Ministry of Education and Science of Ukraine

Ukrainian Medical Stomatological Academy
Microbiology, Virology and Immunology Chair

Microbiology, Virology and Immunology

TRAINING MANUAL
for foreign faculty students
specialty "Medicine"

Part 2

II year _____*group*

Student: _____

Poltava 2019

Training manual on Microbiology, Virology and Immunology for students of faculty of foreign students training specialty "Dentistry" was created by:

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Literature for self-directed work:

Basic

1. Medical microbiology, virology and immunology - T. V. Andrianova, V. V. Bobyr, V. V. Danyleichenko, etc. / Ed. by V. P. Shyrobokov/ - Vinnytsia: Nova Knyha, 2019. - 744 p.
2. Cappuccino G. Microbiology: A Laboratory Manual, Global Edition, 11th Edition / G.Cappuccino, Chad T. Welsh. - Pearson Higher Ed USA, 2017. – 560 p.
3. Medical Microbiology 27 E (Lange) / K. C. Carroll, S. Morse, T. Mietzner [et al.]. - McGraw-Hill Education, 2016. – 864 p.
4. Murray P.R. Medical Microbiology 8th Edition / P.R. Murray, K.S. Rosenthal, M.A. Pfaller. – Elsevier, 2016. - 848 p.
5. Murray P.R. Basic Medical Microbiology 1st Edition / P.R. Murray. - Elsevier, 2018. – 240 p.
6. Medical Microbiology, International Edition, 19 Ed / M.R. Barer, W. Irving, A. Swann [et al.]. - Elsevier, 2018. - 760 p.
7. Engelkirk P.G. Burton's Microbiology for the Health Sciences / P.G. Engelkirk, J. Duben-Engelkirk, R. Fader. - Wolters Kluwer Health, 2015. – 488 p.
8. Hawley L. Microbiology and Immunology (Board Review Series) Sixth Edition / L. Hawley, R.J. Ziegler, B. L. Clarke. - Lippincott Williams & Wilkins, 2014. – 320 p.
9. General Medical Microbiology, Virology and Immunology. Part I. Manual for practical lessons / Comp. by Loban G.A., Hancho O.V. – Poltava, 2005. –153 p.
10. General Medical Microbiology, Virology and Immunology. Part II. Manual for practical lessons/ Comp. by Loban G.A., Hancho O.V. – Poltava, 2007.– 104 p.
11. Pathogenic cocci. Gramnegative intestinal pathogens. Manual for practical lessons /Composed by Hancho O.V. - Poltava, UMSA, 2006. - 113 p.
12. Oral cavity flora. Manual of Microbiology, Virology and Immunology for dental faculty students/ Composed by Hancho O.V.- 2010, Poltava. - 88 p.

Additional

1. Ananthanarayan R., Paniker C.K. Textbook of Microbiology. - International edition. - 2003. – 612 p.
2. MIMs' Medical Microbiology and Immunology 6th / Richard Goering, Hazel Dockrell, Mark Zuckarman [et al.]. – Elsevier, 2019. – 568 p.
3. Harriott M. Microbiology in Your Pocket: Quick Pathogen Review 1st Edition / M. Harriott. – Thieme, 2018. – 330 p.
4. Medical Microbiology / Patrick R. Murray [et al.]. – 4th ed. – An Affiliate of Elsevier Science, 2002. – 826 p.
5. Marsh, P.D. and Martin, M.V. Oral Microbiology, 5th edn. Elsevier: Amsterdam, The Netherlands, 2009. – 300 p.
6. Richard J. Lamont, Robert Burne, Marilyn Lantz, Donald Leblanc. Oral Microbiology and Immunology. Wiley-Blackwell, 2006. – 276 p.

Information resources:

1. President of Ukraine, official website http://www.president.gov.ua/
2. Verkhovna Rada of Ukraine, official web portal http://www.rada.gov.ua/
3. Government Portal, official web portal http://www.kmu.gov.ua/
4. Ministry of Education and Science http://www.mon.gov.ua/
5. Ministry of Ecology and Natural Resources of Ukraine http://www.menr.gov.ua/
6. The State Emergency Service of Ukraine http://www.dsns.gov.ua/
7. National Security and Defense Counsil of Ukraine http://www.rnbo.gov.ua/
8. Permanent Mission of Ukraine to the United Nations http://ukraineun.org/
9. North Atlantic Treaty Organization (NATO) http://www.nato.int/
10. World Health Organization http://www.who.int/en/
11. Microbiology and immunology on-line http://www.microbiologybook.org/
12. On-line microbiology note http://www.microbiologyinfo.com/
13. Centers for diseases control and prevention www.cdc.gov

Date_____ **PRACTICAL SESSION № 1**

Topic: Modern methods of infectious diseases diagnosis

Self-assessment tasks

a) Questions list: theoretical issues:	**b) The list of practical skills and abilities**
1. Reactions with labelled antigens and antibodies: a) chemiluminescence immunoassay CLIA - (direct and indirect); b) enzyme-linked immunosorbent assay - ELISA (direct, indirect, solid-phase, competitive), immunoblotting techniques. c) radioimmunoassay – RIA (competitive, reverse, indirect). 2. Immunoelectron microscopy. 3. Genetic methods in the diagnosis of infectious diseases and the identification of pathogens: a) sequences of DNA, polymerase chain reaction - PCR; b) hybridization of nucleic acids; c) determining the length of nucleic acid fragments. 4. Biocheaps, application in diagnostics.	1. To record and evaluate the results of serological reactions using labelled antigens and antibodies. 2. Ability to evaluate the results of the immunoelectron microscopic test. 3. Ability to interpret the results of genetic methods used to diagnose infectious diseases.

Protocol of practical session

Practical assignments:

Task № 1. Denote the stages of the indirect chemiluminescence immunoassay (CLIA) in order to detect antigens of coronavirus in the cells of the upper respiratory tract epithelium. Conclude.

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Task № 2. Observe and evaluate the results of solid-phase ELISA with a serum of the patient and standard cytomegalovirus antigens. Conclude.

	1	2	3	4	5	6	7	8	9	10	11	12	
A	0,018	0,21 neg	0,012 Neg	0,018 neg	***	***	***	***	***	***	***	***	A
B	0,022 NC2	0,22 neg	0,020 Neg	0,020 neg	***	***	***	***	***	***	***	***	B
C	0,022 NC3	0,143 neg	0,022 Neg	0,025 neg	***	***	***	***	***	***	***	***	C
D	0,021 NC4	0,019 neg	0,020 Neg	0,038 POS	***	***	***	***	***	***	***	***	D
E	2,261 PC1	0,016 neg	0,019 Neg	0,407 POS	***	***	***	***	***	***	***	***	E
F	2,243 PC1	0,027 neg	0,021 Neg	0,380 POS	***	***	***	***	***	***	***	***	F
G	0,018 neg	0,015 neg	0,020 Neg	2,808 POS	***	***	***	***	***	***	***	***	G
H	0,018 neg	0,013 neg	0,018 Neg	2,872 POS	***	***	***	***	***	***	***	***	H

Conclusion:_____

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Task № 3. Evaluate the results of a polymerase chain reaction in order to diagnose infectious mononucleosis.
Keywords:
1. Amplification - massive replication of a target gene or DNA sequence.
2. Primers are short, artificially synthesized DNA molecules that are identical to a particular region of the DNA target and determine the boundaries of the amplified DNA target.

Results of amplificated products electrophoresis:

№№ of wells	1	2	3	4	5	6	7	8
	Clinical samples.					Controls		
						Control "+" № 1	Control "+" № 2 (Master Mix)	Control "-"
Line of the start of DNA electrophoretic separation								
Internal standard								
Target								
Observation								

Stages of Polymerase Chain Reaction (PCR):
The first stage is the separation of double-stranded DNA molecules with the formation of single-stranded molecules (denaturation of DNA).
In the second stage, the primers are joined to the homologous sequences on the DNA target.
The third stage is the synthesis of new DNA chains takes place.
Stages occur with different temperature regimes, and their sequence is one cycle of amplification of certain DNA fragments.
After 30-80 cycles of DNA fragment synthesis, they are identified by electrophoresis.
Possible variants of amplificated products electrophoresis:
1. If there is no amplification of the strip in the column, the reaction does not pass, and the analysis should be repeated.
2. If only the standard strip is visible in the column, the result is negative (in the sample of DNA, the virus of the infectious mononucleosis is absent, or its number is insignificant).
3. If only the strip of target amplitude is visible in the column - the result is positive. It is because a large number of targets suppress standards.
4. If the column shows the standard and the target strips of amplicons, the reaction is positive. The ratio of the intensity of the strips may be different.
5. If the column shows a weak glow of the target and the standard strips, the inhibitor may be present in the reaction. It is necessary to repeat the analysis.
6. In column 6, the positive control of the reaction (C +) was standard and indicated a small number of targets in the sample.
7. In column 7 - control of samples of Master Mix (MM). Positive control of the reaction was normal.
8. In column 8 there is only a strip of the standard, which means that the negative control of the reaction (C-) was normal.

