viruses?
- What method of influenza prevention is most expedient in adults?

Class №20

Topic: "Paramyxoviruses. Laboratory diagnosis of measles, parainfluenza and mumps (epidemic parotitis)"

Actuality of topic:

Paramyxoviruses family includes four genuses — *Paramyxovirus, Morbilivirus, Pneumovirus* and *Rubulavirus*.

The viruses of this group are spherical and size 100-300 nm in diameter. These viruses form syncytium in cell cultures and eosinophilic cytoplasmic inclusions and also has haemolytic activity. The nucleoid has spiral type of symmetries and is enclosed in a lipid-carbohydrate-protein membrane. *Paramyxoviruses* causes the red blood cells to clump (hemagglutinate) or adhere to infected tissue cell cultures. According of antigenic properties they are subdivided by varieties. In human's paramyxoviruses cause parainfluenza, epidemic parotitis, measles, and Newcastledisease.

Morbillivirus is a genus belonging to the *Paramyxoviridae* family of viruses in the order *Mononegavirales*. Many members of the genus cause diseases, such as measles in humans, rinderpest in animals and are highly infectious.

The Respiratory Syncytial Virus (RSV) is a virus that causes infections of the lungs and airways. It commonly appears during the coldest and wettest months of the year, similarly to the influenza virus. It is a major cause of lower respiratory tract infections and hospital visits during infancy and childhood. A prophylactic medication (not a vaccine) exists for preterm birth (under 35 weeks gestation) infants and infants with a congenital heart defect (CHD) or bronchopulmonary dysplasia (BPD). Treatment is limited to supportive care, including oxygen therapy.

Specific objectives:

- To learn the biological properties of parainfluenza, epidemic parotitis and measles viruses.
- To learn the techniques of virological and serological diagnosis of parainfluenza, measles and epidemic parotitis.
- To analyze basic modern principles of the treatment and prevention of parainfluenza, measles and epidemic parotitis.

Basic knowledge, practical skills, experiences needed for study of topic (interdisciplinary integration). See Practical class №16.

A list of key terms, parameters, characteristics that student should learn for the class:

Term	Significance
Family *Paramyxoviridae*	These viruses are similar to orthomyxoviruses according of properties: they have an oval or rounded shape, consisting of a nucleocapsid and an inner membrane. The nucleocapsid has a helical symmetry coated with a membrane consisting of a layer of protein M, a double layer of lipids, and an external

	glycoprotein layer that includes hemagglutinin and neuraminidase. At the same time *Paramyxoviridae* differ from *Orthomyxoviridae* larger sizes (120-300 nm), the ability to cause hemolysis, to form in the cell culture of syncytia.
Parainfluenza	Parainfluenza viruses were first isolated by Chanok in 1956. They are complex viruses ranging in size from 120 to 300 nm, oval in shape and consist of a nucleocapsid and an outer membrane. Membrane has numerous spikes, located radially. The nucleocapsid has a helical symmetry coated with a membrane consisting of protein M, a double layer of lipids, and an external glycoprotein layer that includes hemagglutinin and neuraminidase.
Antigenic structure	The antigenic properties of HN, NP and F proteins distinguish 4 major serotypes of parainfluenza virus: VPIHs-1, VPIHs-2, VPIHs-3, VPIHs-4. All serotypes of parainfluenza virus have hemagglutinins. VPIHs-1 and VPIHs-2 agglutinate erythrocytes of guinea pigs, chickens, monkeys and humans. VPIHs-3 agglutinates guinea pigs, monkeys and humans. VPHs-4 clamping only guinea pig erythrocytes.
Virus epidemic parotitis	The mumps virus belongs to *Paramixovirinae* and the genus *Rubulavirus*. It was first isolated from experimental animals on chicken embryos in 1934 by K. Johnson and E. Goodspacher. The virus morphology and molecular biological properties are the same as those of other paramyxoviruses.
Parotid infection ("mumps")	Acute viral infectious disease, characterized mainly by the affection of the parotid glands, rarely - other glandular organs and the nervous system. The incubation period is 2-3 weeks, sometimes 23-25 days/
Measles virus	The measles virus is a family *Paramyxoviridae* and is a representative genus of Morbillivirus. Viral etiology was established by T. Anderson and J. Goldberger, in 1911. In 1954, J. Enders and T. Peeble discovered a virus from a patient on primary-trypsinized monkey kidney cells and humans.
The spots of Belsky - Filatov - Koplik	Rash in the prodromal period of the measles. On the mucous membrane of the cheeks opposite to the small molars, whitish-grayish elements of 1 to 3 mm in size appear, surrounded by a hyperemic border. Like "a grain of salt on a red background." Do not merge with each other, do not remove with a tampon or spatula. After 48-72 hours they disappear without a trace.

Theoretical questions for the lesson:

- General characteristics and classification of *Paramyxoviruses*. Classification of human parainfluenza viruses.

- General characteristics of parainfluenza, measles, epidemic parotitis viruses and RSV: genome structure and characteristics, chemical composition and antigenic structure.
- Resistance and sensitivity of parainfluenza, measles, epidemic parotitis viruses and RSV viruses to physical and chemical factors.
- Methods of cultivation *Paramyxoviruses*.
- Source of infection of parainfluenza, measles, epidemic parotitis viruses and RSV, and mechanism of its transmission.
- Pathogenesis of parainfluenza, measles, epidemic parotitis viruses and RSV.
- Features of laboratory diagnosis of parainfluenza, measles, epidemic parotitis viruses and RSV.
- Specific prevention and treatment of parainfluenza, measles, epidemic parotitis viruses and RSV.

