Ministry of Education and Science of Ukraine
Міністерство освіти і науки України

Вищий державний навчальний заклад України
«Українська медична стоматологічна академія»
Кафедра мікробіології, вірусології та імунології
Higher State Educational Establishment of Ukraine
"Ukrainian Medical Stomatological Academy"
Microbiology, Virology and Immunology Chair

Microbiology, Virology and Immunology
Manual for foreign medical faculty students

Мікробіологія, вірусологія та імунологія
Посібник для іноземних студентів медичного факультету

Poltava
Полтава
2015

UDC 579 (075.8)
УДК 579 (075.8)

The manual is approved and recommended as a manual for foreign students of Higher Medical Education Institutions of IV accreditiion level by Education and Science Ministry, record №1/11-20654 of 26.12.2014.
Посібник рекомендований Міністерством освіти і науки України як навчальний посібник для іноземних студентів вищих медичних навчальних закладів IV рівня акредитації, протокол №1/11-20654 від 26.12.2014.

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Microbiology, Virology and Immunology (Manual for foreign medical faculty students). – Poltava, HSEE "UMSA", 2015. – 190 p.
Мікробіологія, вірусологія та імунологія (Посібник для іноземних студентів медичного факультету). – Полтава: ВДНЗУ «УМСА», 2015. – 190 с.

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Literature for self work:

1. Levinson W., Jawetz E. Medical microbiology and immunology. - International edition. - 2003. – 614 p.
2. Ananthanarayan R., Paniker C.K. Textbook of Microbiology. - International edition. - 2003. – 612 p.
3. Microbiological application. A laboratory manual in general Microbiology // Benson H.J. – Dubuque, Iowa: Wm. C. Brown Company Publishers, 1983. – 298 p.
4. General Medical Microbiology, Virology and Immunology. Part I. Manual for practical lessons / Comp. by Loban G.A., Hancho O.V. – Poltava, 2005. – 153 p.
5. General Medical Microbiology, Virology and Immunology. Part II. Manual for practical lessons/ Comp. by Loban G.A., Hancho O.V. – Poltava, 2007.– 104 p.
6. Pathogenic cocci. Gramnegative intestinal pathogens. Manual for practical lessons/ Composed by Hancho O.V. - Poltava, UMSA, 2006. - 113 p.
7. Tsyganenko A. Y., Dikiy I. L., Tkachenko V. L., Shevelyova N.Y., Velika M. M., Vasilchenko V. N. Microbiology handbook to laboratory classes in microbiology. – Kharkiv ‹‹Osnova››, 2005. – 210 p.

“Microbiology, Virology and Immunology” discipline structure in medical faculty in 2014 -2015 s.y.

Term	Name of discipline, names of moduls	Normative disciplines	Special disciplines	Quantity of credits	Quantity of hours								Individual class type	Number of discipline (according to typical plan)
					General	Auditory				Individual work	Self work	Practice		
						Lectures	Practical	Laboratory	Seminars					
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**21**
II	Module 1. Morphology and physiology of microorganisms. Infection. Immunity	*		3	90	20	40				30			18
II	Modul 2. Special microbiology.	*		3	90	16	50				24			18
III	Modul 3. General and special virology. Bases of clinical and ecological microbiology. Sanitary microbiology and virology.	*		2	60	14	30				16			18
	All	*		8	240	50	120				70			18

Topical plan of lectures on the discipline

№	TOPIC	Hours
	***Module 1. Morphology and physiology of microorganisms. Infection. Immunity*.**	
1.	Value of Medical microbiology in the doctor`s activity. History of microbiology development. Microbiological methods. Morphology of bacteria	2
2.	Microorganisms classification. Physiology of bacteria	2
3.	Microbiological bases of antimicrobial chemotherapy. Antibiotics	2
4-5.	Conception of an infection	4
6.	History of immunology development. Organism unspecific defence factors	2
7.	Immune system of organism. Antigens. Microorganisms antigens	2
8.	Antibodies, structure. Immunoglobulines classes. Immune responce. Cell mediated immunity	2
9.	Immunepathology. Immuneprevention and immunetherapy	2
10.	Genetics of bacteria and viruses. Biotechnology and geneingenery bases	2
	Total	20
	Module 2. Special microbiology	
1.	Pathogenic cocci	2
2.	Pathogenic Enterobacteria. Esherichia. Salmonella	2
3.	Cholerae and dysentery agents. Campilobacteries. Helicobacteries	2
4.	Mycobacteries. Agents of tuberculosis and mycobacteriosis	2
5.	Corinebacteria diphtheria	2
6.	Pathogenic anaerobic bacteria	2
7.	Pathogenic spirochaetes	2
8.	Chlamidia, Mycoplasma and Rickettsia	2
	Total	16
	Modul 3. General and special virology. Bases of clinical and ecological microbiology. Sanitary microbiology and virology	
1.	General virology, morphology and structure of viruses. Cultivation of viruses.	2
2.	RNA viruses. General characteristics. Orthomyxoviruses. Paramyxoviruses. Picornaviruses	2
3.	Orthomyxoviruses. Paramyxoviruses. Picornaviruses (continuous)	2
4.	Retroviruses. HIV. Oncoviruses	2
5.	Hepatitis viruses	2

6-7.	DNA viruses. General characteristics. Adenoviruses. Herpesviruses	2
	Total	14

Thematic plan of practical training on the discipline

№	Topic	Hours
	Module 1. Morphology and physiology of microorganisms. Infection. Immunity.	
1.	Microbiological laboratory: organization, equipment, purpose. Methods of microscopic examination. Bacterioscopic method for diagnosis of infectious diseases.	2
2.	Morphology of bacteria. Methods of making preparations from cultures of bacteria and pathological material. Simple methods of staining.	2
3.	Structure of bacteria. Staining of bacteria by the Gram method.	2
4.	Structure of the bacterial cell: inclusion, capsule, flagella. Methods of detection. Structure of the bacterial cell. Methods for detection of spores and acid-resistant bacteria.	2
5.	Morphology and structure of spirochetes, actinomyces, fungi and Protozoa. Methods of study of their morphology.	2
6.	Morphology and structure of rickettsia, chlamydia and mycoplasma. Methods of detection.	2
7.	Cultivation of bacteria, culture media . Methods of sterilization, disinfection. Methods for selection of pure cultures of aerobic bacteria (1 - 2-stages). Cultural properties of bacteria.Bacteriological (cultural) method for diagnostics of infectious diseases.	2
8.	Isolation of pure cultures of aerobic bacteria (3^{rd} and 4^{th} stages of the research). Methods for studying the enzymatic activity of bacteria.	2
9.	Methods of Isolation of pure cultures of anaerobic bacteria (1-5 stages of research).	2
10.	Microbiological basis of antimicrobial chemotherapy. Principles of antimicrobial chemotherapy in dentistry. Antibiotics.	2
11.	The doctrine of the infectious process. Biological method of research.	2
12.	The doctrine of the infectious process. Using of biological methods in diagnosis of oral diseases.	2
13.	Types of immunity. Factors of nonspecific protection of the organism and their research methods.	2
14.	Acquired immunity. Antigens and antibodies. Serological methods of microbiological diagnosis of infectious diseases. Application of serological methods in the diagnosis of oral diseases. Reactions of precipitation and neutralization.	2
15.	Agglutination test.	2

16.	The reaction of immune lyses (bacteriolyses, hemolyses). Complement fixation test (RPR)	2
17.	Reactions with the usage of labeled antigens and antibodies.	2
18.	Immunoprophylaxis and immunotherapy of infectious diseases.	2
19.	Immune status of man and methods of assessment. Natural and acquired immunodeficiency states. Test control	2
20.	**Final module control:**	2
	TOGETHER	**40**
	Module 2. Special microbiology.	
1.	Microbiological diagnosis of staphylococcus infections.	2
2.	Microbiological diagnosis of streptococcus infections.	2
3.	Microbiological diagnosis of meningococcus infections.	2
4.	Microbiological diagnosis of gonococcus infections.	2
5.	Microbiological diagnosis of diseases caused by E. coli.	2
6.	Microbiological diagnostics of typhoid and paratyphoids (1^{st} week of disease)	2
7.	Microbiological diagnostics of typhoid and paratyphoids (2^{nd} week of disease)	2
8.	Microbiological diagnostics of typhoid and paratyphoids (3^{rd} and 4^{th} week of disease). Microbiological diagnostics of salmonellosis	2
9.	Microbiological diagnostics of shigellosises	2
10.	Microbiological diagnostics of cholera	2
11.	Microbiological diagnostics of brucellosis and anthrax	
12.	Microbiological diagnostics of plague and tularemia	
13.	Microbiological diagnostics of tuberculosis and actinomycosis	2
14.	Microbiological diagnostics of diphtheria.	2
15.	Microbiological diagnostics of diseases, caused by Bordetella	2
16.	Microbiological diagnostics of anaerobic wounds infection	2
17.	Microbiological diagnostics of tetanus and botulism	2
18.	Microbiological diagnostics of Syphilis	2
19.	Microbiological diagnostics of relapsing typhus and leptospirosis	2
20.	Microbiological diagnostics of the diseases caused by Chlamidia and Mycoplasma.	2
21	Microbiological diagnostics of rickettsiosises	
22.	Elements of medical mycology. Microbiological diagnostics of candidiasis, aspergillosis and penicillosis.	2
23.	Microbiological diagnostics of cutaneous and systemic mycoses	2

24.	Practic skills credit control	2
25	**Final module control:**	2
	TOGETHER	**50**
Modul 3. General and special virology. Bases of clinical and ecological microbiology. Sanitary microbiology and virology		
1.	Methods of cultivation, indication and identification of viruses.	2
2.	Bacteriophages.	2
3.	Laboratory diagnosis of Orthomyxovirusus, Paramyxovirus and Rhabdovirusal infections.	2
4.	Laboratory diagnosis of HIV infection. Defeat mouth under AIDS.	2
5.	Laboratory diagnosis of Enteroviral, Flaviviral and Coronaviral infections.	2
6.	Laboratory diagnosis of hepatitis A, B, C, D, E.	2
7.	Laboratory diagnosis of diseases caused by DNA viruses.	2
8.	Sanitary-microbiological research of water, air, soil and food products	2
9.	Human normal microflora	2
10.	Clinical microbiology. Microbiological research of respiratory organs, blood and CNS	2
11.	Clinical microbiology. Microbiological research of the digestive, urine and genital systems	2
12.	Hospital infections	2
13.	**Practical training**	2
14.	**Final module test control:**	2
15.	**Final module III control:**	2
	TOGETHER	**30**
	Total number of hours of practical training in the discipline, including the final module, control of 3 modules.	**120**

Plan of students' self - training work.(STW)

№	TOPIC	Hours	Type of control
Module 1. Morphology and physiology of microorganisms. Infection. Immunity.			
1.	Preparation for the workshops - theoretical preparation and processing of practical skills.	19,5	Current control on practical
2.	Self-studying of topics that are not included in the plan of classes:		
	Development stages of microbiology.	0,5	The final module control
3.	Individual independent work: a framework of cooperation in the cellular immune response.	1	Current control

4.	Preparing for the final control of the module 1.	5	The final module control
	TOGETHER	26	
	Module 2. Special microbiology.		
1.	Preparation for the workshops - theoretical preparation and processing of practical skills.	21	Current control on practical
2.	Self study topics not included in the plan of classes:		
	Nonclostridial anaerobic bacteria.	1	The final module control
	The causative agent of whooping cough.	1	The final module control
	Nonfermentative Gram-negative bacteria.	1	The final module control
	Other pathogenic bacteria.	1	The final module control
	Medical protozoology.	1	The final module control
	Preparing for the final control of the module 1.	5	The final module control
	TOGETHER	30	
	Modul 3. General and special virology. Bases of clinical and ecological microbiology. Sanitary microbiology and virology		
1.	Preparation for the workshops - theoretical preparation and processing of practical skills.	6,5	Current control on practical
2.	Self study topics not included in the plan of classes:		
	Genetics of viruses.	0,5	The final module control
	Other RNA genomic viruses.	0,5	The final module control
	Ecological group of arboviruses.	0,5	The final module control
	Prions.	0,5	The final module control
3.	Preparing for the final control of the module 3.	5	
4.	Preparing for test control	0,5	The final module
	TOGETHER	14	
	Total number of hours of SSW in the discipline,	**70**	

Microbiological metods of diagnostic of infection diseases

Microscopic (Bacterioscopic, virusoscopic, protozoascpoic).- Manufacturing and coloration of smears of the test material of the patient and studying it under a microscope. It allows to quickly identify the typical morphological

features the causative agent and has a large importance in dianhostycs of gonorrhea, meningococcal meningitis, tuberculosis, leprosy, syphilis, relapsing fever, smallpox, malaria, leishmaniasis, toxoplasmosis and more.
Bacteriological method is to crop material from the patient to the appropriate culture media, allotment of pure cultures of the pathogen and determine its type and, thus, the final diagnosis of the disease. It is critical to in the diagnosis of typhoid fever, dysentery, cholera, diphtheria, plague and other diseases.
Serological methods based on the detection of specific antibodies in the serum of patients with a particular pathogen. For this purpose, various immunological (serological) reaction: agglutination, precipitation, complement fixation and more. For example, on typhoid fever are often held Widal agglutination test, on brucellosis - the Wright reaction, on chronic gonorrhea - complement fixation reaction of Bordeaux - Zhang and others.
Biology (Experimental) method is the infection of susceptible laboratory animals a dedicated pure culture of the pathogen, studied material or introduction of bacterial toxins and reproducing the typical picture of the disease. To do this, use white mice, rats, guinea pigs, rabbits. This method determine the virulence of microbes. For the diagnostic biological sample often used for plague, anthrax, tularemia, tetanus, botulism, anaerobic gas infection, encephalitis, etc.
Allergic method allows to establish the diagnosis by intradermal allergic tests which detect the condition of hypersensitivity to the causative agent or the products of its life activity (allergens). This method is widely used on the diagnosis of tuberculosis (Mantoux test), brucellosis (sample Byurne), tularemia, and many other diseases.
For the understanding, learning and logical application bacterioscopic method of diagnostics has an important value to study the fundamental morphology and ultrastructure of bacteria, methods of simple and complex coloring, detection of separate structures and the inclusion of a bacterial cell. For this purpose laboratories widely used modern microscopes - highly informative optical instruments.

Date:_____

Practical lesson № 1

Topic: Microbiological Laboratory: organization, equipment, purpose. Methods of microscopic examination.

Bacterioscopic method for diagnosis of infectious diseases.

Microscopic (Bacterioscopic, virusoscopic, protozoascpoic).- Manufacturing and coloration of smears of the test material of the patient and studying it under a microscope. It allows to quickly identify the typical morphological features the causative agent and has a large importance in dianhostycs of gonorrhea, meningococcal meningitis, tuberculosis, leprosy, syphilis, relapsing fever, smallpox, malaria, leishmaniasis, toxoplasmosis and more.

Tasks for self – training work:

a) The list of issues to be studied:

1. Subject and tasks of medical microbiology.
The value of microbiology for dentist.
2. Appointment, equipment and organization of the microbiological laboratory.
3. Rules and safety in the microbiology laboratory
4 .Microscopic methods of microorganisms: immersion,
phasecontrast, darkfield, fluorescent, electron microscopy.
5. The structure of the light microscope.
6. Terms of microscopy in the light microscope with immersion lens.

b) The list of practical skills that are necessary to be mastered:

1.Compliance with rules of epidemic profile and safety in the microbiology laboratory.
2. Microscopy preparations in the light microscope with immersion lens.

Rules of using an immersion microscope

I. 1. Work with an artificial light source.
2. Use a flat mirror.
3. Open aperture fully.
4. Lift condenser at the top.
5. Set the maximum lighting in a small increase.

II. 1. Assess the drug visually.
2. Apply 1-2 drops of immersion oil on medication.
3. Place the preparation on the stage.

III. 1. Set in the operating position the immersion lens with the revolver.
2. Lower lens should touch with a covering of glass with macroscrew.

3.Search for pictures of the preparation, slowly raising the lens with macroscrew regulation of image with macroscrew.

IV. 1. After finishing the work raise the lens with macroscrew.

2. Put a microscope in a small increase.

Practical lesson's Protocol

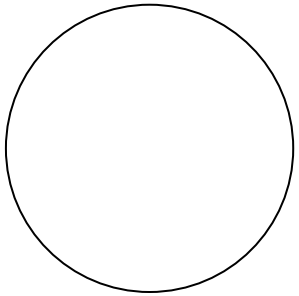
Practical tasks should be done:

Task № 1: To learn the rules of operation and safety in the microbiology laboratory.

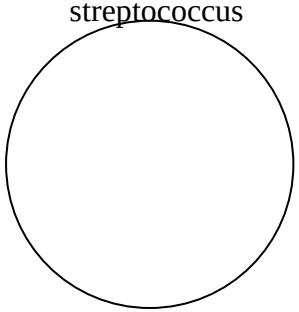
Task № 2: To study the structure of the light microscope and learn techniques of working with immersion lens.

Task № 3: Microscope and sketch slides: 1) staphylococcus, 2) streptococcus, 3) monobacteries, 4) sarcines.

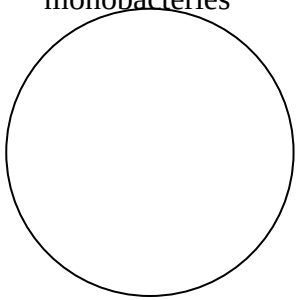
staphylococcus



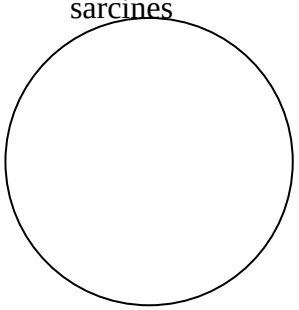
streptococcus



monobacteries



sarcines



Teacher's signature _____

Date:_____

Practical lesson № 2

Topic: Morphology of bacteria. Techniques of making preparations from cultures of bacteria and pathological material. Simple methods of staining.

Tasks for self - training work:

a) The list of issues to be studied:

1. Classification of microorganisms according to the form number and relative position of cells.
2. Steps on making preparations for microscopic examination of cultures of bacteria.
3. Steps on making preparations for microscopic examination of pathological material.
4. Simple methods of staining, their methodology.

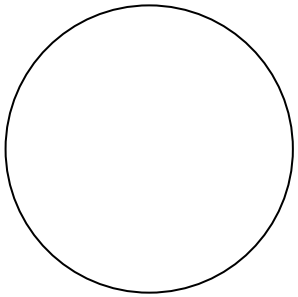
b) The list of practical skills that are necessary to be mastered:

1. Making preparations for microscopic examination.
2. Staining agents by simple methods: aqueous solutions of magenta and methylene blue.
3. Microscope preparations in the light microscope with immersion lens.

Practical lesson's Protocol

Practical tasks should be done:

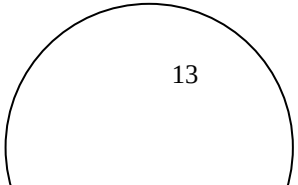
Task № 1: Produce preparation for microscopic studying of bacterial cultures from the solid nutrient medium. Stain with aqueous solution of magenta. To microscope and to sketch.



_____

(Name the organisms according to their shape and arragement of cells)

Task № 2: Produce preparation for microscopic study of bacterial cultures from the solid nutrient medium. Stain with aqueous solution of methylene blue. To microscope and to sketch.



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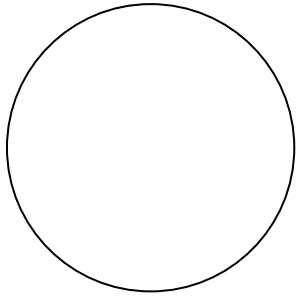
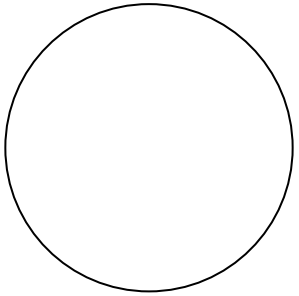
(Name the organisms according to their shape and arragement of cells)

Task № 3: To microscope and to sketch preparations, which are stained by a simple method: 1) diplococcus, 2) vibrios.



diplococcus (staining with methylene blue)



vibrios (simple staining)

Signature of teacher_____

Date_____

Practical lesson № 3

Topic: Structure of the bacterial cell. Complex methods of staining. The method of Gram.

