MINISTRY OF THE PUBLIC HEALTH OF UKRAINE
ZAPOROZHYE STATE MEDICAL UNIVERSITY
CHAIR OF MICROBIOLOGY, VIROLOGY AND IMMUNOLOGY

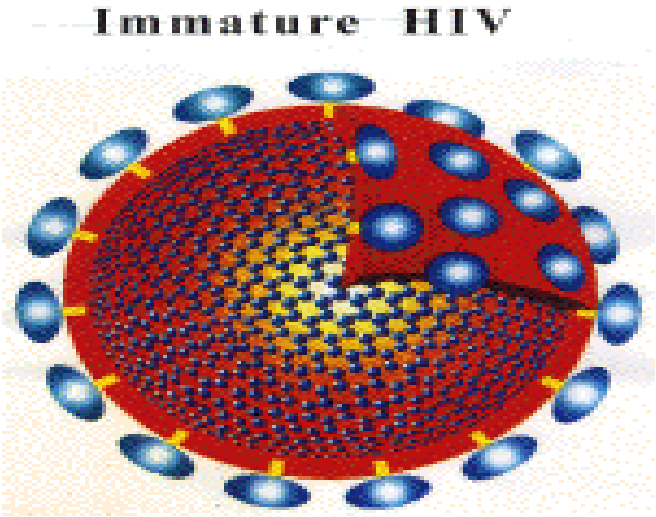
Module I

Collection of methodical recombinations for practical classes

on microbiology, virology and immunology

for the students of 2nd year of the medical faculty.

Part III (Virology).



Zaporizhzhia

2017

МІНІСТЕРСТВО ОХОРОНИ ЗДОРОВ'Я УКРАЇНИ

Запорізький державний медичний університет

Кафедра мікробіології, вірусології та імунології

Модуль І

Збірник методичних рекомендацій

для підготовки практичних занять

з мікробіології, вірусології та імунології

для іноземних студентів ІІ-ІІІ курсів медичних факультетів спеціальності «Лікувальна справа»

ІІІ частина

Збірник методичних рекомендацій до практичних занять з мікробіології, вірусології та імунології (Модуль І. частина Ш.) для іноземних студентів ІІ курсу медичних факультетів спеціальності «Лікувальна справа», які допоможуть студентам краще засвоїти теоретичний матеріал, правильно провести мікробіологічні дослідження на практичному занятті та за допомогою тестових завдань підготуватися до підсумкового тестового контролю.

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The methodical manual for practical lessons on microbiology, virology, immunology for the medical students of II-III year of the study are approved by the Central Methods Board of ZSMU as a methodical manual on practical lessons for students of the medical faculty.

The independent practical work of students is an important part of the syllabus in the course of microbiology, virology and immunology. It helps students to study this fundamental subject.

The systematic independent work enables to reach the final goal in the students' education. It is also important while preparing the students for their future clinic work with patients.

These theoretical material, questions and tests help students to get ready for examination.

Plan

of the lectures in microbiology for foreign students of the medical faculty for spring semester

№	Theme	Amount of hours
1.	Subject and problems of microbiology in historical development. Structure and functions of the bacterial cells. Classification of microorganisms. Physiology of microorganisms. Nutrition, respiratory, growth and reproduction.	2
2.	Antibacterial chemotherapy of an infections diseases. Antibiotics. Genetics of bacteria. Genetic engineering. Biotechnology.	2
3.	The infection. The forms of infections. The infectious process. Pathogenicity and virulence of bacteria.	2
4.	Immunity. Immune system. Antibodies and antigens, their nature and properties. Immune diagnostics.	2
5.	Allergy. Reactions of immunity. Immunoprophylaxis and Immunotherapy of infectious diseases. Immunobiological preparations.	2
6.	History of virology. Morphology, structure, chemical composition, base of classification and reproduction of viruses. Antivirus immunity.	2
7.	Influensavirus and Parainfluenza viruses. Acute respiratory virus infections(respiratory-syncytial virus, reovirus, rhinovirus, adenovirus). Virus of measles. Mumps virus. Rubivirus.	2
8.	Herpesviruses. Virus of chickenpox. Smallpox.	2
9.	Virus of poliomyelitis, ECHO and Coxsackie. Hepatitis Viruses.	2
10.	Viruses of encephalitis, hemorrhagic fever. Viruses of immunodeficiency. AIDS. Principles and methods of the laboratory diagnostics. Oncogenic viruses.	2
	TOTAL	**20**

Plan

of practical classes in microbiology for foreign students of the medical faculty for spring semester

№	Theme	Amount of hours
1.	Microbiological laboratory equipments and instructions for work. Structure of biological light to the microscope and rules of work with him. ***Bacterioscopical method of research.*** Microscopy of the prepared given. Morphology of bacteria. Preparations for a microscopy. Method of staining by Gram.	2,5
2.	Structure of the bacterial cell. Complex methods of staining by Anjesko, Neisser, Burri-Gins and Ziehl-Nilsen. Morphology of spirochetes, riskettsia, fungi and the protozoa.	2,5
3.	***Bacteriological method of research.*** Nutrition of bacteria. Nutrient media and methods of bacteria cultivation. Methods of sterilization. Asepsis and antiseptic. Disinfection. Chemotherapy. Chemotherapeutic preparations.	2,5
4.	Growth and reproduction of bacteria. Methods of isolation and cultivation of pure cultures of aerobes. Respiration of bacteria. Methods of isolation and cultivation of anaerobes. Biochemical properties of microorganisms.	2,5
5.	Genetics of microorganisms. Methods of biotechnology and gene engineering. ***Genetic method of diagnostics.*** Polymerase chain reaction (PCR). Polymerase chain reaction with reverse transcriprase (RT-PCR). Polymerase chain reaction in the real time.	2,5
6.	Ecological microbiology. Microflora of an environment and the human body. Methods of sanitary bacteriological research. Antibiotics. Bacteriophages.	2,5
7.	**Submodule 1. Morphology and physiology of microorganisms.**	**2,5**
8.	Infections, infectious and epidemiological process. Pathogenic factors of microorganisms. Mechanisms of pathogenesis of an infectious diseases. Experimental infection of laboratory	2,5

	animals.	
9.	Immunity. Kinds and forms of immunity. Types of immune answer. Innate immunity. Factors of non specific organism defence. Cells and receptors of innate immunity.	2,5
10.	Adaptive immunity.T- and B-lymphocytes. Description of antigens. Presentation of antigens. Activating of lymphocytes. Antiinfectious immunity.***Immunological method of research*** – serological reactions. Immunoglobulins. Serological reactions of agglutination and precipitation, immune lysis and compliment fixation test, their description and practical use. Coomb's test. Immunohematology. Application of monoclonal antibodies in immunocytochemical and immunohistochemical reactions.	2.5
11.	Immune serum and immunoglobulins. Reaction of flocullation (neutralization). Hypersensitivity. Allergic reactions for immunodiagnosis of infectious diseases. Autoimmune phenomena. Principles of the use of antibodies as medical, preventive and diagnostic preparations.	2,5
12.	Vaccines. Principles of making and application of vaccines. Immunobiological preparations. Immune status of man. Estimation of immune status. Immunomodulators. Immunocorrection. Transplantation immunology.	2,5
13.	Virologic laboratory. Morphology and ultrastructure of viruses. Principles of classification. Virologic methods of research. Cultivation of viruses in culture cells and in chick embryos. Indication of viral reproduction.	2,5
14.	Antiviral immunity. Immunoreactions in virology: hemagglutination reaction, hemagglutination inhibition assay, hemadsorption phenomenon, neutralization test. Radioimmunoassay, direct and indirect immunofluorescence, enzyme immunoassay. Enzyme linked immunosorbent assay (ELISA), immunoelectroblot technigues. Immunochromatography analisis. Immunology of tumours.	2,5
15.	**Submodule 2. Infection, immunity and general virology.**	**2,5**
16.	Laboratory diagnostics of flu and parainfluenza. Laboratory diagnostics of adenoviruses Laboratory diagnostics of mumps,	2,5

	measles and rubella.	
17.	Laboratory diagnostics of chikenpox, smallpox, herpes, zoster, poliomyelitis, Coxsakie and ECHO.	2,5
18.	Laboratory diagnostics of viral hepatitis A, B, C, D, E, F, G, rabies and arboviruses infections.	2,5
19.	Laboratory diagnostics of AIDS. Oncogenic viruses. Viral genetic theory of tumours origin.	2,5
20.	**Final module control I.**	2,5
	TOTAL	**50**

INDEPENDENT WORK
on microbiology for foreign students of II course of the medical faculty for spring semester

№	Theme	Hours quantity
1.	Morphology of microorganisms. Simple and complex methods of coloring the bacteria.	6
2.	Structure of bacterial cell. Complex methods of coloring the bacteria.	4
3.	Morphology of spirochetes and rickets.	4
4.	Morphology of fungi and elementary.	2
5.	Nutrition of microorganisms, nutrient mediums and methods of cultivation bacteria. Devices and methods of sterilization.	4
6.	Action of some physical and chemical factors on microorganisms, disinfection.	2
7.	Antibiotics and Chemotherapy.	6
8.	Genetics of microorganisms. Methods of biotechnology and gene engineering.	4
9.	An infection. Experimental infection of laboratory animals.	4
10.	Immunity, its kinds and forms. Nonspecific factors of protection of an organism.	5
11.	Immune reactoins for infectious diseases.	2
12.	Vaccines. Principles of manufacturing and application of vaccines. Immunobiological preparations.	2
13.	Prion diseases of humans and animals.	1
14.	Modern methods of the laboratory diagnostics of infectious diseases	2
	Total	**48**

The founder of **virology** was ***Dmitry Ivanovsky*** (1864 – 1920).

Russian scientist studied the Mosaic Disease of Tobacco's leafs.

D.I. Ivanovsky proved in 1892 that this disease was caused by an agent, which is a high infectious and has a very specific activity.

This discovery showed that behind cellular forms there are systems which invisible in a simple light microscope and may passed cross small pores filters.

These systems haven't cell structure and couldn't grow on synthetic substation.

Then during 10 years were founded unusual agents of infectious diseases of plants, animals and people. They were named viruses ("virus"- toxin, poison).

The origin of viruses is not known.

Three hypotheses have been proposed:

- Viruses became parasites of primitive cells and two evolved together.
- Viruses evolved from parasitic bacteria.
- Viruses may be the component of host cells that become autonomous.

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

Names of previous disciplines	Otained skills
Human anatomy	To analyze information about the structure of the human body and its organs and systems.
Histology, cytology,	To interpret the microscopic and submicroscopic

embryology	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.

General Characteristics of Viruses

Viruses are strange things that straddle the fence between living and non-living.

Viruses are different from anything else found on earth and are mainly characterized by their size, shape, and half alive/half dead existence.

The big difference between viruses and all else, is that fact that viruses are so small they can not be viewed without the help of an electron microscope.

This is because viruses are, on average, smaller than a regular wavelength of visible light.

In effect, the viruses can hide between light waves, thus making them colorless.

They can not be seen by the naked eye or a regular microscope.

Viruses are so small in fact, that the largest virus is equal in size to the smallest bacteria. The smallest virus measures only 20 nanometers in length.

Because of their incredibly small size, viruses are extremely hard to study and understand.

Shape is also a defining characteristic of viruses. The basic shapes viruses tend to take are rods, filaments, crystals, helixes, polyhedrons and spheres, with added extensions.

Almost all human viruses are close to being spherical. Every virus carry proteins and nucleic acids in a protective coat.

This protective membrane is called the capsid.

Extensions on any virus are called antigens. The antigens allow viruses to identify, attack, and enter its target host.