Note: If negative control passes at variants 6 or 7, it is necessary to find out the source of contamination and repeat the analysis.
Conclusion:

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Teacher's signature: _____

Date_____ **PRACTICAL SESSION №2**

Topic: Morphology and ultrastructure of viruses. Cultivation of viruses. Indication of viral reproduction.

Self-assessment tasks

a) Questions list: theoretical issues:	**b) The list of practical skills and abilities**
1. The kingdom of viruses. Viruses as an aspecial form of life organization. 2. Principles of the structural organization of the viruses. Virion and its components. 3. Simple and complex viruses, types of nucleocapsids symmetry. 4. The chemical composition of the viruses. Their features and functions. Enzymes of viruses, their role, classification. 5. Reproduction of viruses in their interaction with the cell. The main stages of the interaction of viruses with cells in productive infection. 6. Integrative and abortive types of interaction of viruses with the host cell. Persistence of a virus in a cell. Virus interference, defective interfering particles. Viruses- satellites. 7. Cultivation of viruses in the cell cultures, chicken embryos, and laboratory animal organisms. 8. Indication of viral reproduction by hemagglutination reaction (HAR) and hemadsorption reaction (HADR). 9. Methods of detection (indication) of viral reproduction by cytopathogenic action, plaque formation, hemagglutination *(*HAR) and hemadsorption (HADR) reactions, and viral inclusions. 10. Identification of viruses by antigenic properties (NT, HIT (hemagglutination inhibition test), HADIT (hemadsorption inhibition test), PHAT (passive hemagglutination test, Rose-Waaler test), CFT, RIA, ELISA, CLIA). 11. Genetic methods for the detection of viruses and their nucleic components.	1. Microscopy of preparations in a light microscope with an immersive lens. 2. The ability to determine the presence of a virus in the chicken embryo by the hemagglutination reaction and the hemadsorption reaction. 3. Ability to set up, observe and evaluate the results of the reactions used in virology.

Protocol of practical session

Practical assignments:

Task № 1. Draw a scheme of the chicken embryo structure. Then, denote ways in order to infect it.

Task № 2. Observe and evaluate the results of the hemagglutination reaction (HAR) to determine the presence of the parainfluenza virus in an infected chicken embryo. Conclude**.**

Dilution / Ingredients	1:10	1:20	1:40	1:80	1:160	1:320	Control of erythrocytes
Allantoic liquid (ml)	0,1	0,5	0,5	0,5	0,5	0,5	-
Saline solution (ml)	0,9	0,5	0,5	0,5	0,5	0,5	-
1% chicken erythrocytes suspension (ml)	0,5	0,5	0,5	0,5	0,5	0,5	0,5
Incubation 30 minutes at a room temperature							
OBSERVATION							

Conclusion:_____

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Task № 3. Name and briefly characterize the cell cultures used in virology.

1. _____

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2. _____

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3. _____

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Task № 4. Fill in the table on the detection (indication) of viruses in cell cultures

Virus	**Directions of indication**		
	Cytopathic action (CPA), the type of manifestation	**Intracellular inclusions (staining method)**	**Plaque formation phenomenon**
Picornaviruses			
Picornaviruses			
Paramyxoviruses			
Adenoviruses			
Herpesviruses			
Rhabdoviruses			
Poxviruses			
Orthomyxoviruses			

Teacher's signature: _____

Date_____ **PRACTICAL SESSION №3**

Topic: Bacteriophages, morphology and structure. Methods of qualitative and quantitative determination of bacteriophages.

Self-assessment tasks

a) Questions list: theoretical issues:	**b) The list of practical skills and abilities**
1. Morphological types, structure and chemical composition of bacteriophages. 2. Virulent and temperate (moderate, lysogenic) bacteriophages. Stages of productive type of bacteriophages and bacterial cells interaction. 3. Lysogeny and phage conversion. 4. Specificity of bacteriophages action. 5. Practical use of bacteriophages in microbiology and medicine to identify bacteria, prevent and treat infectious diseases and evaluate the environmental objects microbial contamination.	1. Be able to determine phage-type of bacteria. 2. Taking water samples for sanitary-bacteriological research.

Protocol of practical session

Practical assignments:

Task № 1. Draw a scheme of the T4 coliphage structure. Make the appropriate notations.

Task № 2. Denote the features of the phages and bacteria interaction' types.

1. Productive type of interaction: _____

_____

_____

2. Integrative type of interaction: _____

_____

_____

3. Abortive type of interaction: _____

_____

_____

Task № 3. Mark in the table possible types of interaction between the phages and the sensitive bacteria.

Type of interaction / Bacteriophages	Productive type	Integrative type	Abortive type
Virulent			
Temporate			

Task № 4. Observe and evaluate the results of the phage identification of the hemoculture isolated from the patient with suspicion of typhoid fever. Conclude.

Specific standard phages / Explored culture	Typhoid bacteriophage			Paratyphoid A bacteriophage		Paratyphoid B bacteriophage	
Hemoculture	Explored culture	Control of culture	Control of bacteriophage	Explored culture	Control of bacteriophage	Explored culture	Control of bacteriophage

Note:
"+" - turbidity (growth of hemoculture)
"-" - no turbidity
Conclusion:_____

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Task № 5. Observe and evaluate the results of the intestinal bacteriophage titration in the water from the open-water by the Apelman method. Conclude.

Tube number / Ingredients (ml)	1	2	3	4	5	6	7	8	9	10	Control	
											phages'	cultures'
											11	12
MPB	4,5	4,5	4,5	4,5	4,5	4,5	4,5	4,5	4,5	4,5	4,5	4,5
Investigated phage	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	-
0,85 % NaCI	-	-	-	-	-	-	-	-	-	-	-	0,5
Broth culture of bacteria	0,05	0,05	0,05	0,05	0,05	0,05	0,05	0,05	0,05	0,05	-	0,05
Dilution	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	10^{-1}	–
Observation												

0,5м

"+" - the presence of lysis; "-" - no lysis.

Conclusion:_____

_____

Task № 6. Observe and evaluate the results of the staphylococcies' pure culture phagotyping. Fill in the results in the table, Conclude.

Standard phage	The presence of lysis zones
3A	
3B	
3C	
55	
71	

"+" - the presence of a lysis

"-" - no lysis

Conclusion:_____
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Teacher's signature: _____

Date_____ **PRACTICAL SESSION №4**

Topic: Serological reactions in virology

Self-assessment tasks

a) Questions list: theoretical issues:	**b) The list of practical skills and abilities**
1. Features of serological reactions used in virology. 2. A double-antibody technique with the paired serums. 3. Features of the standard viral antigens. 4. Complement fixation test (CFT) in virology. 5. Reactions used exclusively in virology - hemagglutination reactions (HAR) and hemadsorption (HADR), the reaction of virus neutralization. 6. Identification of viruses by antigenic properties NT, HIT (hemagglutination ingibition test), HADIT (hemadsorption ingibition test), PHAT (passive hemagglutination test, Rose-Waaler test), CFT, RIA, ELISA, CLIA).	1. To observe and to evaluate the results of the complement reaction. 2. Be able to observe and to evaluate the results of neutralization reactions (color test). 3. Be able to set up, to observe and to evaluate the results of the reactions used in virology.

Protocol of practical session

Practical assignments:

Task № 1. Observe and evaluate the results of the complement fixaction reaction (CFT), performed as a double-antibody technique with the paired serums of the patient and the standard specific adenoviral antigen. Conclude.

Tube number / Ingredients		1	2	3	4	5	Control of sera	Control of antigen
Dilutionserums(ml)		1: 16 0,25	1:32 0,25	1: 64 0,25	1:128 0,25	1: 256 0,25	0,25	-
standard antigen (ml) (+) - presence		+ 0,5	+ 0,5	+ 0,5	+ 0,5	+ 0,5	-	0,5
Complement (ml) (+) - absence		+ 0,5	+ 0,5	+ 0,5	+ 0,5	+ 0,5	+ 0,5	+ 0,5
Saline solution (ml) (+) - presence		-	-	-	-	-	0,5	0,5
Incubation at 37°C for 60 minutes								
Hemolytic system (ml) (+) - presence		+ 1,0	+ 1,0	+ 1,0	+ 1,0	+ 1,0	+ 1,0	+ 1,0
Incubation at 37°C for 30 minutes								
Observation	serum №1							
	serum №2							

Conclusion:_____

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Task № 2. Observe and evaluate the results of the neutralization reaction (NR) - color test, in order to identify the isolated strain of the poliomyelitis virus. Conclude.