Tasks for self - training work:

a) The list of issues to be studied:

1. Structure of the bacterial cell. Cell wall, neroplazm, cytoplasm membrane, cytoplasm, nuclide, ribosomes, mezosoms, plasmids.
2. Chemical composition and functions of the structural components of bacterial cells.
3. Polymorphism of bacteria. Properties of L-form bacteria.
4. Complex methods of staining. The method of Gram.
5. Mechanisms of interaction of dyes with the structures of bacterial-cell
6. Factors affecting the color of bacteria by Gram.

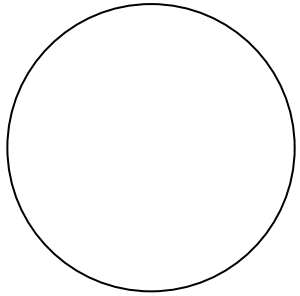
b) The list of practical skills that are necessary to be mastered:

1. Making preparations for microscopic examination of pathological material.
2. Staining preparations with sophisticated method: stain by Gram.
3. Microscopy of preparations in the light microscope with immersion lens.
4. Differentiation of microorganisms by morphological and tinctorial properties.

Practical lesson's Protocol

Practical tasks should be done:

Task№ 1: Produce smear of microbial associations of bacteria, stained by the method of Gram. To microscope and to sketch

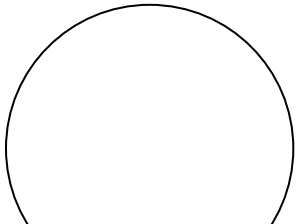


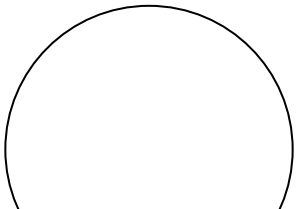
Steps of staining by Gram (modification of Syniov):

1.Solution of hentsianviolet - 2 min. (filter paper, impregnated with dye and dried).
2. Solution of Lugol – 1 min.
3. Ethyl alcohol- rectified - 30 sec.
4. To rinse with water.
5. Magenta of Pfeiffer - 2 min.
6. To rinse with water, to dry.
7. To microscope

(To name the detected microorganisms with regard to the shape, mutual arrangement of cells and tinctorial properties)

Task № 2: To microscope and to sketch preparations, which are stained by Gram: 1) streptobacillus, 2) diplococci..





Grampositive streptobacillus Gramnegative diplococcus

Teacher's signature_____

Date_____

Practical lesson № 4

Topic: Structure of the bacterial cell: inclusion, capsule, flagella. Methods of detection. Methods for detection of spores and acid bacteria.

Tasks for self - training work:

a) The list of issues to be studied:

1.Include: chemical composition, functions, practical importance. Methods for detection of inclusions.
2. Capsules of bacteria: structure, chemical composition, functional significance. Methods of detection. Staining by Hins-Burri.
3. Flagella, cilia: structure, location on the surface of the bacterial cells, functional significance. Methods of re-appearance of flagella. Staining by the method of Loeffler.
4. Detection of motion of bacteria. Preparation of drugs "hanging" drop and "crushed" drop.

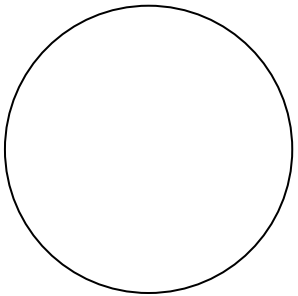
b) The list of practical skills that are necessary to master:

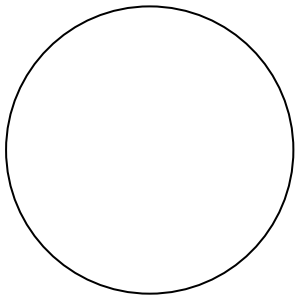
1. Making preparations "crushed" drop and "hanging" drop for microscopic examination.
2. Staining preparations by sophisticated method.
3. Microscopy of preparations on the light microscope with immersion lens.
4. Differentiation of microorganisms by morphological and tinctorial properties.

Practical lesson's Protocol

Practical tasks should be done:

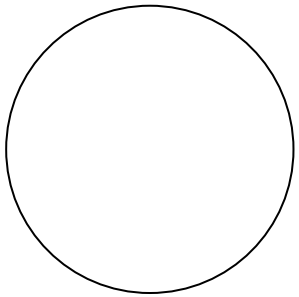
Task №1: Study microscopic visualization and to sketch grains in the cytoplasm of corynebacteria of diphtheria.





grains(staining by Loeffler (staining by Neisser)

Task № 2: Study microscopic visualization and sketch it.



capsulars of bacteria (staining by Hins-Burri)

Task № 3: Make preparation " hanging" drop from one day culture of choleric vibrios. To microscope and to identify the mobility of bacteria.

Task № 4: Study microscopic visualization and sketch preparations of spore-forming bacteria that are stained by the methods of Ogeshco, Peshkov,Gram

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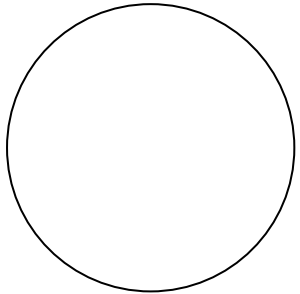

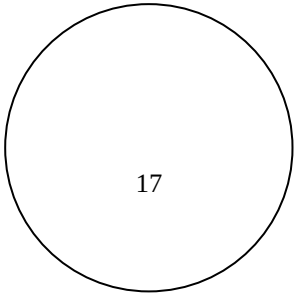

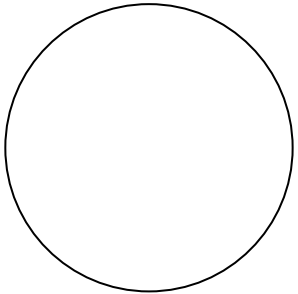

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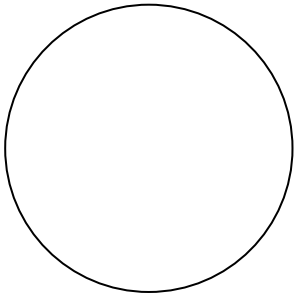
(To describe microorganisms by morphological features, specify a method of staining)

Task № 5: Produce preparation of sputum of the patient, stained by Ziehl-Nielsen. To microscope and to sketch



Acid fast bacteria

Teacher's signature_____

Date_____

Practical lesson № 5

Topic: Morphology and structure of spirochetes, actinomyces, fungi and Protozoa. Methods of study of their morphology.

Tasks for self - training work:

a) The list of issues to be studied:

1. Classification, morphology and structure of spirochetes. Methods of studying of their morphology. Pathogenic representatives.
2. Classification, morphology and structure of fungi. Methods of study of their morphology. Pathogenic representatives.
3. Actinomyces, morphology and structure. Methods of study of their morphology. Pathogenic representatives.
4. Classification, morphology and structure of the simplest. Methods of study of their morphology. Pathogenic representatives.

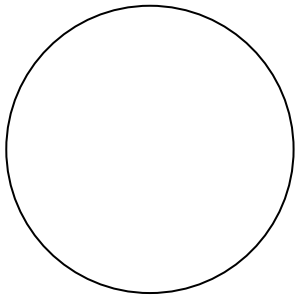
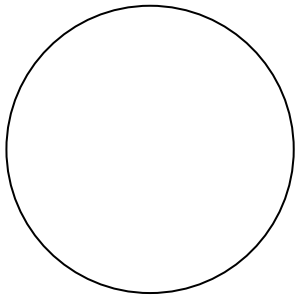
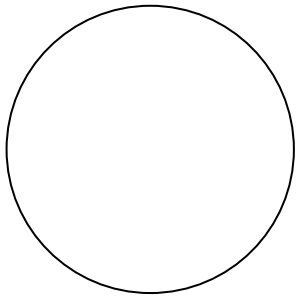
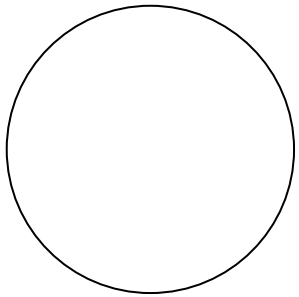
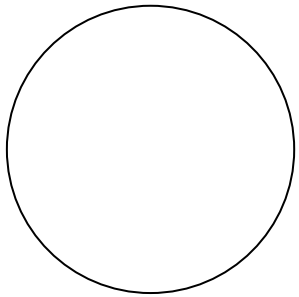
b) The list of practical skills that are necessary to master:

1. Making preparations for microscopic examination of pathological material.
2. Staining preparations by complex methods (Gram).
3. Microscopy preparations on the light microscope with immersion lens.
4. Differentiation of microorganisms by morphological and tinctorial signs.

Practical lesson's Protocol

Practical tasks should be done:

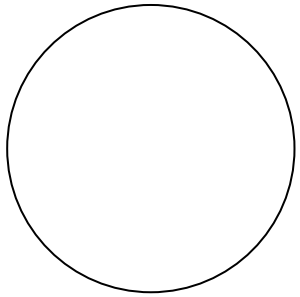
Task № 1: To microscope and to sketch preparations of fungi and actinomyces.

Mucor Penicillium Aspergillus Candida actinomyces

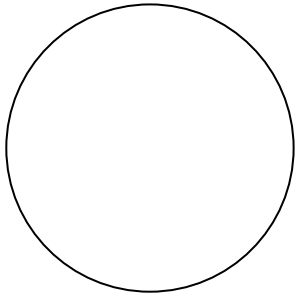
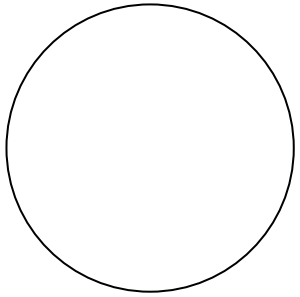
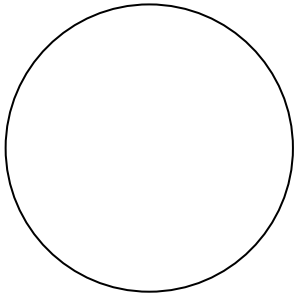
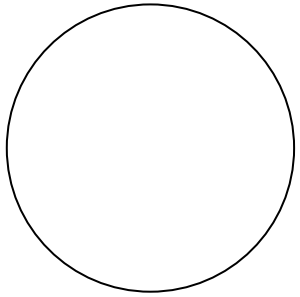
(To mark morphological and tinctorial properties of the microorganisms)

Task№ 2: Make preparation of plaque by the method of Burri. To microscope and to sketch.



spirochetes in plaque

Task№ 3: Study microscopic visualization and sketch preparations of Protozoa : 1) trypanosome, 2) Trichomonas, 3) leishmania,,4) malaria plasmodium.

Trypanosome (stained by Romanovsky –Giemza)

Trichomonas (Stained with methylen blue)

Leishmania (stained by Romanovsky –Giemza)

malaria plasmodium (stained by Romanovsky –Giemza)

Teacher's signature_____

Date_____

Practical lesson № 6

Topic: Morphology and structure of rickettsia, chlamydia, and mycoplasma. Methods of detection.

Tasks for self - training work:

a) The list of issues to be studied:

1. Classification, morphology and structure of rickettsia. Methods of detection.
2. Chlamydia and mycoplasma: morphology and structure. Methods of detection.

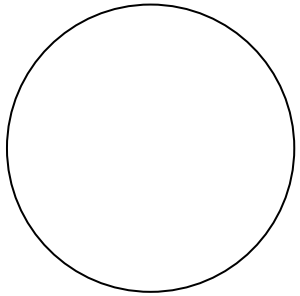
b) The list of practical skills that are necessary to master:

1. Determination of bacteria.
2. Microscopy preparations on the light microscope with immersion lens.

Practical lesson's Protocol

Practical tasks should be done:

Task № 1: Study microscopic visualization and sketch rickettsia in the preparation, which is stained by Zdrodovsky



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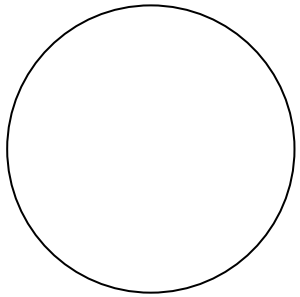
(mark morphological properties of microorganisms)

Task № 2: Study microscopic visualization and sketch inclusion of Chlamydia in infected cells (staining by Romanovsky-Giemza).



(mark infected cells)

Teacher's signature _____

Date_____

Practical lesson № 7

Topic: Cultivation of bacteria culture media. Methods of sterilization, disinfection. Methods for Isolation of pure cultures of aerobic bacteria (Stage 1-2 study). Bacteriological (cultural) method for diagnostics of infectious diseases.

Bacteriological method is to crop material from the patient to the appropriate culture media, allotment of pure cultures of the pathogen and determine its type and, thus, the final diagnosis of the disease. It is critical to in the diagnosis of typhoid fever, dysentery, cholera, diphtheria, plague and other diseases.

Tasks for self - training work:

a) The list of issues to be studied:

Rules for working with bacterial cultures and safety in the bacteriological laboratory.

1. Power microorganisms, classification by type of power. Mechanisms of transport of nutrients into bacterial cells.
3. Cultivation of bacteria. Nutrient media, classification for purpose, consistency, origin and number of components.
4. Sterilization. Methods of sterilization, assessment of sterilization.
5. Asepsis, antisepsis, disinfection.

6. Bacteriological (cultural) method for diagnostics of infectious diseases.
7. Mixed and pure cultures of bacteria. Isolation of pure cultures of aerobic bacteria (Stage 1).
8. Growth and reproduction of microorganisms. Vegetative form and rest of microbes.
9. Phase propagation of microbes in liquid nutrient medium under stationary conditions.
10. Colonies, particularly their formation in different species of bacteria. Formation of pigment.
11. Isolation of pure cultures of aerobic bacteria (2-stage study).

b) The list of practical skills that are necessary to master:

1. Compliance with rules of epidemic profile and safety in the bacteriological laboratory.
2. Disinfection of infected material, antiseptic treatment of hands, contaminated by the investigated or culture of microbes.
3. Making preparations for microscopic examination of pathological material.
4. Staining preparations with complex method (by Gram).
5. Microscopy preparations in the light microscope with immersion lens.
6. Differentiation of microorganisms by morphological and tinctorial signs.
7. Sowing the investigated material with swab, pipette and loop on solid, semi-solid and liquid culture media.
8. Be able to prepare plates, nutrient medium for sterilizing.

Practical lesson's Protocol

Practical tasks should be done:

Task № 1: Familiarize with the equipment used for sterilization. Bring the results to the table.

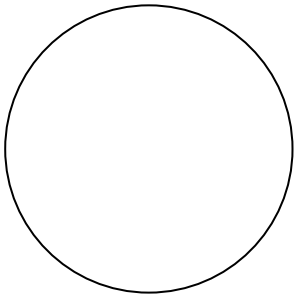
Type of sterilization	Equipment	Sterilization mode	Objects to be sterilized	Results
Burning	Flame			
Boiling	Sterilizer			
Dry heat	Oven of Pasteur			
Pressure	Autoclave			
Pasteurization	Water bath			

Tindolization	Water bath			
Fluid couple	Koch machine, autoclave			
Filter	Filter of Zeitz			
Ultraviolet rays	Sterilizing lamp			
Gamma radiation	In production conditions			

Task№ 2: Familiar with the kinds of culture media, which are used for cultivating bacteria. Bring the results to the table, to indicate their type and purpose.

Type of nutrient medium	Purpose	Examples of culture media
		MPB, MPA
		Sugar MPB, serum MPB, blood MPA, ascitic MPA, Kitt-Tarozzi medium
		Medium of Hiss, MPG, Endo, Levine, Russell, Olkenytskiy
		Gall MPB, alkaline peptone water, alkaline MPA, Aronson media, flat timber, blood-agar
		Glycerol mixture

Task № 3: Make preparation of pathological material of from patients, stained by Gram, study microscopic visualization.



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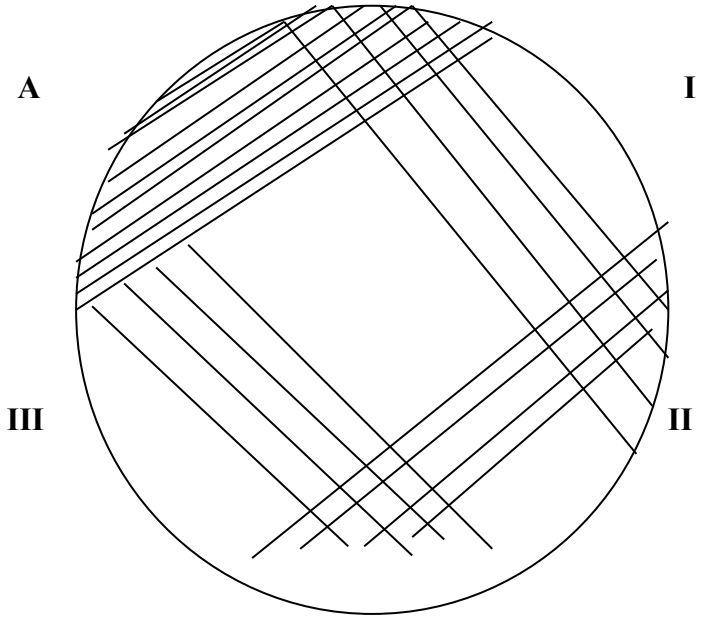
(mark morphological and tinctorial properties of the microorganisms)

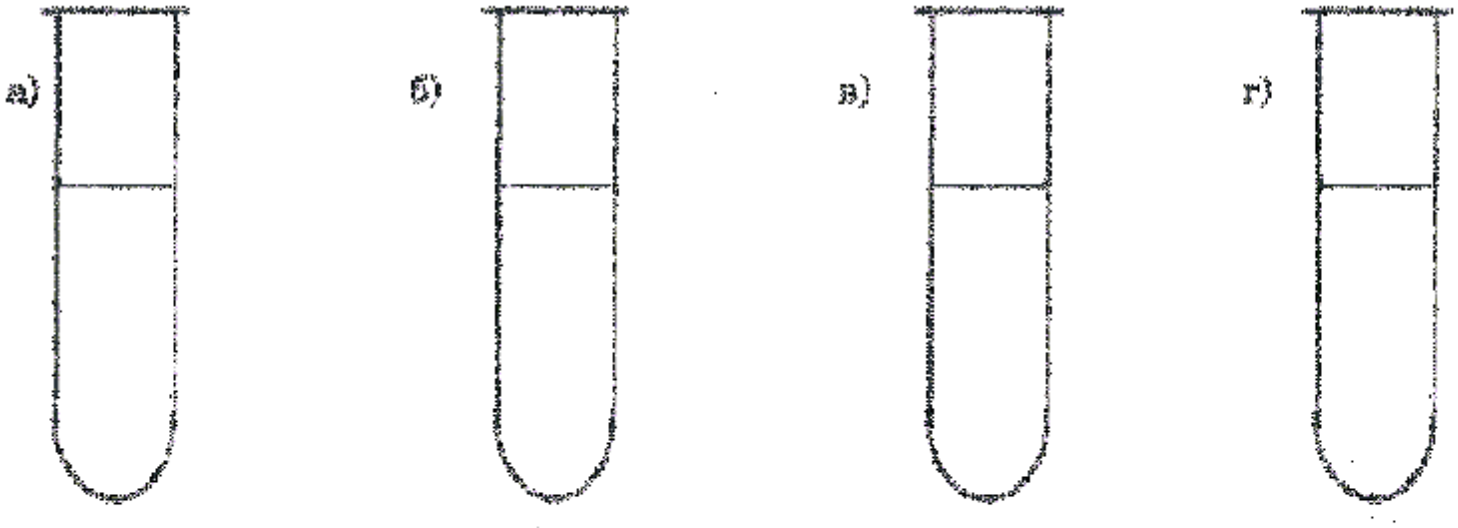
Task № 4: Sow pathological material in a Petri plate with meat and peptone agar (MPA) by the sector method (method of Gold) to obtain isolated colonies.



The scheme of sowing

Task№ 5: View the cultural properties of different types of microorganisms:

a) vibrio cholerae in alkaline peptone water; b) the streptococcus in the sugar and meat peptone broth (sugar MPB);
c) leptospiras in Ulenhut medium; d) staphylococci in meat peptone broth (MPB).
Stain and specify the nature of the growth of microorganisms in liquid nutrient medium.