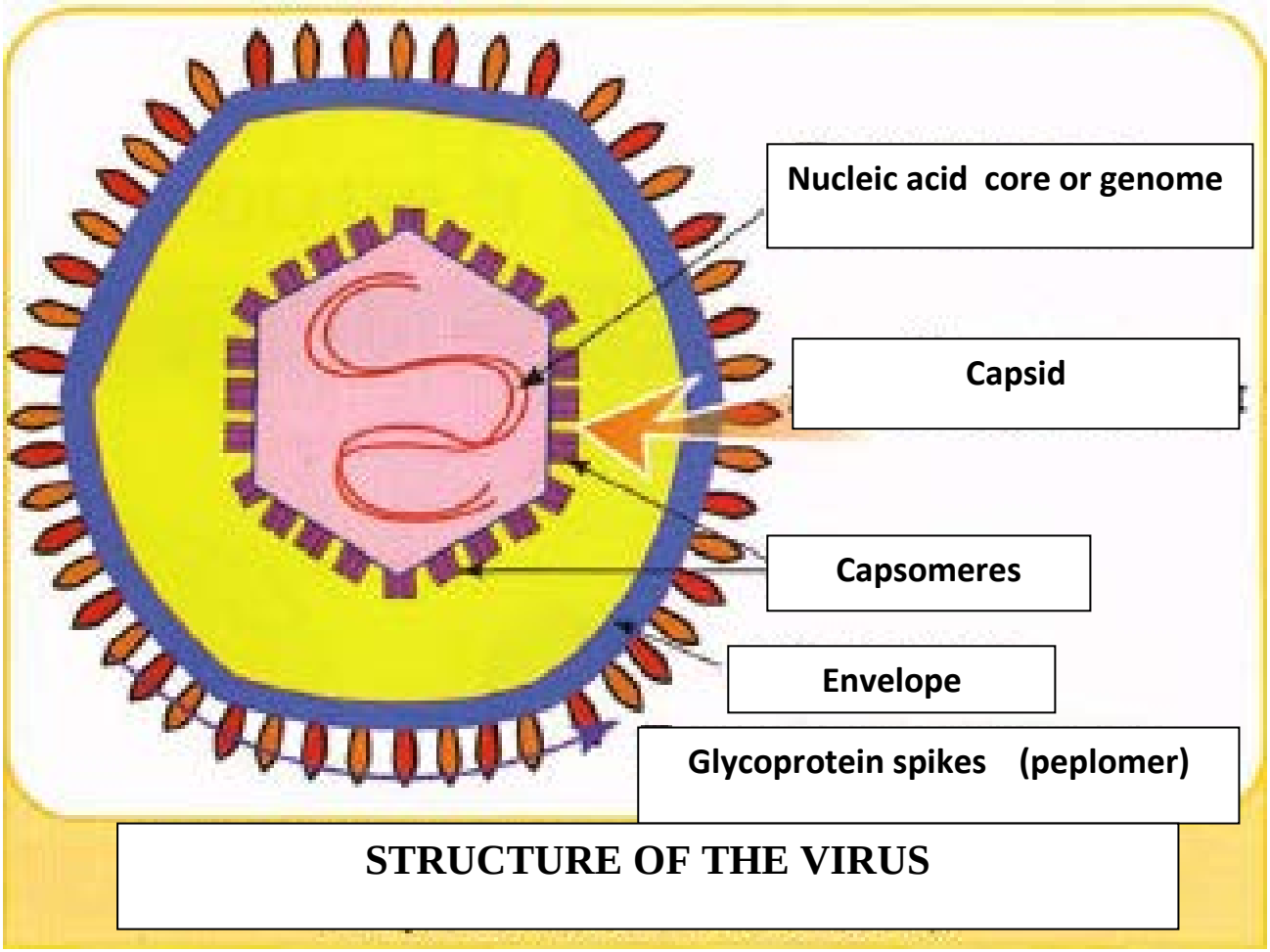
A virus is basically a tiny bundle of genetic material—either DNA or RNA—carried in a shell called the viral coat, or capsid, which is made up of bits of protein called capsomeres.

Some viruses have an additional layer around this coat called an envelope. That's basically all there is to viruses.

1. Depending on one's viewpoint, viruses may be regarded as exceptionally complex aggregations of **nonliving** chemicals or as exceptionally simple **living** microbes.

2. Viruses contain a single type of **nucleic acid** (DNA or RNA) and a **protein coat**, sometimes enclosed by an **envelope** composed of lipids, proteins, and carbohydrates.

3. Viruses are **obligatory intracellular parasites**. They multiply by using the host cell's synthesizing machinery to cause the synthesis of specialized elements that can transfer the viral nucleic acid to other cells.



STRUCTURE OF THE VIRUS

Host Range:

1. Host range refers to the spectrum of host cells in which a virus can multiply. (**narrow** vs. **broad**)

2. Most viruses infect only specific types of cells in one host species, so they do not generally **cross species barriers**.

3. Host range is determined by the **specific attachment site** on the host cell's surface and the availability of host cellular factors.

Size:

1. Viral size is ascertained by electron microscopy.
2. Viruses range from 20 to 14,000 nm in length.

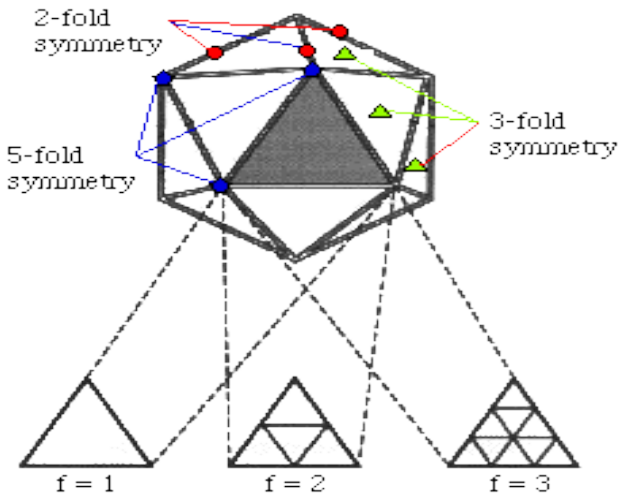
Viral Structure

A **virion** is a complete, fully developed viral particle composed of **nucleic acid surrounded by a coat**.

General Morphology

1. **Helical** viruses (for example, Ebola virus) resemble long rods and their capsids are hollow cylinders surrounding the nucleic acid.

2. **Polyhedral** viruses (for example, adenovirus) are many-sided. Usually the capsid is an **icosahedron**.



3. **Enveloped** viruses are covered by an envelope and are roughly **spherical** but highly **pleomorphic** (for example, *Poxvirus*). There are also **enveloped helical** viruses (for example, *Influenzavirus)* **and enveloped polyhedral** viruses (for example, *Herpesvirus).*

4. **Complex** viruses have complex structures. For example, many **bacteriophages** have a polyhedral capsid with a helical tail attached.

Nucleic Acid

1. Viruses contain either **DNA or RNA**, never both, and the nucleic acid may be **single- or double-stranded**, **linear or circular**, or divided into several **separate molecules**.

2. The proportion of nucleic acid in relation to protein in viruses ranges from about 1% to about 50%.

Capsid and Envelope

1. The **protein coat** surrounding the nucleic acid of a virus is called the **capsid**.

2. The **capsid** is composed of subunits, **capsomeres**, which can be a single type of protein or several types.

3. The capsid of some viruses is enclosed by an **envelope** consisting of lipids, proteins, and carbohydrates.

4. Some envelopes are covered with carbohydrate-protein complexes called **spikes**.

Taxonomy of Viruses

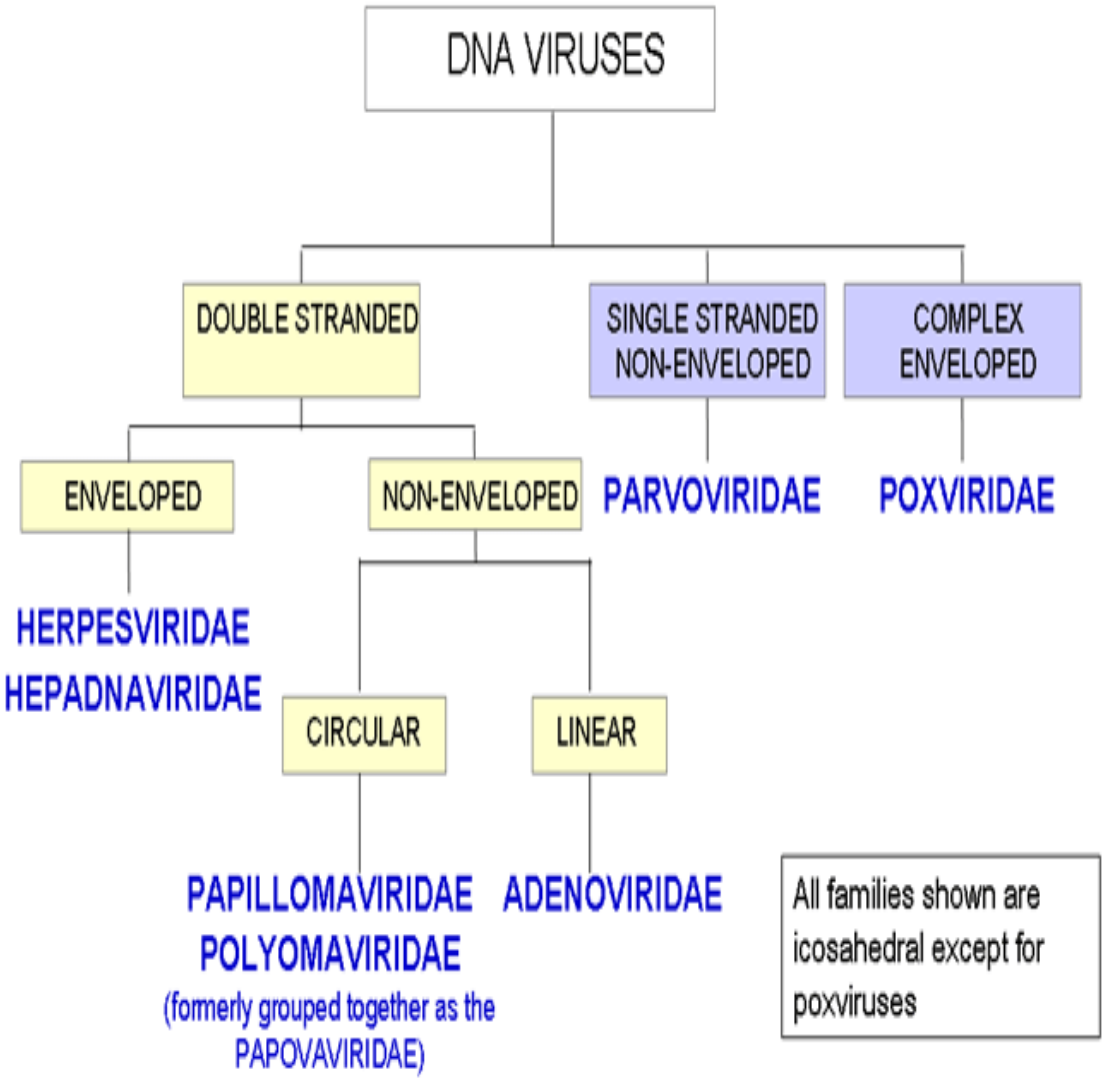
1. Classification of viruses is based on type of **nucleic acid**, strategy for **replication**, and **morphology**.

2. Virus **family** names end in **-viridae***;* **genus** names end in ***-virus****;* specific epithets have not been assigned.