Ingredients, ml	Tubes						Control of:	
	1	2	3	4	5	6	serum	antigen
Phosphate buffer solution	0,25	0,25	0,25	0,25	0,25	0,25	0,25	0,25
Specific serum	0,25→	0,25→	0,25→	0,25→	0,25→	0,25↓	0,25	-
Dilution	1:100	1:200	1:400	1:800	1:1600	1:3200	1:100	-
Virus-contained material	0,25	0,25	0,25	0,25	0,25	0,25	0,25	0,25
Incubation at 37°C for 30 minutes								
Cell culture suspension	0,25	0,25	0,25	0,25	0,25	0,25	0,25	0,25
Incubation at 37°C for 6-8 hours								
Observation								

Conclusion:

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Teacher's signature: _____

Date_____ **PRACTICAL SESSION №5**

Topic: Orthomyxoviruses. Laboratory diagnosis of influenza.

Homeland Orthomyxoviridae

Genera: Influenzavirus

Representative: Influenza viruses A, B, C

Self-assessment tasks

a) Questions list: theoretical issues:	**b) The list of practical skills and abilities**
1. General characteristics and classification of orthomyxoviruses. 2. Human influenza viruses. Virion structure. Features of the genome. Cultivation. Sensitivity to physical and chemical factors. 3. Characteristics of human influenza virus antigens. Hemagglutinins, neutrinidases, functional activity. Classification of human influenza viruses. Types of antigenic variability, its' mechanisms. 4. Epidemiology and pathogenesis of influenza. Role of the virus persistence in the human and animal organisms in the preservation of epidemically significant strains. Immunity. 5. Methods of the influenza laboratory diagnosis. 6. Specific prevention and treatment of influenza.	1. Microscopy of preparations in a light microscope with an immersive lens. 2. The ability to determine the presence of a virus in a chicken embryo by the hemagglutination reaction, in a cell culture by the cytopathogenic action. 3. Ability to set up, to observe and to evaluate the results of the reactions used in virology.

Protocol of practical session

Practical assignments:

Task № 1. Observe and evaluate the results of the hemagglutination inhibition reaction (HIT) in order to identify the strain of the virus by antigenic properties. Conclude.

Tube numbers / Ingredients	Investigated well								
	1	2	3	4	5	6	7	Control of serum	Control of erythrocytes
Saline solution (ml)	-	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2
Standard serums(1:10)									
H1N1(1 row)	0,2	0,2						0,2	-
H2N2 (2 row)	0,2	0,2						0,2	-
H3N2(3 row)	0,2	0,2						0,2	-
Investigated viruses	0,2	0,2	0,2	0,2	0,2	0,2	0,2	-	-
Dilution	1:10	1:20	1:40	1:80	1:160	1:320	1:640	-	-
Incubation at 18-20 ° C for 30 minutes									
1% suspension of chicken red blood cells	0,4	0,4	0,4	0,4	0,4	0,4	0,4	0,4	0,4
Incubation at 18-20 ° C for 1 hour									
Observation									
Serums: H1N1									
H2N2									
H3N2									

Conclusion:

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Task №2. Observe and evaluate the results of the hemagglutination inhibition reaction (HIT), made performed as a double-antibody technique with the paired serums of the patient and standard viral antigens (standard strain of H1N1 influenza virus). Conclude.

Tube number / Ingredients		1	2	3	4	5	6	7	Control of serum	Control of erythrocytes
Dilution serum / ml		1:10 0,2	1:20 0,2	1:40 0,2	1:80 0,2	1:160 0,2	1:320 0,2	1:640 0,2	1:10 0,2	-
Standard viral antigens (ml)		0,2	0,2	0,2	0,2	0,2	0,2	0,2	-	-
Saline solution (ml)		-	-	-	-	-	-	-	0,2	0,2
Incubation at a room temperature for 30 minutes										
1% suspension of chicken red blood cells		0,4	0,4	0,4	0,4	0,4	0,4	0,4	0,4	0,4
Incubation at a room temperature for 60 minutes										
Observation	serum №1									
	serum №2									

Conclusion:_____

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Task: №3. Characterize biological preparations for specific prophylaxis and treatment of infections caused by orthomyxoviruses.

Preparations	Type	Purpose of use	Type of formed immunity
For active immunization			
For passive immunization			

Teacher's signature: _____

Date_____ **PRACTICAL SESSION №6**

Topic: Family Paramyxoviridae - parainfluenza, measles, epidemic parotitis, respiratory syncytial viruses.

Family: Paramyxoviridae

Genera: Paramyxovirus, Morbillivirus, Pneumovirus

Representatives: parainfluenza virus, measles, mumps, respiratory syncytial viruses

Self-assessment tasks

a) Questions list: theoretical issues:	**b) The list of practical skills and abilities**
1. Paramyxoviruses. General characteristics and classification of paramyxoviruses. 2. Paramyxoviruses (parainfluenza virus, measles, mumps, respiratory syncytial virus). The structure of the virions. Antigens. Cultivation. Sensitivity to physical and chemical factors. 3. Epidemiology and pathogenesis of paramyxoviral infections. 4. Immunity in conditions of paramyxoviral infections. Persistence of paramyxoviruses. 5. Methods of laboratory diagnosis of paramyxoviral infections. 6. Specific prevention and treatment of paramyxoviral infections.	1. Microscopy of preparations in a light microscope with an immersive lens. 2. The ability to determine the presence of a virus in a chicken embryo by the hemagglutination reaction, in a cell culture by the cytopathogenic action. 3. Ability to set up, to observe and to evaluate the results of the reactions used in virology.

Protocol of practical session

Practical assignments:

Task № 1. Observe and evaluate the results of the hemagglutination inhibition reaction (HIT), performed as a double-antibody technique with the paired serums of the patient and standard parotitis' antigens. Conclude.

Tube number / Ingredients		1	2	3	4	5	6	7	Control of serum	Control of erythrocytes
Dilution serum / ml		1:10 0,2	1:20 0,2	1:40 0,2	1:80 0,2	1:160 0,2	1:320 0,2	1:640 0,2	1:10 0,2	-
Standard parotitis' antigen (ml)		0,2	0,2	0,2	0,2	0,2	0,2	0,2	-	-
Saline solution (ml)		-	-	-	-	-	-	-	0,2	0,2
Incubation at a room temperature for 30 minutes										
1% suspension of chicken red blood cells		0,4	0,4	0,4	0,4	0,4	0,4	0,4	0,4	0,4
Incubation at a room temperature for 60 minutes										
Observation	serum №1									
	serum №2									

Conclusion:_____

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Task № 2. Compose an algorithm of the parainfluenzas' virological diagnosis (scheme or notes of the main stages of the virological exploration).

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Task №3. Mark the methods of express diagnosis of measles and their purpose:

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Task: №4. Characterize biological preparations for specific prophylaxis and treatment of infections caused by paramyxoviruses.

Preparations	Type	Purpose of use	Type of formed immunity
For active immunization			
For passive immunization			

Teacher's signature: _____

Date_____ **PRACTICAL SESSION №7**

Topic: Family of Rabdoviruses, properties of viruses. Specific prevention of rabies.

Family: Rhabdoviridae
Genera: Rhabdovirus
Representative: Rabies virus, Vesicular Stomatitis Virus

Self-assessment tasks

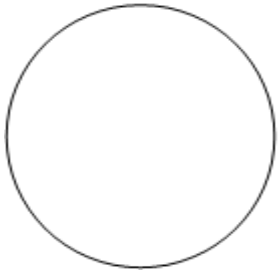
a) Questions list: theoretical issues:	**b) The list of practical skills and abilities**
1. General characteristics and classification of Rhabdoviruses. 2. Rhabdoviruses (rabies virus). The structure of the virions. Antigens. Cultivation. Sensitivity to physical and chemical factors. 3. Epidemiology and pathogenesis under conditions of rhaboviral infections. 4. Methods of laboratory diagnosis of rhaboviral infections. 5. Specific prevention and treatment of rhabdoviral infections. 6. The Genus Vesiculovirus. Vesicular stomatitis virus, Its Role in human pathology, diagnosis.	1. Microscopy of preparations in a light microscope with an immersive lens. 2. To observe and evaluate the results of the chemiluminescence immunoassay.

Protocol of practical session

Practical assignments:

Task № 1. Draw the scheme of the rabies virus structure.

Task № 2. Microscopy and draw inclusions (Babesh-Negri bodies) stained by Turevich in the brain cells in a case of the rabies.



Mark the inclusions

Task № 3. Characterize the rabies and the vesicular stomatitis viruses.