Practical tasks performed in the classroom:

- To learn the scheme of virological diagnosis of parainfluenza, measles, epidemic parotitis viruses and RSV.
- To perform CFT the in paired sera for the serological diagnosis of measles take into account the results and make conclusion.
- To learn diagnostic, prophylactic and therapeutic drugs aused for parainfluenza, measles, epidemic parotitis viruses and RSV.

Content of the topic.

At practical class the students learn classification, morphological, physical and chemical properties, ultrastructure and antigenic structure of the family *Paramyxoviridae*; methods of laboratory diagnosis of parainfluenza, mumps, measles and respiratory syncytial viral infection; principles of treatment, prevention of parainfluenza, mumps, measles and respiratory syncytial viral infection; to perform CFT for the purpose of serological diagnosis of measles in paired sera, create a scheme for laboratory diagnosis of parainfluenza, mumps, measles and respiratory syncytial viral infection, study drugs used for the diagnosis and specific prevention of parathyroiditis, viral infection. Students complete the tasks in the protocol and sign it with the teacher. The students write down the results of completed tasks and teacher signs it.

Recommendations for the registration of protocol.

Paramyxoviridae

Paramyxovirinae	***Pneumovirinae***
Morbillivirus (measles virus)	**RS virus**
Respirovirus (VPIHs-1 and VPIHs-3**)**	
Rubulavirus (VPIHs-2; VPIHs-4 and mumps)	**Metapneumovirus**

Laboratory diagnosis of parainfluenza

1. Express diagnostics

-Ag in nasal passages cells (IT, ELISA)

2. Virological method

wash with nasopharynx

↓

cell culture

↓

IAdsT, CPE

↓

CFT, HAIT, NT

3. Serological method

- Detection of antibodies in paired sera (HAIT, CFT, NT)

Laboratory diagnosis of measles

Serological diagnosis

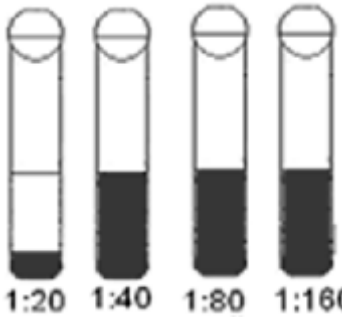
- Using high-sensitivity enzyme-linked immunosorbent assay (ELISA) IgM antibodies are detected in 90% of measles 3 days after the onset of rash.
- The level of IgM antibodies peaks after 7-10 days, then rapidly decreases. Later 6-8 weeks IgM antibodies are very rare.
- The level of Ig G antibodies rises for 4 weeks and persists for a long time after infection (4x increase in titer).
- Serum and secretory IgA antibodies are also produced.

Virological diagnostics

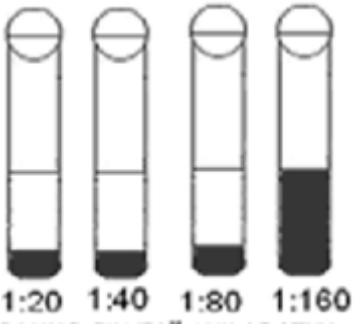
- Is the isolation of the virus from the blood and nasopharyngeal flushing in cell culture?
- Characteristic CPE - the formation of giant multinucleated cells with cytoplasmic inclusions, a week after infection - intra-nuclear inclusions. Staining by Romanovsky-Gimze.
- Hemadsorption test (HAdsT) with monkey erythrocytes.
- IAdsT with serum of immunized animals.
- Neutralization test of CPE, HAIT and ELISA.

Task 1. To perform CFT the in paired sera for the serological diagnosis of measles take into account the results and make conclusion.

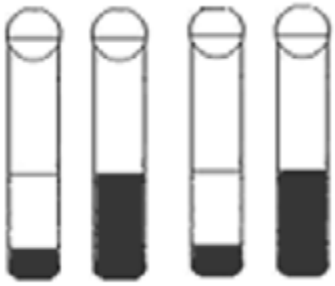
Scheme of CFT for serological diagnosis of measles



Dilution of the first serum



Dilution of the second serum



Control

Task 2. To learn diagnostic, prophylactic and therapeutic drugs aused for parainfluenza, measles, epidemic parotitis viruses and RSV.

Questions for self-control.

- What family belong parainfluenza, measles, epidemic parotitis viruses and RSV?
- What is the morphology of parainfluenza, measles, epidemic parotitis viruses and RSV?
- What is the strategy of the genome of parainfluenza, measles, epidemic parotitis viruses and RSV?
- What proteins determine the strain specificity of parainfluenza, measles, epidemic parotitis viruses and RSV?
- What is the mechanism of pathogenesis of parainfluenza, measles, epidemic parotitis viruses and RSV?
- What biological systems use for detection of parainfluenza, measles, epidemic parotitis viruses and RSV?
- What method of parainfluenza, measles, epidemic parotitis viruses and RSV prevention is most expedient in adults?

Class №21

Topic: "Picornaviruses. Laboratory diagnosis of enteroviruses infections"

Actuality:

Family *Picornovirudae* is a typical representative of RNA-containing simple icosahedral viruses. High resistance to physical and chemical agents is typical for these viruses.