3. A viral species is a group of viruses sharing the same genetic information and ecological niche.

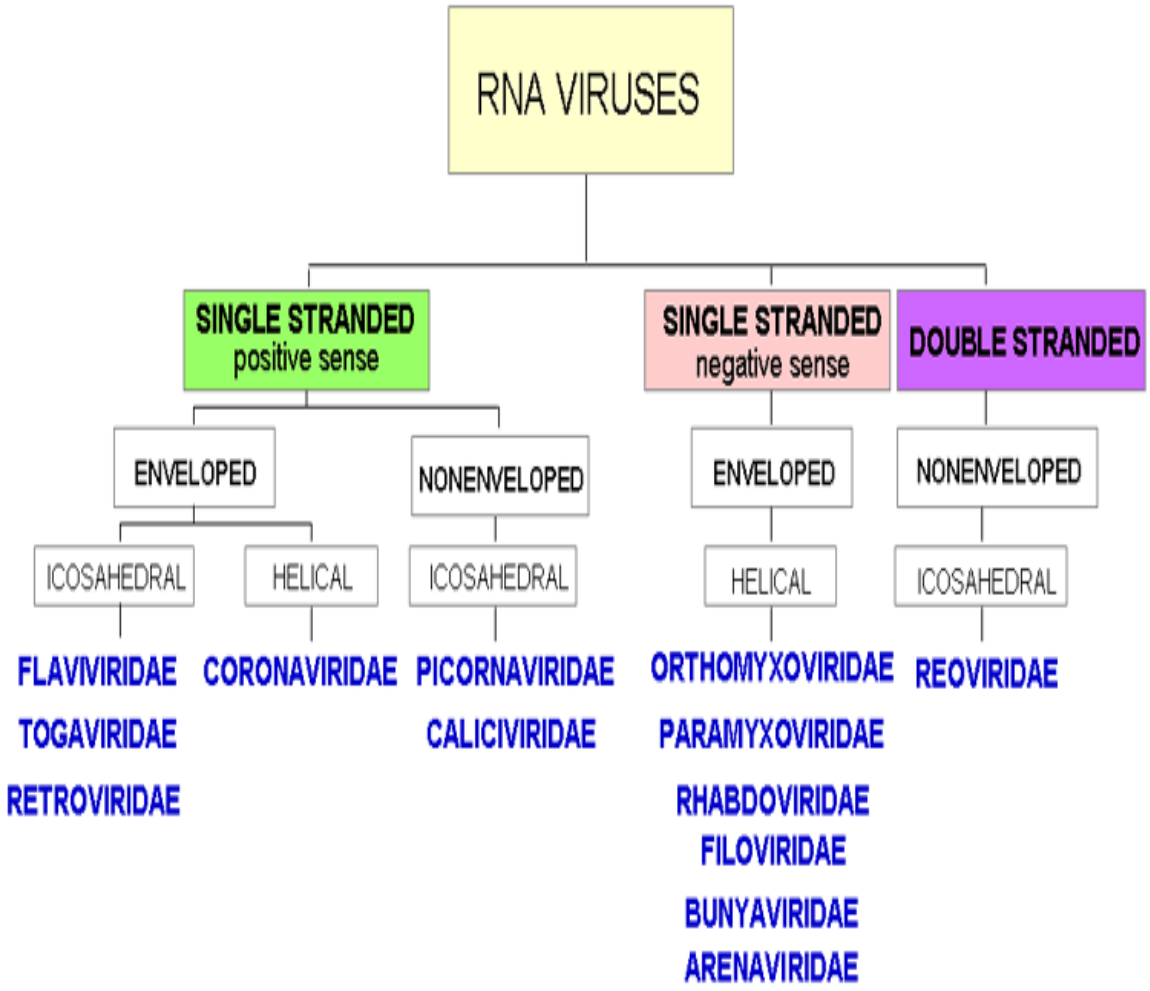
RNA & DNA VIRUSES

Families of DNA viruses



Modified from Volk et al., Essentials of Medical Microbiology, 4th Ed. 1991

Families of RNA viruses



Modified from Volk et al., Essentials of Medical Microbiology, 4th Ed. 1991

Isolation, Cultivation, and Identification of Viruses

1. Viruses **must be grown in living cells**.

2. The easiest viruses to grow are bacteriophages.

Growth of Bacteriophages in the Laboratory

1. The **plaque method** mixes bacteriophages with host bacteria and nutrient agar.

2. After several viral multiplication cycles, the bacteria in the area surrounding the original virus are destroyed; the **area of lysis is called a plaque**.

3. Each plaque originates with a single viral particle; the **concentration of viruses** is given as **plaque-forming units** (**PFUs**).

Growth of Animal Viruses in the Laboratory

1. Cultivation of some animal viruses requires whole animals.

2. Simian AIDS and feline AIDS provide models for study of human AIDS.

3. Some animal viruses can be cultivated in **embryonated eggs**.

4. **Cell cultures** are cells growing in culture media in the laboratory.

5. **Primary cell lines** and **embryonic diploid cell lines** grow for a **short time *in vitro***.

6. **Continuous cell lines** can be **maintained *in vitro* indefinitely**.

7. Signs of viral infections are **called cytopathic effects** (**CPE**).

CPE are visible under the microscope, morphological changes of cells resulting in intracellular virus reproduction.

Viral growth can cause **cytopathic effects** in the cell culture. Some viruses cause **cytocidal effects** (cell death), and others cause **noncytocidal effects**.

Cytopathic effects include the stopping of mitosis, lysis, the formation of inclusion bodies, cell fusion, antigenic changes, chromosomal changes, and transformation.

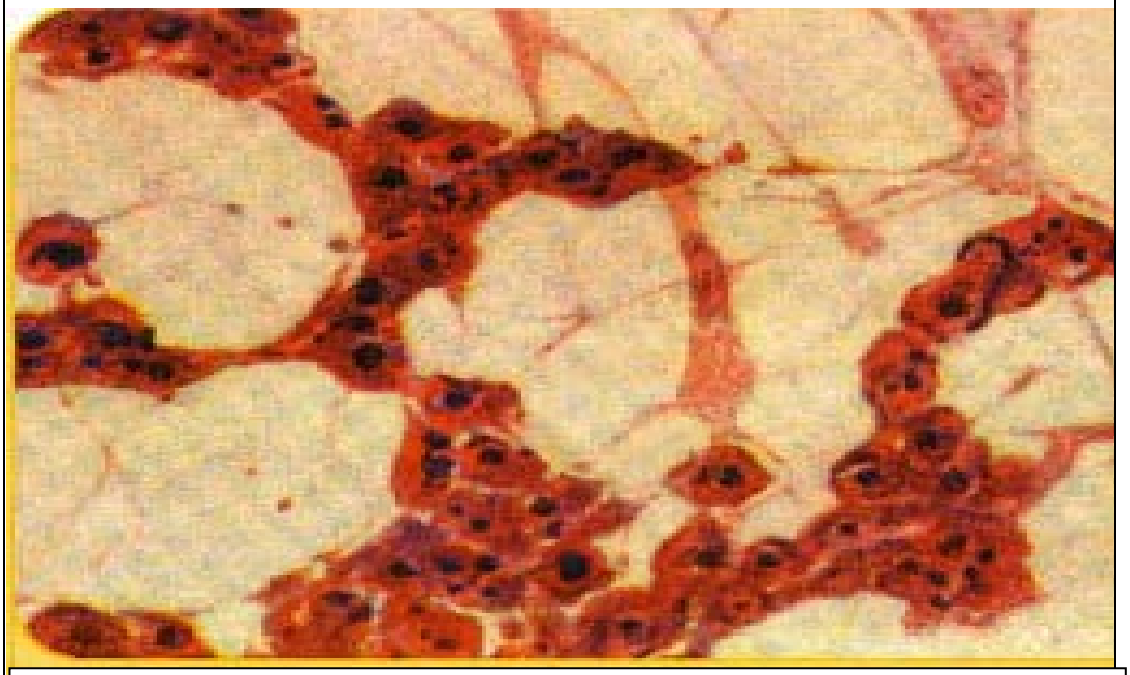
Classification of cell cultures and tissues that used in virology

Tissue or organ culture			
Embryonic tissue organ or part of it that are supported in vitro and retain the cell differentiation and their functions			
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)

Term	Significance
Cytopathic effect of the virus (CPE)	 **Cytopathic effect are visible under the microscope, morphological changes of cells resulting in intracellular virus reproduction.**
Inclusions	***Inclusions*** 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 viruses 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.

Hem-agglutination test	Hemagglutination test is based on the ability of some viruses to cause agglutination of erythrocytes by viral glycoprotein spikes - hemagglutinins.
Reaction of hem-adsorbtion	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.

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.

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.

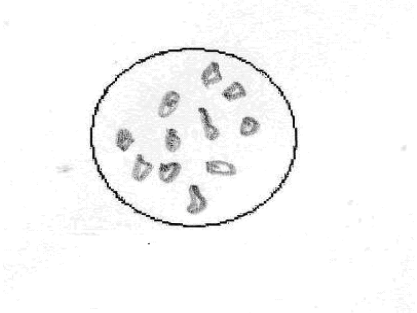
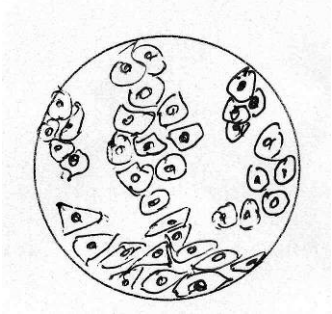
Methods of viruses indication

1. Identification of the cytopathic action of viruses in cell culture.

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

<table>
<tr><th colspan="2">Types of cytopathic effect</th><th>Viruses that cause cytopathic effect</th></tr>
<tr><td colspan="2">Complete degeneration</td><td>Poliovirus
Coxsackie viruses
ECHO viruses</td></tr>
<tr><td rowspan="3">Partial degeneration</td><td>Type of grapes form</td><td>Adenoviruses</td></tr>
<tr><td>Type of focal destruction</td><td>Smallpox virus
Influenza virus</td></tr>
<tr><td>Type of symplast generating</td><td>Measles virus
Mumps virus
Parainfluenza virus
RS-virus
Herpes simplex virus</td></tr>
<tr><td colspan="2">Proliferation</td><td>Oncogenice viruses</td></tr>
</table>

Different types of virus CPE with demonstration preparations.

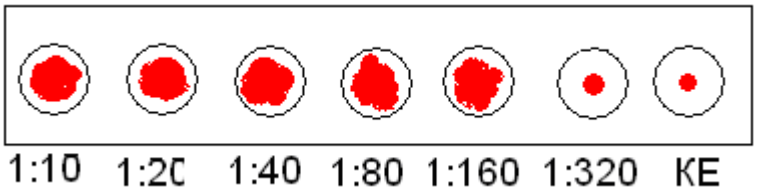
Type of CPE	Viruses that cause CPE	Type CPE
Complete degeneration	Polioviruses, Coxsackie viruses, ECHO viruses	 Complete degeneration
Type as grapes-like clusters	Adenoviruses	 CPE type as grapes form
Type of symplast generating (giant cells or syncytia)	Measles virus, mumps virus, parainfluenza virus, RS - virus, herpes virus.	 CPE as symplast generating

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

3. Plaque reacts under the agar and bentonite.

4. Hemagglutination test.

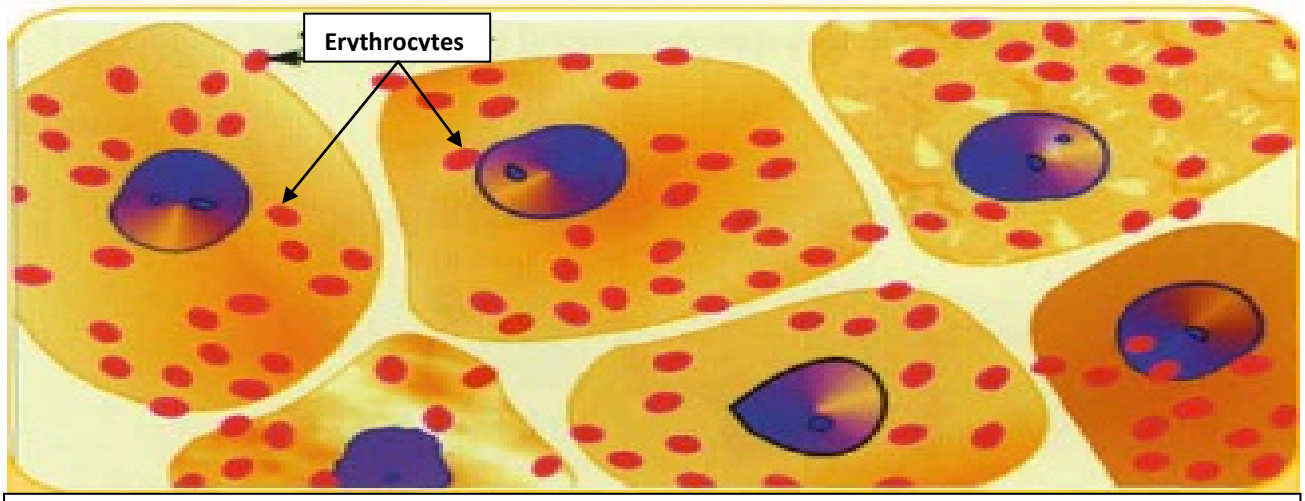
HA is agglutination of erythrocytes under the action of viruses.