Properties	**Pathogen of rabies**	**Vesicular Stomatitis pathogen**
1. Morphology		
2. Genome		
3. Reservoir of pathogen		
4. Modes and vectors of transmission		
5. Susceptible host		
6. Portal of entry		
7. Pathogenesis and clinical manifestations		
8. Specimen for testing		
9. Laboratory diagnosis		

Task: №4. Characterize biological preparations for specific prophylaxis and treatment of infections caused by rhabdoviruses.

Preparations	Type	Purpose of use	Type of formed immunity
For active immunization			
For passive immunization			

Teacher's signature: _____

Date_____ **PRACTICAL SESSION № 8**

Topic: Pikornaviruses. Laboratory diagnosis of enteroviral infections

Family: Picornaviridae
Genera: Enterovirus
Representatives: poliomyelitis viruses, Coxakie, ECHO, numbered enteroviruses of 68-72 types.

Self-assessment tasks

a) Questions list: theoretical issues:	**b) The list of practical skills and abilities**
1. Pikornaviruses. General characteristics and family classification. Medical importance genera. 2. Enterovirus genus. Classification: polio, coxsackie, echo, enteroviruses 68-72 viruses. Characteristics of virions. Antigens. Cultivation. Sensitivity to physical and chemical factors. 3. Significance of genetic heterogeneity of enterovirus populations in the development of the disease. The role of enteroviruses in human pathology. 4. Epidemiology, pathogenesis of poliomyelitis and other enteroviral infections. Immunity. 5. Laboratory diagnosis of enteroviral infections. 6. Specific prevention and treatment of enteroviral infections.	1. Microscopy of preparations in a light microscope with an immersive lens. 2. Ability to set up, to observe and to evaluate the results of the reactions used in virology.

Protocol of practical session

Practical assignments:

Task № 1. Observe and evaluate the results of the neutralization reaction (NR) - a color test, performed as a double-antibody technique with the paired serums of the patient and standard strains of the poliomyelitis virus of type 1. Conclude.

Tube number / Ingredients		1	2	3	4	5	6	7	Control	
									Antigen	Serum
Sera dilution / ml		1:10 0.25	1:20 0.25	1:40 0.25	1:80 0.25	1:160 0.25	1:320 0.25	1:640 0.25	-	1:10 0.25
Nutrient medium (ml)		-	-	-	-	-	-	-	0,25	0,25
1 type virus 100 CPA_{50}(ml)		0.25	0,25	0,25	0,25	0,25	0,25	0,25	0,25	-
Cell culture suspension 300000 – 4000000 (ml)		0,25	0,25	0 , 2 5	0,25	0,25	0,25	0,25	0,25	0,25
Incubation at 37°C for 4-7 days										
Observation	serum № 1									
	serum №2									

Note:

(+) - the presence of a single-cell culture (the color of the medium is yellow);

(-) - absence of a single-layer cell culture (the color of the medium is raspberry).

Conclusion:_____

_____

Task № 2. Characterize of enterovirus antigens, their names, specificity and methods of detection:

1._____
_____
_____

2._____
_____
_____

Task № 3. Specify features of the enterovirus cytopathic action in the infected cell cultures:

_____

_____

Task № 4. Characterize biological preparations for specific prophylaxis and treatment of enterovirul infections infections.

Preparations	Type	Purpose of use	Type of formed immunity
For active immunization			
For passive immunization			

Teacher's signature: _____

Date_____ **PRACTICAL SESSION № 9**

Topic: Retroviruses. HIV. Laboratory diagnosis of HIV infection

Family: Retroviridae
Genera: Lentivirus
Representatives: HIV-1, HIV-2

Self-assessment tasks

a) Questions list: theoretical issues:	**b) The list of practical skills and abilities**
1. Retroviruses. General characteristics. Classification. 2. Human immunodeficiency virus (HIV). Morphology and chemical composition. 3. Features of the HIV genome. Variability, its' mechanisms. Types of HIV. 4. Stages of the HIV interaction with sensitive cells. 5. Sensitivity of HIV to physical and chemical factors. 6. Epidemiology and pathogenesis of HIV infection. Target cells in the human body. 7. Mechanisms of the immunodeficiency development, AIDS - associated pathology (opportunistic infections and tumors) 8. Laboratory diagnosis of HIV infection. 9. Treatment of HIV infection (etiotropic, immunomodulatory, immunorestorative). Prospects for specific prevention of HIV infection.	1. Ability to set up, to observe and to evaluate the results of the reactions used in virology.

Protocol of practical session

Practical assignments:

Task № 1. Draw the sheme of the structure of the human immunodeficiency virus. Make the appropriate denotements.

Task № 2. Observe and evaluate the results of the enzyme-linked immunosorbent assay (ELISA) made with the sera of the patients in order to detect antibodies to antigens of HIV (anti gp 120). Conclude.

	1	2	3	4	5	6	7	8	9	10	11	12	
A	0.005 NCl	-0.005 neg	0.0120 neg	0.002 neg	0.006 neg	0.006 neg	0.000 neg	****	****	****	****	****	A
B	00.96 COl	0,002 neg	0,004 neg	0,003 neg	0,002 neg	0,004 neg	0,005 neg	****	****	****	****	****	B
C	0.266 CO2	0,003 neg	0,003 neg	0,004 neg	0,002 neg	0,005 neg	****	****	****	****	****	****	C
D	0.209 CO3	0,000 neg	0,016 neg	0,000 neg	-0,001 neg	0,221 **POS**	0,004 neg	****	****	****	****	****	D
E	0.338 PC1	0,002 neg	0,007 neg	0,003 neg	0,270 **POS**	0,004 neg	0,002 neg	****	****	****	****	****	E
F	0,314 **POS**	-0,005 neg	0,003 neg	0,005 neg	0,002 neg	0,005 neg	0,003 neg	****	****	****	****	****	F
G	0,002 neg	0,002 neg	0,015 neg	0,001 neg	0,004 neg	0,007 neg	0,005 neg	****	****	****	****	****	G
H	0,017 neg	0,003 neg	0,005 neg	-0,004 neg	0,003 neg	0,003 neg	0,004 neg	****	****	****	****	****	H
	1	2	3	4	5	6	7	8	9	10	11	12	

*****INDICATES VALUE OUT OF RANGE
#####INDICATES COBINED DATA
POS INDICATES A POSITIVE REACTION
пeg INDICATES A NEGATIVE REACTION
??? INDICATES EQUAL TO OR BETWEEN LIMITS
31. INDICATES VALUE OUT OF RANGE
# INDICATES COMBINED DATA
Conclusion:_____

_____

Task № 3. Observe and evaluate the results of the polymerase chain reaction (PCR), made with the sera of the patients in order to detect HIV infection. Conclude:

_____

_____

Task № 4. Characterize biological preparations for specific prophylaxis and treatment of infections caused by HIV.

Preparations	Type	Purpose of use	Type of formed immunity
For active immunization			
For passive immunization			

Teacher's signature: _____

Date_____ **PRACTICAL SESSION №10**

Topic: Other RNA genomic viruses (Corona-, Rubi-, Reo-, Filoviruses).

Family Reoviridae Rotavirus Genera (A, B, C) **Family Togavirida** Genera Rubivirus	**Family Coronaviridae** Genera Coronavirus, (SARS and MERS). **Family Filoviridae** Genera Marburgvirus Genera Ebolavirus

Self-assessment tasks

a) Questions list: theoretical issues:	**b) The list of practical skills and abilities:**
1. **Reoviruses** (family Reoviridae) General characteristics. Classification. The role of human pathology. The Genera Rotavirus (Rotavirus). Classification, properties. The role in human pathology. Laboratory diagnosis. 2. **Togaviridae** (family Togaviridae). The Genera Rubi virus (Rubivirus). Rubella virus - morphology and chemical composition, antigenic structure. The role of human pathology. Laboratory diagnosis. Specific prevention. 3.**Coronaviruses** (the family of Coronaviridae). General characteristics. Atypical pneumonia virus (SARS-CoV), characteristic of the pathogen' structure, epidemiology, clinical manifestations, virological diagnosis. The Middle Eastern Respiratory Syndrome Virus (MERS). The role of human pathology. Laboratory diagnosis. 4.**Filoviruses** (the Genera Filoviridae). Ebola and Marburg fever viruses. Epidemiology. Pathogenesis of diseases. Laboratory diagnosis. 5. Emerging and reemerging infections.	1. Ability to set up and evaluate the results of serological reactions used in virology.

Protocol of practical session

Practical assignments:

Task № 1. Observe and evaluate the results of the hemagglutination inhibition reaction (HIT), performed as a double-antibody technique with the paired serums of the patient and standard antigens of respiratory coronaviruses. Conclude.