Picornaviruses have a wide range of hosts, a significant diversity of clinical manifestations of the diseases and they are widely spreading in the environment.

The genus of enteroviruses is a major genus of this family with potential pathogenicity for human. Coxsackie, ECHO viruses and poliovirus are included to this genus.

Physicians have to know the biological properties of enteroviruses; pathogenesis, clinical manifestations and immunogenesis of infections, methods of laboratory diagnostics, principles of treatment and prevention of specific infections. All this underlies of actuality of practical class topic and urges to formation of positive motivation for learning.

Specific objectives:

- To analyze the physical and chemical properties of Picornaviruses family generally and genus of enteroviruses particularly.
- To form the scheme of the laboratory diagnostics of enteroviruses infections.
- To identify the ways to compare two cell cultures in the two tubes: in the first unmodified monolayer of cell culture is present, and in the second - cell culture with poliovirus cytopathic effect as complete degeneration is present.
- To use the complement fixation test for serological diagnosis of poliomyelitis.
- Top use virus neutralization test for serological identification of enteroviruses selected from a sick child with suspicion of poliomyelitis.
- To examine the preparations used for diagnosis, specific prevention and therapy of enteroviral infections.

Basic knowledge, skills, experiences needed for study of topic (interdisciplinary integration). See a class №16.

A list of key terms, parameters, characteristics that student should learn for the class:

Term	Significance
Picornoviridae	Picornaviruses are small (24-30 nm in diameter), simple icosahedral RNA-genomic viruses that are highly resistant to physical and chemical factors. Many members of this family are highly pathogenic for humans. According to the modern classification the family consists of 9 genera.

Enterovirus	Enterovirus is a typical genus of *Picornoviridae* characterized by tropism to enterocytes and cells of the nervous system; stability in wide pH range (from 2.0 to 10.0); stabilization of cationic ions Ca^{++}, Mg^{++}, Al^{+++} to thermal inactivation.
Polioviruses	*Polioviruses* are the representatives of Enterovirus genera of human that exist as three serotypes. They are distinguished distinctly and infect the cell by binding with specific receptors PVR: CD 155.
Salk's vaccine	Salk's vaccine is an inactivated poliovaccine for parenteral introduction. Vaccines do not create local immunity.
Sebin's vaccine	Sebin's vaccine - a live poliovaccine made from attenuated strains of three serotypes of polioviruses, oral introduction. It forms general and local immunity.
Coxsackie Viruses	Coxsackie Viruses – enteroviruses were isolated in Coxsackie city of New York State (USA). It is characterized by multiorgan tropism. It"s divided into groups A and B by antigenic structure, cytopathogenic effect of cell culture and formation of paralysis in newborn mice. They are 23 serotypes of Coxsackie A and 6 serotypes of Coxsackie B.
ECHO Viruses	Viruses ECHO - abbreviated name from English words: Enteric - Cytopatogenic – Human – "Orphans". They are 28 serotypes.

Theoretical questions for the lesson:

- 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.

Practical tasks performed in the classroom:

- Form a scheme of the laboratory diagnosis of enteroviruses infections.
- Take account of the results of serological and virological diagnosis of poliomyelitis (demonstration).
- Study the preparations for diagnosis, specific prevention and therapy of enteroviruses infections.

Content of the topic:

In practice the students study the biological properties of *Picornaviridae* family, *Enterovirus* genera and some of it's 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 and perform serological identification of enteroviruses selected from a ill child with suspicion of poliomyelitis. At the same time the students study the differentiation of "wild" and attenuated strains of polioviruses with PCR, ELISA, NT with monoclonal antibodies.

Bentonite test is used for type I of *Poliovirus* (virulent polioviruses have characteristic as Abent (-) and avirulent as Abent (+).

They accomplish accounting of complement fixation test for serological diagnosis of poliomyelitis. The students write down the results of completed tasks and teacher signs it.

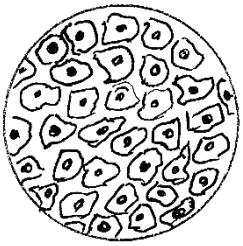
Recommendations for the registration of protocol.

Task 1. The students make up a scheme of laboratory diagnostics of enteroviruses infections and draw it to protocol. under the guidance of teachers,

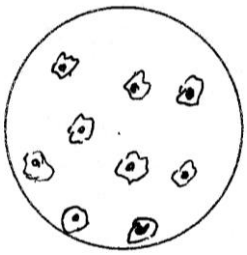
Laboratory diagnosis

- SAMPLES:
  - Throat washing, Stool, Nasal secretion, CSF, Conjunctival swab
- VIRUS ISOLATION
  - Monkey kidney cell line, human embryonic lung fibroblast cell
- NUCLEIC ACID DETECTION
  - Reverse transcriptase PCR
- SEROLOGY
  - Neutralizing antibody appears early in life
  - Immunofluorescence test is done with antibody against infected cell culture

Task 2. To identify the unchanged cells culture (control) on the demonstration preparations and cytopathic effect of poliovirus as type of complete degeneration. Draw a microscopic picture to the protocol.