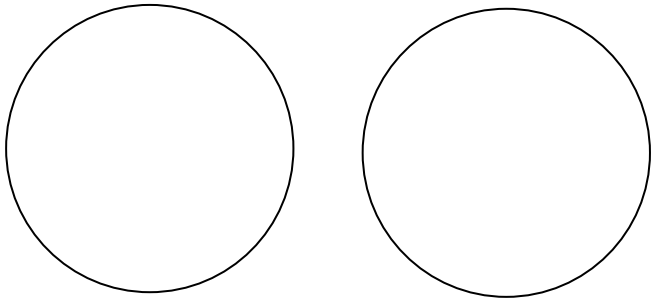
Task № 6. Describe the cultural properties of bacteria, given the nature of the growth of isolated colonies on solid nutrient medium (complete table).

Cultural properties	Column №1	Column №2
Research in the transmitted light		
Size (diameter)		
The form of outlines		
The degree of transparency		

Research in reflected light		
Color of colonies		
The nature of the surface		
The position on the nutrient medium		
Microscopic examination		
The nature of the land		
Structure		
Other cultural properties		
Consistence		

Task № 7: Make preparations of isolated colonies culture of number 1 and number 2, isolated from a patient with catarrhal stomatitis, stained by Gram, to microscope and sketch.

colony № 1 colony № 2



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(mark morphological and tinctorial properties of the microorganisms)

Task № 8: Resow isolated colonies of number 1 and number 2 on the beveled MPA to the accumulation of pure cultures of bacteria.

Teacher's signature_____

Date_____

Practical lesson № 8

Topic: Isolation of pure cultures of aerobic bacteria (3rd and 4th stages of the research).
Methods for studying the enzymatic activity of bacteria.

Tasks for independent work:

a) The list of issues to be studied:

1. Enzymes of bacteria and their classification.
2. Methods for studying the enzymatic activity of bacteria and their use for identification of bacteria.
3. Differential diagnostic culture media, their composition and purpose.
4. Methods for identification of selected crops. The concept of serovaries, morfovaries, biovaries, phagovaries.
5. Modern methods for identification of bacteria by automated enzymatic identification systems.
6. Isolation of pure cultures of aerobic (3rd and 4th stages).

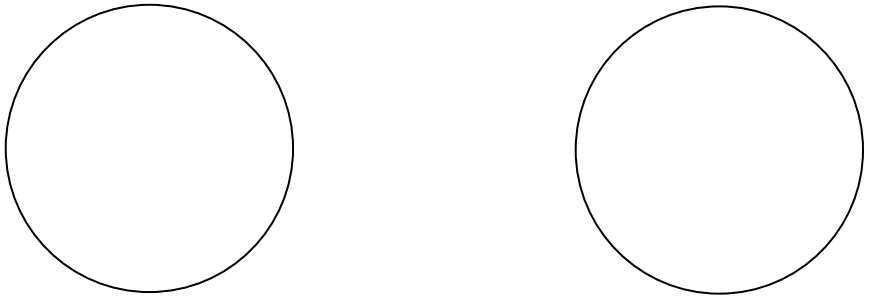
b) The list of practical skills that are necessary to master:

1. Compliance with rules epidemic profile and safety in the bacteriological laboratory.
2. Disinfection of infected material, antiseptic treatment of hands, contaminated by the investigated material or culture microbes.
3. Making preparations for microscopic examination.
4. Staining agents by complex method (by Gram).
5. Microscope preparations in the light microscope with immersion lens.
6. Sowing the investigated material with loop and pipette for solid, semi-solid and liquid culture media.
7. Isolation of pure cultures of aerobic microorganisms.

Practical lesson's Protocol
Practical tasks should be done:

Task № 1: Make products with pure cultures of bacteria isolated from patients with catarrhal stomatitis, stained by Gram, to microscope and sketch.

№1: №2:



(mark morphological and tinctorial properties of the microorganisms, estimation of culture purity)

Task № 2: Resow pure culture in meat peptone broth, meat peptone gelatin, milk and medium of short colorful range for the study of enzymatic activity of bacterias.

Task № 3: Inoculate the researched material from the patient with wound into Kitt-Tarozzi medium.

Task №4: Study the circuit stages of Isolation of pure cultures of aerobic bacteria, state the purpose of each stage.

I stage	II stage	III stage	IV stage
Researched material Microscopic study Staining (by Gram) 37°C 24gr Nutrient medium	1) macro- and microscopic study of cult 2) Staining (by Gram and other methods) 3) 37°C 24gr	1) Estimation of culture purity: a) macroscopic b) microscopic Staining (by Gram) 2) Sowing of differential diagnostic medium 3) Infection of laboratory animals, studying of toxin formation 4) Statement of serological tests with diagnostic serums 5) Setting of antibiotic-grams 6) Study of sensitivity to phages	**Accounting of the studied properties:** 1) Morphological 2) Tinctorial 3) Cultural 4) Biochemical (enzymatic) 5) Biological (toxigenity virulence, etc.) 6) Antigenic 7) Sensitivity to antibiotics
Aim:	Aim:	Aim:	Aim:

Teacher's signature_____

Date_____

Practical lesson № 9

Topic: Methods of Isolation of pure cultures of anaerobic bacteria (1-5 stages of research).

Tasks for self - training work:

a) The list of issues to be studied:

1. Respiration of microorganisms. Types of breathing.
2. Ways to create anaerobic conditions of cultivation of bacteria.
3. Nutrient medium for the cultivation of anaerobes.
4. Isolation of pure cultures of anaerobic bacteria (1-5 stages of research).

b) The list of practical skills that are necessary to master:

1. Compliance with rules of epidemic profile and safety in the bacteriological laboratory.
2. Disinfection of infected material, antiseptic treatment of hands, contaminated by the investigated material or culture microbes.
3. Making preparations for microscopic research.
4. Staining agents by complex method (by Gram).
5. Microscope preparations in the light microscope with immersion lens
6. Differentiation of microorganisms by morphological and tinctorial properties.
7. Sowing the investigated material with loop and pipette for solid, semi-solid and liquid culture media.
8. Isolation of pure cultures of aerobic and anaerobic bacteria identification of morphological, tinctorial, cultural, enzymatic properties.

Practical lesson's Protocol

Practical tasks should be done:

Task № 1: Conduct consideration of enzymatic properties of selected pure cultures of aerobic bacteria.

Culture of bacterias	Lactose	Glucose	Saccharose	Maltose	Manitol	MPG	Milk	Indol	H_2S
№1									
№2									

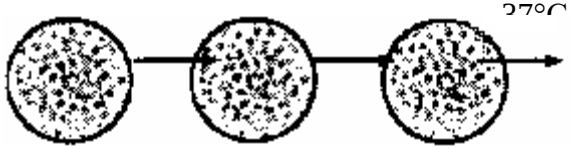
Fill in the table. Specify the character of breakdown carbohydrates (to acid - "A" or to the acid and gas - "AG").

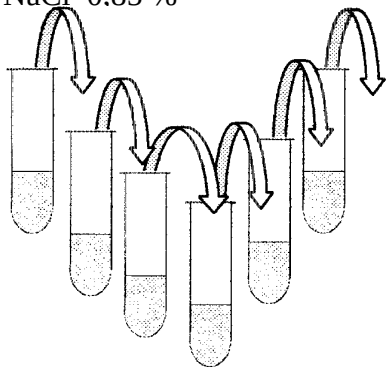
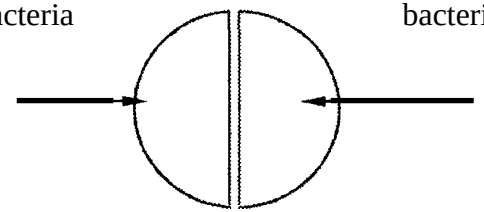
Task № 2: Identify isolated pure culture of bacteria to the genus by the properties.

Properties	Culture № 1	Culture № 2
Morphological		
Tinctorial		
Cultural		
Enzymatic		
Conclusion	Genus	Genus

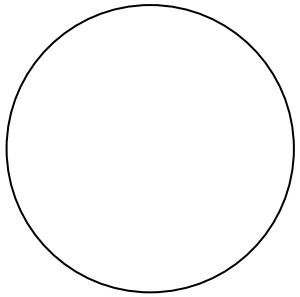
Task № 3: To familiar with the equipment used for cultivation of anaerobic bacteria.

Task № 4: Learn how to obtain isolated colonies of anaerobic bacteria by Zeysler, Weinberg, Fortner. Indicate the name of the method

1)	Method: _____  24 – 28 hours	Sequential distribution of the mixture of bacteria on the surface of a glass spatula blood sugar agar in 3 Petri dish. Crops placed in anaerostat or other devices.

2)	Method: _____ NaCl 0.85 % MPA 	Culture from the medium with Pasteur pipette are soldered to the end and successively transferred to the 1st, 2nd, 3rd test tubes with 10 ml 0.85% sodium chloride solution and continue in the fourth, fifth, sixth tube of melted and cooled to 50 ° C and meat peptone agar. Crops are put in a thermostat.
3)	Method: _____ aerobic bacteria anaerobic bacteria 	In nutrient medium in Petri dish a strip of agar is cut. At one-half cup default cultures of aerobic bacteria are spread to another - culture anaerobes investigated. Petri dish covers, sealed by molten paraffin and after cooling put the cup in the thermostat.

Task № 5: Make preparations from cultures of bacteria grown in a medium of Kitt Tarotstsi, stained by Gram, to microscope and sketch.



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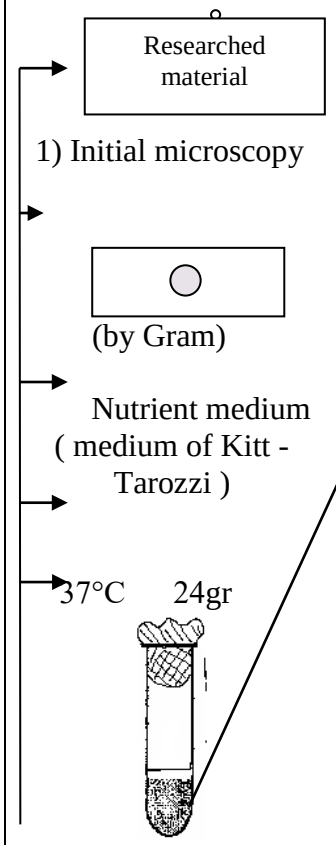
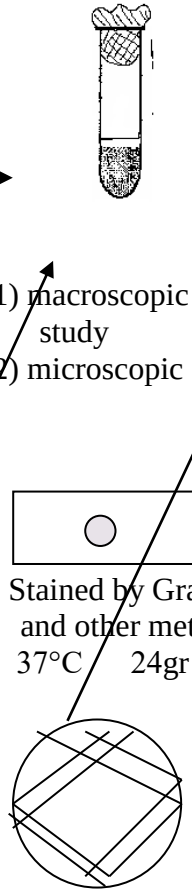
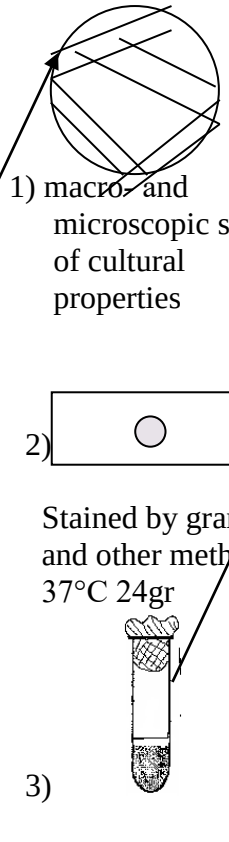
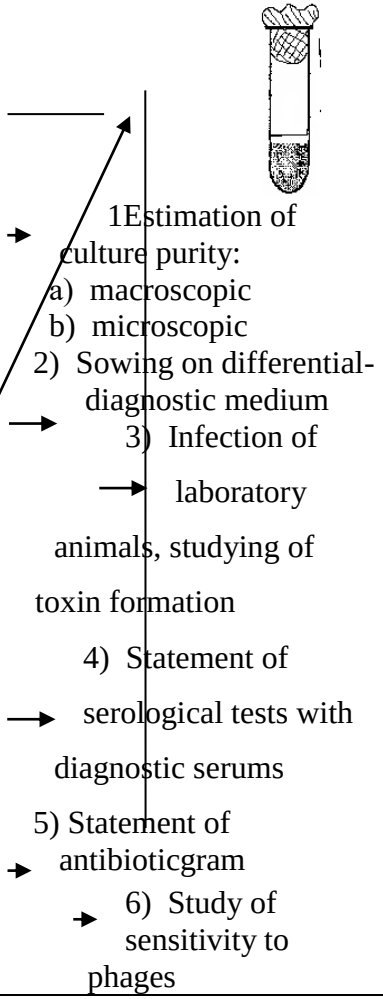
(mark morphological and tinctorial properties of the microorganisms)

Task № 6: Study the allocation scheme of pure cultures of anaerobic bacteria. Specify the purpose of each stage.

I	II	III	IV	V
				**Consideration of the studied properties:** 1) Morphological 2) Tinctorial 3) Cultural 4) Biochemical (enzymatic) 5) Biological (toxigenity, virulense,etc) 6) Antigenic 7) Phaguelizable 8) Sensitivity to antibiotics
Aim:	Aim:	Aim:	Aim:	Aim:

Teacher's signature _____

Date_____

Practical lesson № 10

Topic: Microbiological basis of antimicrobial chemotherapy. Principles of antimicrobial chemotherapy in dentistry. Antibiotics.

Tasks for independent work:

a) The list of issues to be studied:

1. The concept of chemotherapeutic drugs. Chemotherapeutic index.
2. The phenomenon of antagonism in bacteria. Antibiotics, Definitions, concepts.
3. Classification of antibiotics in origin, variety acts, the nature of antimicrobial action and mechanism of action.
4. Units of antimicrobial activity of antibiotics.
5. Methods of determining the sensitivity of bacteria to antibiotics: the method of standard drives and serial dilutions method.
6. Complications of antibiotic therapy. Disbacteriosis and their prophylaxis.
8. Natural and acquired resistance of microorganisms to antibiotics. Genetic and biochemical mechanisms of antibiotic resistance. The role of plasmids and transposons in the formation of drug resistance in bacteria.
9. Ways to prevent the formation of resistance in bacteria to antibiotics. Principles of rational antibiotic therapy.

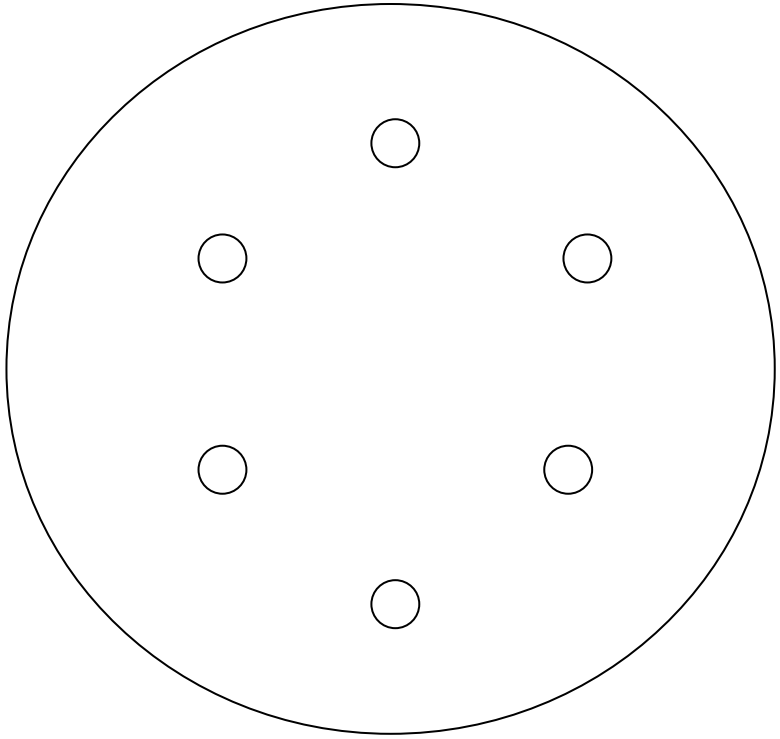
b) The list of practical skills that are necessary to master:

1. To determine the sensitivity of microorganisms to antibiotics.

Practical lesson's Protocol

Practical tasks should be done:

Task № 1: Conduct consideration of sensitivity of pure culture of Streptococcus to antibiotics determined by the standard disks. Mark the picture area of stunted growth. The results add to the table (accounting antibiotic-gram). Make a conclusion.



№	Name of antibiotic	Diameter of zone of stunted growth (mm)	Sensitivity
1.			
2.			
3.			
4.			
5.			
6.			

Conclusion:

_____
_____
_____

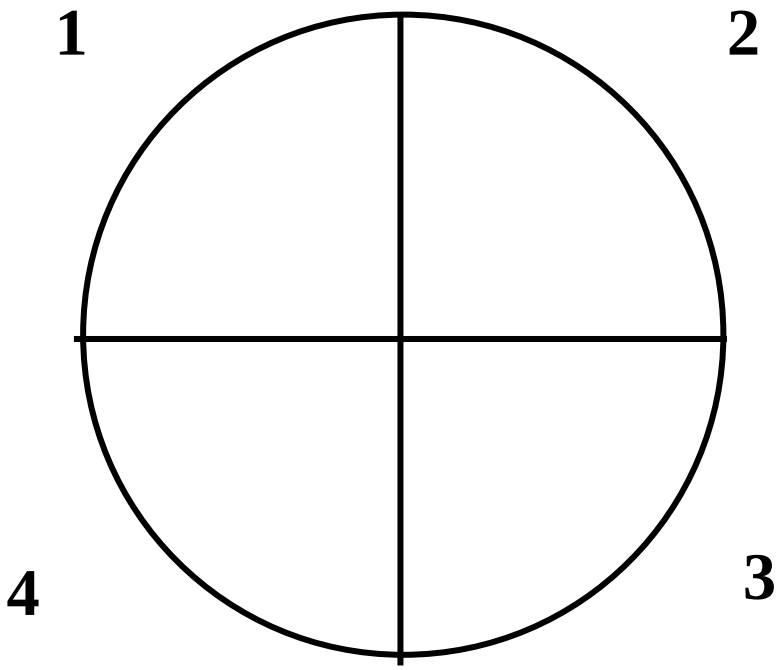
Task № 2. Determine the minimum inhibitory concentrations of cefazolin for Staphylococcus culture. Make a conclusion.

№ tubes / **Ingridients**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9** control of culture	**10** control of antibiotics
MPB	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0	0,5
Antibiotic solution 16 mkg/ml	1,0 →	1,0 →	1,0 →	1,0 →	1,0 →	1,0 →	1,0 →	1,0	-	0,5
Broth culture of bacteria	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2	—
Concentration of antibiotics мkg/мml	8	4	2	1	0,5	0,25	0,125	0,0625	—	8
Consideration										

1 ml

"+"-presence of growth
"-"- absence of growth
Conclusion_____
_____

Task № 3: Determine the minimum bactericidal concentration of cefazolin for Staphylococcus culture. Mark in the picture the presence of bacterial growth (resow in sectors carried out test from tubes 1, 2, 3, 4 -, see task number 2). Make a conclusion.



Conclusion_____
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Teacher's signature _____

Date_____

PRACTICAL LESSON № 11

Topic: The doctrine of the infectious process. Biological method of research.

Biology (Experimental) method is the infection of susceptible laboratory animals a dedicated pure culture of the pathogen, studied material or introduction of bacterial toxins and reproducing the typical picture of the disease. To do this, use white mice, rats, guinea pigs, rabbits. This method determine the virulence of microbes. For the diagnostic biological sample often used for plague, anthrax, tularemia, tetanus, botulism, anaerobic gas infection, encephalitis, etc.