Tube number / Ingredients		1	2	3	4	5	Controlserums	Control standard antigen y
Dilutionserum		1 :10	1:20	1:40	1:80	1:160	1:10	
standard antigen (“+”) - внесення		+	+	+	+	+	-	+
Інкубація при кімнатній температурі протягом 1 години								
Suspension erythrocytes 1% (“+”) -		+	+	+	+	+	+	+
Інкубація при кімнатній температурі протягом 45 хвилин								
Observation	serum № 1							
	serum № 2							

Conclusion: _____

_____

Task № 2. Characterize the properties of the viruses:

Properties of viruses	**Reoviruses**	**Togaviruses**	**Coronaviruses**	**Filoviruses**
1. Morphology				
2. Genome				
3. Reservoir of pathogen				
4. Modes and vectors of transmission				
5. Susceptible host				
6. Portal of entry				
7. Pathogenesis and clinical manifestations				
8. Specimen for testing				
9. Laboratory diagnosis				

Task № 3. Characterize biological preparations for specific prophylaxis and treatment of infections caused by Reo-, Toga-, Corona, and Phio- viral infections.

Preparations	Type	Purpose of use	Type of formed immunity
For active immunization			
For passive immunization			

Teacher's signature_____

Date_____ **PRACTICAL SESSION №11**

Topic: Test computer control of topics 1-10

In order to be ready for testing repeat information about tasks for self-directed work from the Topics 1-10, prepare Krok-tests from the book-collection and from the electronic collection at the site of the chair.

Teacher's signature: _____

Date_____ **PRACTICAL SESSION № 12**

Topic: Pathogens of viral hepatitis. Laboratory diagnosis of hepatitis A and E.

Family: Picornaviridae
Genera: Hepatovirus
Representative: HAV
Genera: Hepavirus
Representative: HEV

Self-assessment tasks

a) Questions list: theoretical issues:	**b) The list of practical skills and abilities**
1. Virus of hepatitis A. Virion structure. Sensitivity to physical and chemical factors. 2. Epidemiology and pathogenesis of hepatitis A. Immunity. Approaches to specific prevention. 3. Laboratory diagnosis of hepatitis A. 4. Specific prevention and treatment of hepatitis A. 5. Virus of hepatitis E. Virion structure. Sensitivity to physical and chemical factors. 6. Epidemiology and pathogenesis of hepatitis E. Immunity. 7. Laboratory diagnosis of hepatitis E.	1. Ability to set up, to observe and to evaluate the results of the reactions used in virology.

Protocol of practical session

Practical assignments:

Task № 1. Observe and evaluate the results of the enzyme-linked immunosorbent assay (ELISA), made with patients' serum in order to detect Ig M against the antigens of the hepatitis A virus.

	1	2	3	4	5	6	7	8	9	10	11	12	
A	0.005 NCl	-0.005 neg	0.0120 neg	0.002 neg	0.006 neg	0.006 neg	0.000 neg	****	****	****	****	****	A
B	00.96 COl	0,002 neg	0,004 neg	0,003 neg	0,002 neg	0,004 neg	0,005 neg	****	****	****	****	****	B
C	0.266 CO2	0,003 neg	0,003 neg	0,004 neg	0,002 neg	0,005 neg	****	****	****	****	****	****	C
D	0.209 CO3	0,000 neg	0,016 neg	0,000 neg	0,270 **POS**	0,004 neg	0,004 neg	****	****	****	****	****	D
E	0,314 **POS**	0,002 neg	0,007 neg	0,003 neg	-0,001 neg	0,221 **POS**	0,002 neg	****	****	****	****	****	E
F	0.338 PC1	-0,005 neg	0,003 neg	0,005 neg	0,002 neg	0,005 neg	0,003 neg	****	****	****	****	****	F
G	0,002 neg	0,002 neg	0,015 neg	0,001 neg	0,004 neg	0,007 neg	0,005 neg	****	****	****	****	****	G
H	0,017 neg	0,003 neg	0,005 neg	-0,004 neg	0,003 neg	0,003 neg	0,004 neg	****	****	****	****	****	H
	1	2	3	4	5	6	7	8	9	10	11	12	

Conclusion:

Task № 2. Give the description of hepatitises caused by hepatitis A and E. viruses.

Properties	Hepatitis A virus	hepatitis E virus
1. Morphology		
2. Genome		
3. Reservoir of pathogen		
4. Modes and vectors of transmission		
5. Susceptible host		
6. Portal of entry		
7. Pathogenesis and clinical manifestations		
8. Specimen for testing		
9. Laboratory diagnosis		

Task № 3. Characterize biological preparations for specific prophylaxis and treatment of infections caused by hepatitis A, E viruses.

Preparations	Type	Purpose of use	Type of formed immunity
For active immunization			
For passive immunization			

Teacher's signature: _____

Date_____ **PRACTICAL SESSION № 13**

Topic: Pathogens of viral hepatitis. Laboratory diagnosis of viral hepatitis B, C and D.

Family: Flaviviridae Genera: Hepacivirus Representative: HCV **Genera: Deltavirus** Representative: HDV	**Family: Hepadnaviridae** Genera: Orthohepadnavirus Representative: HBV

Self-assessment tasks

a) Questions list: theoretical issues:	**b) The list of practical skills and abilities**
1. Hepatitis B virus. The structure of the virion. Sensitivity to physical and chemical factors. 2. Antigens: HBs - surface antigen, Dane particle**.** Internal antigens: HBc, HBe, their characteristics. 3. Epidemiology and pathogenesis of hepatitis B. Persistence. Immunity. 4. Laboratory diagnosis of hepatitis B. Methods of detection and diagnostic value of the hepatitis B markers (antigens, antibodies, nucleic acids). 5. Specific prevention and treatment of hepatitis B. 6. Virus of hepatitis C. The virion structure. Sensitivity to physical and chemical factors. 7. Epidemiology and pathogenesis of hepatitis C. Immunity. 8. Laboratory diagnosis of hepatitis C. 9. Hepatitis D virus. Virion structure. Sensitivity to physical and chemical factors. 10. Epidemiology and pathogenesis of hepatitis D. Immunity. 11. Laboratory diagnosis of hepatitis D. 12. Other pathogens of hepatitis (G, TTV, SENV**),** their taxonomic position, properties. 13. The Role of Hepatitis G Viruses, TTV, SENV in human Pathology. 14. Methods of laboratory diagnosis of hepatitis caused by viruses G**,** TTV, SENV.	1. Ability to set up, to observe and to evaluate the results of the reactions used in virology.

Protocol of practical session

Practical assignments:

Task № 1. Draw the sheme of the hepatitis B virus structure. Denote its' antigens.

Task № 2. Analyze various combinations of hepatitis B serum markers during of the sera examination from the patients No 1 and 2. Fill in the results of the explorations and their analysis in the table (for the analysis of the obtained results see the appendix).

Serological markers / Examined sera	Hbs Ag	Hbe Ag	Anti HBc	Anti Hbe	Anti HBs	Analysis of results	Infectiousness of blood
1							
2							

Task № 3. Observe and evaluate the results of the enzyme-linked immunosorbent assay (ELISA), made with the sera of the patients to detect Hepatitis B virus antigens.

	1	2	3	4	5	6	7	8	9	10	11	12	
A	0.005 NCl	-0.005 neg	0.0120 neg	0.002 neg	0.006 neg	0.006 neg	0.000 neg	****	****	****	****	****	A
B	00.96 COl	0,002 neg	0,004 neg	0,003 neg	0,002 neg	0,004 neg	0,005 neg	****	****	****	****	****	B
C	0.266 CO2	0,003 neg	0,003 neg	0,004 neg	0,002 neg	0,005 neg	****	****	****	****	****	****	C
D	0.209 CO3	0,000 neg	0,016 neg	0,000 neg	-0,001 neg	0,004 neg	0,004 neg	****	****	****	****	****	D
E	0,314 PC1	0,002 neg	0,007 neg	0,003 neg	0,270 **POS**	0,221 **POS**	0,002 neg	****	****	****	****	****	E
F	0.338	-0,005 neg	0,003 neg	0,005 neg	0,002 neg	0,005 neg	0,003 neg	****	****	****	****	****	F
G	0,002 neg	0,002 neg	0,015 neg	0,001 neg	0,004 neg	0,007 neg	0,005 neg	****	****	****	****	****	G
H	0,017 neg	0,003 neg	0,005 neg	-0,004 neg	0,003 neg	0,003 neg	0,256 **POS**		****	****	****	****	H

Conclusion: _____

_____

Task № 4. Characterize the properties of the viruses:

Properties of viruses	Virus of hepatitis B	Virus of hepatitis C	Virus of hepatitis D
1. Morphology			
2. Genome			
3. Reservoir of pathogen			
4. Modes and vectors of			
5. Susceptible host			
6. Portal of entry			
7. Pathogenesis and clinical manifestations			
8. Specimen for testing			
9. Laboratory diagnosis			

Appendix:

Analysis of various combinations of serological markers during HBV (F.Dainhard, I.D.Gast, 1982)

HBsAg	HBeAg	**Anti-HBc**	**Anti -HBe**	**Anti -HBs**	Analysis of the results	Infectiousness of blood
+	-	-	-	-	Acute stage or chronic carrier	++
+	+	-	-	-	Incubation period and early acute stage	++
+	+	+	-	-	Acute chronic hepatitis or chronic carrier	++
+	-	+	+	-	Late stage of acute hepatitis B or chronic hepatitis	+
-	-	+	+	+	Convalescence after acute hepatitis	-
-	-	+	-	+	Convalescence after carring one in past VHB	-
-	-	-	-	+	After immunization, after the contact with HbsAg without development of infection, convalescence after carring one in past VHB	-
	-	+	-	-	Convalescence after carring one in past HB, without detection the anti-HBs, early stage of convalescence or chronic infection	+ -

Note:

1. All HBsAg-positive individuals are infected by HBV.

2. All individuals with anti-HBs are immune to Hepatitis B.

3. Anti-HBs titre and (or) immunoglobulin class anti-HBs can distinguish the recovery stage, persistent carrier and chronic infection.