Dilutions of virus-containing material

CE- control of erythrocytes.

5. Reaction of hemadsorbtion.



Reaction of hemadsorbtion **is the ability of infected by viruses culture cells to adsorb red blood cells on the surface.**

6. "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).

7. 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

Viral Identification

1. **Serological tests** are used most often to identify viruses.

2. Viruses may be identified by **RFLPs** and **PCR**.

Serological reactions in virology.

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 study.

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.

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.

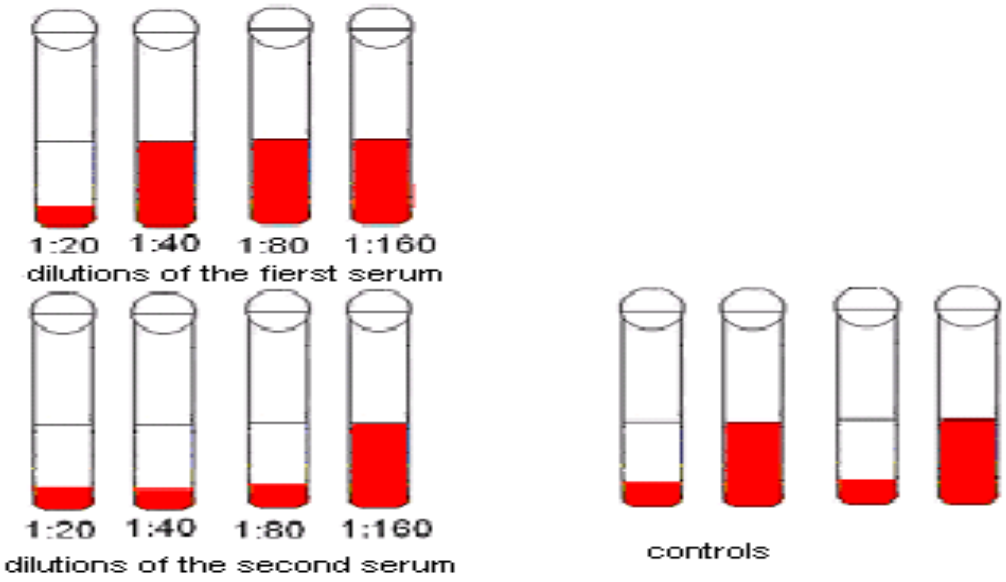
They take into account the complement fixation test - to detect antiviral antibodies, virus neutralization test - to identify viruses.

The features of serological diagnosis of viral infections and take into account CFT perfomed for serological diagnosis of viral infection.

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 of CFT in viral infections



The content of tubes:

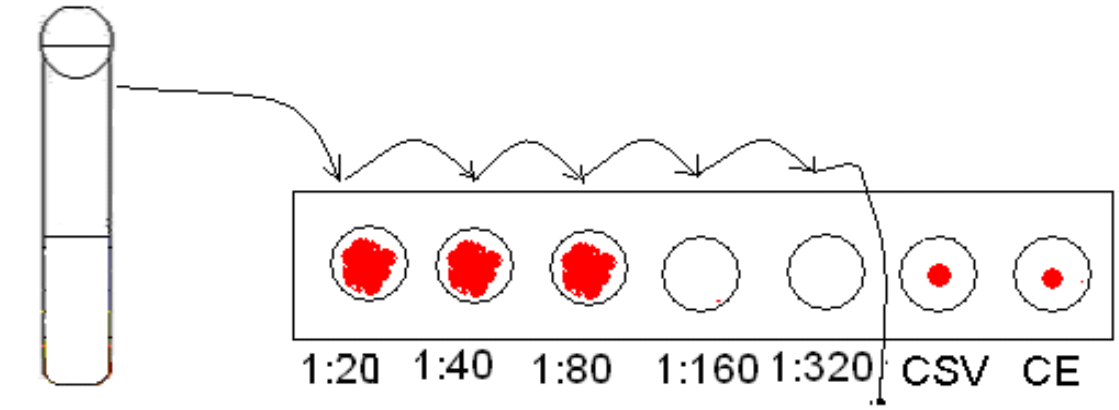
1- 4 – dilutions of investigated serum (serum + antigen + complement),

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



Diagnostic serum by influenza B virus titer 1:160

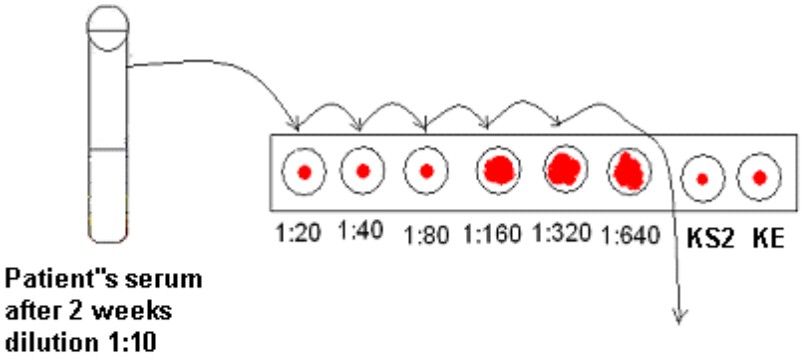
add to each 0,25 ml of physiological sol.

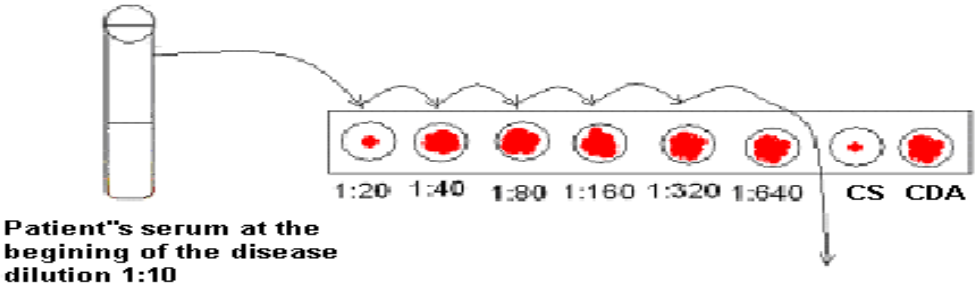
add to each 0,25 ml of maked virus

add to each 0,25 ml of 1% chicken erythrocytes

Scheme of hemagglutination inhibition test (HIT)

for serological diagnosis of influenza





Viral Multiplication

1. Viruses **do not contain enzymes** for energy production or protein synthesis.

2. For a virus to multiply, it must invade a host cell and direct the **host's metabolic machinery** to produce viral enzymes and components.

Multiplication of Bacteriophages

1. During a **lytic cycle**, a phage causes **the lysis and death** of a host cell.

2. Some viruses can either cause lysis or have their **DNA incorporated as a prophage** into the DNA of the host cell. The latter situation is called **lysogeny**.

3. The T-even bacteriophages that infect *E. coli* have been studied extensively.

4. During the **attachment phase** of the lytic cycle, sites on the **phage's tail fibers** attach to **com-plementary receptor sites** on the bacterial cell.

5. During the **penetration phase**, phage **lysozyme** opens a portion of the bacterial cell wall, the tail sheath contracts to force the tail core through the cell wall, and **phage DNA enters** the bacterial cell. The capsid remains outside.

6. During **biosynthesis**, **transcription of phage DNA** produces **mRNA coding for proteins** necessary for phage multiplication**. Phage DNA is replicated**, and **capsid proteins are produced**. During the **eclipse period**, separate phage DNA and protein can be found.

7. During **maturation**, phage DNA and capsids **assemble** into complete viruses.

8. During **release**, phage **lysozyme** breaks down the bacterial cell wall, and the multiplied phages are released.

9. The time from phage adsorption to release is called **burst time** (20 to 40 minutes). **Burst size**, the number of newly synthesized phages produced from a single infected cell, ranges from 50 to 200.

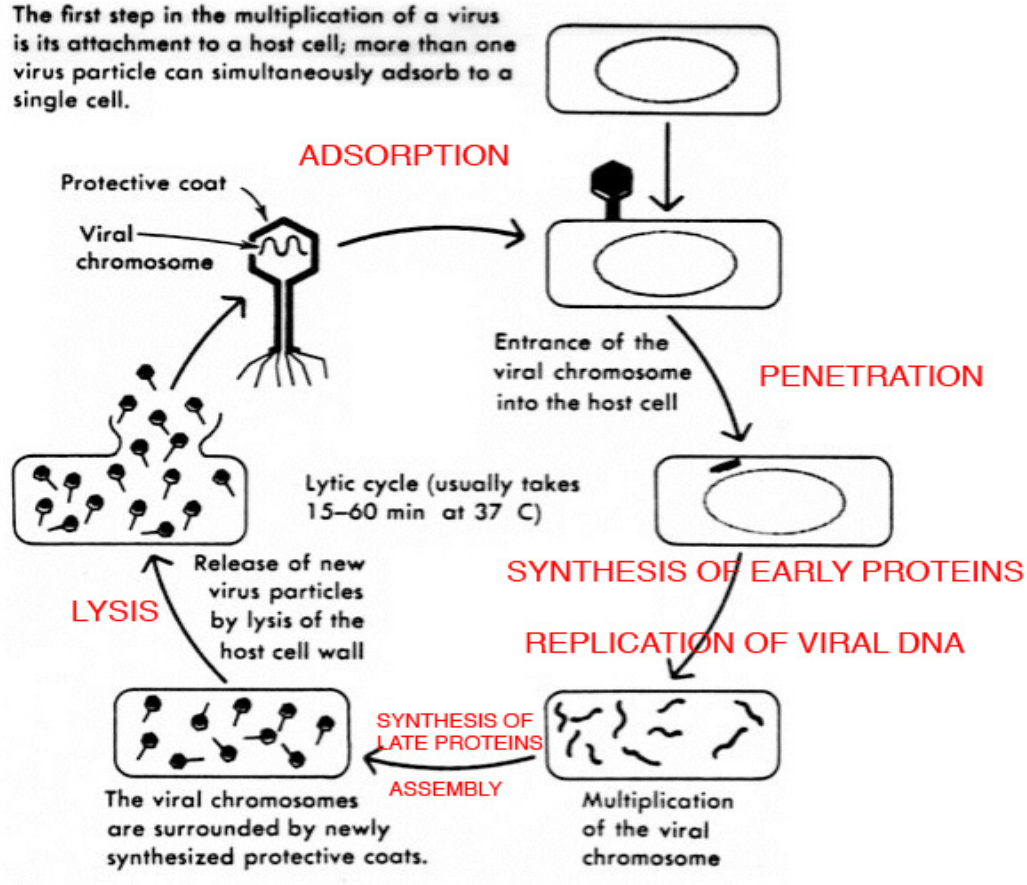
10. During the **lysogenic cycle**, **prophage** genes are regulated by a **repressor** coded for by the prophage. The **prophage** (**temperate phage**) is **replicated** each time the cell divides.

11. Exposure to certain **mutagens** can lead to **excision** of the prophage and **initiation of the lytic cycle**. Lysogenic phage may also become lytic through **spontaneous** random events.