Tasks for self - training work:

a) The list of issues to be studied:

1.The definition of "infection", "infectious process" "infectious disease".
2. Appearing the infection conditions.
3. The role of microorganisms in the infectious process. Pathogenicity of microbes, definition. Obligate pathogens, conditionally pathogenic, pathogenic microorganisms.
4.Virulence, determination. Units of virulence.
5. Factors of microorganisms: is pathogenicity adgezins, invazins, pathogenicity of enzymes, structure and substance of bacteria that inhibits phagocytosis, endotoxins, protein toxins (exotoxins).
6. Pathogenic properties of rickettsia, Chlamydia, mycoplasma, fungi and protozoa. Obligatory intracellular parasitism.
7. Biological method of research.
8. Laboratory animals, linear animals. Methods of experimental infections of laboratory animals.

b) The list of practical skills that are necessary to master:

1. Compliance with rules of epidemic profile and safety in the bacteriological laboratory.
2. . Disinfection of infected material, antiseptic treatment of hands, contaminated by the investigated material or culture microbes.

Practical lesson's Protocol

Practical tasks should be done:

Task № 1: Conduct comparative analysis of bacterial toxins. Bring the results to the table.

	Exotoxins	Endotoxins
Producer		
Localization		
Chemical nature		
Stability at 100 C°		
Inactivation of formaldehyde		
Neutralization by homologous AT		
Biological activity		
Toxicity		

Task № 2: Determine the presence of factors of pathogenicity in staphylococci studied cultures, bring the results to the table.

Factors of pathogenicity	*Culture № 1*	*Culture № 2*
Hemolysin		
Plazmocoagulaze		
Lecitynaze		

Note: "+" – presence of factor of pathogenicity; "-" – its absence.

Task № 3: Conduct intraperitoneal infection of white mice of these materials.

Teacher's signature _____

Date_____

PRACTICAL LESSON № 12

Topic: The doctrine of the infectious process. Biological method of research.

Tasks for self - training work:

a) The list of issues to be studied:

1. The role of macro-organisms, the external environment and social conditions in the origin and development of infections.
2. Stages of epidemiological chain.
3. The concept of the pathogenesis of infectious disease.
3. The spread of germs and their toxins in the body.
4. Dynamics of infections.
5. Forms of infections.
6. Biological research method, its use in studying the etiology, pathogenesis, immunogenesis, diagnosis, treatment and prevention of infectious diseases.
7. Microbiological study of dead animals.

b) The list of practical skills that are necessary to master:

1. Compliance with rules of epidemic profile and safety in the bacteriological laboratory.
2. Disinfection of infected material, antiseptic treatment of hands, contaminated by the investigated material or culture microbes.
3. Making preparations of pathological material stained by Gram, microscopy of preparations in the light microscope with immersion lens.

Practical lesson's Protocol

Practical tasks should be done:

Task № 1: To establish a correspondence between the degree of intensity of the epidemic process and its definition. Bring the results to the table. Definitions that characterize the epidemic process: an epidemic, sporadic disease, endemia, pandemic, quarantine (convectional) disease.

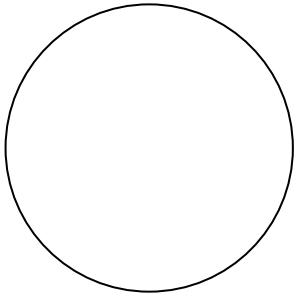
№	The degree of intensity of the epidemic process	Definition
1	The ordinary level of disease of this nosological form in this area at this historical time period (eg., disease of typhoid fever in the city B in 1988. was 2 per 100 thousand population)	
2	The level of disease of this nosological form in the area at a particular period of time is dramatically higher than in sporadic disease (eg, incidence of typhoid fever in the city B in 1994. was 200 per 200 thousand population)	
3	The level of disease of this nosological form in the area at a particular period of time that sharply higher than the epidemic level and includes countries and continents	

Task № 2: To establish a correspondence between certain forms of infections and their names. Bring the results to the table. The names of infections: monoinfection, reinfection, superinfection, mixed infection, recurrence, manifest infection, inaparant infection autoinfection.

№ п/п	The name of infectious process	Signs of infectious process
1		Re-infection of the body with the same stimulus occurs before recovery
2		Re-infection of the body with the same agent after recovery because of the absence of sustained immunity
3		Manifestation of symptoms that occur after clinical recovery without re-infection by pathogens that remain in the body
4		Infection occurs as a result of the weakening of immunity against a background of primary infection and can be caused by other pathogens
5		Development of infectious process that caused by its own (usually pathogenic) microflora when it gets from one habitant to another as a result of autoinfection
6		The simultaneous occurrence of two infectious processes caused by various microorganisms

Task № 3: Conduct an autopsy of the deceased experimentally infected white mice.

Task№ 4: Prepare smears-imprints of internal organs of the dead animals, stained by Gram. To microscope and sketch.



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(mark morphological and tinctorial properties of the microorganisms)

Teacher's signature _____

Date_____

PRACTICAL LESSON № 13

Topic: Types of immunity. Factors of nonspecific protection of the organism and their research methods.

Tasks for self - training work:

a) The list of issues to be studied:

1. The concept of "immunity". Classification of immune origin, the orientation and mechanism of action.
2. Factors of nonspecific protection of the body: cellular and tissue, humoral, functional - physiological.
3. Phagocytosis, the concept of opsonins. Classification of phagocytic cells. The main stages of phagocytosis. Complete and incomplete phagocytosis.
4. Methods for studying of phagocytic activity: identification percentage of phagocytic neutrophils, phagocytes number.
5. Humoral factors of nonspecific protection. Methods of study.
6. Mechanical, chemical and biological factors of nonspecific resistance in the oral cavity (saliva, normal microflora, lysozyme and other enzymes in saliva, complement, β-lysine, etc.). Features of phagocytosis in the mouth.

b) The list of practical skills that are necessary to master:

1. Conduct consideration and estimate the results of the titration reaction of lysozyme.
2. To be able to determine the percentage of phagocytic neutrophils, phagocytic number.
3. Microscopy of preparations in the light microscope with immersion lens.

Practical lesson's Protocol

Practical tasks should be done:

Task№ 1: Determine the titer of saliva lysozyme.

Ingredients \ Number of tube	1	2	3	4	5	6	7	8 Control of culture
Ph.solution (ml)	1.8	1	1	1	1	1	1	1
Saliva (ml)	0.2	1	1	1	1	1	1	
Dilution	1:10	1:20	1:40	1:80	1:160	1:320	1:640	-
Test-culture Micrococcus lysodeikticus (мл)	1	1	1	1	1	1	1	1
Consider								

"+"- lyses of test culture; "-"- absence of lyses

Conclusion:

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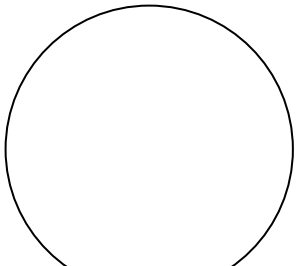
Task № 2: Examine under a microscope and stain preparation, demonstrate the phenomenon of phagocytosis. Make appropriate notations.



(stained by Romanovskiy- Giemza)

Task № 3: Determine the percentage of phagocytic neutrophils and phagocytic number in the examined blood smears .

The number of phagocytic neutrophils	The number of "empty" neutrophils	The number of captured particles by neutrophils		
		1-10	11-20	21 and more
a	b	c	d	e

The percentage of phagocytic neutrophils =

Phagocytic number (number of particles in one cell) = $\frac{5 \cdot c + 15 \cdot d + 25 \cdot e}{a}$ =

Teacher's signature _____

Date_____

PRACTICAL LESSON № 14

Topic: Acquired immunity. Antigens and antibodies. Serological methods of microbiological diagnosis of infectious diseases. Reactions of precipitation and neutralization.

Serological methods based on the detection of specific antibodies in the serum of patients with a particular pathogen. For this purpose, various immunological (serological) reaction: agglutination, precipitation, complement fixation and more. For example, on typhoid fever are often held Widal agglutination test, on brucellosis - the Wright reaction, on chronic gonorrhea - complement fixation reaction of Bordeaux - Zhang and others.

Tasks for self - training work:

a) The list of issues to be studied:

1. Antigens: definition, description, classification.
2. Antigenic structure of microorganisms. Location, chemical composition and specificity of antigens of bacteria, viruses, enzymes, toxins. The role of microbial antigens in the infectious process and development of the immune response.
3. Histocompatibility antigens of man, their characteristics and functions.
4. Antibodies: definition, structure, classification, synthesis. The concept of valence antibodies. Antigenic structure of immunoglobulins: iso-, alo-, idiotypovi determinants. Practical applications.
5. Dynamics of antibody formation. Primary and secondary immune response, their features.
6. Immunoglobulins in saliva. The role of secretory immunoglobulins.
7. The concept of immunological memory and immunological tolerance.
8. Serological reaction, their mechanisms and practical application.
9. The main components of serological reactions. Diagnostic immune serum, diagnostics. Monoclonal antibodies and their use.
10. Application of serological methods in the diagnosis of infectious diseases under specific localization process in the oral cavity (syphilis, gonorrhea, diphtheria, herpes infection, etc.)..
11. Reactions based on the phenomenon of precipitation: ring precipitation, flocculation, precipitation in gels. Practical applications.
12. Neutralization (toxins, viruses, rickets). Practical applications.

b) The list of practical skills that are necessary to master:

1. To be able to make consideration and estimate the results of precipitation reactions and neutralization.

Antigen-antibody reaction are useful in laboratory diagnosis of various diseases and in the identification of infection agents in epidemiological survey. Antigen-antibody reactions in vitro are called serological reactions.

Precipitation reactions: when a soluble antigen combines with in presence of electrolytes (NaCI) at a suitable temperature and complex forms insoluble precipitate.

User of precipitation reaction

1. Identification of bacteria, e.g. detection of group specific polysaccharides substance in streptococci in Lancefield grouping, etc.
2. Identification of antigenic component of bacteria in infected animal tissue, e.g. Bacillus anthracis.
3. Standardization of toxin and antitoxins.
4. Demonstration of antibody in serum, e.g. Kahn’s test for the diagnosis of syphilis.
5. Serological methods for detection of blood, serum, etc.

Techniques of precipitation reaction

1. *Ring test.* The antigen is layered over serum in a narrow tube. The reaction is visible as a white zone at the junction of two clear fluids.
2. *Slide test.* When a drop of ahtigen and antiserum is placed on a slide and mixed by shaking, floccules appear.
3. *Tube test.* The Kahn test for syphilis is an example of tube flocculation test.

4. *Gel diffusion.* The main advantages of this metod are:
   - The precipitate is relatively fixed by agar medium and is easily visible.
   - If antigen or antiserum contains more than one factor then each factor produces separate precipitin line.
   - Antigen and antibodies can be compared for common antigenic determinants.

Practical lesson's Protocol

Practical tasks should be done:

Task № 1: Set the reaction of thermal ring precipitation(by Ascoli) with precipitated anthrax serum and extract, which is obtained from the bodies of dead animals. Make consideration and estimate the results.

Number of tube / Ingredients (ml)	Research	Control	Control	Control
	1	2	3	4
Antianthrax serum	0,5		0,5	0,5
Investigated extract	0,5	0,5		
Normal serum		0,5		
Anthrax extract				0,5
Extract without anthrax antigens			0,5	
Consideration				

Conclusion:

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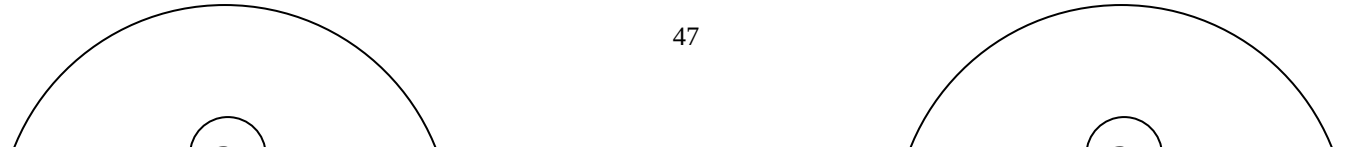
Task № 2: Make consideration and estimate the results of gel precipitation reaction with demonstration agents.



positive/negative reaction *(delete incorrect)* positive/negative reaction *(delete incorrect)*

1. Specific immune precipitated serum (antidiphtheriae);
2. Known antigen (toxigenicity culture of diphtheria pathogen Corynebacterium diphtheriae);
3. Normal serum;
4. Unknown antigen (investigated cultures Corynebacterium diphtheriae 4a i 4b).

Conclusion:

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Teacher's signature_____

Date_____

PRACTICAL LESSON № 15

Topic: Agglutination test.

Tasks for self - training work:

a) The list of issues to be studied:

1. Central and peripheral organs of the immune system.

2. Immunocompetent cells. Characteristics of populations of T- and B-lymphocytes.
3. Surface markers and receptors of immune cells.
4. Cooperation between immunocompetent cells in the process of immune response. The concept of immunomodulators, immunostimulants and Immunosuppressors. Interleukins.
5. Regulation of immune responses (physiological and genetic).
6. Reactions based on the agglutination phenomenon: direct and indirect agglutination, indirect hemagglutination inhibition reaction, the reaction of reverse indirect hemagglutination, Coombs reaction - antiglobulin test. Ingredients, aim.
7. Practical use of agglutination test.

b) The list of practical skills that are necessary to master:
1. To be able to seet, to make consideration and estimate the results of agglutination test on glass.
2. To be able to make consideration and estimate the results of extended agglutination test.
3. To be able to make consideration and estimate the results of indirect hemagglutination reaction.

.**Agglutination Reaction:** when a particulate antigen is mixed with its antibody in presence of electrolytes at a suitable temperature and pH, then the particles are clumped or agglutinated. It is more sensitive than precipitation for the detection of antibodies.

Uses of agglutination reaction

1. Indefication of bacteria, e.g. serotyping of salmonella and shigella with known antisera.
2. Serological diagnosis of infection, e.g. Widal test for typhoid fever, etc.
3. Haemagglutination test, e.g. Rose Waaler, Paul Bunnel.

Techniques of agglutination reaction

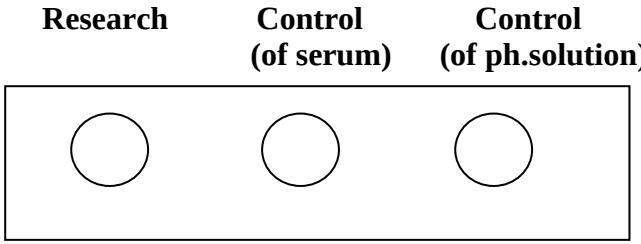
1. *Microagglutination:* It is carried on a clean slide by mixing of antiserum and antigen suspension a drop each. Reaction occurs immediately. It is used for detecting bacterial antigen, blood grouping and typing, etc.
2. *Macroagglutination:* It is carried out as a quantitative test to estimate the titre of antibody and to confirm the result of microagglutination. The following types of agglutination are observed with bacterial antigen:
   - Flagella antigen or H-type of agglutination is seen when a formalized suspension of motile bacteria in treted with antiserum. It forms floccular, snowy flakes like deposit. Agglutination appears 2 to 4 hours after incubation at 52 C.
   - Somatic O-type of agglytination occurs when heat liked or alcohol treated suspension of bacteria is treated with homologous antiserum. The agglutination is compact with fine granulation/ The reaction appears 18 to 24 hours after incubation at 37C.
   - Vi-agglutanation is similar to O-agglutination and occurs slowly at 37C.

Co-agglutination: Here the Fc-fragment of any antibody gets attached to protein A of staphylococci. Thus staphylococci with a known attached antibody are agglutinated when mixed with the specific antigen.

Practical lesson's Protocol

Practical tasks should be done:

Task № 1: Set the agglutination reaction on glass with diagnostic agglutinated typhoid serum (dilution 1:10) and daily investigated culture of bacteria. Make consideration, sketch and estimate the results.



Conclusion:

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Task № 2: Make consideration and estimate the results of expanded agglutination reaction (PPA) with the patient's serum and typhoid diagnostics.

Number of tube / Ingridients	1	2	3	4	5	6 Control of diagnostics	7 Control of serum
Ph.solution (ml)	_	1	1	1	1	1	—
Patient's serum 1 :50 (ML)	1	1	1	1	1	—	1
Dilution of serum	1:50	1:100	1:200	1:400	1:800	—	1:50
Diagnostics (drops)	5	5	5	5	5	5	—
Consideration							

„+" - formation of sludge, undersludge liquid is transparent;
„-" - absence of sludge, cloudy liquid.
Conclusion:

_____

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Task № 3: Make consideration and estimate the results of reaction of indirect hemagglutination, put the patient's serum and erythrocyte diagnostics.

Ingredients \ Number of well	1	2	3	4	5	6 Control of diagnostics	7 Control of serum
Ph.solution (ml)	0,25	0,25	0,25	0,25	0,25	0,25	—
Patient's serum 1:50 (ml)	0,25	0,25	0,25	0,25	0,25	—	0,25
Dilution of serum	1:100	1:200	1:400	1:800	1:1600	_	1:50
Diagnostics (ml)	0,25	0,25	0,25	0,25	0,25	0,25	—
Visual estimation of results (sketch)							
Consideration							

„+" - precipitate of large diameter, granular, with a rough edge ("mat");
„-" - precipitate of small diameter, dense, homogeneous, with straight edge ("button").

Conclusion:
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Signature of teacher_____

Date_____

PRACTICAL LESSON № 16

Topic: The reaction of immune lysis (bacteriolysis, hemolysis). Complement fixation test (CFR, CBT).

Tasks for independent work:

a) The list of issues to be studied:

1. Cellular immune response. Types of immune responses of cell type.
2. Humoral immune response and its stages.
3. The reaction of immune lysis: components, mechanism, practical application.
4. The reaction bacteriolysis; components, methods of production, estimation, practical application.
5. The reaction of immune hemolyses: components, methods of production, consideration and estimation. Application.
6. Complement fixation test (CFT): Components, mechanism, method of production, consideration and estimation reaction, the practical application.

b) The list of practical skills that are necessary to master:

1 . To make consideration and estimate the results of complement fixation reaction.

Complement-Fixation Test (CFT): this is a very sensitive test and is capable of detecting 0.04 mg of antibody nitrogen and 0.1 mg of antigen. It is used for serological diagnosis of diseases: gonorrhoea, brucellosis, syphilis (Wasserman reaction), typhus fever, viraldiseases like lymphogranuloma venereum, etc.

Principle of Complement-Fixation Test **:** the ability of antigen antibody complex to fix complement.

Technique of Complement-Fixation Test **:** heat the patient's serum at 56C for 30 minutes to destroy its own complement. Patient serum, complement (guinea pig serum) and antigen are incubated at 37C for one hour. Now sensitized sheep RBC are added as indicator system. The whole mixture is incubated at 37C for 1 hour.

Interpretation of resau lts thisserological reaction: if complement has been used up, there would not be haemolysis. It means antigen antibody reaction has taken place. Test is reported as positive.

If sensitized CFT are lysed it means complement has not been fixed and test is reported as negative.

Practical lesson's Protocol

Practical tasks should be done:

Task № 1: To make consideration and estimate the results of complement fixation reaction (RPR) on patient's serum and gonococcal diagnostics .

Ingredients (ml) / Number of tubes	Investigated serum (dilution1:10)	Antigen (working dose)	Complement (working dose)	Ph.solution	37ºC – 1 hour	Hemolytic system		37ºC – 1 hour	Consideration	
						Hemolytic serum	Erythrocytes of ram		Hemolyses	CFT
1 (research)	0,5	0,5	0,5	-		0,5	0,5			
2 (control of serum)	0,5	-	0,5	0,5		0,5	0,5			
3 (control of antigen)	-	0,5	0,5	0,5		0,5	0,5			

«+» - positive result

«-» - negative result

Conclusion:

_____

_____

_____

Teacher's signature_____

Date_____

PRACTICAL LESSON № 17

Topic: Reactions with the usage of labeled antigens and antibodies.

Tasks for independent work:

a) The list of issues to be studied:

1. The reaction of immunofluorescence (IF): direct and indirect.
2. Enzyme immunoassay (ELISA): direct, indirect, solid, competitive, imunobloting.
3. Radiomune Analysis (RIA): competitive, reverse, non-direct.
4. Imunoelectronic microscopy.
5. Practical use of these methods of investigation.

b) The list of practical skills that are necessary to master:

1. To make consideration and estimate the results of immunofluorescence, ELISA.

Practical lesson's Protocol

Practical tasks should be done:

Task № 1: To sketch the scheme of direct and indirect immunofluorescence reaction (IFR).

direct IFR **indirect IFR**

Task № 2: To sketch the scheme of direct and indirect ELISA.

direct ELISA **indirect ELISA**

Task № 3: To make consideration and estimate the results of ELISA to detect antibodies to antigens of the causative agent of syphilis. To bring research results to the table.