Unchanged cells culture

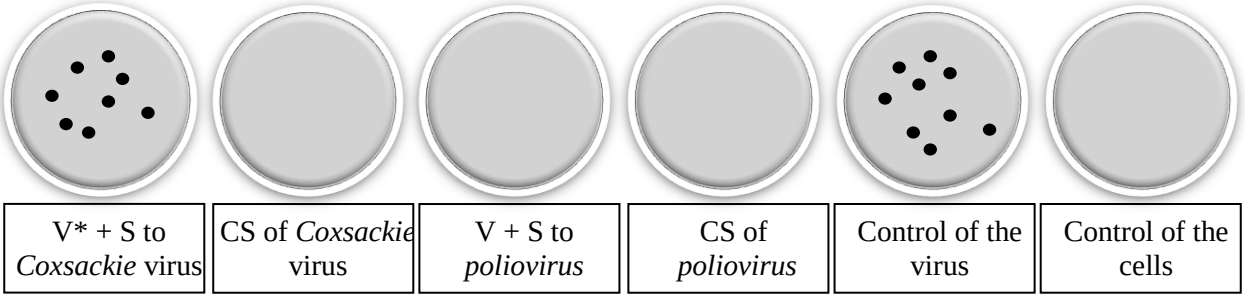


Cytopathic effect of poliovirus

Differentiation of enteroviruses with cytopathic effect

Virus	CE
Poliovirus	+
Coxsackie A	±
Coxsackie B	+
ECHO	+

Task 3. By demonstration preparation to take account of the virus neutralization test for serological identification of viruses isolated from an ill child with suspicion of poliomyelitis.



V* - virus S – serum CS – control of the serum

Task 4. To take account of complement fixation test for serological diagnosis of poliomyelitis.

Task 5. To study the preparation for diagnosis, specific prevention of Enterovirus infections. Students write completed tasks to the protocol.

Questions for self-control.

- What type of nucleic acid does *Picornoviridae* contain?
- What structure do picornoviruses have?
- What are the defining features of *Enterovirus* genera?
- What viruses does genera *Enterovirus* include?
- What is the antigenic structure of enteroviruses?
- Wtat methods of cultivation of *Enterovirus* can be used?
- What are the stages of interaction between enteroviruses and sensitive cells?
- How many serological types of polio viruses are there?
- What is the pathogenesis of poliomyelitis?
- What are the methods of laboratory diagnosis of poliomyelitis?
- What vaccines can be used for prevention of poliomyelitis? What are the positive properties and negative properties of live and inactivated vaccines?
- What are the criteria for differentiation between Coxsackie A viruses and Coxsackie B viruses?

Class №22

Topic: "Herpesviruses, adenoviruses. The laboratory diagnostics of diseases caused by Herpesviruses and Adenoviruses infections"

Topic relevance:

Human pathogenic DNA viruses belong to the six families: *Adenoviridae, Herpesviridae, Parvoviridae, Poxsviridae, Hepadnaviridae, Papovaviridae*.

Compared with the RNA viruses the DNA viruses are more genetically conservative which means less variable and usually persist in the host organism for a long time. The most of DNA viruses are reproducing in the nucleuses of cells.

The representatives of the family *Adenoviridae* are the pathogenic agents of infectious diseases of respiratory tracts and other organs of human, monkeys, cattle, dogs, mice and birds.

Specific objectives:

- To create the scheme of laboratory diagnostic of adenoviruses infections and herpesviruses.
- To assess the indirect hemagglutination reaction performed with the purpose of serological diagnostics of adenoviruses infectious.
- To learn the cytopathic effect caused by herpesvisuses.
- To learn the medication used for the diagnostic and specific prophylaxis of adenoviruses infections and herpesviruses.

Basic knowledge, skills, needed to study topic (interdisciplinary integration). See a class №16.

A list of key terms, parameters, characteristics that student should learn for the class:

The term	The definition
DNA viruses	The human pathogenic viruses which contain the deoxyribonucleic acid (DNA) belong to the six families: *Adenoviridae, Herpesviridae, Parvoviridae, Poxsviridae, Hepadnaviridae, Papovaviridae*.
Herpesviruses	The family Herpesviridae includes the 3 sub-families: *Alphaherpesvirinae*, *Bethaherpesvirinae*, *Gammaherpesvirinae*.
Adenoviruses infections	The family *Adenoviridae* is divided to the two genus: *Mastadenovirus* – mammals' adenoviruses, including more than 40 serovariants caused the human diseases, *Aviadenovirus* – 14 serovariants caused the birds' diseases.

The theoretical questions for the classes:

- The general characteristic of DNA viruses, its classification.

- The morphology and particularity of reproduction of *Herpesviruses* and *Adenoviruses*.
- Cultivation.
- The pathogenesis, clinical symptoms and immunogenesis of *Herpesviruses* and *Adenoviruses* infection.
- The principles and methods of laboratory diagnostics of *Herpesviruses* and *Adenoviruses* infection.
- The principles of treatment and prophylaxis of adenoviruses and herpesviruses infections.

The practical tasks which are being performed during the classes:

- To create the scheme of laboratory diagnostic of *Herpesviruses* and *Adenoviruses* infections.
- To investigate the results of reproduction of *Herpesviruses* and *Adenoviruses* assessing the cytopathic effect.
- To study the scheme of experiment and assess the indirect hemagglutination reaction performed with the purpose of serological diagnostics of adenoviruses infections.
- To familiarize with the diagnostic, treatment-prophylactic medications prescribed for *Herpesviruses* and *Adenoviruses* infection.