12. Because of lysogeny, lysogenic cells become immune to reinfection with the same phage and may undergo **phage conversion** (**acquiring new properties**).

13. A **lysogenic phage can transfer bacterial genes** from one cell to another through **transduction**. Any genes can be transferred in **generalized transduction**, and specific genes can be transferred in **specialized transduction**.

Viral reproduction

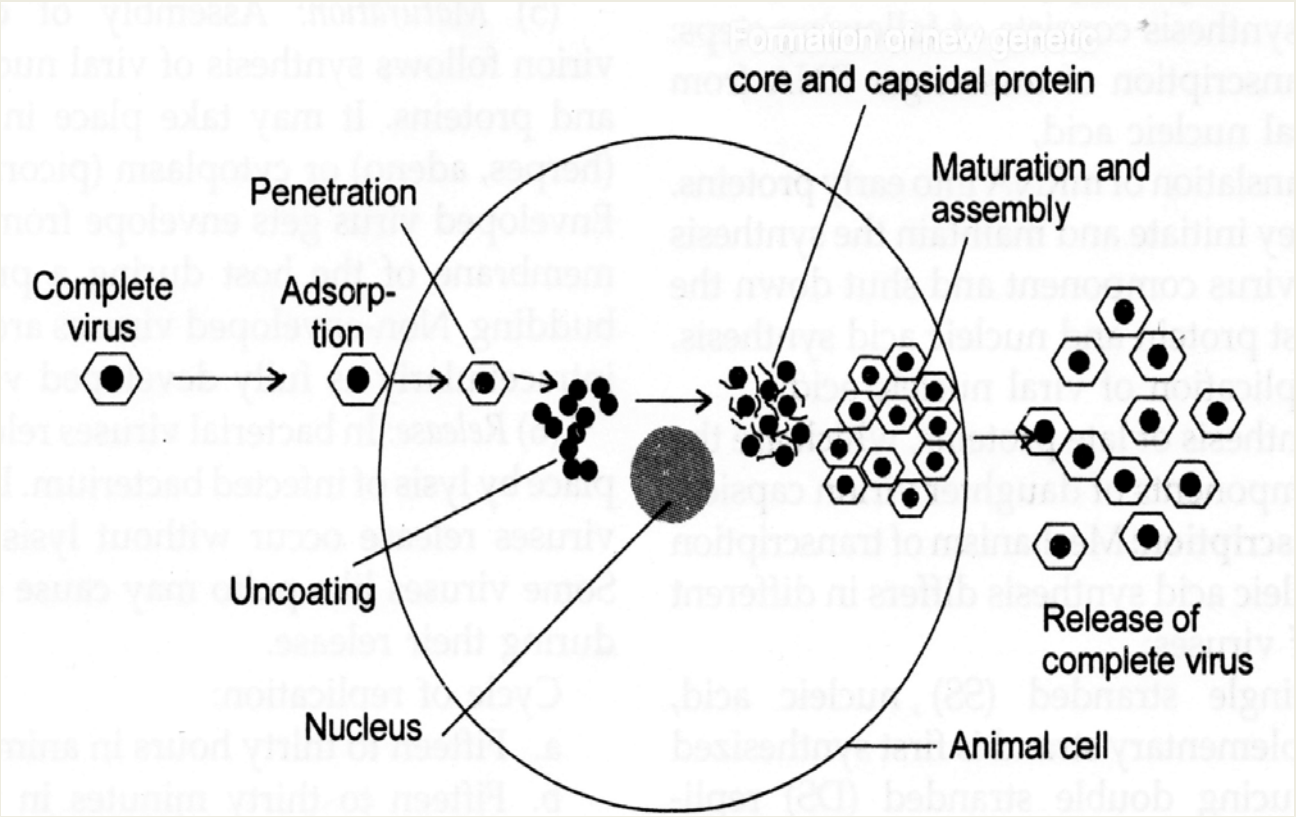


Multiplication of Animal Viruses

1. Animal viruses **attach** to the **plasma membrane** of the host cell.

2. **Penetration** occurs by **endocytosis or fusion**.

3. Animal viruses are **uncoated** by viral or host cell **enzymes**.



4. The DNA of most DNA viruses is released into the nucleus of the host cell. **Transcription** of viral DNA and **translation** produce viral DNA and, later, capsid protein. **Capsid protein** is synthesized in the cytoplasm of the host cell.

5. **DNA viruses** include members of the families Adenoviridae, Poxviridae,

Herpesviridae, Papovaviridae, and Hepadnaviridae.

6. Multiplication of **RNA viruses** occurs in the cytoplasm of the host cell**. RNA-dependent RNA polymerase** synthesizes a double-stranded RNA.

7. **Picornaviridae + strand RNA** acts as mRNA and directs the synthesis of RNA-dependent RNA polymerase.

8. **Togaviridae + strand RNA** acts as a template for RNA-dependent RNA polymerase, and mRNA is transcribed from a new - RNA strand.

9. **Rhabdoviridae - strand RNA** is a template for viral RNA-dependent RNA polymerase, which transcribes mRNA.

10. **Reoviridae** (double capsid) are digested in host-cell cytoplasm to release **double-stranded RNA** for viral biosynthesis.

11. **Retroviridae** carry **reverse transcriptase** (RNA-dependent DNA polymerase), which transcribes DNA from RNA.

12. After **maturation**, viruses are **released**. One method of release (and envelope formation) is **budding**. **Nonenveloped** viruses are released through **ruptures** in the host cell membrane.

HOW VIRUSES INFECT

Viruses do not possess any life sustaining characteristics, and do not require any nutrients. In fact, without a proper host viruses lie dormant indefinitely. Infection takes place when a virus comes in contact with its intended host. As soon as a virus encounters its victim, it attaches itself to the organism.

Furthermore, most viruses prefer a certain type of host cell and a specific mode of entrance. Naked viruses, those without a structured casing, directly enter the cells while other types of viruses fuse themselves to the outsides of their victims and inject their genetic material inside the cell. Once the genetic material of a virus is transferred to the host cell, the virus can 'take over' by incorporating its DNA into the hosts DNA much like they used to do in the prehistoric days. The infected cell is essentially a factory in charge of virus manufacturing. In a process called budding, mature viruses leave the cell a few at a time.

Lysis is the much more devastating cousin of budding. In lysis, the cell membrane of the host is completely destroyed,

killing the cell. The new viruses are unleashed instantaneously.

Almost all viral infections result in the death of the host, but in rare cases viruses leave their host cells alive. When this happens the cells are normally damaged beyond repair. With each successive transmission between hosts a virus is able to replicate itself thousands of times, and ensure the continuance of its reign of terror.

Distinctions of viruses from bacteria

- Viruses have small sizes
- Viruses contain DNA or RNA
- Viruses have not a cellular organisation
- Viruses have multiplication or reproduction
- Viruses are obligate intracellular parasites
- Viruses are sensitive to interferon.

SPECIAL MICROBIOLOGY

Theme: **Laboratory diagnostic of influenza and parainfluenza. Acute respiratory virus infections (adenovirus, rhinovirus, reovirus, respiratory-syncytial virus).**

Question for the learning.

1. Antiviral immunity. Features of specific protection of organism. Interferon, its properties.
2. Influenza viruses, their properties. Classification and variability, cultivation. Antigenic structure of influenza viruses. Pathogenesis, mechanism of contamination, clinical development of influenza. Role of specific and non

specific mechanisms in antiflue immunity. Laboratory diagnostic, specific treatment and prophylaxis of influenza.

3. Parainfluenza viruses, main properties, cultivation. Pathogenesis and clinical manifestation, immunity. Laboratory diagnostic, treatment of parainfluenza.

4. Adenovuruses and their characteristic. Mechanism of infection, pathogenesis, immunity. Laboratory diagnostic and specific prophylaxis.

5. Rhinoviruses, reoviruses and respiratory-syncytial viruses, their characteristic.

❖ **Actuality of topic.**

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 absolutely 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:**

• To learn the biological properties of influenza viruses.

• To learn the techniques of virological and serological diagnosis of influenza.

• To analyze basic modern principles of the treatment and prevention of influenza.

Basic terms, parameters, characteristics that a student has to learn during preparing to the lesson:

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 change.

Practical tasks performed in the classroom:

1. 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.
2. To perform Hemagglutonation Inhibition test for serological diagnosis of influenza, take into account the results and make conclusion.
3. To learn diagnostic, prophylactic and therapeutic drugs aused 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, subunit vaccines, normal human immunoglobulin, various types of interferon and write to the protocol.

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

Influenza Virus

Disease: Influenza. Influenza A virus is the main cause of world-wide epidemics (pandemics).

Characteristics: Enveloped virus with a helical nucleocapsid and segmented, single-stranded RNA of negative polarity.

RNA polymerase in virion. The two major antigens are the hemagglutinin and the neuraminidase on separate surface spikes. Antigenic shift in these proteins as a result of re-assortment of RNA segments accounts for the epidemics of influenza caused by influenza A virus.

Influenza A viruses of animals are the source of the new RNA segments. Antigenic drift due to mutations also contributes.

The virus has many serotypes because of these antigenic shifts and drifts. The antigenicity of the internal capsid protein determines whether the virus is an A, B, or C influenza virus.

Transmission: Respiratory droplets.

Pathogenesis: Infection is limited primarily to the epithelium of the respiratory tract.

Laboratory Diagnosis: Virus grows in cell culture and embryonated eggs and can be detected by hemadsorption or hemagglutination.

It is identified by hemagglutination inhibition or complement fixation. Antibody titer rise in convalescent-phase serum is diagnostic.

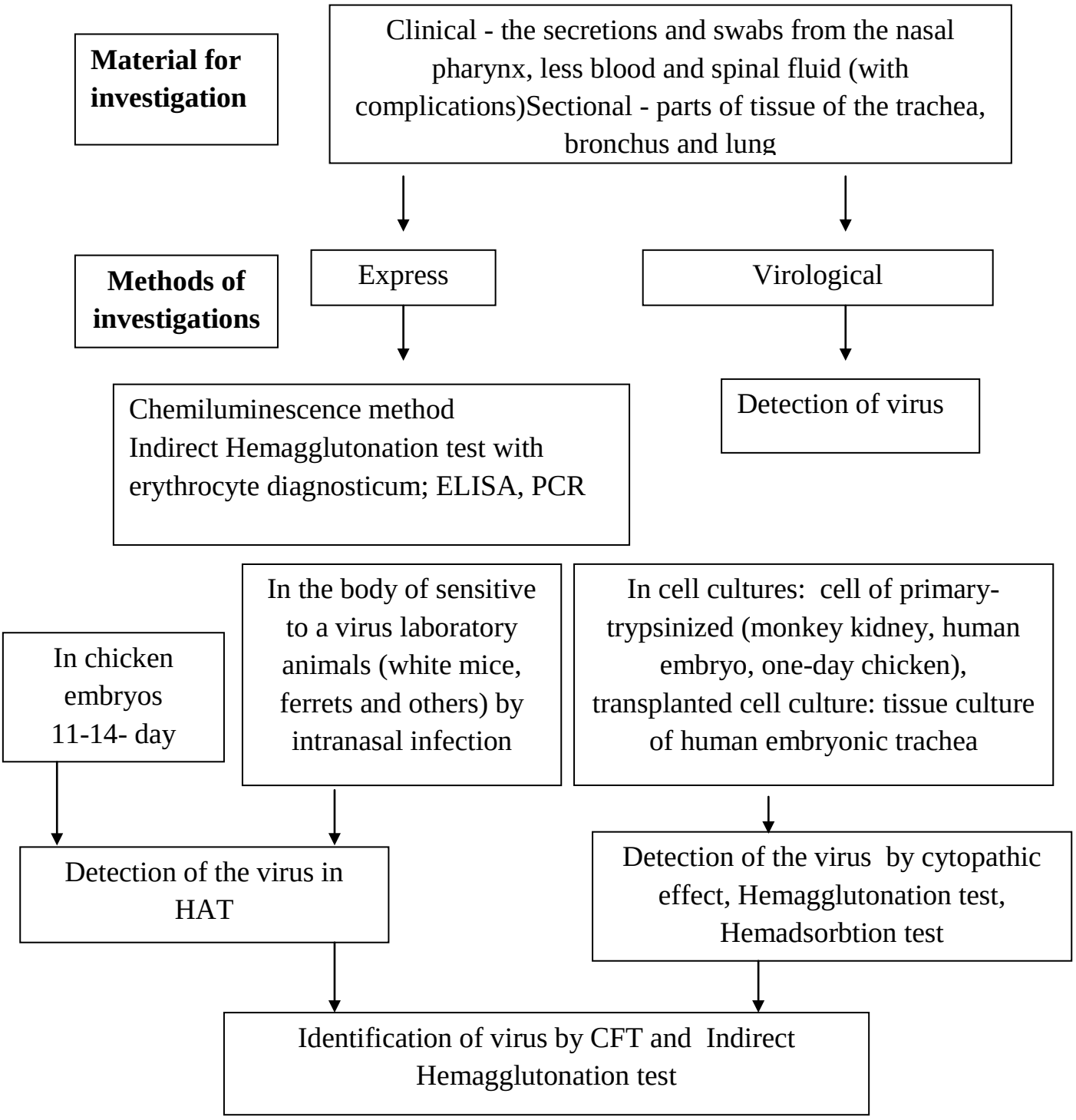
Treatment: Amantadine is available but infrequently used.