Photometry of samples

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Conclusion:

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Teacher's signature _____

Date_____

PRACTICAL LESSON № 18

Topic: Immunoprophylaxis and immunotherapy of infectious diseases.

Tasks for independent work:

a) The list of issues to be studied:

1. Active and passive immunoprophylaxis and immunotherapy.
2. Vaccines: types, receipt, evaluation of efficiency and control role. Adjuvant.
3. Vaccine and vaccinotherapy. Autovaccine.
4. Contraindications and complications observed in vaccinoprophylaxis and vaccinotherapy. Prevention of complications.
5. Serum: classification, principles of receiving, treatment and control serum and immunoglobulins.
6. Seroprophylaxis and serotherapy.
7. Complications of serotherapy and seroprophylaxis. Prevention of complications.

b) The list of practical skills that are necessary to master:

1. To make consideration and estimate the results of serological tests.

Practical lesson's Protocol

Practical tasks should be done:

Task № 1: To make consideration and estimate the results of flocculation reaction (RF). To initialize flocculation determine the immunogenic units (IU) in 1 ml of toxoid, using the scheme below of toxoid, antitoxic serum of known strength (800 AO in 1 ml) and explanation.

Ingredients	Tubes					
	1	2	3	4	5	6
Anatoxin	2,0 ml	2,0 ml	2,0 ml	2,0 ml	2,0 ml	2,0 ml
Antitoxic serum	0,1 ml	0,2 ml	0,3 ml	0,4 ml	0,5 ml	0,6 ml
Result(flocculation)						

Tubes maintained at a temperature 45^0C and note that tube, where earlier, than it was in other ways (+)
Initialize flocculation (the most intensive and earlier) comes with complete neutralization of antigen and absence of unused antibody. Thus, in the tube, where are flocculation is, the number of antitoxic units (AU) of serum equivalent to immunogenic units (IU) of toxoid are utilized:

IU in 2 ml of toxoid = AU in ____ ml of antitoxic serum;
AU in ____ml of serum = AU in 1 ml of serum (800 AU) x_____ml of serum
IU in 1 ml of toxoid = IU in 2 ml of toxoid: 2 = _____ IU
Conclusion:_____

Task№ 2: To make consideration and estimate the results of flocculation reaction (RF). To initialize flocculation determine the strength of antitoxic serum (number AU in 1 ml), using the scheme below of toxoid, antitoxic serum and explanation.

Ingredients	Tubes					
	1	2	3	4	5	6
Anatoxin	2,0 ml	2,0 ml	2,0 ml	2,0 ml	2,0 ml	2,0 ml
Antitoxic serum	0,1 ml	0,2 ml	0,3 ml	0,4 ml	0,5 ml	0,6 ml
Result(flocculation)						

Tubes can be maintained at a temperature 45^0C and note that tube, where can be earlier, than in others flocculation.
To initialize flocculation (the most intensive and earlier) comes with complete neutralization of antigen and an absence of unused antibody. Thus, in the tube, where initialize flocculation came, the number of antitoxic units (AU) of serum equivalent to immunogenic units (IU) of toxoid:
It is necessary an antitoxic serum the number of DLM, which containes 1 ml of toxin and DLM, which neutralizes 1 AU of antitoxic serum. We need to titrate the diphtheritic antitoxic serum.
It is known that in 1 ml of toxin containes 5000 DLM, and 100 DLM of diphtheria toxin is neutralized by 1 AU of diphtheria antitoxic serum. Thus, 10000 DLM, contained in two milliliters of toxin will be neutralized by 100 AU of diphtheria serum. Thus, in the tubes, where is initialized flocculation by the appropriate volume of antitoxic serum would contain 100 AO.

Strength of antitoxic serum = $\frac{100\ AU}{\text{volume (in ml) of antitoxic serum}}$ = _____AU
(number of AU in 1 ml)

Conclusion:_____
_____
_____
_____
_____

Task № 3: To familiar with the specific immunobiological preparations that are designed for specific prophylaxis and treatment of infectious diseases. Features of considered preparations should be brousht to the relevant tables.

Vaccines

	Vaccine №1	Vaccine №2	Vaccine №3
Name			
Type			
Composition			
Appointment			
The form of immunity			

Sera

	Serum № 1	Serum № 2	Serum№ 3
Name			
The degree of purification			
The composition (nature of antibodies)			
Appointment			
The form of immunity			

Signature of teacher_____

Date_____

PRACTICAL LESSON № 19

Topic: Human immune status and methods of assessment. Natural and acquired immunodeficiency status.

Tasks for independent work:

a) The list of issues to be studied:

1. The concept of immune status. Immune status as a dynamic balanced system.
2. Immunodeficiency status and its causes.
3. Primary and secondary immunodeficiency status. Features of the immune response (reactivity) in violation of the most vulnerable parts of the immune system.
4. Indicators of the immune system of the human body (immunogram):
   a) non-specific parameters (macrophages, normal killer cells, complement, interferon, lysozyme);
   b) specific performance (immunoglobulins, T-and B-lymphocytes and their subpopulation, mitogen stimulation index, etc.).
5. Methods of assessing the general condition of the immune system and the reasons for their choice:
   a) immunological tests of the first level (approximately): determination of titer of complement, phagocytic activity of neutrophils score, the concentration of the major classes of immunoglobulins (IgA, IgM, IgG), total lymphocytes, T-and B-lymphocytes;
   b) immunological tests of the second level (analytical): NBT-test, determination of LKB, the number of T-and B-lymphocytes and their subpopulations (CD4, CD8, etc.), specific IgE, circulating immune complexes (CIC), the functional activity of lymphocytes (reaction of blasttransformation lymphocytes (RBTL).
6. General rules, which should comply with the interpretation of immunogram.
7. The practical importance of evaluation immunogram.

b) The list of practical skills that are necessary to master:

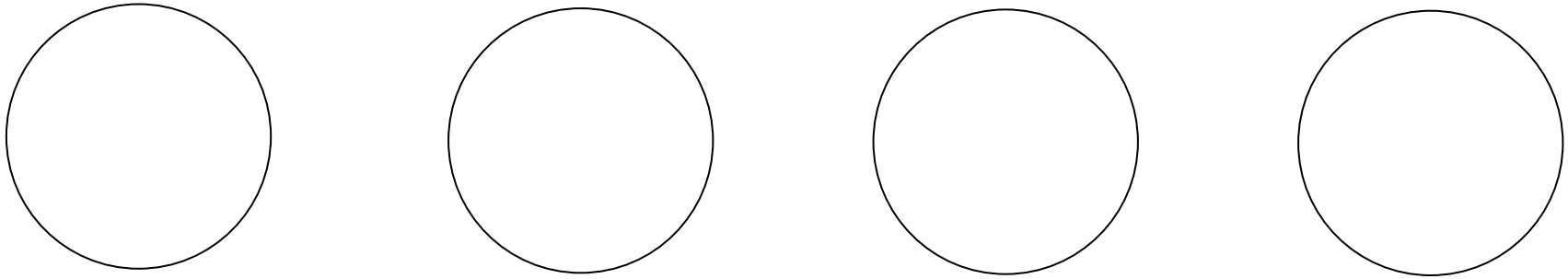
1. Learn to fill in forms of immunogram.
2. Be able to estimate the immunogram.

Practical lesson's Protocol

Practical tasks should be done:

Task № 1: Microscope the display of products for the determination of NBT-test, stain neutrophils of different groups (depending on the number of granules of dyformazane in the cytoplasm).

0 1 2 3



Task № 2: Estimate oxygen-activating ability of neutrophils by NBT-test in the examined people using the count results of neutrophils in blood smears and their distribution in groups (see appendix p. 71-72):

	Examined	
	№1	№2
O - neutrophils without granules		
1- neutrophils isolated from granules or with the area of stained cytoplasm to 25-30%		
2 – neutrophils, which cytoplasm of 30-70% filled with granules of dyformazane		
3 - neutrophils, which cytoplasm of 100% filled with granules dyformazane		

Calculate the average of Cytochemical coefficient (ACC), bring to the forms of immunogram.
Examined № 1 ACC =
Examined № 2 ACC =
Conclusion:_____
_____

Task № 3: Determine the concentration of immunoglobulin classes A, M, and G in serum examined by immunoassay method , according to the results of photometry and control of samples, using inversely-proportional calculation and taking into account the concentration of IgG in the control samples (see appendix p. 68-72):

IgA -1,59 mg/ml; IgM -1,32 mg/ml; IgG - 8,95 mg/ml.

To bring the results of photometry to the table.

To determine the concentrations *Ig* (A, M, G) bring to the forms of immunogram.

	1	2	3	4	5	6	7	8	9	10	11	12
A	б	3 0,103	7	11	б	3 0,104	7	11	б	3 0,041	7	11
B	б	3 0,104	7	11	б	3 0,106	7	п	б	3 0,039	7	11
C	кс 0,152	4	8	12	кс 0,119	4	8	12	кс 0,108	4	8	12
D	кс 0,150	4	8	12	кс 0,120	4	8	12	кс 0,110	4	8	12
E	1 0,138	5	9	13	1 0,036	5	9	13	1 0,043	5	9	13
F	1 0,140	5	9	13	1 0,037	5	9	13	1 0,045	5	9	13
G	2 0,112	б	10	14	2 0,130	б	10	14	2 0,092	6	10	14
H	2 0,114	б	10	14	2 0,132	6	10	14	2 0,094	6	10	14
	IgA				IgM				IgG			

	Examined №1	Examined №2
IgA		
IgM		
IgG		

Conclusion:_____

Task № 4: Bring to the forms of immunogram the results of patient's examination, estimate the results.

Immunogram

Indicators	*Contents in 1 mkl (%)*			*Examined № 1*	*Examined № 2*
The absolute number of leukocytes	4500-7000 (100 %)				
Including: neutrophils	4000 (65%)				
Eosinophils	200-400 (4%)				
The absolute number of lymphocytes	1500-2000 (25%)				
-CD3 (T-general)	800-1200				
-CD4 (T-helpers)	500-900				
-CD8 (T-killers)	400-600				
-CD16 (NK)	170-400				
-CD20 (B-cells)	200-400				
HLA II	340-720				
Imunoglobulins					
IgG	8-12 г/л				
IgM	0.5-1,9 г/л				
IgA	1,4-4,2 г/л				
IgE	20-100 КЕ/л				
CIC, (умов.од.)	20-80				
Phagocitosis					
	Spontaneous	Stimulated	Index of stimulation		
NBT-test	70-120	150-200	1,2-2		
Phagocitosis (%)	48-88				
Index of phagocitosis	1,3-3				
Adhesion (%)	40-55	70-80			

The reaction blasttransformation					
	PHA	PWM			
RBTL	20-100	5-20			
Complement					
C1q		100-250			
C3		700-1800			
C4		200-500			
C5a		0,01-0,03			

Conclusion:_____
_____

Teacher's signature_____

Appendix

1.Determination the number of leukocytes in the blood.

The method is based on the count of white blood cells per unit volume (liter or ml) of blood at a constant dilution of blood and specified volume of the chamber for counting. Counting of leukocytes are in small increase of the microscope (objective x8, x10 eyepiece), dark field of view (omitted condenser or restricted diaphragm) in 100 large squares of Horyayev camera, received number multiplied on 50, expressed as a • 109 / L or thousands / ml.

2. Determination the number of lymphocytes in the blood.

Determining the number of lymphocytes in the blood conducted by counting the leukocyte formulas, determine the percentage of leukocytes in blood smears, stained by Romanovsky-Giemza or Papenheym. Knowing the percentage of lymphocytes and the total number of leukocytes per unit volume of blood, we can find the absolute number of lymphocytes in the blood (in 1 liter or ml).

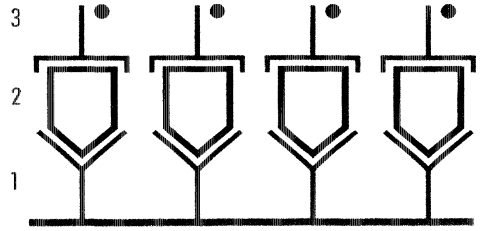
3.Determination of subpopulation contest of blood lymphocytes by the method of indirect immunofluorescence.

The principle of the method: specific monoclonal antibodies bind to membrane antigens (receptors CD^3 , CD^4 , CD^8, CD^{16}, CD^{20} and etc.) living cells (lymphocytes) that are in suspension.. For detection of the complex antibodies IgG are used, labeled by fluorochrom. In fluorescent microscopy preparations determine the percentage of lymphocytes of specific subpopulation, and then calculate their absolute number and ratio of specific subpopulations (CD^4/CD^8, CD^3/CD^{20}).

4. Determination of concentration Ig A, M, G.

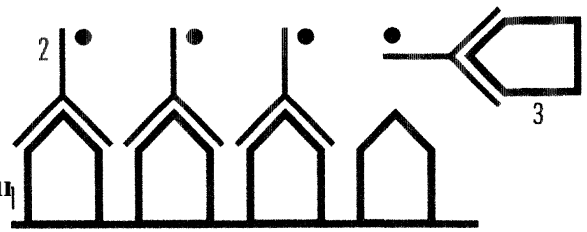
For the quantitative determination of IgG in serum and other biological fluids ,solid human enzyme immunoassay method (ELISA) is used.

Direct solid ELISA method based on the principle of "sandwich". analysis is conducted in two stages.
On the first stage of the control samples with known concentrations of IgG (A, M, G) and incubating samples investigated in holes of polistirile tablet with immobilized monoclonal antibodies (mAbs resulted) and immunoglobulins (A, M, G). Then the tablet "laundered" (removal of the systems of other, non-specifically associated components of monoclonal antibodies).
On the second stage the immunoglobulin (A, M, G), that touched in the hole, treated by conjugate of mAbs resulted in Ig (A, M, G) a person with peroxidase (mAbs resulted in the conjugate and immobilized in the wells of tablet specific to mAbs resulted in different parts of the molecule Ig (A, M, G). After a "clean" excess conjugates immune complexes "immobilized mAbs -1g (A, M, G) - conjugate" exhibit enzymatic reaction of peroxidase with hydrogen peroxide in the presence of chromogen. The color intensity is proportional to the concentration of chromogen Ig (A, M, G) in the studied sample. After stopping the reaction of peroxidase with stop reagent results are recorded with the samples of photometry (measuring the optical density of holes in the tablet at 492 nm).



1 - MAbs resulted in Ig (A, M, G), immobilized in the wells of tablet;
2 - Ig (A,M,G) of investigated samples
3 - conjugate (mAbs resulted in Ig (A, M, G) with the enzyme labeled.

Competitive solid ELISA method.
In the wells of polystirile tablet with immobilized human immunoglobulin (IgA – 1-4 rows, IgM - 5-8 rows, IgG - 9 -12 rows) bring control serum ("cs") with a known concentration of Ig (A, M, G), investigated samples (14) and phosphate buffer saline ("b" is used for dilution of samples, controls, conjugative, washing tablet). Immediately after it the holes are making with the solutions of conjugative `(` conjugate A).
(MAbs resulted in IgA enzyme label - peroxidase) - in 1-4 rows, conjugate M – in 5-8 rows, conjugate G in 9-12 rows). Immunoglobulins contained in the test sample compete with immobilized on solid phase immunoglobulins for communication with the conjugate. The degree of connection imposed by binding of mAbs resulted immunoglobulins of solid phase decreases (they are "recaptured" by immunoglobulins of samples). After incubation the tablet is washed. Contact mAbs resulted in the conjugate with immobilized immunoglobulins assessed by enzyme reaction of peroxidase with hydrogen peroxide in the presence of chromogen. To do this, make a hole with substrate mixture (substrate - chromogen and H2O2) and again incubated. After stopping stop-reagent enzymatic reaction the results are brought to photometry samples.



1 - immobilized immunoglobulins in the holes of tablet (A,M,G);
2 - MAbs resulted in Ig (A, M, G) with the enzyme labeled.
3 - immunoglobulins (A,M,G) of investigated samples
.

The concentration of immu samples is determined by gauge chart or using an inverse calculation:

$$\frac{P_к}{P_x} = \frac{C_x}{C_к}$$, де

$P_к$ - optical density of control sample
P_x - optical density of the investigated sample,
$C_к$ - immunoglobulin concentration in the reference sample,
C_x - concentration of immunoglobulin in the test sample.
Based on it:

$$C_x = \frac{P_к \cdot C_к}{P_x}$$

5. **Determination of circulating immune complexes (CIC)**.

It is based on the ability of the solution (PEG) precipitate from serum aggregated immunoglobulins and immune complexes. Low concentrations of PEG precipitated complexes of large size, high concentrations cause precipitation of low molecular weight compounds. Changing the density of solutions is recorded on a spectrophotometer at a wavelength of 280 nm.

6. **Determination of phagocytic activity of neutrophils**.

It is based on the ability of phagocytes (neutrophils) to capture particles of latex, which are stained by Romanovsky-Giemza in blue. Under the microscope 100 leukocytes (neutrophils) are seen and determined the number of particles captured by them, absorbed an average of one percentage of phagocytic neutrophils and neutrophils - so, number of neutrophil of 100 that showed phagocytic activity (a).

Number of phagocytic neutrophils	Number of "empty" neutrophils	Number of particles captured by neutrophil		
		1-10	11-20	21 and more
a	b	c	d	e

Phagocytic number (number of particles in one cell)= $\frac{5 \cdot c + 15 \cdot d + 25 \cdot e}{a}$

where *5, 15, 25* - number of particles in one neutrophil; *c, d, e* – number of neutrophils

7. Determination of oxygen-activating ability of neutrophils by NBT-test.

The method is based on the ability of mature granulocytes recover by reactive oxygen species (super-oxydanionradical, released during the activation of neutrophils respiratory explosion) pinocitated by light yellow dye of tetrazole row- nitroblue tetrazole (NBT) to insoluble form - dyformazane that looks like dark blue granules in the cytoplasm of neutrophils.

Applied spontaneous and stimulated (killed culture of staphylococcus or zymozanom) NBT-test. In a blood smear with immersion microscopy count 100 neutrophils, distributing them into groups depending on the number of dyformazane granules in the cytoplasm.

0 - neutrophils without granules;
1 – neutrophils with isolated granules or with the area of stained cytoplasm to 25-30%;
2 - neutrophils with the cytoplasm on 30-70% filled with granules of dyformazan;
3 - neutrophils with the cytoplasm on 100% filled with granules of dyformazan. Count the average
Cytochemical coefficient by the formula:

$$ACK = \frac{0 \times a + 1x\ b + 2x\ c + 3x\ d}{100}$$

where *a, b, c, d, e*- number of neutrophils of one group; *0, 1, 2, 3* – group of neutrophils.
If spontaneous and stimulated NBT-test is used, the stimulation index is calculated:

$$IS = \frac{\text{ACK of stimulated NBT-test}}{\text{ACK of spontaneous NBT-test}}$$

8. Determination of lysosomal cationic protein (LCP).
Cationic proteins – are not enzyme protein, inflammatory mediators, which are localized in lysosomes of granulocytes and play an important role in the bactericidal function of neutrophils. LCT - a method that quickly determines the shift a level of nonspecific resistance and assess esvearity of disease.
In the basis of cytochemical studies of cationic proteins is the usage of diagram anionic dyes.
Lysosomes of neutrophilic, eosinophilic granulocytes and bacteria that died under the influence of cationic proteins, stained in one color (depending on the dye used: zabuferen alcoholic solution of durable green - in green, blue bromfenol - in blue), and cellular elements (core) and viable bacteria - in other (while the application of AZUR A - in lilac and blue colors , safranin - orange and red). In immersion microscopy of preparation (smear blood, bone marrow, sputum, drug-print on the surface of the fire of inflammation, bronchial washings from) counted 100 neutrophils, distributing them into groups depending on the presence of a positive reaction to BC and their intensity:

0 - do not give a positive reaction to cationic proteins;
1 - give a mild positive reaction;
2 - give a marked positive reaction;
3 - give the strong positive reaction.