The content of theme:

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

Recommendations on the protocol completion

Human Herpesvirus (HHV) classification

Subfamily	Genus	Name	Pathophysiology
Alphaherpesvirinae	*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

Betaherpesvirinae	*Cytomegalo virus*	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)	
Gammaherpesvirinae	*Lymphocrypto virus*	Epstein-Barr virus (EBV), lym phocryptovirus	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*

- Icosahedral symmetry of nucleocapsid;
- Large viruses 150-250 nm in diameter; enveloped with lipid bilayer membrane;
- Linear DNA genomes encoding 100-200 genes; molecular weight 54-94 million daltons;
- Capsid consists of 162 capsomeres: 150 hexons; 12 pentons on the tops;
- Tegument – protein layer called containing both viral proteins and viral mRNAs;
- May persist a long time in the nervous system.

The main properties of *Adenoviruses*.

- Icosahedral symmetry of nucleocapsid;
- Capsid consists of 252 capsomeres: 240 hexons; 12 pentons with fibers associated with each penton;
- Double-stranded linear DNA genome; encoding 30 genes.

Scheme of laboratory diagnostics of *Adenoviruses* infections.

<table>
<tr><td rowspan="3">1.Isolation and identification of virus
→</td><td colspan="2">Samples from the patient
↓ ↓</td></tr>
<tr><td>Human and monkey primary and passaging cell cultures.
↓
5 -14 days
↓ ↓</td><td rowspan="2">Fluorescence rhinocytoscopy</td></tr>
<tr><td>Cytopathic effect (CPE)</td><td>Serological identification
1.Reaction of neutralization;
2.Reaction of inhibition of hemagglutination</td></tr>
<tr><td>2.Serological diagnostics</td><td colspan="2">Pair serums samples of the patient
1. Reaction of neutralization (RN);
2. Complement fixation test (CFt);
3. Reaction of inhibition of hemagglutination;
4. Reaction of indirect hemagglutination.</td></tr>
<tr><td>3. Genetic diagnostics</td><td colspan="2">PCR</td></tr>
</table>

Scheme of laboratory diagnostics of Herpesviruses infections.

Samples which contain viruses

I. Microscopy

a) Detection of intranuclear inclusions (Kaudri bodies) in the swab-impressions;

b) Detection of specific antigene using direct and indirect methods of immunofluorescence.

II. Isolation of virus

↓ ↓ ↓

In the cell culture (assessing CPE) | Chick Embryo. (plaque appearance on the amniochorial surface) | Biological test

↓ ↓ ↓

Identification of isolated virus

↓ ↓

immunofluorescence | reaction of neutralization;

↓ ↓

Diagnosis: herpesvirus infection.

III. Serological diagnostics: detection of antibodies titer increasing in RN, CFT, reaction of inhibition of hemagglutination, reaction of direct hemagglutination.

Medications for herpes virus treatment and prophylaxis:

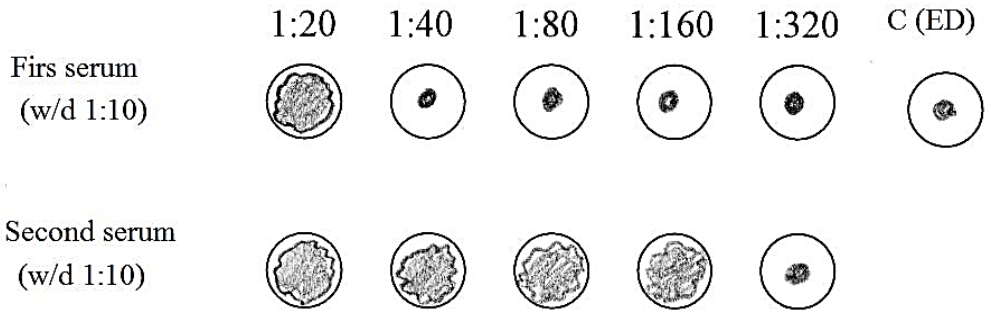
- modified nucleosides which inhibit the virus replication (interferon inductors, acyclovir);
- gamma-globulins against the Varicella zoster virus;

- immunomodulators (Levamisole).
- Vaccines:
- inactivated vaccine of Herpes virus strains of I and II types;
- alive attenuated vaccine against the human cytomegalovirus.

Medications for treatment and prophylaxis of Adenoviruses infections.

- interferon;
- DNAse;
- Oxolin ointment and etc.;
- Alive vaccines from 4,7,21 serotypes (already proposed).

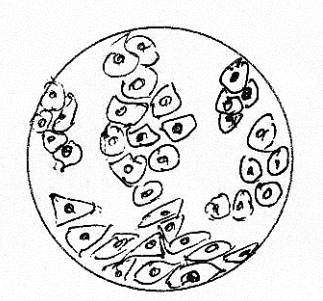
Task 1. To assess the results of passive hemagglutination reaction performed with the purpose of serological diagnostics of adenoviruses infections (the demonstration).



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

Task 2. To learn the changes in cells developed as a result of herpes virus and adenovirus reproduction. Please draw the picture to the protocol.

The symplast formation caused by herpes viruses



Bunch-shaped destruction caused by adenoviruses

Questions for the self-control:

- The general characteristic of DNA viruses, its classification.
- The morphology and particularity of reproduction of Herpesviruses and Adenoviruses.
- Cultivation.