Task № 5. Characterize biological preparations for specific prophylaxis and treatment of infections caused by hepatitis B, C and D viruses.

Preparations	Type	Purpose of use	Type of formed immunity
For active immunization			
For passive immunization			

Teacher's signature:_____

Date_____ **PRACTICAL SESSION № 14**

Topic: Herpesviruses. Laboratory diagnosis of herpes viral infections.

Family: Herpesviridae, Representatives: Herpessimplex virus 1
Herpes simplex virus 2
Variocella-zoster virus
Cytomegalovirus
Epstein-Barr virus

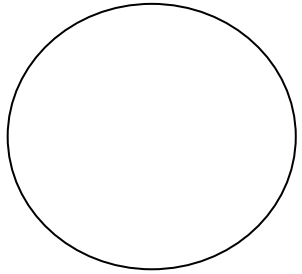
Self-assessment tasks

a) Questions list: theoretical issues:	**b) The list of practical skills and abilities**
1. Herpesviruses. General characteristics and classification. The structure of virions, antigens, cultivation, sensitivity to physical and chemical factors. 2. Herpes viruses, pathogenic to humans: Herpes simplex virus type 1 and type 2, varicella (chickenpox) zoster (shingles, zona) virus, Epstein-Barr virus, Cytomegalovirus. Epidemiology and pathogenesis of diseases caused by herpesviruses. 3. Immunity. The mechanism of the herpes virus persistence. 4. Laboratory diagnosis. 5. Specific prevention and treatment of herpes infections.	1. Microscopy of preparations in a light microscope with an immersive lens. 2. Ability to set up, to observe and to evaluate the results of the reactions used in virology. 3. Reading and rating of the request forms with the results of virological research.

Protocol of practical session

Practical assignments:

Task № 1. Microscopy and draw the preparation of a single-cell culture infected with a herpes simplex virus staned by Romanovsky – Gimza, mark a cytopathogenic effect (CPE).



_____

Specify the type of CPE

Task № 2. Specify the methods of rapid diagnosis of herpes simplex and the purpose of their application:

_____

_____

_____

_____

_____

Task № 3. Observe and evaluate the results of the enzyme-linked immunosorbent assay (ELISA) serum pregnant women in order to detect IgM to antigens HSV-2. Conclude.

	1	2	3	4	5	6	7	8	9	10	11	12	
A	0.005 NCL	-0.005 neg	0.0120 neg	0.002 Neg	0.006 neg	0.006 neg	0.000 neg	****	****	****	****	****	A
B	00.96 CO1	0.002 neg	0.004 neg	0.270 POS	0.002 neg	0.004 neg	0.005 neg	****	****	****	****	****	B
C	0.266 CO2	0.003 neg	0.003 neg	0.004 Neg	0.002 neg	0.005 neg	****	****	****	****	****	****	C
D	0.209 CO3	0.000 neg	0.016 neg	0.000 Neg	0.002 neg	0.004 neg	0.004 neg	****	****	****	****	****	D
E	0.314 **POS**	0.002 neg	0.007 neg	0.003 Neg	-0.001 neg	0.002 neg	0.002 neg	****	****	****	****	****	E
F	0.338 PC1	-0.005 neg	0.003 neg	0.005 Neg	0.002 neg	0.005 neg	0.003 neg	****	****	****	****	****	F
G	0.002 neg	0.002 neg	0.015 neg	0.001 Neg	0.004 neg	0.007 neg	0.005 neg	****	****	****	****	****	G
H	0.017 neg	0.003 neg	0.270 **POS**	-0.004 Neg	0.003 neg	0.003 neg	0.004 neg	****	****	****	****	****	H

Conclusion:

_____

_____

Task № 4. Observe and evaluate the results of the enzyme-linked immunosorbent assay (ELISA) made with the sera **to detect IgG to antigens HSV-1** of the patients in order to detect IgG to antigens HSV-1. Conclude.

	1	2	3	4	5	6	7	8	9	10	11	12	
A	0,018	0,21 neg	0,012 neg	0,018 Neg	***	***	***	***	***	***	***	***	A
B	0,022 NC2	0,22 neg	0,020 neg	0,020 Neg	***	***	***	***	***	***	***	***	B
C	0,022 NC3	0,143 neg	0,022 neg	0,025 Neg	***	***	***	***	***	***	***	***	C
D	0,021 NC4	0,019 neg	0,020 neg	0,038 POS	***	***	***	***	***	***	***	***	D
E	2,261 PC1	0,016 neg	0,019 neg	0,407 POS	***	***	***	***	***	***	***	***	E
F	2,243 PC1	0,027 neg	0,021 neg	0,380 POS	***	***	***	***	***	***	***	***	F
G	0,018 neg	0,015 neg	0,020 neg	2,808 POS	***	***	***	***	***	***	***	***	G
H	0,018 neg	0,013 neg	0,018 neg	2,872 POS	***	***	***	***	***	***	***	***	H

Conclusion:_____

_____

Task № 5. Characterize biological preparations for specific prophylaxis and treatment of infections caused by herpesviruses.

Preparations	Type	Purpose of use	Type of formed immunity
For active immunization			
For passive immunization			

Teacher's signature: _____

Date_____ **PRACTICAL SESSION № 15**

Topic: Adenoviruses. Laboratory diagnosis of adenoviral infections. Poxviruses.

Family: Adenoviridae Genera: Mastadenovirus	Family: Poxviridae Genera: Orthopoxvirus

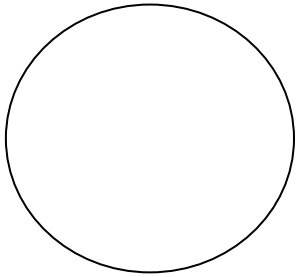
Self-assessment tasks

a) Questions list: theoretical issues:	**b) The list of practical skills and abilities**
1. Adenoviruses. General characteristics and classification. The structure of virions, antigens, their localization and specificity, cultivation, sensitivity to physical and chemical factors. 2. Epidemiology and pathogenesis of diseases caused by adenoviruses. Immunity. 3. Persistence, oncogenic serotypes of adenoviruses. Intestinal adenoviruses. 4. Laboratory diagnosis, specific prevention and treatment of adenoviral infections. 5. Poxviruses. General characteristics and classification. The structure of virions, antigens, their localization and specificity, cultivation, sensitivity to physical and chemical factors. 6. Epidemiology and pathogenesis of smallpox. Immunity. 7. Laboratory diagnosis, specific prophylaxis of smallpox.	1. Microscopy of preparations in a light microscope with an immersive lens. 2. Ability to set up, to observe and to evaluate the results of the reactions used in virology. 3. Reading and rating of a reques forms with the results of virological research.

Protocol of practical session

Practical assignments:

Task № 1. Microscopy and draw inclusions (the bodies of Gvarnieri) in cells of a single-layered culture of fibroblasts or in the cornea of the rabbit infected with the smallpox virus, staining by Romanovsky - Gimza.



Mark the inclusion

Task № 2. Observe and evaluate the results of the response of passive (indirect) hemagglutination test (PHAT), performed as a double-antibody technique with the paired serums of the examined and standard erythrocytic adenoviral antigen type 41. Conclude.

№ test tubes / Ingredients		1	2	3	4	5	6	7	Serum control	Diagnostic control
Dilution of the patients' serum (ml)		1:10		1:40	1:80	1:160	1:320	1:640	1:10	-
Standard antigen (ml)		0,2	0,2	0,2	0,2	0,2	0,2	0,2	-	-
Physiological solution (ml)		-	-	-	-	-	-	-	0,2	0,2
Incubation at the room temperature for 30 minutes										
Observation	Serum № 1									
	Serum № 2									

Conclusion:_____

_____

Task № 3. Characterize biological preparations for specific prophylaxis and treatment of infections caused by adenoviruses and smallpox viruses.