Date_____

PRACTICAL LESSON № 20

Topic: Final control of module 1. "Morphology and physiology of microorganisms. The infection an immunity. "
Questions for final module control:

1. Subject and tasks of medical microbiology. Stages of development of microbiology. The value of microbiology for the dentist. Methods of microbiological examination.
2. Appointment, equipment and organization of the microbiological laboratory.
3. Rules and safety at the microbiology laboratory.
4. Microscopic methods of microorganisms: immersion, phase contrast, darkfield, fluorescent, electron microscopy.
5. The structure of the light microscope.
6. Terms of microscopy in the light microscope with immersion lens.
7. Classification of microorganisms according to the form, number and relative position of cells.
8. Steps in making preparations for microscopic examination of cultures of bacteria.
9. Steps in making preparations for microscopic examination of pathological material.
10. Simple methods of staining, their methodology.
11. Structure of the bacterial cell. Cell's wall, periplazm, cytoplasm membrane, cytoplasm, nucleoid, ribosomes, mezosoms, plasmids.
12. Chemical composition and functions of the structural components of bacterial cells.
13. Polymorphism of bacteria. Properties of L-form bacteria.
14. Complex methods of staining. Gram's method.
15. Mechanisms of interaction of dyes with structures of bacterial cells.
16. Factors affecting the color of bacteria by Gram.
17. Chemical composition, functions, practical importance. Methods of detection of inclusions.
18. Capsules of bacteria: structure, chemical composition, functional significance. Methods of detection. Hins - Burri's method.
19. Flagella: structure, location on the surface of bacterial cells, the functional significance. Detection of flagella. Staining by the method of Loeffler.
20. Detection of motion of bacteria. Preparation of drugs "hanging drop and "crushed" drop.
21. Spore, chemical composition, dynamics, functional significance. Pathogenic spore-formation.
22. Factors that provide high resistance of microorganisms to environmental factors.
23. The color of spores by methods of Ojzeszko and Peshkov.
24. Acid bacteria, am chemical composition. Pathogenic representatives.
25. Method of staining by methods Ziehl-Nielsen.
26. Classification, morphology and structure of spirochetes. Methods of their morphology. Pathogenic representatives.
27. Classification, morphology and structure of fungi. Methods of study of their morphology. Pathogenic representatives.

28. Actinomycetes, morphology and structure. Methods of study of their morphology. Pathogenic representatives.
29. Classification, morphology and structure of Protozoa. Methods of study of their morphology. Pathogenic representatives.
30. Classification, morphology and structure of rickettsia. Methods of detection.
31. Chlamydia and mycoplasma: morphology and structure. Methods of detection.
32. Rules for working with bacterial cultures and safety at the bacteriological laboratory.
33. Cultivation of bacteria. Nutrient medium, classification for purpose, consistency, origin and number of components.
34. Sterilization. Methods of sterilization, assessment of sterilization.
35. Asepsis, antisepsis, disinfection.
36. The evolution of microorganisms. Taxonomy, classification and nomenclature of microorganisms.
37. Genetics of bacteria. Fundamentals of biotechnology and genetic engineering.
38. Bacteriological (cultural) method of diagnosis of infectious diseases.
39. The role of bacteriological methods in the differential diagnosis of dental diseases.
40. Features collection of material for biological research in dental practice
41. Mixed and pure cultures of bacteria. Isolation of pure cultures of aerobic bacteria (Stage 1).
42. Growth and reproduction of microorganisms. Vegetative form and rest of microbes.
43. Phase propagation of microbes in liquid nutrient medium under stationary conditions.
44. Colonies; formation in different species of bacteria. Pigment formation.
45. Isolation of pure cultures of aerobic bacteria (2-stage study).
46. Enzymes of bacteria and classification.
47. Methods of the enzymatic activity of bacteria and their use of identification of bacteria.
48. Differential diagnostic culture media, their composition and purpose.
49. Methods for identification of selected crops. The concept of serovaries, morfovaries, biovaries, phagevaries.
50. Modern methods of identification of bacteria by automated enzymatic identification systems.
51. Isolation of pure cultures of aerobic (3rd and 4th stages).
52. Respiration of microorganisms. Types of breath.
53. Ways to create anaerobic conditions of cultivation of bacteria.
54. Nutrient medium for the cultivation of anaerobes.
55. Isolation of pure cultures of anaerobic bacteria (1-5 stages of research).
56. The role of bacteriological methods in the differential diagnosis of dental diseases.
57. Features collection of material for biological research in dental practice (in terms of caries, stomatitis, periodontitis and others.).
58. Principles for Isolation of nutrient media for culturing microorganisms - causative agents of dental diseases
59. The concept of chemotherapeutic drugs. Chemotherapeutic index.
60. The phenomenon of antagonism in bacteria. Antibiotics, definitions, concepts.
61. Classification of antibiotics in origin, variety acts, the nature of antimicrobial action and mechanism of action.
62. Units of antimicrobial activity of antibiotics.

63. Methods of determining the sensitivity of bacteria to antibiotics: the method of standard and serial dilutions.
64. The use of chemotherapeutic drugs in dental diseases: antibacterial (including antianaerobics and osteotropic), antifungal, anti-virus.
65. Complications of antibiotic therapy. Disbacteriosis and its prevention.
66. Natural and acquired resistance of microorganisms to antibiotics. Genetic and biochemical mechanisms of antibiotic resistance. The role of plasmids and transposons in the formation of drug resistance in bacteria.
67. Ways to preventive the formation of resistance in bacteria to antibiotics. Principles of rational antibiotic therapy.
68. The definition of "infection", "infectious process", "infectious disease".
69. Terms of infection.
70. The role of microorganisms in the infectious process. Pathogenicity of microbes definition. Obligate pathogens, conditionally pathogenic, pathogenic microorganisms.
71. Virulence, determination. Units of virulence.
72. Factors of pathogenicity of microorganisms: adhezyns, invasins, pathogenicity enzymes, structure and substance of bacteria that inhibit phagocytosis, endotoxins, protein toxins (exotoxins).
73. Pathogenic properties of rickets, Chlamydia, mycoplasma, fungi and protozoa. Obligatory intracellular parasitism of viruses.
74. Biological method of research.
75. Laboratory animals. Methods of experimental infection of laboratory animals.
76. The role of macro-organisms, the external environment and social conditions in the origin and development of infection.
77. The level of epidemiological chain.
78. The concept of the pathogenesis of infectious disease.
79. The spread of germs and toxins in the body.
80. Dynamics of infection.
81. Forms of infections.
82. Biological research method, etiology, pathogenesis, immunogenesis, diagnosis, treatment and prophylaxis of infectious diseases.
83. Microbiological study of dead animals.
84. The concept of "immunity". Classification of immune origin, the orientation and mechanism of action.
85. Factors of nonspecific protection of the body: cell and tissue, humoral, functional and physiological.
86. Phagocytosis, the concept of opsonins. Classification of phagocytic cells. The main stages of phagocytosis. Complete and incomplete phagocytosis.
87. Methods for studying phagocytic activity: identification percentage of phagocytic neutrophils, phagocytes number.
88. Humoral factors of nonspecific protection. Methods of study.
89. Mechanical, chemical and biological factors of nonspecific resistance in the oral cavity (saliva, normal microflora, lysozyme and other enzymes in saliva, complement, β-lysine, etc.).. Features of phagocytosis in the mouth.
90. Antigens: definition, description, classification.
91. Antigenic structure of microorganisms. Location, chemical composition and specificity of antigens of bacteria, viruses, enzymes, toxins. The role of microbial antigens in the infectious process and development of the immune response.

92. Histocompatibility antigens of man, their characteristics and functions.
93. Antibodies: definition, structure, classification, synthesis. The concept of valence of antibodies. Antigenic structure of immunoglobulins: iso-, alo-, idiotypic determinants. Practical applications.
94. Dynamics of antibody formation. Primary and secondary immune response.
95. Immunoglobulins in saliva. The role of secretory immunoglobulins
96. The concept of immunological memory and immunological tolerance.
97. Forms of immunity against infection: the communication and agent (sterile and non-sterile), the circumference of the body (general and local), the mechanism (humoral, cellular, mixed), the orientation (antitoxic, antibacterial, antiviral, anti fungal, against parasitic).
98. Serological reaction mechanisms and their practical application.
99. The main components of serological reactions. Diagnostic immune serum diagnostics. Monoclonal antibodies and use.
100. Application of serological methods in the diagnosis of infectious diseases under specific localization process in the oral cavity (syphilis, gonorrhea, diphtheria, herpes, etc.).
101. Reactions based on the phenomenon of precipitation: ring precipitation, flocculation, precipitation in gels. Practical applications.
102. Neutralization (toxins, viruses, rickets). Practical applications.
103. Central and peripheral organs of the immune system.
104. Immunocompetent cells. Characteristics of populations of T-and B-lymphocytes.
105. Surface markers and receptors of immune cells.
106. Cooperation between immunocompetent cells in the process of immune response. The concept of immunomodulators, immunostimulants and immunosuppressors. Interleukins.
107. Regulation of immune responses (physiological and genetic).
108. Mechanisms of specific immunity of the oral cavity.
109. Reactions based on the agglutination phenomenon: direct and indirect agglutination, indirect hemagglutination inhibition reaction, the reaction of reverse indirect hemagglutination, Coombs reaction - antihlobulin test. Ingredients goal.
110. Practical use of agglutination test.
111. Cellular immune response. Types of immune responses of cell type.
112. Humoral immune response and its stages.
113. The reaction of immune lyses: components, mechanisms, practical applications.
114. The reaction of bacteriolyses: components, methods of production, evaluation, practical application.
115. The reaction of immune hemolysis: components, methods of production, and evaluation. Application.
116. Complement fixation test (RPR): Components, mechanism, method of production, recording and evaluation of reaction, the practical application.
117. The reaction of immunofluorescence (IF) test: direct and indirect.
118. Enzyme immunoassay (ELISA): direct, indirect, solid, competitive, immunobloting.
119. Radiomune Analysis (RIA): competitive, reverse, indirect.
120. Imunoelectronic microscopy.

121. Practical use of these methods.
122. The concept of immune status. Immune status as a dynamic balanced system.
123. Immunodeficiency status and its causes.
124. Primary and secondary immunodeficiency status. Features of the immune response (reactivity) in violation of the most vulnerable parts of the immune system.
125. Indicators of the immune system of the human body (immunogram): a) non-specific parameters (macrophages, normal killer cells, complement, interferon, lysozyme), b) specific performance (immunoglobulins, T-and B-lymphocytes and their subpopulation, mitogen stimulation index and others).
126. Methods of assessing the general condition of the immune system and the reasons for their choice: a) immunological tests and the level of (approximately): determination of titer of complement, phagocytic activity of neutrophils score, the concentration of the major classes of immunoglobulins (IgA, IgM, IgG), total lymphocytes, T-and B lymphocytes, b) immunological tests Tier II (analytical): NBT-test, determination of LKP, the number of T-and B-lymphocytes and their subpopulations (CD4, SD8, etc.), specific IgE, circulating immune complexes (CIC), the functional activity of lymphocytes (lymphocyte reaction of blasttransformation (RBTL).
127. General rules, which should comply with the interpretation of immunogram.
128. The practical importance of evaluation immunogram.
129. Active and passive immunoprophylaxis and immunotherapy.
130. Vaccines: types, receipt, evaluation of efficiency and control role. Adjuvant.
131. Vaccine and vaccinotherapy. Autovaccine.
132. Contraindications and complications observed in vaccinprophylaxis and vaccinotherapy. Prevention of complications.
133. Sera: classification, principles of receiving, treatment and control sera and immunoglobulins.
134. Seroprophylaxis and serotherapy.
135. Complications of serotherapy and seroprophylaxis. Prevention of complications.
136. Immunological basis of allergic reactions. Allergens. Skin allergy tests.
137. Allergic situational problems.

Questions for final module control knowledge in practical training:

1. Microscope preparation ,to conduct the color method ,morphology and properties of tinctorial bacteria. (Preparations for microscopy: 1) Staphylococcus, 2) streptococcus, 3) monobacterias Gr-, 4) capsular bacteria, 5) spores by Ozeszko, 6) spores by Peshkov, 7) spores by Gram, 8), yeast fungi, 9) incomplete phagocytosis diplococcus).
2. Make the preparation of culture of bacteria grown on dense media, stain by Gram-Synov. Microscope, determine the morphology and tinctorial properties.
3. Make the preparation of culture of bacteria grown on dense nutrient medium, staining by the simple method. Microscope ,conduct the morphology.
4. Make the preparation of patient specimens, stain by Ziehl-Nielsen, microscope, conduct the morphology.
5. Principal structure and mechanism of action of Endo media. Practical application.

6. Principal structure and mechanism of action of Levin media. Practical application.
7. Principal structure and mechanism of action Ploskyrev media. Practical application.
8. Practical application of Kitt-Tarozzi media , a principal structure and mechanism of action. Practical application.
9. Conduct consideration of biochemical properties of selected clean cultures of bacteria. Make a conclusion.
10. To identify the sensitiveness of culture of staphylococcus to antibiotics using diagnostic discs. Conduct consideration, to make a conclusion.
11. To identify the minimum inhibitory concentrations of cefazolin for Staphylococcus aureus cultures by the method of serial dilutions. Conduct consideration, to make a conclusion.
12. Set reaction of termoringprecypitation by Ascoli to detect antigens of anthrax pathogen in tested extract of animal raw materials. Conduct consideration, to make a conclusion.
13. Set agglutination reaction on glass with an unknown culture and typhoid diagnostic agglutinated serum. Conduct consideration, to make a conclusion.
14. CBT with serum patient and gonococcal diagnostics, to make a conclusion
15. Describe the cultural properties of bacteria on nutrient dense medium.
16. Determine the titer of saliva lysozyme by the method of serial dilutions.
17. Make consideration and estimate the results of gel precipitation test, set to determine the toxigenicity studied cultures of corynebacteria diphtheria.
18. Conduct consideration and estimate the results of extended agglutination test with serum of the patient and typhoid diagnostics.
19. Conduct consideration and estimate the results of indirect hemagglutination reaction, the set of patient serum and erythrocyte diagnostics.
20. Conduct consideration and estimate the results of enzyme immunoassay (ELISA) for detection of antibodies to antigens of excitation manual pages of syphilis.

Contest

Pages.

1. Microbiological Laboratory: organization, equipment, purpose. Methods for microscopic examination. Bacterioscopic method for infectious diseases diagnosis……..…13
2. Morphology of bacteria. Techniques of making preparations from cultures of bacteria and pathological material. Simple methods of staining……..……..…...15
3. Structure of the bacterial cell. Complex methods of staining. The method of Gram. ……...…….……..…..…...17
4. Structure of the bacterial cell: inclusion, capsule, flagella. Methods of detection. Methods for detection of spores and acid bacteria.……..…… ……...…....18
5. Morphology and structure of spirochetes, actinomyces, fungi and the simplest. Methods of study of their morphology.. …….…….....21
6. Morphology and structure of rickettsia, Chlamydia and mycoplasma. Methods of detection.……..…….....23
7. Cultivation of bacteria culture media. Methods of sterilization, disinfection. Methods for Isolation of pure cultures of aerobic bacteria (1-2-stages). Cultural properties of bacteria.....24
8. Isolation of pure cultures of aerobic bacteria (3rd and 4th stages of the research).Methods for studying the enzymatic activity of bacteria.....30
9. Methods of Isolation of pure cultures of anaerobic bacteria (1-5 stages of research)..…… ……..…....33
10. Microbiological basis of antimicrobial chemotherapy. Antibiotics ……...……....37
11. The doctrine of the infectious process. Biological method of research. ……. ……41
12. The doctrine of the infectious process. Biological method of research. The use of biological methods in diagnosis of infectious diseases……...43
13. Types of immunity. Factors of nonspecific protection of the organism and their research methods. …….….45
14. Acquired immunity. Antigens and antibodies. Serological methods of microbiological diagnosis of infectious diseases. Application of serological methods in the diagnosis of oral diseases. Reactions of precipitation and neutralization……..48
15. Agglutination test. …….....……..…….....51
16. The reaction of immune lyses (bacteriolyses, hemolyses). Complement fixation test (CBT)……..……..…54
17. Reactions with the usage of labeled antigens and antibodies. ……..……...…….....56
18. Immunoprophylaxis and immunotherapy of infectious diseases……...60
19. Immune status of man and his methods of assessment. Natural and acquired immunodeficiency state…...…….…….…….64
20. Final control……..…….....…72

Date:_____

Practical lesson №21

Topic : Microbiological diagnostics of staphylococcal infections.

Family: *Micrococcaceae*
Genus: *Staphylococcus*
Species: *Staphylococcus aureus, S.epidermidis, S. saprophyticus*

Tasks for independent work:

a) The list of issues to be studied:

1. General characteristic of coccal bacteria group.
2. Classification. Biological properties of staphylococci. Pathogenicity factors of staphylococci.
3. The role of staphylococcus in human pathology, epidemiology and pathogenesis of infection posed by them.
4. The role of staphylococcus in the progress of hospital infections.
5. Immunity and its features in staphylococcal diseases.
6. Methods of microbiological diagnosis of staphylococcal diseases.
7. Prophylaxis and treatment of staphylococcal infections. Preparations for specific prevention and therapy.

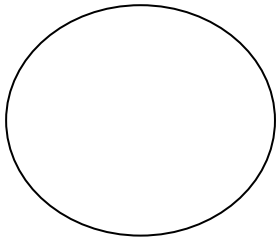
b) The list of practical skills that are necessary to master:

1. Compliance with the rules of antiepidemic regiment and safety in the microbiology laboratory.
2. Making preparations for microscopic research of pathological material (pus).
3. Staining preparations by sophisticated methods (by Gram).
4. Microscope preparations in the light microscope with immersion lens.
5. Crop the investigated material by loop into solid and liquid media.
6. Filling in the directions of the investigated material in the laboratory for bacteriological research.

Practical lesson's Protocol

Practical tasks should be done:

Task №1. To prepare preparation from a pus, to stain by Gram, to microscope and to sketch.



To mark morphological and tinctorial properties of the microorganisms

Task №2. To inoculate the pus on bloody and yolk-salt agar with the purpose of receipt of the isolated colonies.

Task №3. Fill in the direction to bacteriological laboratory of researched material from a patient with a diagnosis sepsis.

Direction №___

For microbiological (bacteriological, virological, parasitological) study

"_____" _____20___ _____ o' clock _____ minutes

(Date and time of capture of biomaterial)

To _____ laboratory

Surname, Name, Patronimic _____ Age _____

Medical card № _____ Institution_____ Department _____

Address of permanent / temporary residence (with indication of S., N., O. of a person, where the subject lives)

_____

Place of work, training (name of child care facility, school _____

Diagnosis, date:_____

Indications for examination: the patient, convalescents, bacteria-, virus-, parasitecarring, contact, preventive inspection

(underline, write other)

Material: blood, urine, sputum, feces, duodenal content, cerebrospinal fluid, punctate, pus discharge from wound exudate, sectional material, swab of the mucosa, etc. _____ _____

(underline, write in, from where the material got)

Aim and tname of research: _____

(which infections research)

Post, name and signature of the person who sent material _____

Task №4. To inoculate the patient blood with sepsis in saccharine broth (MPB) for the isolation of haemoculture.

Task № 5. To describe immunobiological preparations for a specific prophylaxis and treatment of staphylococcal infections.

Preparations	**Type**	**Purpose of application**	**Orientation of the immunity, that is created**

For active immunization			
For passive immunization			

Signature of teacher _____

Date:_____

Practical lesson № 22

Topic: Microbiological diagnostics of streptococcal infections.
Family**:** *Streptococcaceae*
Genus**:** *Streptococcus*
Species: *Streptococcus pyogenes, S.pneumoniae, S.mutans, S.faecalis*
Tasks for independent work:
a) The list of issues to be studied:
1. Biological properties of streptococci. Classification. Serological group of streptococci that inhabit the mouth's cavity.
2 Characteristics of factors streptococcal pathogenicity.