- The pathogenesis, clinical symptoms and immunogenesis of Herpesviruses and Adenoviruses infection.
- The principles and methods of laboratory diagnostics of Herpesviruses and Adenoviruses infection.
- The principles of treatment and prophylaxis of adenoviruses and herpesviruses infections.

Class №23

Topic: “Agents of viral hepatitis. Laboratory diagnostics of viral hepatitis”

Topic relevance:

Viral hepatitis (VH) — group of the diseases, which are caused by different types of viruses, characterized by different mechanisms of transmission of infection and difference in pathogenesis, but due to their tropism to hepatocytes are similar in main clinical symptoms (jaundice, intoxication, hepatomegaly, splenomegaly).

The great increase in incidence of viral hepatitis is marked over the past decade, in particular by parenteral transmission. It is believed that about 1 billion people on the planet infected with at least one of the viruses, which caused viral hepatitis. According to official information VH take second place after influenza and other acute respiratory infections by the number of affected, they greatly outnumbered them by the number of severe, economic costs, resulting in death. And this despite the fact that anicteric forms which are often passed by the doctors. Per one day the VH and their complications caused equal number of deaths to those caused by AIDS per year. To stop VH expansion is one of the World Health Organization priority tasks.

The role in the pathology of liver is currently established for at least 6 viruses (A - E and G). 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.

Knowledge about the characteristics of viral hepatitis causative agents, the ability to choose adequate methods of laboratory diagnosis of viral hepatitis and to interpret the results are necessary for the formation of students' understanding of the methods for diagnosis of viral diseases.

The specific aims:

- To analyze the biological properties of viral hepatitis causative agents.
- To explain the role of causative agents of viral hepatitis in human pathology.
- To treat methods for diagnosis of viral hepatitis, to make conclusions by results.
- To analyze the preparations which are used for specific prophylaxis of viral hepatitis.

The general knowledge, abilities, skills necessary for theme learning (the inter-disciplines integration). See a class №16.

A list of the main terms, parameters, characteristics which should be learnt by the student during the preparation to the classes:

Term	Definition
Viral hepatitis	Viral hepatitis (VH) — a group of diseases caused by different viruses, characterized by different transmission mechanisms and pathogenesis, but all of them are hepatotropic pathogens which can be similar in cause of the

	major clinical symptoms (jaundice, intoxication, hepatosplenomegaly).
Australian antigen	Australian antigen — original name of the surface antigen of hepatitis B virus existed prior to discovering of hepatitis B virus and its antigens. The term originated because of discovery of this antigen in serum of Australian aborigines. Later it was identified as the antigenic component of the hepatitis B virus - HBsAg.
Diagnostic markers	Diagnostic markers (markers of infection) — antigens, antibodies and nucleic acids of viruses, detection of which allows to determine the etiology of viral hepatitis and / or the presence of the virus, to characterize the progress of infection, to predict its result, to evaluate the effectiveness of treatment, to perform a retrospective analysis of the previous meeting with the virus that causes hepatitis, and to estimate a level of post-vaccination immunity.
Viral persistence	Viral persistence — a presence of the virus in functionally active condition in cells of an organism or cell cultures for longer periods then during typical acute infections. Infections caused by this phenomenon are known as persistent viral infections.
Rapid, immunochromatgraphic assays for the detection of HBsAg and anti-HBs	Rapid, immunochromatgraphic assays for the detection of HBsAg and anti-HBs allow to obtain research results within 5-15 minutes without using the complex laboratory equipment. The basis of the test is a nitrocellulose membrane on the surface of which HBs or antigens encoded by HCV RNA are firmly sorbed. The process contains next stages: the binding of HBsAg or anti-HCV we are looking for, the washing and addition of conjugate (for example anti-HBs marked with colloidal gold, an enzyme or marked antiserum that presipitate IgG), the presence of markers is defined by the corresponding substrate.
Groups of risk	The group of risk — a part of population united by principle of high probability of contamination by various infection diseases because of more often, close and durable contacts with the pathogen.
Hepatitis viruses	Hepatitis viruses — viruses that can cause specific injury of liver – hepatitis. Its belong to different taxonomic groups and have different biological properties but can cause human hepatitis. The most common causative agents of viral hepatitis are the hepatitis A virus, hepatitis B virus, hepatitis C virus, hepatitis D virus or delta-hepatitis virus, hepatitis E virus, hepatitis G virus and hepatitis F virus. Existence of other unidentified hepatitis viruses is being considered.

Theoretical questions for the classes:

- Obligate and facultative pathogens of viral hepatitis, their properties and classification.
- Viral hepatitis A. Etiology, epidemiology, pathogenesis, clinical manifestations. Characteristics of laboratory diagnostics. Specific prevention, postinfectious and postvaccination immunity.
- Hepatitis B virus structure specifics. Characteristics of antigens. Reproduction.
- Epidemiology, pathogenesis and clinical picture of hepatitis B. Postinfectious immunity.
- Laboratory diagnosis. Dynamics of the appearance of serological markers of hepatitis B. The interpretation of serological data.
- Prevention and treatment of hepatitis B.
- Viral hepatitis C: etiology, epidemiology, pathogenesis, clinical symptoms. Laboratory diagnostics. Specific prevention, postinfectious and postvaccination immunity.
- Viral hepatitis D: etiology, epidemiology, pathogenesis, clinical symptoms. Laboratory diagnostics. Specific prevention, postinfectious and postvaccination immunity.
- Viral hepatitis E: etiology, epidemiology, pathogenesis, clinical symptoms. Laboratory diagnostics. Specific prevention, postinfectious and postvaccination immunity.
- Causative agents of Viral hepatitis G, TTV, SenV.