Preparations	Type	Purpose of use	Type of formed immunity
For active immunization			
For passive immunization			

Teacher's signature: _____

Date_____ **PRACTICAL SESSION № 16**

Topic: Ecological group of arboviruses. Flaviviruses, bunyaviruses and filoviruses. Laboratory diagnosis of flaviviral infections.

Family: Flaviviridae Genera: Flavivirus Representative: tick-borne encephalitis virus	Family: Filoviridae Genera: Filovirus Representative: Ebola virus Family: Bunyaviridae

Self-assessment tasks

a) Questions list: theoretical issues:	**b) The list of practical skills and abilities**
1. Flavivirus. General characteristics. Classification. The main representatives of the human pathogenic viruses are tick-borne group (tick-borne encephalitis, Omsk hemorrhagic fever) and mosquito-borne group (Japanese encephalitis, yellow fever, and Dengue fever). 2. Features of pathogenesis. Natural focuses - limited geographic zones (endemic infections). 3. Antigens, cultivation. Sensitivity to physical and chemical factors. 4.Tick-borne encephalitis virus: epidemiology, immunity, pathogenesis. Approaches to specific prevention. 5. Laboratory diagnosis of flavivirus infections. 6. Specific prevention and treatment. 7. Filoviruses. Virion structure. Sensitivity to physical and chemical factors. 8. Ebola virus: epidemiology, pathogenesis and clinic of the Ebola fever. 9. Laboratory diagnosis of Ebola fever. 10. Prospects for specific prevention and treatment. 11. Bunyaviruses. General characteristics. Classification. The main representatives of human pathogens.	1. Ability to set up, to observe and to evaluate the results of the reactions used in virology.

Protocol of practical session

Practical assignments:

Task № 1. Observe and evaluate the results of the of hemagglutination inhibition reaction, performed as a double-antibody technique with the paired serums of the patient and standard antigens of the tick-borne encephalitis. Conclude.

№ test tubes / Ingredients		1	2	3	4	5	Serum control	Antigen control
Dilution of serum		1 :1 0	1:20	1:40	1:80	1:160	1:10	
Standard antigen (+) - insertion		+	+	+	+	+	-	+
Incubation at room temperature for 1 hour								
Erythrocytes suspension 1% (+)		+	+	+	+	+	+	+
Incubation at room temperature for 45 minutes								
Observation	Serum № 1							
	Serum № 2							

Conclusion:_____

_____

Task № 2. Give a comparative description of viruses.

Properties of viruses	Pathogenic agents				
	Virus of tick-borne encephalitis	Yellow fever virus	Dengue fever virus	Japanese encephalitis virus	Ebola fever virus
1. Morphology					
2. Genome					
3. Reservoir of pathogen					
4. Modes and vectors of transmission					
5. Susceptible host					
6. Portal of entry					
7. Pathogenesis and clinical manifestations					
8. Specimen for testing					
9. Laboratory diagnosis					

Task № 3. Characterize biological preparations for specific prophylaxis and treatment of infections caused by flaviviruses, filoviruses and bunyaviruses.

Preparations	Type	Purpose of use	Type of formed immunity
For active immunization			
For passive immunization			

Teacher's signature: _____

Date_____ **PRACTICAL SESSION № 17**

Topic: Oncoviruses

Self-assessment tasks

a) Questions list: theoretical issues:	**b) The list of practical skills and abilities**
1. History of the development of the id about the role of viruses in carcinogenesis. 2. Signs of a transformed cell. Mechanisms of the oncogenic viruses transforming action. 3. The concept of "oncogene". Theories of the oncogenes origin. The virus-genetic theory of the tumour origin by L.O. Zilber. 4. Oncogenic DNA-containing viruses from the families Papovaviruses, and Herpesvirus. General characteristics, participation in viral carcinogenesis in humans. 5. Oncogenic RNA-containing viruses from the Retroviruses family - representatives of the subfamily Oncovirinae. Morphology, classification. The role in human carcinogenesis. 6. Oncogenic viruses of other taxonomic groups (representatives of families Adenoviridae, Poxviridae, Hepadnoviridae). General characteristics. 7. Endogenous retroviruses.	1. Ability to set up, observe and evaluate the results of the reactions used in virology.

Protocol of practical session

Practical assignments:

Task № 1. Fill in the table: **oncogenic viruses**."

	Taxonomic groups					
	Retroviridae	Papillomaviridae	Polyomaviridae	Herpesviridae	Hepadnoviridae	Poxviridae
Representatives	Deltaretrovirus	Papillomavirus	Polyomavirus	Simplexvirus	Orthohepadnavirus	Molluscipoxvirus
1. Morphology						
2. Genome						
3. Reservoir of pathogen						
4. Modes and vectors of transmission						
5. Susceptible host						
6. Portal of entry						
7. Pathogenesis and clinical manifestations						
8. Specimen for testing						
9. Laboratory diagnosis						

Task № 2. Compose the algorithms for the western blot in order to detect possible oncoviruses carriers (HTLV-1) whith:
1) positive result;
2) negative result.
Conclude.

1) _____

_____

2) _____

_____

Task № 3. Observe and evaluate the results of the CFT, performed as a double-antibody technique, with the paired serums a patient with recurrent herpessimplex infection (herpes simplex virus type 2). Conclude.

№ of test tubes / Ingredients		1	2	3	4	5	6	Serum control	Antigen control
Serum dilution		1 :10	1:20	1:40	1:80	1:160	1:320	1:10	-
Herpetic standard antigen (+) - insertion		+	+	+	+	+	+	-	+
Incubation at room temperature for 1 hour									
Hemolytic system (+) - insertion		+	+	+	+	+	+	+	-
Incubation at room temperature for 45 minutes									
Observation	Serum № 1								
	Serum № 2								

Conclusion:_____

_____

Teacher's signature: _____

Date_____ **PRACTICAL SESSION №18**

Topic: Test computer control by topics 1-17.

In order to be ready for testing repeat information about tasks for self-directed work from the Topics 1-17, prepare Krok-tests from the book-collection and from the electronic collection at the site of the chair.

Date_____

PRACTICAL SESSION №19

Topic: Final control of practical skills.

Question to the final module control of practical SESSION:

1. Observe and evaluate the results of the hemagglutination reaction (HR) to determine the presence of the parainfluenza virus in an infected chicken embryo. Conclude.
2. Observe and evaluate of the results of phage identification of the hemoculture isolated from the patient with suspicion of typhoid fever. Conclude.
3. Observe and evaluate the results of the titration of the intestinal bacteriophage in the sample of an open water by the method of Apelman. Conclude
4. Observe and evaluate the results of the hemagglutination inhibition reaction (HAIT), made with paired ed sera of the examined and standard parotitis antigen. Conclude.
5. Observe and evaluate the results of the enzyme-linked immunosorbent assay (ELISA) made with the sera of the patients in order to detect antibodies to antigens of HIV (anti-gp 120). Conclude.
6. Observe and evaluate the results of the neutralization reaction (NR) - a color test, performed as a double-antibody technique, with the paired serums of the examined and standard strain of the poliomyelitis virus type 1. Conclude.
7. Observe and evaluate the results of the complement fixation reaction (CFT), performed as a double-antibody technique with the paired serums of the examined and standard specific adenoviral antigen. Conclude.

8. Observe and evaluate the results of the neutralization reaction (NR) - color test, in order to identify the selected isolated strain of the poliomyelitis virus. Conclude.

9. Observe and evaluate the results of the hemagglutination inhibition reaction (HAIT), made with paired sera of the examined and standard reference strain of H1N1 influenza virus. Conclude.

10. Observe and evaluate the results of the hemagglutination inhibition reaction (HIT), made for the purpose of serotyping the isolated strain of the influenza virus. Conclude.

11. Observe and evaluate the results of enzyme-linked immunosorbent assay (ELISA) of the pregnant women serum in order to detect IgM against antigens of herpes simplex virus. Conclude.

12. Observe and evaluate the results of the enzyme-linked immunosorbent assay (ELISA) made with the sera of the patients in order to detect IgG to antigens HSV-1. Conclude.

13. Observe and evaluate the results of enzyme-linked immunosorbent assay (ELISA), made with serum of patients in order to detect Ig M to antigens of the hepatitis A virus.

Date_____ **PRACTICAL SESSION №20**

Topic: Final module control: theoretical SESSION. Module 2.