3. The role of streptococcus in human pathology; epidemiology and pathogenesis of disease that are caused by them.
4. Etiological and pathogenetic role of streptococci group A under conditions of erysipelas, scarlet fever and rheumatism. Scarlet fever stomatitis.
5. Inflammatory processes in the mouth caused by streptococci without group antigen.
6. Immunity and its features with streptococcal infections.
7. Methods of microbiological diagnosis of streptococcal diseases
8. Prevention and treatment of streptococcal infections

b) The list of practical skills that are necessary to master:

1. Isolation of clean cultures of aerobic bacteria, identification of selected crops.
2. Making preparations for microscopic examination of pathological material.
3. Sophisticated staining of preparations (by Gram).
4. Microscope of preparations in the light microscope with immersion lens.
5. Differentiation of microorganisms by morphological and tinctorial characteristics.
6. Crop the investigated material by loop on solid media.
7. Determine the sensitiveness of isolated cultures to antibiotics.
8. Reading and evaluation forms with the results of microbiological research.

Practical lesson's Protocol

Practical tasks should be done:

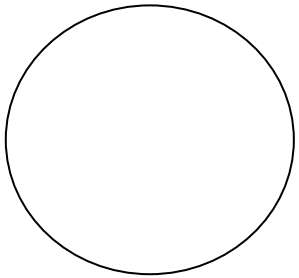
Task № 1. To study macro-and microscopic properties of the isolated colonies on bloody and yolk-salt MPA agar

Cultural properties	Bloody MPA	Yolk-salt MPA
Research in transmitted light		
Size (diameter)		
Form of outlines		
Degree of transparency		
Research in reflected light		
Color of colony		
Character of surface		
Position on a media		
Microscopic research		
Character of edge		

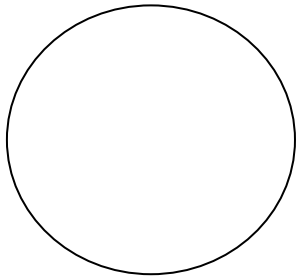
Structure		
Other properties		
Consistency		
Hemolytic activity		
Lesitinaze activity		

Task № 2. To prepare preparations from the isolated colonies, to stain by Gram, to microscope and to sketch.

Bloody MPA



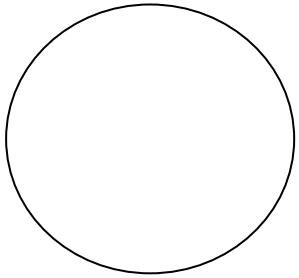
Yolk-salt MPA



To mark morphological and tinctorial properties of the detected microorganisms

Task №3. To inoculate of the isolated colonies on MPA for accumulation of pure culture.

Task №4. To study macro- and microscopic properties of haemoculture into saccharine MPB.



To mark morphological and tinctorial properties of the detected microorganisms

Task №5 . To inoculate the culture on differential-diagnostic media. To indicate:

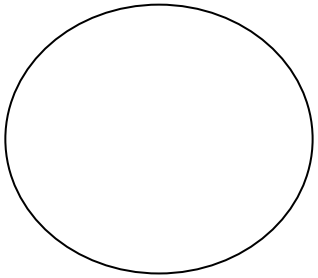
1) media for the study of sacharolytic activity:_____

2) media for the study of proteolytic activity
:_____

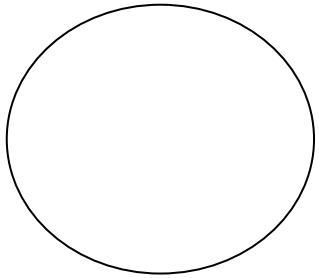
Task №6. To put the antibioticogramm of the selected clean culture by the method of diagnostic disks.
List of antibiotics: _____

Task №7. To prepare preparations from a mucouse of the patient with pneumonia, to stain by Gram and Bouri-Gins, microscope and to sketch.

Staining by by Gram



Staining by Burri-Gins



_____

To mark morphological and tinctorial properties of the detected microorganisms

Task №8. To describe immunobiological preparations for a specific prophylaxis and treatment of streptococcal infection.

Preparations	Type	Purpose of application	Immunity
For active immunization			
For passive immunization			

Signature of teacher _____

Date:_____

Practical lesson № 23

Topic: Microbiological diagnostics meningococcal infections.
Family: *Neisseriaceae*
Genus: *Neisseria*
Species: *Neisseria meningitidis*

Tasks for independent work:

a) The list of issues to be studied:

1. Biological properties of Neisseria. Classification.
2. Biological properties of meningococci, their classification. Factors of pathogenicity of meningococci.
3. Epidemiology and pathogenesis of meningococcal disease. Bacteriocarrier.
4. Immunity at meningococcal disease.
5. Methods of microbiological diagnosis of meningococcal disease and bacteriocarrier state.
6. Differentiation of meningococcal and gramnegative diplococcus of nasopharynx.
7. Prophylaxis and therapy of meningococcal infections.

b) The list of practical skills that are necessary to master:

1. Making preparations for microbiological research of pathological material.
2. Staining preparations by simple and complex methods: water liquid of methylene blue by Gram.
3. Microscope preparations in the light microscope with immersion lens.
4. Differentiation of microorganisms by morphological and tinctorial characteristics .
5. Determine the sensitiveness of isolated cultures to antibiotics.
6. To be able to carry out accounting and evaluate the results of serological tests (reaction of complement fixation).
7. Reading and evaluation forms with the results of microbiological research.

Practical lesson's Protocol
Practical tasks should be done:

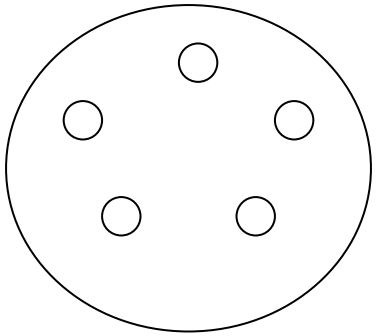
Task № 1. To define the fermentative properties of the selected clean culture of bacteria from patient's pus with abscess in submandibular area.
Results write down to table.

Index / Specific name	Glucose	Lactose	Maltose	Saccharose	Manit	Milk	MPG	H_2S	Indol

Task № 2. To identify the selected clean culture of bacteria from patient's pus with abscess in submandibular area, considering morphological, tinctorical, cultural and fermentative properties (see p.6-7, task 1,2).
Conclusion:_____

_____

Task № 3. To define the antibioticogramm, indicating the name of antibiotic and delay of growth of area of the selected staphylococcus strain. To make a conclusion.



№ p/p	The name of antibiotic	Diameter of area of delay of growth (mm)	Sensitiveness
1			
2			
3			
4			
5			

Conclusion:_____
_____
_____

Task № 4. Fill in the blank with the results of microbiological research of pathological material (pus) from a patient with submandibular abscess area.

The result of microbiological research №____

_____
(specify exactly the research)

"_____" _____20___.
(date of taking the biomaterial)

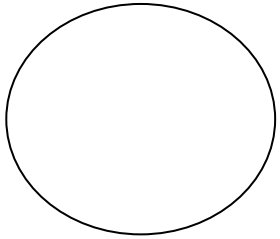
Surname, N., P. _____Age _____
Establishment_____ Department_____
Medical card № _____

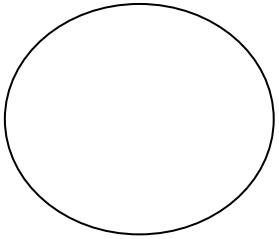
During the research(specify the material) _____
_____
_____
_____

"__" _____ 20___. **Surname, N., P.**_____
(date of analysis) (signature)

Task № 4. To microscope and to sketch the preparations from the spinal liquid stained by methylen blue and by Gram.

Staining with methylen blue Staining by Gram





_____
To mark morphological and tinctorial properties of the microorganisms

Task № 5. To describe immunobiological preparations for a specific prophylaxis and treatment of meningococcal infections.

Preparations	Type	Purpose of application	Orientation of action of Immunity, that is created

For active immunization			
For passive immunization			

Signature of teacher _____

Date:_____

Practical lesson № 24

Topic: Microbiological diagnostics of gonococcus infections.

Family: *Neisseriaceae*
Genus: *Neisseria*
Species: *Neisseria gonorrhea*

Tasks for independent work:

a) The list of issues to be studied:

1. Biological properties of gonococcus, their variability.
2. Pathogenicity for humans. Epidemiology and pathogenesis of gonorrhea. Acute and chronic gonorrhea.
3. Immunity at gonorrhea.
4. Methods of microbiological diagnosis of gonorrhea.
5. Prophylaxis and therapy of gonorrhea and honoblenorrhea

b) The list of practical skills that are necessary to master:

1. Making preparations for microbiological research of pathological material.
2. Staining preparations by simple and complex methods: water liquid of methylene blue by Gram.
3. Microscope preparations in the light microscope with immersion lens.
4. Differentiation of microorganisms by morphological and tinctorial characteristics.

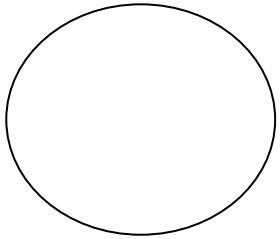
5. Determine the sensitiveness of isolated cultures to antibiotics.
6. To be able to carry out accounting and evaluate the results of serological tests (reaction of complement fixation).
7. Reading and evaluation forms with the results of microbiological research.

Practical lesson's Protocol

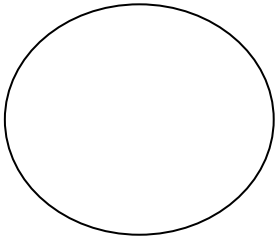
Practical tasks should be done:

Task № 1. To microscope and to sketch preparations of urethral pus, stained with methylen blue and by Gram. To make a conclusion.

Staining with methylen blue



Staining by Gram



To mark morphological and tinctorial properties of the microorganisms

Conclusion (to indicate the microscopic signs of preparations that are the basis for the diagnosis: acute gonorrhea

Task № 2. To put of the reaction of connecting of complement (RCC) with the sera or inspected patient and gonococcus diagnosticum, for confirmation of diagnosis: chronic gonorrhoea.

Ingredients (ml)	The explored sera	Antigen (working dose)	Complement (working dose)	Solution		Haemolitic system	37°C - 1 hour	Cosideration

87

№ Test tubes	ml	ml	ml	ml	37^0 C - 1 hour	Haemolitic sera ml	Erythrocytes of ram ml	Haemolyzis	CBT
1 (experiment)	0,5	0,5	0.5	-		0.5	0.5		
2 (control of serum)	0.5	-	0.5	0.5		0.5	0.5		
3 (control of antigen)	-	0.5	0.5	0.5		0.5	0.5		

Note: "-" negative "+" – positive results

Conclusion:_____

_____

_____

Task № 3. Fill in the blank the results of research of blood sera of patient with chronic gonorrhea.

The result of microbiological research №____

_____

(specify exactly the research)

"_____" _____20___.

(date of taking the biomaterial)

Surname, N., P. _____Age _____

Establishment_____ Department_____

Medical card № _____

During the research(specify the material) _____

_____

_____

_____
_____
_____

"__" _____ 20___. **Surname, N., P.**_____
(date of analysis) (signature)

Task № 4. To describe immunobiological preparations for a specific prophylaxis and treatment of gonococcal infections.

Preparations	Type	Purpose of application	Immunity
For active immunization			
For passive immunization			

Signature of teacher _____

Date:_____

Practical lesson №25

Topic: Microbiological diagnostics of the diseases caused by colon bacilla.

Family: *Enterobacteriaceae*
Genus: *Escherichia*
Species: *Escherichia coli*

Tasks for independent work.

a) ***The list of issues that must be studied:***

1. Classification and general characteristics of the family Enterobacteriaceae.
2. Biological properties of the genus Escherichia. Classification.
3. Antigenic structure of pathogenicity factors of colon bacilla.

4. Epidemiology and pathogenesis of diseases caused by Escherichia coli. Immunity.
5. Role of E. coli in the etiology of purulent-inflammatory diseases.
6. Role of intestinal rod in causing hospital infections.
7. Methods of microbiological diagnostics of esherihiosis infections.
8. Prophylaxis and treatment of esherihiosis.

b) ***The list of practical skills that are necessary to master:***

1. Making preparations for microscopic research of pathological material.
2. Staining preparations by complex methods(by Gram).
3. Microscope preparations in the light microscope with immersion lens**.** **.**
4. Differentiation of microorganisms by morphological and tinctorial characteristics .
5. Isolation of clean cultures of aerobic microorganisms, identification of isolated cultures on morphological, tinctorial, cultural, biochemical, antigenic properties.
6. Production, consideration and evaluation of reaction on glass agglutination.

Practical lesson's Protocol

Practical tasks should be done:

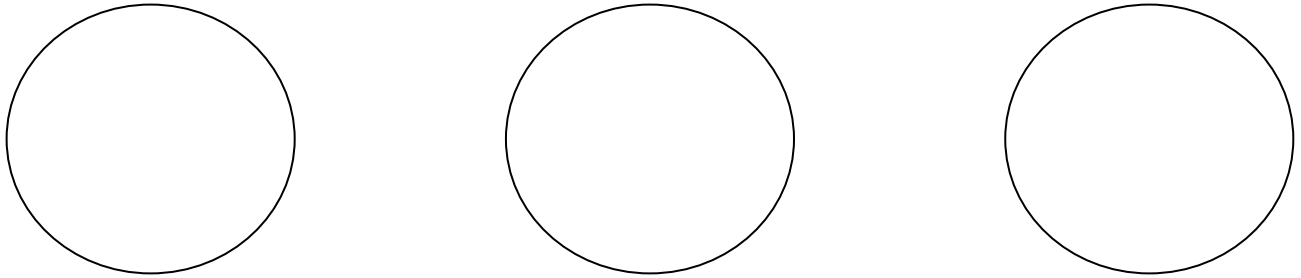
Task № 1. To conduct macro- and microscopic study of the isolated colonies on differential-diagnostic Endo, Levin and Ploskirev's media.

Cultural properties	Endo's media	Levin's media	Ploskirev's media
Research in transmitted light			
Size(diameter)			
Form of outlines			
Degree of transparency			
Research in reflected light			
Color of colony			
Character of surface			
Position on a media			
Microscopic research			
Character of edge			
Structure			

Other properties			
Consistency			

Task № 2. To prepare preparations from lactosepositive and lactosenegative colonies, that grew on differential-diagnostic media of Endo, Levin and Ploskirev, to stain by Gram, to microscope and to sketch.

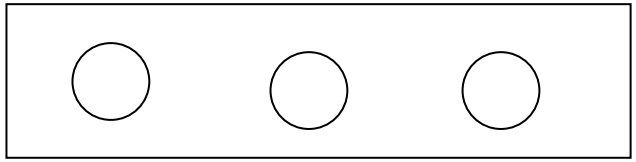
Endo's media Levin's media Ploskirev's media



To mark morphological and tinctorial properties of the microorganisms.

Task № 3. To put the reaction of agglutination on glass with the bacteria of the explored lactopositive colonies and mixture of standard esherihiosis serums (026, 055, 0111). To conduct consideration and make a conclusion. Results were got to sketch.

Experiment **Control (of serum)** **Control (of solution)**



Conclusion:

_____

_____

Task № 4. To conduct consideration of biochemical properties of selected clean cultures of bacteria from patient with coli-enteritis. The results were got bring to table.

Index / Species name	Glucose	Lactose	Maltose	Saccharose	Manit	Milk	MPG	H_2S	Indol

Task № 5. To identify the selected clean cultures of bacteria, including morphological, tinctorial, cultural, fermentative and antigenic properties.
Conclusion:_____
_____

Task № 6. To describe immunobiological preparations for a specific prophylaxis and medical treatment of esherihiosis.

Preparations	Type	Purpose of application	Immunity
For active immunization			
For passive immunization			

1st stage of clean culture selection of microorganisms from the blood of patient with typhoid (task №7)

Task № 7. To inoculate haemoculture from bilious broth on an Ploscirev`s media with the purpose of the isolated colonies receiption.

Signature of teacher_____

Date: _____

Practical lesson № 26

Topic: Microbiological diagnostics of typhoid and paratyphoids B and A (1st and 2nd week of disease))
Family: *Enterobacteriaceae*
Genus: *Salmonella*
Species: *Salmonella typhi, Salmonella paratyphi, Salmonella schottmulleri.*

The tasks for independent work:

a)The list of issues that must be studied:

1.General characteristics of the genus Salmonella. Classification of the genus Salmonella bacteria by biochemical and antigenic properties of the structure (Kauffman – White table).
2. Biological properties of the causative agents of typhoid and paratyphoid A and B. Antigenic structure factors of pathogenicity.
3. Epidemiology and pathogenesis of typhoid and paratyphoid A and B. Phase of the pathogenesis. Bacteria.
4. Immunity at typhoid and paratyphoid A and B. The dynamics of accumulation of O-, H-, Vi-antibodies in the serum of the patient.
5. Methods for microbiological diagnosis of typhoid and paratyphoid A and B on the 1st and 2nd week of illness.

b)The list of practical skills that are necessary to master:

1. Making preparations for microscopic research of pathological material.
2. Staining preparations by complex methods(by Gram).
3.Microscope preparations in the light microscope with immersion lens**.**
4. Differentiation of microorganisms by morphological and tinctorial characteristics.
5. Isolation of clean cultures of aerobic microorganisms, identification of isolated cultures on morphological, tinctorial, cultural, biochemical, antigenic properties.
6. Production, consideration and evaluation of reaction on glass agglutination.

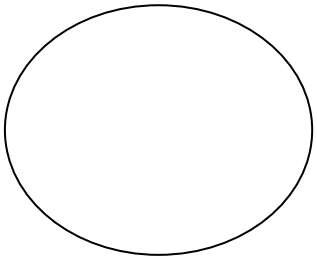
Practical lesson's Protocol

Practical tasks should be done:

Task №1. To define the macro- and microscopic properties of the isolated colonies on a differential-diagnostic Ploskirev`s media.

Cultural properties	Ploskirev's media
Size (diameter)	
Form of outlines	
Degree of transparency	
Color of colony	
Character of surface	
Position on media	
Microscopic research	
Character of edge	
Structure	
Other properties	
Consistency	

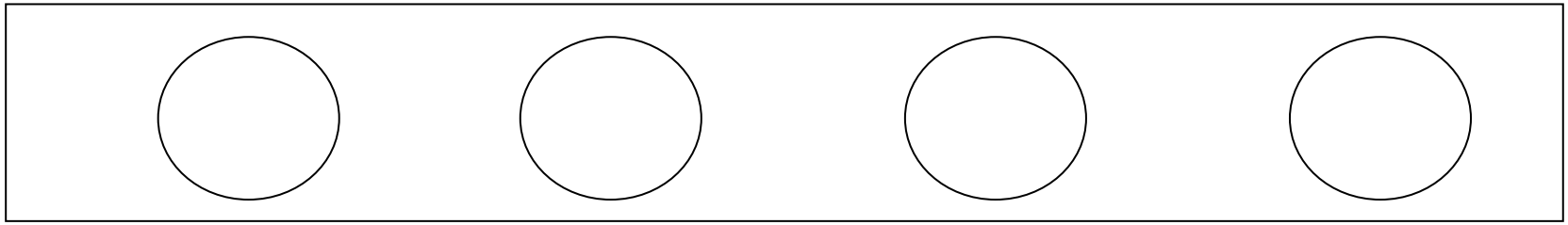
Task №2. To prepare preparations from colonies, to stain by Gram, microscope and to sketch.



To mark morphological and tinctorial properties of the microorganisms.

Task №3. To put the reaction of agglutination on glass with the bacteria of the explored colonies and diagnostical serums**.** Perform accounting and conclude.

Control The thyphoid serum Paratyphoid A serum Paratyphoid B serum



Conclusion;_____

_____

_____

Task №4. Reinoculate the investigated lactosonegative colony from Ploskirev`s media to MPA for pure culture accumulation.

Task №5. Perform accounting of Widal`s test with the patient serum and the typhoid-O, paratyphoid A O- , paratyphoid B O- diagnosticums; typhoid H-, paratyphoid A H-, paratyphoid B H - diagnosticums. Conclusion.