Prevention: Vaccine contains inactivated strains of A and B virus currently causing disease.

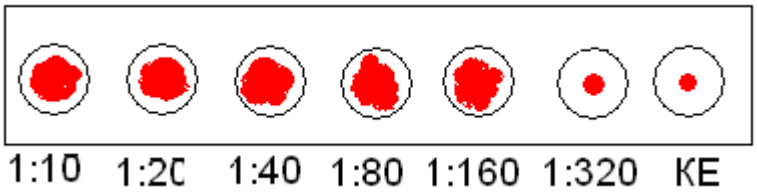
The vaccine is not a good immunogen and must be given annually. Recommended for people older than age 65 years and for those with chronic diseases, especially of the heart and lungs.

Amantadine provides good prophylaxis in unvaccinated people who have been exposed.

Scheme of laboratory diagnosis of influenza



***Task*. To use the Indirect Hemagglutonation test to detect the virus with ability of hemagglutination**.



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

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

Parainfluenza Virus

Disease: Bronchiolitis in infants, croup in young children, and the common cold in adults.

Characteristics: Enveloped virus with helical nucleocapsid and one piece of single-stranded, negative-polarity RNA. RNA polymerase in virion. Unlike influenza viruses, the antigenicity of its hemagglutinin and neuraminidase is stable. There are four serotypes.

Transmission: Respiratory droplets.

Pathogenesis: Infection and death of respiratory epithelium without systemic spread of the virus. Multinucleated giant cells caused by the viral fusion protein are a hallmark.

Laboratory Diagnosis: Isolation of the virus in cell culture is detected by hemadsorption. Im-munofluorescence is used for identification. A 4-fold or greater rise in antibody titer is diagnostic in primary infections, but the heterotypic response limits its usefulness in repeated infections.

Treatment: None.

Prevention: No vaccine or drug is available.

The main properties of Adenoviruses.

1. Icosahedral symmetry of nucleocapsid;

2. Capsid consists of 252 capsomeres: 240 hexons; 12 pentons with fibers associated with each penton;

3. Double-stranded linear DNA genome; encoding 30 genes.

Scheme of laboratory diagnostics of Adenoviruses infections.

<table>
<tr><td rowspan="4">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="3">Fluorescence rhinocytoscopy</td></tr>
<tr><td>Cytopathic effect (CPE)</td><td>Serological identification
1.Reaction of neutralization;
2.Reaction of</td></tr>
</table>

		inhibition of hemagglutination	
2. Serological diagnostics	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.		
3. Genetic diagnostics	PCR		

Rotavirus

Disease: Rotavirus causes gastroenteritis (diarrhea), especially in young children.

Characteristics: Naked double-layered capsid with 10 or 11 segments of double-stranded RNA. RNA polymerase in virion.

Rotavirus is resistant to stomach acid and hence can reach the small intestine. There are at least six serotypes.

Transmission: Rotavirus is transmitted by the fecal-oral route.

Pathogenesis: Rotavirus infection is limited to the gastrointestinal tract, especially the small intestine.

Laboratory Diagnosis: Detection of rotaviras in the stool by ELISA. Isolation of the virus is not done from clinical specimens.

Treatment: No antiviral drug is available.

Prevention: A vaccine containing live, attenuated virus was available but has been withdrawn because of side effects.

Rhinoviruses

Disease: Common cold.

Characteristics: Naked nucleocapsid viruses with single-stranded, positive-polarity RNA. No virion polymerase.

There are more than 100 serotypes. Rhinoviruses are destroyed by stomach acid and do not replicate in the gastrointestinal tract, in contrast to other picornaviruses such as po-liovirus, coxsackievirus, and echovirus, which are resistant to stomach acid.

Transmission: Aerosol droplets and hand-to-nose contact.

Pathogenesis: Infection is limited to the mucosa of the upper respiratory tract and conjunctiva. The virus replicates best at the low temperatures of the nose and less well at 37 °C, accounting for its failure to infect the lower respiratory tract.

Laboratory Diagnosis: Laboratory tests are rarely used clinically.

The virus can be recovered from nose or throat washings by growth in cell culture. Serologic tests are not useful.

Tratment: No antiviral therapy is available.

Prevention: No vaccine is available because there are too many serotypes.

Respiratory Syncytial Virus

Diseases: Bronchiolitis and pneumonia in infants. Otitis media in older children.

Characteristics: Enveloped virus with a helical nucleocapsid and one piece of single-stranded, negative-polarity RNA. RNA polymerase in virion.

Unlike other paramyxoviruses, it has only a fusion protein in its surface spikes. It has no hemagglutinin. It has a single serotype.

Transmission: Respiratory droplets.

Pathogenesis: Infection involves primarily the lower respiratory tract in infants without systemic spread.

Immune response probably contributes to pathogenesis.

Laboratory Diagnosis: Isolation in cell culture. Multinucleated giant cells visible.

Immunofluo-rescence is used for identification. Serology is not useful for diagnosis in infants.

Treatment: Aerosolized ribavirin for sick infants.

Prevention: No vaccine or prophylactic drug is available.

Protocol of practical lesson

Theme: **Laboratory diagnostic of influenza and parainfluenza. acute respiratory virus infections (adenovirus, rhinovirus, reovirus, respiratory-syncytial virus).**

<u>Question for the learning.</u>

1. Antiviral immunity. Features of specific protection of organism. Interferon, its properties.
2. Influenza viruses, their properties. Classification and variability, cultivation. Antigenic structure of

influenza viruses. Pathogenesis, mechanism of contamination, clinical development of influenza. Role of specific and non specific mechanisms in antiflue immunity. Laboratory diagnostic, specific treatment and prophylaxis of influenza.

3. Parainfluenza viruses, main properties, cultivation. Pathogenesis and clinical manifestation,

immunity.Laboratory diagnostic, treatment of parainfluenza.

4. Adenovuruses and their characteristic. Mechanism of infection, pathogenesis, immunity. Laboratory

diagnostic and specific prophylaxis.

5. Rhinoviruses, reoviruses and respiratory-syncytial viruses, their characteristic.

Independent work.

1. Draw the structure of the influenza virus.

_____

_____

_____

2. Name taxonomy of the influenza virus and parainfluenza virus.

	Influenza	**Parainfluenza**
Family		
Genus		

3. Name morphological properties of the influenza virus and parainfluenza virus.

	Influenza	**Parainfluenza**
Genome		
Shape		
Size of virion		
Site of synthesis of ribonucleoprotein		
Antigenic stability		

4. Explain the terms "Antigenic drift" and "Antigenic shift".

Antigenic drift_____

_____

_____

Antigenic shift_____

_____

5. Fill in the table.Virological investigations of influenza and parainfluenza.

	Specimens	**Object for cultivation of the virus**	**Indication of the virus**	**Identification of the virus**

Influenza				
Parainfluenza				

6. Enumerate biological preparations for treatment of influenza and parainfluenza.

_____

_____

7. Enumerate biological preparations for specific prophylaxis of influenza and parainfluenza.

_____

_____

8. Enumerate non specific factors of antiviral immunity and give examples.

_____

_____

9. What is the interferon? Its mean properties.

_____

_____

10. Name taxonomy of the adenovirus, rhinovirus, respiratory-syncytial virus.

	Adenovirus	Rhinovirus	Respiratory-syncytial virus
Family			
Genus			

11. Fill in the table. **Virological investigations of the adenovirus, rhinovirus, respiratory-syncytial virus infections.**

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

Respiratory-syncytial virus infection				

Theme: **Laboratory diagnostics of measles, mumps and rubella.**

1. Measels virus, mumps virus and rubivirus.
2. Main biological properties, cultivation.
3. Pathogenesis and clinical manifestation, immunity.
4. Laboratory diagnostics, specific prophylaxis measels, mumps and rubella.

Virus of Measles.

Measles is acute high contagious infectious disease of children. The measles - is characterized by fever, catarrhal effect and rash.
Virus of Measles was excreted in 1954.

Family ***Paramyxoviridae*** Genus ***Morbillivirus***

Morphology: Virus of Measles has spherical form, medium size, contains RNA.

Antigenic structure: Virus of Measles contains few antigens: external and surface antigens. Antigen has a hemagglutinin activity.

Cultivation: The cultivation of viruses lays in cells cultures, where one can see cytopathogenic activity of virus, appearing of inclusions, hemadsorption phenomenon.

Resistance: Virus died from direct sunshine, UV-rays.

Epidemiology: It is the rise of epidemic. All the age groups may become ill. Sick becomes contagious at the last days of incubation period and at the first days of the rash appearance.The main way of transmission is respiratory way.

Pathogenesis and clinical symptoms: Virus of Measles penetrates through mucous membrane of upper respiratory tracts (it is the place of its reproduction), then gets into blood and strikes vessel's cells. Incubation period lasts for 8-21 days.

Prodromaliny period runs like acute respiratory disease. Then the rash appears on skin and mucous membrane, which spreads from top to down. Disease lasts for 7-9 days. Pneumonia and acute encephalitis are the variants of complications. In the development of complications the main role plays ability of virus to inhibit T-lymphocyte's activity and cause the weakening of immunity reactions.

Immunity: Stable, for whole life.

Laboratory diagnosis: For analysis there are taken nasopharynx smears, blood, urine, and skin's tests.

Express-diagnostic is based on finding specific antigens with help of immunofluorescence, finding antigens type IgM with help of immune-enzyme analysis. It's possible to excreted virus of Measles on cells culture.

Identification is made with help of immunofluorescence reaction, hemagglutination-inhibition reaction and neutralization reaction in cells culture. Serum from sick people is researched with help of neutralization reaction, complement-fixing reaction, hemagglutination-inhibition reaction.

Specific prophylaxis is made by living vaccines A.A. Smorodinceva and M.P. Chumakova. It is entered to 1-year children into vein. Gammaglobulins are used in the center of infection. Passive immunity lasts for 1 month.
Treatment – symptomatic treatment.

Epidemical Parotiditis.

Parotiditis is acute infectious disease of children and characterized by lesion of parotids salivary glands. Virus of Parotiditis was extracted in 1934.

Family ***Paramyxoviridae*** Genus ***Paramyxovirus***

Morphology : Virus of Parotiditis has oval form, medium size, contains RNA.

Antigenic structure: Virus of Parotiditis possesses a hemagglutinin activity.

Cultivation: Chicken's embryos, cells cultures are used for cultivation.
Resistance: Virus isn't stability.