Practical tasks which are being performed during the classes:

- To study the classification and differential properties of the hepatitis viruses.
- To assess results of laboratory diagnosis of hepatitis B by ELISA. Make a conclusion.
- Familiarize with the diagnostic test systems, treatment and preventive medications that are used for hepatitis.

The content of theme:

In practice, students are introduced to modern methods of laboratory diagnosis of viral hepatitis; acquainted with enzyme immunoassay method for diagnosis of hepatitis B; acquainted with drugs for specific prevention of hepatitis A and hepatitis B (vaccine). Completed protocols are signed by the teacher.

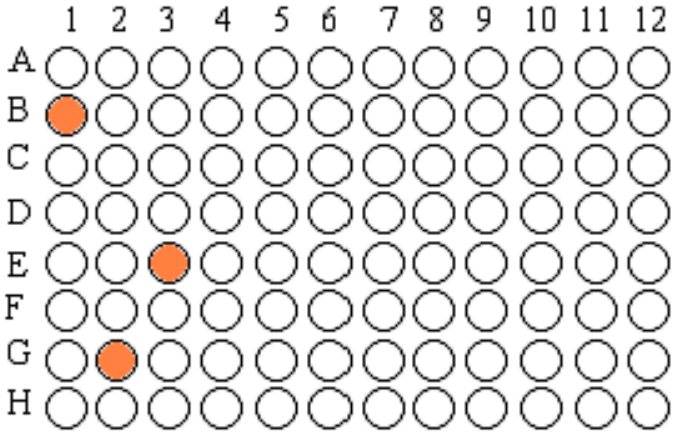
Recommendations for design of the protocol

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*	*Hepadnaviridae*	*Flaviviridae*	*Deltavirus*	*Caliciviridae*	*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	–	–	–	?
Pathogenicity for animals	Chimpanzees, marmozets	Chimpanzees	Chimpanzees	Chimpanzees	Chimpanzees	?
Replication in hepatocytes	Cytoplasm	Nucleus	Cytoplasm	Nucleus	Cytoplasm	?
Antigenic variants	Viral specific antigen	HBsAg, HBcAg, HBeAg, HBxAg	Few subtypes	Two forms: small, big	Heterogeneous	Five phylogenetic groups
Oncogenicity	-	+	+	+	-	+
Association with other HV				HBV		HCV HAV, 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, hemodialysis patients	Patients with hepatitis B, doctors, recipients, drug users	Young people from Asia, Africa	Doctors, blood recipients, drug users, sexual partners, hemodialysis patients
Prevention	Inactivated and live vaccine	Plasma vaccine (from blood of HBsAg-carriers) Genetically engineered 3.Recombinant from poxvirus	Interferon	Not developed	Not developed	Not developed

Work 1. To assess results of laboratory diagnosis of hepatitis B by ELISA.



Conclusions: ***A1, E3, G2 – seropositive samples.***

Work 2. Familiarize with preparations for the specific prophylaxis of viral hepatitis.

1. Combined vaccine for hepatitis A and hepatitis B prevention TWINRIX.
2. Recombinant yeast vaccine for the specific prophylaxis of viral hepatitis B – ENGERIX.

Questions for the self-control

- 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?
- Which markers of acute hepatitis B can be detected in the blood of the patient?
- Which markers of acute hepatitis A can be detected in the blood of the patient?
- Which marker can be detected in blood after vaccination against hepatitis B?
- What medications are used for specific prophylaxis of hepatitis B?
- What is the minimum infectious dose of hepatitis B virus during parenteral enters?
- Which hepatitis viruses can cause the development of primary hepatocellular carcinoma?
- What are the reasons of high percentage of chronic cases and resistance to antiviral therapy during viral hepatitis C.
- What measures are used for prophylaxis of hepatitis B?
- Which viral hepatitis can be diagnosed in Ukraine? What methods of laboratory diagnosis should be used?
- 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?

Class №24

Topic: “Retroviruses. HIV. Laboratory diagnosis of HIV-infection”

Topic relevance.

AIDS is called the plague of the XX century, which takes more and more human victims. To prevent further spreading of HIV and its impact on social and economic processes in our country the Law of Ukraine "On Prevention of Acquired Immune Deficiency Syndrome (AIDS) and social protection" was approved, the program of AIDS prevention and drug addiction was approved and implemented, the program "Safety of donor blood" was introduced.

Every day, an estimated 2500 young people are newly infected with HIV, according to a global report on HIV prevention. Acquired immune deficiency syndrome or acquired immunodeficiency syndrome (AIDS) is a disease of the human immune system caused by the human immunodeficiency virus (HIV). The illness interferes with the immune system making people with AIDS much more likely to get infections, including opportunistic infections and tumors that do not affect people with working immune systems. In 2009, the World Health Organization (WHO) estimated that there are 33,4 million people worldwide living with HIV/AIDS, with 2,7 million new HIV infections per year and 2,0 million annual deaths due to AIDS. Monthly HIV/(AIDS)-infection involved more than 1,200 Ukrainian citizens. Ukraine has one of the highest rates of increase of HIV/AIDS cases in Eastern Europe. All this show the relevance of lessons topic and create positive motivation for its learning.