The questions for the final module control of the theoretical knowledge

1. Modern methods of infectious diseases diagnosing. Reactions with labeled antigens and antibodies: Immunofluorescence Reaction (direct and indirect). Practical use of the reaction.
2. Modern methods of infectious diseases diagnosing. Reactions with labeled antigens and antibodies: ELISA (direct, indirect, solid-phase, competitive). Immunoblotting. Practical use of reactions.
3. Modern methods of diagnosing infectious diseases. Reactions with Labeled antigens and antibodies: Radioimmune Assay (competitive, reverse, indirect). Practical use of reactions.
4. Genetic methods in the diagnosis of infectious diseases and the identification of pathogens: a) DNA sequences, polymerase chain reaction;

b) hybridization of nucleic acids. Biochips. Application in diagnosis.

5. The history of the discovery and the main stages of the virology development. Contribution of ukrainian scientists. Methods of viruses studying, their Observation.
6. Principles of viruses' classification. The main properties of humans' and animals' viruses.
7. Vira kingdom. Viruses as special forms organization of the life. Principles of viruses' structural organization.
8. Virion and its' components. Nucleocapsid, capsid, capsomers, supercapsid (peplos), peplomers.
9. Simple and complex viruses.
10. Chemical composition of viruses: nucleic acids, proteins, lipids, polysaccharides. Their features and functions. Enzymes of viruses, their role, classification.

11. Reproduction of viruses in the process of their interaction with the cell. The main stages of the interaction of viruses with cells in productive infection.
12. Integrative and abortive types of virus interaction with the host cell.
13. Methods of viruses cultivating in chicken embryos, in laboratory animals.
14. Indication of viral reproduction by hemagglutination reaction. Mechanism, practical value, use, diagnosis value.
15. Indication of viral reproduction by hemadsorption reaction. Mechanism, practical value, use, diagnostic value.
16. Methods of viruses cultivating in cells. Classification of cell cultures used in virology, their characteristics.
17. Methods of viral reproduction detection (indication) by cytopathogenic action, plaque formation under agar coating, viral inclusions.
18. Genetic methods for the viruses and their nucleic components detection.
19. The serological reactions used in Virology. The double-antibody technique with the paired serums.
20. Features of viral standard antigens. Complement fixation reaction and its' peculiarities in virology. Complement fixation reaction, its' essence, Observation. Features of the complement fixation reaction in viral infections diagnosis.
21. Hemagglutination ingibition test, mechanism, conditions of set upting, principles of use, diagnostic value.
22. Virus neutralization reaction, mechanism, conditions of set upting, principles of use, diagnostic value.
23. Non-specific factors of macroorganism protection from viral agents, their characteristics. Interferons, mechanism of action, interferonogens.
24. Virus vaccines, classification, principles of obtaining, requirements for them, control, Observation of effectiveness.
25. Bacteriophage, the history of studying. Structure, classification of phages by morphology. Chemical composition.

26. Virulent and moderate phages.
27. Stages of productive type of interaction the bacteriophages with bacterial cells.
28. Bacteriophages. Lysogenecity and phage conversion.
29. Practical use of bacteriophages in microbiology and medicine.
30. Ocogenic viruses, classification. L. O. Zilber's Virus-Genetic theory of tumor. Mechanisms of viral carcinogenesis.
31. Practical use of bacteriophages in medicine for the prevention and treatment of infectious diseases.
32. Family of the Orthomyxoviruses. General characteristics, biological properties, classification. Human influenza viruses. Virion structure. Features of the genome. Cultivation. Sensitivity to physical and chemical factors.
33. Characteristics of human influenza virus antigens. Hemagglutinins, neuraminidases, functional activity. Classification of human influenza viruses. Types of antigenic variability of influenza viruses, its' mechanisms.
34. Pathogenesis of influenza, immunity. The role of specific and nonspecific mechanisms of the anti-influenza immunity.
35. Methods of the influenza laboratory diagnosis and their Observation. The problem of specific prevention and treatment of influenza. Drugs and their Observation.
36. Family of Paramyxoviruses, general description. Parainfluenza viruses, their biological properties. The role in the development of human pathology. Laboratory diagnosis of Paramyxoviral infections.
37. Family of Paramyxoviruses (Paramyxoviridae). Epidemic Mumps Virus. The role in human pathology. Immunity. Specific prevention.
38. Morbillivirus Genera. Measles virus, biological properties. Pathogenesis of the disease. Immunity and specific prevention.
39. Family of Paramyxoviruses (Paramyxoviridae). Genera of pneumoviruses (Pneumovirus). Respiratory syncytial human virus. Biological properties. Pathogenesis of the disease. Immunity.

40. Genera Picornavirus, general characteristics. Antigenic structure. Biological features of Coksaki viruses, ECHO, 68 - 71 types of enteroviruses, properties. Importance in the development of human pathology.
41. Polio Viruses, characteristics, classification. Pathogenesis and immunogenesis of infection.
42. Laboratory diagnosis, specific prevention of the poliomyelitis. The problem of polio eradication in the world.
43. Rhinoviruses, biological properties. Classification. The role in human pathology. Methods of laboratory diagnosis of infections caused by Rhinoviruses.
44. Aphtovirus Genera. Hand-foot-and-mouth disease virus. Biological properties. Classification. Pathogenesis of infection in humans. Laboratory diagnosis, specific prevention.
45. Reoviruses (family Reoviridae). General characteristics. Classification. The role in human pathology. The Genera Rotavirus (Rotavirus). Classification, properties. The role in human pathology. Laboratory diagnosis.
46. Family of Rhabdoviruses. The rabies virus. Virion structure. Cultivation. Sensitivity to physical and chemical factors. Pathogenicity for humans and animals. Laboratory diagnosis. Intracellular inclusions (Babesh-Negri bodies). Specific prevention.
47. The Genera Vesiculovirus. Vesicular Stomatitis Virus. Role in Human Pathology. Diagnosis.
48. General characteristics of the ecological group of Arboviruses. Virus of tick-borne encephalitis. Japanese encephalitis viruses. Biological properties, methods of laboratory diagnosis, specific prophylaxis.
49. Togaviridae (Togaviridae family). The Genera Rubivirus. Rubella virus. The role in human pathology. Laboratory diagnosis. Specific prevention.
50. Retroviruses (the family of Retroviridae). General characteristics. Biological properties. Classification. Representatives of the subfamilies: Oncovirinae, Lentivirinae, Spumavirinae.
51. Human Immunodeficiency Virus (HIV). Morphology and chemical composition. Features of the genome. Variability, its' mechanisms. HIV Types. Origin and evolution.

52. Human Immunodeficiency Virus (HIV). Cultivation, stages of interaction with sensitive cells. Sensitivity to physical and chemical factors.

53. Pathogenesis of HIV infection. Target cells of the human body, characteristic of surface receptors. Mechanism of immunodeficiency development.

54. AIDS-associated pathology (opportunistic infections and tumors).

55. HIV infection. Principles of treatment. Prospects for specific prevention.

56. Human Immunodeficiency Viruses (HIV). Methods of laboratory diagnosis (immunological, genetic).

57. Herpes viruses, pathogenic to humans: Herpes Symplex virus 1st and 2nd types. Biological properties. Role in pathology.

58. Herpes viruses, pathogenic to humans: Chickenpox. Biological properties. Role in pathology.

59. Herpesvirus: cytomegaly (CMV). Biological properties. Role in pathology.

60. Herpes viruses, pathogenic to humans: Epstein-Barr (EBV) - a pathogen of infectious mononucleosis, human cancers. Herpes virus of 6, 7, 8th types. Biological properties. Role in pathology.

61. Family of Adenoviruses. Biological properties. Antigenic structure. Cultivation. Pathogenesis and laboratory diagnosis of adenoviral infections. Immunity. Specific prevention.

62. Poxvirus. Pathogenesis of infection. Methods of diagnosis and specific prophylaxis. Vaccine virus. Elimination of smallpox in the world.

63. Viral hepatitis A, properties and classification of viruses. Pathogenesis of diseases. Laboratory diagnosis. Specific prevention.

64.Viral hepatitis B, properties and classification of viruses. Pathogenesis of diseases. Laboratory diagnosis. Specific prevention.

65. Viral hepatitis C, properties and classification of viruses. Pathogenesis of diseases. Laboratory diagnosis. Prospects for specific prevention.

66. Viral hepatitis D viruses, properties and classification of viruses. Pathogenesis of diseases. Laboratory diagnosis. Specific prevention.
67. Viral hepatitis E: pathogens' properties and classification of viruses. Pathogenesis of diseases. Laboratory diagnosis. Prospects for specific prevention.
68. Coronaviruses. Properties of SARS virus. Methods of diagnosis. Middle Eastern Respiratory Syndrome Virus (MERS). Methods of diagnosis.
69. Filovirus. Marburg and Ebola viruses. Properties. Treatment, prevention.
70. Emergening and reemerging infections.
71. Prions. Properties of animal's prion diseases (scrapie, spongiform encephalopathy of cows) and humans (Creutzfeld-Jacob disease, kuru etc.). Pathogenesis of prion diseases. Diagnosis.