Epidemiology: It is the rise of epidemic. Children from 3 to 15 years. Sick becomes contagious at the last days of incubation period and at the first days of the rash appearance. The main way of transmission is respiratory way.
Pathogenesis and clinical symptoms: Virus of Parotiditis gets on mucous membranes of upper respiratory tracts, oral cavity, bulbar conjunctiva, then viruses gets into the blood (after reproduction) and spreads throughout the organism. Virus of Parotiditis possess the tropism of glandular organs and nervous system. Incubation period lasts for 18-21 days.

Parotiditis cause the fever, inflammation and intumescences of salivary glands, especially parotid glands. The disease lasts for 7-10 days. There are lesions of other glandular organs and central nervous system.

Orchitis, meningitis, encephalitis are able to become a complication.

Immunity. Stable, for whole life.

Laboratory diagnosis: For analysis there are taken saliva, nasopharynx smears, blood, urine, cerebrospinal fluid. Virus of Parotiditis is extracted on cells cultures and chickens embryos. Identification is made with help of immunofluorescence reaction, neutralization reaction, hemagglutination-inhibition reaction, complement-fixation reaction.

Specific prophylaxis is made by living vaccines A.A. Smorodinceva for children before 1 year.
Treatment – symptomatic treatment.

Rubivirus

Rubella is acute infectious disease, characterized by fever and rash. There is a risk of birth a deformity or died child from pregnant woman, sick for rubella. Rubivirus was extracted in 1961.

Family **T*ogaviridae*** Genus ***Rubivirus***

Morphology: Rubivirus has spherical form, medium size, contains RNA.

Antigenic structure: Rubivirus contains few antigens: external and surface antigens. Antigen has a hemagglutinin activity.

Cultivation: The cultivation of viruses lays in primary and interweaves cells cultures, where one can see cytopathogenic activity of virus, appearing of inclusions.

Epidemiology: It is the rise of season epidemic in winter and spring. All the age groups may become ill (children from 3 to 6 and adults).

The way of transmission is respiratory way. Rubivirus passes through placental barrier and infects the child.

Pathogenesis and clinical symptoms: Rubivirus gets on mucous membrane of upper respiratory tracts. It has its reproduction in cervical lymphatic nodes. Then gets into the blood (after reproduction) and spreads throughout the organism. Incubation period lasts for 11-22 days.

The disease begins with rise in temperature, appearing spots of rush, inclusion of cervical lymphatic nodes. The run of disease is easy, complications are uncommon. Rubella is dangerous for pregnant women, in a cause of risk of fetus wastage.

Immunity. Immunity is stable.

Laboratory diagnosis: For analysis there are taken nasopharynx smears, blood, urine, feces. The extraction of virus is made in a cells culture.

Identification is made with help of hemagglutination-inhibition reaction. Serum diagnostics is made with help of reaction of immunofluorescence, immunoenzyme analysis, and radioimmunoassay.

Specific prophylaxis is made by living and inactivated vaccines.
Treatment – symptomatic treatment.

Protocol of practical lesson

Theme: **Laboratory diagnostics of measles, mumps and rubella.**

Name taxonomy of the measles, mumps and rubella viruses.

	Measles virus	Mumps virus	Rubella virus
Family			
Genus			

Name morphological properties of the measles, mumps and rubella viruses.

	Measles virus	Mumps virus	Rubella virus
Genome			
Shape			
Size of virion			
Site of synthesis of ribonucleoprotein			
Antigenic stability			

Fill in the table. **Virological investigations of the measles, mumps and rubella.**

	Specimens	Object for cultivation of the virus	Indication of the virus	Identification of the virus
Measles				

Mumps				
Rubella				

Enumerate biological preparations for treatment of the measles, mumps and rubella viruses infections.

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Enumerate biological preparations for specific prophylaxis of the measles, mumps and rubella viruses infections.

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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.

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 latent virus.

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 immunocompromised individuals.

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

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.

Smallpox Virus

Disease: Smallpox (eradicated in 1977).

Characteristics: Poxviruses are the largest viruses. Enveloped virus with linear double-stranded DNA. DNA-dependent RNA polymerase m virion. One serologic type.

Transmission: By respiratory droplets or direct contact with the virus from skin lesions.

Pathogenesis: The virus infects the mucosal cells of the upper respiratory tract, then spreads to the local lymph nodes and by viremia to the liver and spleen and later the skin. Skin lesions progress in the following order: macule, papule, vesicle, pustule, crust.

Laboratory Diagnosis: Virus identified by CPE in cell culture or "pocks" on chorioallantoic membrane. Electron microscopy reveals typical particles; cytoplasmic inclusions seen in light microscopy.

Viral antigens in the vesicle fluid can be detected by precipitin tests. A 4-fold or greater rise in antibody titer in the convalescent-phase serum is diagnostic.

Treatment: None.

Prevention: Vaccine contains live attenuated vaccinia virus. Vaccine is no longer used except by the military, because the disease has been eradicated.

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.

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 inactivated 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.

Protocol of practical lesson

Theme: **Laboratory diagnostic of smallpox, chickenpox (varicella), herpes simplex, zoster, poliomyelitis, Coxsackie and ECHO.**

Question for the learning.

1. Smallpox viruses, their characteristic. Mechanism of infection. Pathogenesis, immunity. Laboratory diagnostic and specific prophylaxis. Liquidation of smallpox all over the world.
2. Herpesviruses. Characteristic, cultivation. Pathogenesis, clinical manifestation, immunity. Laboratory diagnostic, treatment and prophylaxis.
3. Virus of the chicken pox (varicella) and zoster. Characteristic, cultivation. Pathogenesis, clinical manifestation, immunity. Laboratory diagnostic, treatment and prophylaxis.

4. Picornoviruses, viruses of Coxsackie and ECHO. Viruses of poliomyelitis, their properties, classification. Pathogenesis, clinical manifestation, immunity. Laboratory diagnostic, treatment and prophylaxis.

Independent work.

1. Name taxonomy of the smallpox, chickenpox, herpes simplex, zoster viruses.

	Smallpox virus	**Herpes simplex virus**	**Chickenpox virus**	**Zoster virus**
Family				
Genus				

2. Name morphological properties of the smallpox, chickenpox, herpes simplex, zoster viruses.

	Smallpox virus	**Herpes simplex virus**	**Chickenpox virus**	**Zoster virus**
Genome				
Shape				
Size of virion				
Site of synthesis of ribonucleoprotein				
Antigenic stability				

3. Fill in the table. **Virological investigations of the smallpox, chickenpox (varicella), herpes simplex, zoster.**

	Specimens	**Object for cultivation of the virus**	**Indication of the virus**	**Identification of the virus**

Smallpox				
Chickenpox (varicella)				
Herpes simplex				
Zoster				

5. **Biological preparations for treatment of the smallpox,chickenpox(varicella),herpes simplex, zoster.**

5. Fill in the table. **Classification of human herpesviruses**

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

6. Name taxonomy of the viruses of poliomyelitis, Coxsackie, ECHO.

	Poliovirus	Coxsackie virus	ECHO virus
Family			
Genus			

7. Name morphological properties of the viruses of poliomyelitis, Coxsackie, ECHO.

	Poliovirus	Coxsackie virus	ECHO virus
Genome			
Shape			
Size of virion			

Site of synthesis of ribonucleoprotein			
Type of symmetry			

8. Fill in the table. Virological investigations of poliomyelitis, Coxsackie, ECHO.

	Specimens	**Object for cultivation of the virus**	**Indication of the virus**	**Identification of the virus**
Poliomyelitis				
Coxsackie				
ECHO				

9. Enumerate biological preparations for treatment of poliomyelitis, Coxsackie, ECHO.

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10. Enumerate biological preparations for specific prophylaxis of poliomyelitis, Coxsackie, ECHO.

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11. Fill in the table.

Clinical features of poliomyelitis, Coxsackie, ECHO .

Poliomyelitis	Coxsackie	ECHO

Hepatitis A Virus

Disease: Hepatitis A.

Characteristics: Naked nucleocapsid virus with a single-stranded, positive-polarity RNA. No virion polymerase. Virus has a single serotype.

Transmission: Fecal-oral route.

Pathogenesis: The virus replicates in the gastrointestinal tract and then spreads to the liver during a brief viremic period.

The virus is not cytopathic for the hepatocyte. Hepatocellular injury is caused by immune attack by cytotoxic T cells.

Laboratory Diagnosis: The most useful test is IgM antibody. Isolation of the virus from clinical specimens is not done.

Treatment: No antiviral drug is available.

Prevention: Vaccine contains killed virus. Administration of immune globulin during the incubation period can mitigate the disease.

Hepatitis B Virus

Diseases: Hepatitis B; implicated as a cause of hepatocellular carcinoma.

Characteristics: Enveloped virus with incomplete circular double-stranded DNA; ie, one strand has about one-third missing and the other strand is "nicked" (not covalently bonded). DNA polymerase in virion. HBV-encoded polymerase acts as a reverse transcriptase by using viral mRNA as the template for the synthesis of progeny genome DNA.

There are three important antigens: the surface antigen, the core antigen, and the e antigen, which is located in the core. In the patient's serum, long rods and spherical forms composed solely of HBsAg predominate.

HBV has one serotype based on the surface antigen.

Transmission: Transmitted by blood, during birth, and by sexual intercourse.

Pathogenesis: Hepatocellular injury due to immune attack by cytotoxic (CD8) T cells. Antigen-antibody complexes cause arthritis, rash, and glomerulonephritis. About 5% of HBV infections result in a chronic carrier state.

Chronic hepatitis and cirrhosis can occur.

Hepatocellular carcinoma may be related to the integration of part of the viral DNA into hepatocyte DNA.

Laboratory Diagnosis: HBV has not been grown in cell culture. Three serologic tests are commonly used: surface antigen (HBsAg), surface antibody (HBsAb), and core antibody (HBcAb).

Detection of HbsAg for more than 6 months indicates a chronic carrier state. The presence of e antigen indicates a chronic carrier who is making infectious virus.

Treatment: No specific treatment.

Prevention: There are three main approaches:

(1) vaccine that contains HBsAg as the immuno-gen;

(2) hyperimmune serum globulins obtained from donors with high titers of HBsAb; and

(3) education of chronic carriers regarding precautions.

Hepatitis D Virus

Disease: Hepatitis D (delta).

Characteristics: Defective virus that uses hepatitis B surface antigen as its protein coat. HDV can replicate only in cells already infected with HBV; ie, HBV is a helper virus for HDV. Genome is one piece of single-stranded, negative-polarity, circular RNA. No polymerase in virion.

1- transactivation of transcription

HDV has one serotype.

Transmission: Transmitted by blood, sexually, and from mothej; to child.

Pathogenesis: Hepatocellular injury probably caused by cytotoxic T cells. Chronic hepatitis and chronic carrier state occur.

Laboratory Diagnosis: Serologic testing detects either delta antigen or antibody to delta antigen.

Treatment: Alpha interferon mitigates symptoms but does not eradicate the carrier state.

Prevention: Prevention of HBV infection by using the HBV vaccine and the HBV hyperimmune globulins will prevent HDV infection also.

Hepatitis C Virus

Disease: Hepatitis C; associated with hepatocellular carcinoma.

Characteristics: Enveloped virus with one piece of single-stranded, positive-polarity RNA. No polymerase in virion. HCV has multiple serotypes.

Transmission: Most transmission is via blood. Sexual transmission and transmission from mother to child probably occurs as well.

Pathogenesis: Hepatocellular injury probably caused by cytotoxic T cells. HCV does not cause a cytopathic effect. More than 50% of infections result in the chronic carrier state.

The chronic carrier state predisposes to chronic hepatitis and to hepatocellular carcinoma.

Laboratory Diagnosis: Serologic testing detects antibody to HCV.

Treatment: Alpha interferon mitigates chronic hepatitis but does not eradicate the carrier staie.

Prevention: Posttransfusion hepatitis can be prevented by detection of antibodies in donated blood. There is no vaccine, and hyperimmune globulins are not available.

Rabies Virus

Disease: Rabies.