The specific aims:

- To learn the basic biological properties of retroviruses.
- To learn modern methods of laboratory diagnosis of HIV infection and AIDS.
- To analyze the prospects of effective antiretroviral therapy drugs creating and modern approaches to prevention and treatment of HIV infection.

Basic knowledge, skills, needed to study topic (interdisciplinary integration). See a class №1.

A list of the main terms, parameters, characteristics which should be learnt by the student during the preparation to the classes:

Term	Definition
HIV-infection	HIV-infection – a disease caused by human immunodeficiency virus (HIV).
AIDS	AIDS – final stage of HIV infection, characterized by various pathological symptoms, caused by a deep lesion of the immune system by HIV.
Revertase (reverse transcriptase, RNA-dependent DNA polymerase)	Revertase (reverse transcriptase, RNA-dependent DNA polymerase) – enzyme that is directly linked to viral RNA, it defines the strategy of the genome in the cell, providing some

	stages of viruses reproduction (creates a copy of DNA using viral RNA as the matrix).
Screening	Screening (in the case of HIV – study of serum of some people populations (donors, pregnant women, drug users etc.), for the purpose of identifying sera that with high probability contain antiviral antibodies and exclusion of sera that contain no specific antiviral antibodies.
AIDS-associated diseases	AIDS-associated diseases – diseases that occur in the later stages of HIV-infection and are considered as the symptoms of AIDS.
Polymerase chain reaction (PCR)	Polymerase chain reaction (PCR) – method of detection and identification of viruses and bacteria in the investigated materials. The principle of the method is based on numerous copying (selective amplification) of target nucleic acid by the enzyme DNA-polymerase.

The theoretical questions:

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

The practical tasks which are being performed during the classes:

- To write the scheme of the laboratory diagnosis of HIV-infection into the protocol.
- To assess the results of laboratory diagnosis of HIV-infection by ELISA and to make conclusions.
- Familiar with the diagnostic test systems, medical-prophylactic medications used in case of HIV-infection.

The content of the theme.

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.

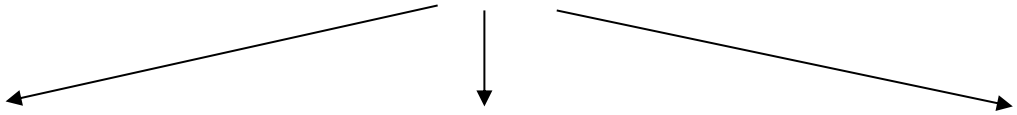
Recommendations for design of the protocol

Classification of retroviruses (*Retroviridae* family)

Genus	Representatives
Alpharetrovirus	Rous sarcoma virus, avian leukosis virus, and avian myeloblastosis virus.
Betaretrovirus	Mouse mammary tumour virus, Mason-Pfizer monkey virus
Gammaretrovirus	Feline leukemia virus, murine leukemia virus
Deltaretrovirus	Bovine leukemia virus, human T-lymphotropic virus (HTLV-1, HTLV-2)
Epsilonretrovirus	Walleye epidermal hyperplasia virus
Lentivirus	HIV, simian immunodeficiency virus, feline immunodeficiency virus, puma lentivirus, equine infectious anemia, bovine immunodeficiency virus, caprine arthritis encephalitis virus, Maedi-Visna virus
Spumavirus	Simian foamy virus, human foamy virus

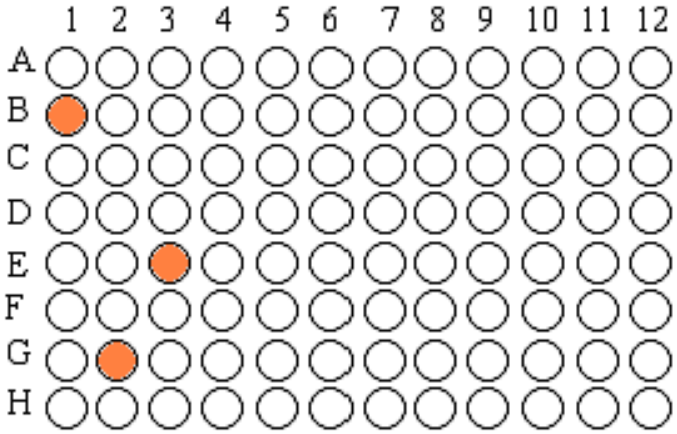
Work 1. To write the scheme of the laboratory diagnosis of HIV / AIDS in the protocol.

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* • *electronic 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*

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



Conclusions: *A1, E3, G2 – seropositive samples.*

Work 3. To read and add to the protocol the main drugs used for treatment of HIV/AIDS.

- Nucleoside analog of reverse transcription inhibitors (zidovudine, lamivudine, stavudine etc.).
- Non-nucleoside reverse transcriptase inhibitors (efavirenz, nevirapine, delavirdine etc.).
- Protease inhibitors (indinavir, ritonavir, ABT-378).

Questions for the self-control:

- What is the structure of human immunodeficiency virus?
- What are the enzymes HIV having?
- Which cell receptor interacts with HIV?
- What physical factors are harmful to HIV?
- What are the violations of cellular immunity observed in patients with AIDS?
- What are the mechanisms of HIV transmission?

RECOMMENDED LITERATURE

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