Characteristics: Bullet-shaped enveloped virus with a helical nucleocapsid and one piece of single-stranded, negative-polarity RNA. RNA polymerase in virion.

The virus has a single serotype.

Transmission: Animal bite, usually by wild animals such as skunks, raccoons, and bats. In the United States, dogs are infrequently involved, but in developing countries they are often involved.

Pathogenesis: Viral receptor is the acetylcholine receptor on the neuron. Replication of virus at the site of the bite, followed by ascension up the nerve to the central nervous system.

After replicating in the brain, the virus migrates peripherally to the salivary glands, where it enters the saliva.

When the animal is in the agitated state as a result of encephalitis, virus in the saliva can be transmitted via a bite.

Laboratory Diagnosis: Tissue can be stained with fluorescent antibody or with various dyes to detect inclusions called Negri bodies.

The virus can be isolated in newborn mice, but because this procedure takes 1 or 2 weeks, it cannot be used to determine whether a person should receive the vaccine. Serologic testing is useful only to make the diagnosis in the clinically ill patient; it does not help the person who has been bitten.

It is also used to evaluate the antibody response to the vaccine given before exposure to those in high-risk occupations.

Treatment: No antiviral therapy is available.

Prevention: Preexposure prevention of rabies consists of the vaccine only. Postexposure prevention consists of (1) washing the wound; (2) giving immune serum, mostly into the wound; and (3) giving the inactivated vaccine made in human cell culture.

The decision to give the immune serum and the vaccine depends on the circumstances.

Prevention of rabies in dogs and cats by using a killed vaccine has reduced human rabies significantly.

Protocol of practical lesson

Theme: **Laboratory diagnostics of hepatitis A, B, C, D, E, G, F.**
Laboratory diagnostic of rabies, encephalitis, hemorrhagic fevers.

Question for the learning.

1. Hepatitis viruses. Hepatitis A, B, C, D, E, G. Biological characteristic of viruses. Sources of infections, mechanism of transmission. Pathogenesis and clinical manifestations. Laboratory diagnostic. Treatment and specific prophylaxis.

2. Rabies virus, their properties, cultivation. Mechanism of man's infection and pathogenesis. Laboratory diagnostic of rabies. Antirabic immunoglobulin and vaccine. Pasteur's merits in creation of antirabic vaccine.

3. Infections, caused by arthropod borne viruses (encephalitis). Morphology, antigenic structure of viruses. Virus of hemorrhagic and yellow fevers.

Independent work.

1. Name taxonomy of the viruses of hepatitis A, B, C, D, E, G, F.

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

2. Draw the structure of Hepatitis B virus.

3. Fill in the table.

	Source of infection	Transmission of infection
Hepatitis A		
Hepatitis B		
Hepatitis C		
Hepatitis D		
Hepatitis E		
Hepatitis F		
Hepatitis G		

4. Fill in the table.

Virological investigations of hepatitis A, B, C, D, E, G, F.

	Specimens	Object for cultivation of the virus	Indication of the virus	Identification of the virus
Hepatitis A				
Hepatitis B				
Hepatitis C				
Hepatitis D				

Hepatitis E				
Hepatitis F				
Hepatitis G				

6. Enumerate biological preparations for treatment of hepatitis A, B, C, D, E, G, F.

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6. Enumerate biological preparations for specific prophylaxis of hepatitis and give characteristic.

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7. Name peculiarity of the morphological structure and reproduction of the Hepatitis D virus.

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8. Name taxonomy of the viruses of rabies, encephalitis, hemorrhagic fevers.

	Rabies	**Encephalitis**	**Hemorrhagic fevers**
Family			
Genus			

9. Fill in the table. Clinical features of rabies, encephalitis, hemorrhagic fevers.

Rabies	Encephalitis	Hemorrhagic fevers

Virological investigations of rabies, encephalitis, hemorrhagic fevers.

	Specimens	Object for cultivation of the virus	Indication of the virus	Identification of the virus
Rabies				
Encephalitis				
Hemorrhagic fevers				

12. Enumerate biological preparations for treatment and specific prophylaxis of rabies.

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13. Draw inclusion bodies.

Negri bodies in the cytoplasm of neurons in rabid dog brain. Mann's stain.

14. Fill in the table. Arboviruses associated with different clinical syndromes

Virus	Distribution	Vector	Reservoir
E n c e p h a l i t i s			
Eastern equine encephalitis			
Western equine encephalitis			
St. Louis encephalitis			
Murray Valley encephalitis			
Japanese encephalitis			
H e m o r r h a g I c F e v e r			
Yellow fever			
Omsk hemorrhagic fever			
Hemorrhagic fever			

15. Enumerate biological preparations for treatment of encephalitis, hemorrhagic fevers.

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16. Enumerate biological preparations for specific prophylaxis of encephalitis, hemorrhagic fevers.

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Human Immunodeficiency Virus

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 many serotypes.

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, eg, astrocytes, are infeoted 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 ddC inhibit 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 no vaccine.

Protocol of practical lesson

Theme: **Laboratory diagnostic of HIV-infection. AIDS. Oncogenic viruses.**

Questions for the learning.

1. Viruses of immunodeficiency. HIV-infection and AIDS. Mechanism of transmission, clinical

manifestations, pathogenesis. Laboratory diagnostic of disease. Treatment and prophylaxis. Prospects of specific prophylaxis and treatment.

2. Oncogenic viruses, general characteristic, classification. Mechanism of viral carcinogenesis. Virusogenetic theory of oncogenesis. Works by Zilber in questions of development of doctrine on role of viruses in occurrence of new formations.

Independent work.

1. **Decipher HIV**_____
2. **Decipher AIDS** _____
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3. **Draw the structure of HIV.**

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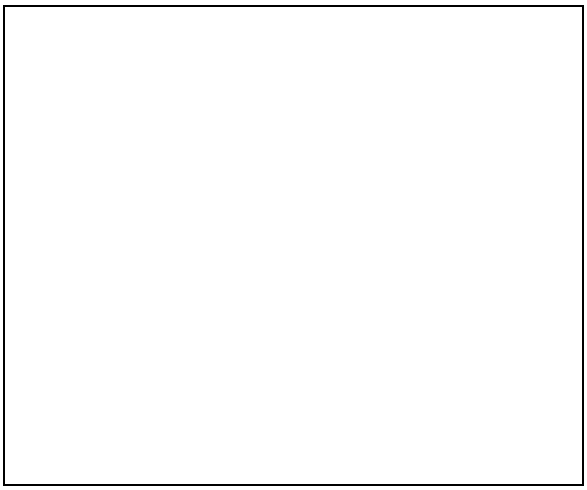

4. Name taxonomy of HIV.

	H I V
Family	
Genus	

5. Name morphological properties of HIV.

	H I V
Genome	
Shape	
Size of virion	
Site of synthesis of ribonucleoprotein	
Type of symmetry	

6. Name major antigens of HIV.

Major antigens of HIV

A._____ **C._____**

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_____ **D._____**

B._____ **_____**

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7. Reproduction of HIV.

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8. Pathogenesis of AIDS.

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9. Modes of transmission.

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10. Enumerate biological preparations for treatment of AIDS.

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11. Enumerate biological preparations for prophylaxis of AIDS.

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12. Fill in the table.

Virological investigations of AIDS.

Specific tests for HIV infections	Laboratory tests for detection of specific antibodies in HIV infections

13. Name the classification of oncogenic viruses and oncogenic diseases.

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Semantic module. General virology.

1. History of discovery and the main stages in the development of virology. The contribution of national scientists. Methods of study of viruses and their evaluation.
2. Morphology and ultrastructure of viruses. Symmetry types of viruses. Chemical composition and function of components of the virus.
3. Bacteriophage, the history of studying. Structure, classification of phages by morphology. Methods of qualitative and quantitative determination of bacteriophages. Practical use of bacteriophages.

4. Forms of interaction between bacteriophage and bacterial cell. Virulent and moderate phages. Characteristics of productive interaction. Lysogenity and phage conversion.
5. Modern views on the nature and origin of viruses. Place of viruses in the live system.
6. Principles of viruses classification. Basic properties of human and animal viruses.
7. Methods of cultivation of viruses and their evaluation.
8. Reactions of viral hemagglutination and hemadsorbtion. The mechanism, practical importance, use and diagnostic value.
9. Serological tests that are used in virology. Reaction of virus neutralization, mechanism, principles of use, diagnostic value.
10. Hemagglutination inhibition test, its mechanism, principles of use, diagnostic value.
11. Complement fixation test, its essence, evaluation. Features of complement fixation test in viral infection diagnosis.
12. Reactions with labeled antibodies and antigens in virology. Immunofluorescence reaction (IFR).
13. Usage of cell cultures in virology. Classification of cell cultures. Growth medium for cells culturing.
14. Types of interaction between viruses and cells. Characteristics of productive interaction, phases.
15. Features of the pathogenesis of viral infections. Acute and persistent viral infections.
16. Immunological features of viral infections. Factors of antiviral immunity.
17. Detection of viruses in cell culture and their evaluation. Cytopathogenic action of viruses, its form.
18. Nonspecific factors of macroorganism protection from viral agents and their characteristics. Interferons, mechanism of action, interferon-stimulated genes.

19. Viral vaccines, classification, principles receiving, the requirements for them, control, evaluation of effectiveness.

Special virology.

1. Orthomyxoviridae family. History of discovery, biological properties, classification.
2. Methods of laboratory diagnosis of influenza and its estimation.
3. Antigenic structure and types of antigenic variability of influenza virus. Modern hypotheses explaining antigenic variability 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 diagnosis of parainfluenza infections.
7. Measles virus biological properties and cultivation. Pathogenesis of infection. Laboratory diagnosis and specific prophylaxis.
8. Mumps (epidemic parotitis) virus. Pathogenesis of infection. Laboratory diagnosis 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.
11. Picornaviridae family, general characteristics. Antigenic structure. Biological features of Coxsackie viruses, properties. The value in the development of human pathology.

12. Polioviruses, characteristics, classification. Pathogenesis and immunogenesis of infection. Laboratory diagnosis and specific prophylaxis. The problem of polio worldwide eradication.
13. Enterovirus genus, general characteristics, classification. Laboratory diagnosis of infections cauaed by enteroviruses.
14. Rhinovirus genus, biological properties. Classification. Role in human pathology. Methods of laboratory diagnosis of infections caused by rhinoviruses.
15. Rabdoviridae family. Rabies virus, biological properties. The pathogenesis of the disease. Laboratory diagnosis. Differentiation of fixed and wild rabies virus. Specific prophylaxis of rabies.
16. General characteristics of the arbovirus ecological group. Tick-borne and japanese encephalitis viruses. History of discovery and study of these viruses. Biological properties, methods of laboratory diagnosis, specific prophylaxis.
17. Rubivirus genus. Rubella virus. Biological properties. Pathogenesis of disease. Immunity. Laboratory diagnosis and specific prophylaxis.
18. Retroviridae family, biological properties. Classification. The mechanism of viral carcinogenesis.
19. Herpesviridae family, biological properties, the value in the development of human pathology. Laboratory diagnosis of diseases. Genetic methods for diagnosis.
20. Adenoviridae family. Biological properties. Antigenic structure. Cultivation. Pathogenesis and laboratory diagnosis of infection caused by adenoviruses. Immunity. Specific prevention.
21. Variola virus. Pathogenesis of infection. Methods of diagnosis and specific prophylaxis. Vaccinia virus. Smallpox eradication worldwide.
22. Causative agents of viral hepatitis, properties and classification of viruses. Pathogenesis of diseases. Laboratory diagnosis. Prospects of specific prevention.
23. Oncogenic virus classification. Virus-genetic tumor formation theory by L.A. Zilber. Mechanisms of viral carcinogenesis.

24. Human immunodeficiency virus (HIV). Properties. Role in human pathology. Pathogenesis of AIDS. Methods of laboratory diagnosis (immunological, genetic). Prospects for of specific preventive and therapy.
25. Cardioviruses. General characteristics.
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