№ test tubes			1	2	3	4	5	6	Control of serum	Control of diagnosticouma
Serum of patient (1:50) (ml)			1	1 ⇨	1 ⇨	1 ⇨	1 ⇨	1	1	-
NaCl solution (ml)			-	1	1	1	1	1	-	1
Dilution			1:50	1:100	1:200	1:400	1:800	1:1600	1:50	-
Diagnosticum (ml)			1	1	1	1	1	1	-	1
O L AND C	O-diagnosticum	Typhoid O-diagnosticum								
		Paratyphoid A O-diagnosticum								
		Paratyphoid B O-diagnosticum								

	H-diagnosticum	Typhoid H-diagnosticum								
		Paratyphoid A H-diagnosticum								
		Paratyphoid B H-diagnosticum								

Conclusion:

_____

_____

Signature of teacher_____

Date:_____

Practical lesson № 27

Topic: Microbiological diagnostics of typhoid and paratyphoids B and A (3rd and 4th week of disease)

Family: *Enterobacteriaceae*

Genus: *Salmonella*

Species: *Salmonella typhi, Salmonella paratyphi, Salmonella schottmulleri.*

The tasks for independent work:

a) The list of issues that must be studied:

1. Pathogenesis of typhoid and paratyphoid A and B (3rd and 4th week of the disease) .
2. Methods of microbiological diagnosis of typhoid and paratyphoid A and B on the 3rd and 4th week of the disease.
3. Microbiological diagnosis of bacteria carring.
4. Salmonella are pathogens of acute enterocolitis . Features of the epidemiology, pathogenesis.
5. Salmonella are pathogens of nosocomial salmonellosis . Features of the nosocomial strains.
6. Methods for microbiological diagnosis of salmonellosis .
7. Prevention and treatment of typhoid, paratyphoid A and B and salmonellosises.

b)The list of practical skills that are necessary to master:

1. Making preparations for microscopic research of pathological material.
2. Staining preparations by complex methods(by Gram).
3. Microscope preparations in the light microscope with immersion lens**.**
4. Differentiation of microorganisms by morphological and tinctorial characteristics.

5. Isolation of clean cultures of aerobic microorganisms, identification of isolated cultures on morphological, tinctorial, cultural, biochemical, antigenic properties.
6. Production, consideration and evaluation of reaction on glass agglutination.

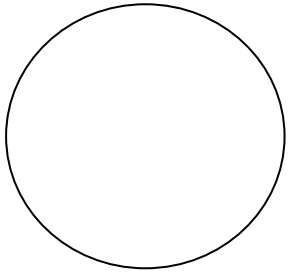
Practical lesson's Protocol

Practical tasks should be done:

III -IV stages of pure bacterial cultures isolation from blood of a patient with suspected typhoid (task number 1, 2, 3)

Task №1. To define macro– and microscopic properties of the selected culture of microorganisms (haemoculture).

Microscopy:



To mark morphological and tinctorial properties of the microorganisms

Task №2. To make calculations of the reaction of agglutination (RA) of Haemoculture with typhoid and paratyphoid A and B diagnostic serums. To do a conclusion.

№ test tubes		1	2	3	4	Control of serums	Control of cultures
Dilution of diagnostic serums		1:500	1:1000	1:2000	1:4000	1:500	-
Consideration	The typhoid serum						
	Paratyphoid A serum						
	Paratyphoid B serum						

Conclusion:

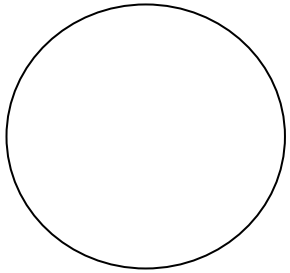
_____

Task №3. To define the biochemical properties of the pure culture of microorganisms (haemoculture). The obtained results are presented in the table.

.Index	Glucose	Lactose	Maltose	Saccharose	Mannit	Milk	MPG	H_2S	Indol

Task №4. To identify haemoculture including morphological, tinctirial, cultural, enzymatic and antigenic properties

Task №5. To prepare preparation from S. typhimurium culture, to stain it by Gram, microscope and to sketch.



To mark morphological and tinctorial properties of the microorganisms

Task №6. To describe immunobiological preparations for a specific prophylaxis and treatment of typhoid and paratyphoids A and B.

Preparations	Type	Purpose of using	Immunity
For active immunization			
For passive immunization			

Signature of teacher_____

Date:_____

Practical lesson № 30

Topic: Microbiological diagnostics of shigellosis

Family: *Enterobacteriaceae*

Genus: *Shigella*

Species: *Shigella dysenteriae; Shigella sonnei; Shigella flexneri; Shigella boydii*

The tasks for independent work:

a) The list of issues that must be studied:

1. Biological properties of the genus Shigella. Classification.
2. Shigella virulence factors.
3. Epidemiology, pathogenesis, clinical manifestations of shigellosis.
4. Immunity at shigellosis.
5. Methods for microbiological diagnosis of shigellosis.
6. Prevention and treatment of shigellosis. The problem of specific prophylaxis. Specific therapy.

b)The list of practical skills that are necessary to master:

7.Making preparations for microscopic research of pathological material.
8.Staining preparations by complex methods(by Gram).
9.Microscope preparations in the light microscope with immersion lens**.**
10. Differentiation of microorganisms by morphological and tinctorial characteristics.
11. Isolation of clean cultures of aerobic microorganisms, identification of isolated cultures on morphological, tinctorial, cultural, biochemical, antigenic properties.
12. Production, consideration and evaluation of reaction on glass agglutination.

Practical lesson's Protocol

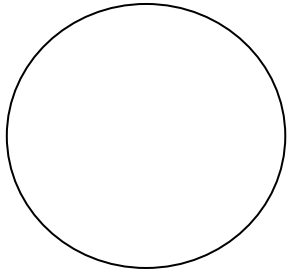
Practical tasks should be done:

Task №1. To conduct macro- and microscopic study of the isolated lactosenegative bacteria on a differential-diagnostic Ploskirev`s media.

Cultural properties	
Size (diameter)	
Form of outlines	

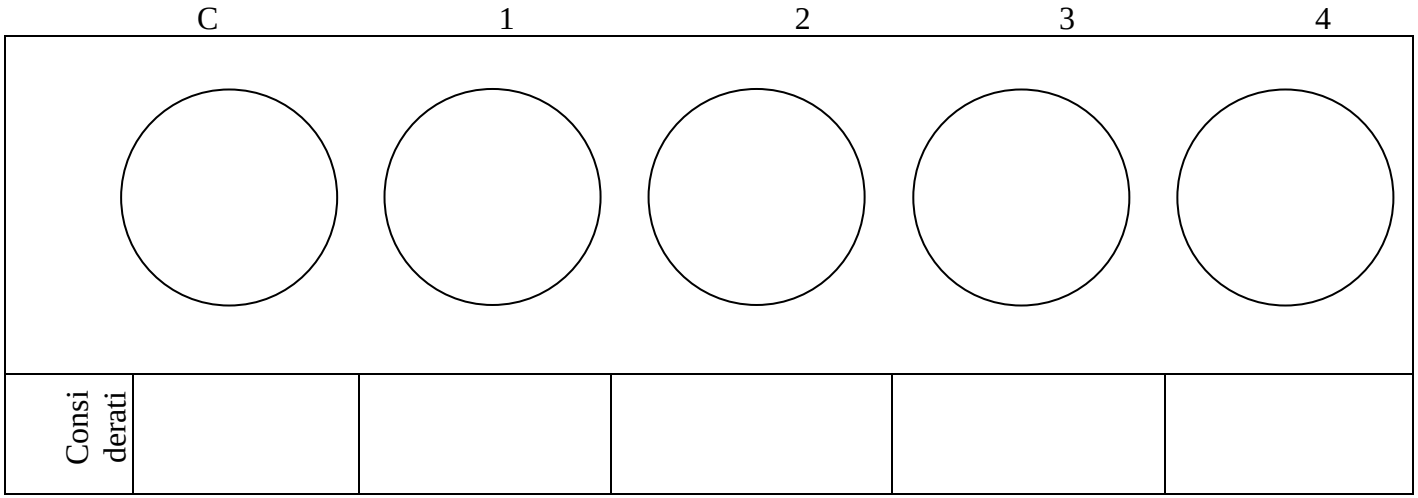
Degree of transparency	
Color of colony	
Character of surface	
Position on a media	
Character of edge	
Structure	
Other properties	
Consistency	

Task №2. To prepare preparations from the explored isolated colonies that grew on differential diagnostic Ploskirev`s medium , stain by Gram, microscope and sketch .



To mark morphological and tinctorial properties of the microorganisms

Task №3. To put the reaction of agglutination on glass with the bacteria of the lactosenegative colonies and diagnostic specific serums: 1- S. dysenteriae; 2 - S. sonnei; 3 - S. flexneri; 4 - S. boydii; C - Control. To conduct consideration and do a conclusion.



Conclusion:

_____

_____

Task №4. To conduct consideration of biochemical properties of Shigella isolated cultures.

.Index / Species	Glucose	Lactose	Maltose	Saccharose	Mannit	Milk	MPG	H_2S	Indol
S.dysenteria									
S.sonnei									
S.flexneri									
S.boydii									

Task №5. To describe immunobiological preparations for a specific prophylaxis and treatment of shigelllosis.

Preparations	Type	Purpose of application	Immunity
For active immunization			
For passive immunization			

Signature of teacher_____

Date:_____

Practical lesson №29

Topic: Microbiological diagnostics of cholera.

Family: *Vibrionaceae*

Genus: *Vibrio*

Species: *Vibrio choleare.* Biovaries (*classical* and *El Tor*)

Tasks for independent work.

a) The list of issues that must be studied:

1. General characteristics of vibrios. Classification, mechanism of action.
2. Cholera vibrios (Vibrio cholerae). Biovary (classical and El Tor), their differentiation.
3. Classification of vibrios by Heyberg. Antigenic structure, biovary. Factors of cholera vibrios virulency. Cholerogen, mechanism of action.
4. The spread of cholera. Epidemiology, pathogenesis, main clinical manifestations of cholera. Immunity. Methods of microbiological diagnostics of cholera.
5. Prophylaxis and treatment of cholera.

b) The list of practical skills that are necessary to master:

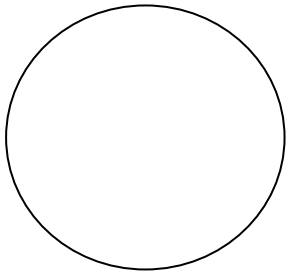
1. Making preparations for microscopic research of pathological material.
2. Staining preparations by complex methods(by Gram).
3. Microscope preparations in the light microscope with immersion lens**.**
4. Differentiation of microorganisms by morphological and tinctorial characteristics.

5. Isolation of clean cultures of aerobic microorganisms, identification of isolated cultures on morphological, tinctorial, cultural, biochemical, antigenic properties.
6. Production, consideration and evaluation of glass agglutination reaction.

Practical lesson's Protocol

Practical tasks should be done:

Task № 1. To prepare the preparations from cultures of cholerical vibrios, to stain by Gram, to microscope and to sketch.



_____

To mark morphological and tinctorial properties of the microorganisms.

Task № 2. To identify the mobility of vibrios in the preparation "hanging" drop.

Task № 3. To conduct consideration of agglutination reaction with the purpose of rapid exposure of choleraic vibrios in a drinking-water. To do a conclusion.

№ test tubes	1	2	3	4	5	6	Control of serum	Control of water
Dilution of O-cholerae serums	1:100	1:200	1:400	1:800	1:1600	1:3200	1:100	-
Consideration								

Conclusion:_____

Task № 4. To describe immunobiological preparations for a specific prophylaxis and treatment of cholera.

Preparations	Type	Purpose of application	Immunity
For active immunization			
For passive immunization			

Signature of teacher_____

Date:_____

Practical lesson №30

Topic: Microbiological diagnosis of brucellosis and anthrax.

Family: *Bacillaceae*
Genus: *Bacilla*
Species: *Bacilla antracis*

Family: *Brucellaceae*
Genus: *Brucella*
Species: *Brucella abortus, B. melitensis, B. ovis, B. canis*

Tasks for independent work:

a) The list of issues that must be studied:

1. Ecology of anthrax pathogens.
2. Biological properties of anthrax pathogens. Classification. Resistance. Pathogenicity factors. Pathogenicity for humans and animals.
3. Epidemiology and pathogenesis. The main clinical manifestations of anthrax in humans.
4. Immunity at anthrax.
5. Biological properties of brucellosis pathogens. Virulence factors. Classification.
6. Epidemiology, pathogenesis and clinical forms of brucellosis.
7. Immunity at brucellosis.

8. Methods of microbiological diagnosis of anthrax and brucellosis.
9. Principles of prophylaxis and treatment of anthrax and brucellosis. Specific prophylaxis and treatment.

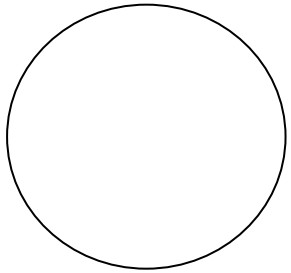
b) The list of practical skills that are necessary to master

1. Compliance with the rules of anti-epidemic regiment and safety in the microbiology laboratory working with agents of especially dangerous infections.
2. Microscope preparations in the light microscope with immersion lens.
3. Differentiation of microorganisms by morphological and tinctorial characteristics.
4. Ability to perform consideration and evaluate the results of serological reactions (reactions of precipitation, agglutination).

Practical lesson's Protocol

Practical tasks should be done:

Task №1. To prepare preparations from patient feces, to stain by Gram, microscope and to sketch.



_____

To mark morphological and tinctorial properties of the microorganisms.

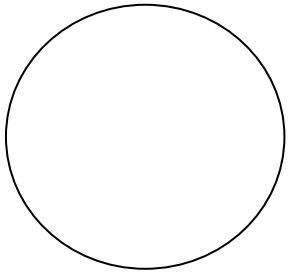
Task №2. To conduct consideration and estimate the results of the agglutination test (Wrayt`s reaction) put with the serum of patient and brucellosis diagnosticum. To do a conclusion.

№ test tubes	1	2	3	4	5	Control of serum	Control diagnosticoumou
Solubilization of serum	1: 50	1: 100	1: 200	1: 400	1: 800	-	-
Consideration							

Conclusion:_____

_____

Task №3. To microscope and to sketch the preparation from the anthrax culture, stained by Gram.



To mark morphological and tinctorial properties of anthrax pathogen

Task №4. To put and to conduct consideration of precipitation reaction by Ascoli. To make a conclusion.

№ test tubes / Ingredients	1	2	3	4
Precipitated anthrax serum (ml)	0.5		0.5	0.5
The explored extract (ml)	0.5	0.5		
Normal serum (ml)		0.5		
Extract from normal organs (ml)			0.5	
Extract of anthrax (ml)				0.5
Consideration				

Conclusion:_____
_____

Task №4. To describe immunological preparations for a specific prophylaxis and medical treatment of anthrax and brucellosis.

Preparations	Type	Purpose of application	Immunity
For active immunization			

For passive immunization			

Signature o teacher_____

Date:_____

Practical lesson №31

Topic: Microbiological diagnosis of plague and tularemia.

Family: *Enterobacteriaceae*
Genus: *Yersinia*
Species: *Yersinia pestis, Y.pseudotuberculosis, Y.enterocolitica*

Family: *Francisellaceae*
Genus: *Francisella*
Species: *Francisella tularensis*

Tasks for independent work:

a) The list of issues that must be studied:

1. Biological properties of plague pathogens. Virulence factors. Classification.
2. Epidemiology, pathogenesis and clinical forms of plague.
3. Immunity under the plague.
4. Ecology of tularemia pathogen.
5. Biological properties of tularemia pathogen. Classification. Resistance. Pathogenicity factors. Pathogenicity for humans and animals.
6. Epidemiology and pathogenesis. The main clinical manifestations of tularemia in humans.
7. Immunity at tularemia.
8. Methods of microbiological diagnosis of tularemia and plague.
9. Principles of prophylaxis and treatment of tularemia and plague. Specific prophylaxis and treatment.

b) The list of practical skills that are necessary to master

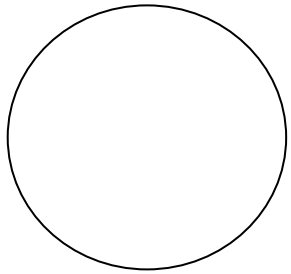
1. Compliance with the rules of anti-epidemic regime and safety in the microbiology laboratory when working with agents of especially dangerous infections.

2. Microscope preparations in the light microscope with immersion lens.
3. Differentiation of microorganisms by morphological and tinctorial characteristics.
4. Ability to perform consideration and evaluate the results of serological reactions (reactions of agglutination).

Practical lesson's Protocol

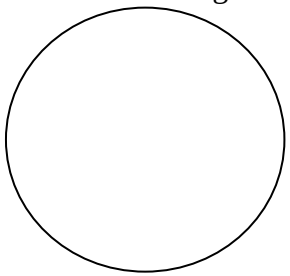
Practical tasks should be done:

Task №1. To microscope and to sketch preparation of "Yersinia pestis", stained with methylen blue



To mark morphological and tinctorial properties of plague pathogen

Task №2. To microscope and to sketch the preparation from the tularemia agent culture, stained by Gram.



To mark morphological and tinctorial properties of tularemia pathogen

Task №3. To conduct consideration and estimate the results of the agglutination test put with the serum of patient and tularemia diagnosticum. To do a conclusion.

№ test tubes	1	2	3	4	5	Control of serum	Control diagnosticum
Solubilization of serum	1: 50	1: 100	1: 200	1: 400	1: 800	-	-
Consideration							

Conclusion:_____
_____

Task №4. To describe immunological preparations for a specific prophylaxis and treatment of plague and tularemia.

Preparations	Type	Purpose of application	Immunity
For active immunization			
For passive immunization			

Signature of teacher_____

Date:_____

Practical lesson №32

Topic: Microbiological diagnostics of tuberculosis and actinomycosis.

Family: *Mycobacteriaceae*
Genus: *Mycobacterium*
Species: *Mycobacterium tuberculosis, M.bovis, M.africanum, M.microti*

Family: *Actinomycetaceae*
Genus: *Actinomyces*
Species: Actinomyces israelii, A.bovis

Tasks for independent work*:*

a) The list of issues that must be studied:

1. Pathogenic and saprophytic mycobacteria.
2. Biological properties of the agents of tuberculosis.
3. Variability of tuberculosis bacteria, pathogenicity factors. Tuberculin.
4. Epidemiology and pathogenesis of tuberculosis.
5. Patterns of immunity, the role of cellular mechanisms under conditions of tuberculosis.
6. Pathogens of mycobacterioses. Classification, properties. Role in human pathology. Mycobacterioses as a manifestation of HIV infection.
7. General characteristics of the genus of actinomycetes.
8. Pathogens of actinomycosis. Ecology. Resistance. Properties.
9. Epidemiology and pathogenesis of actinomycosis. Immunity.
10. Methods of microbiological diagnosis of tuberculosis and actinomycosis. Material for research.
11. Prophylaxis and treatment of tuberculosis and actinomycosis.

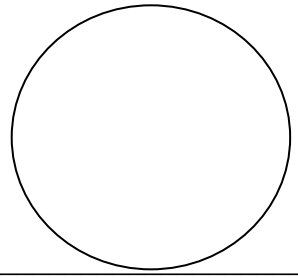
b) The list of practical skills that are necessary to master:

1. Making preparations for microscopic examination of pathological material (mucus).
2. Staining preparations by complex methods (by Ziehl – Neelsen)
3. Microscope preparations in the light microscope with immersion lens.
4. Differentiation of microorganisms by morphological and tinctorial characteristics.

Practical lesson's Protocol

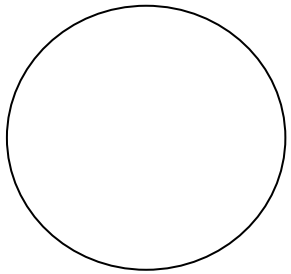
Practical tasks should be done:

Task №1. To prepare preparations from a mucus of patient with tuberculosis,to stain by Ziehl- Neelsen, to microscope and to sketch.



To mark acid fast bacteria

Task №2. To microscope and to sketch actinomycetes in the preparation, produced from patient's pus with maxillo-facial actinomycosis. Stained by Gram.



To mark morphological and tinctorial properties of microorganisms

Task №3. To describe immunological preparations for a specific prophylaxis and medical treatment of tuberculosis and actinomycosis.

Preparations	Type	Purpose of application	Orientation of action of Immunity, that is created
For active immunization			
For passive immunization			

Signature of teacher_____

Date:_____

Practical lesson №33

Topic: Microbiological diagnostics of diphtheria.

Family: *Corynebacteriaceae*
Genus: *Corynebacterium*
Species*: Corynebacterium diphtheriae, C.ulcerans, C.xerosis, C. pseudodiphtheriticum*

Tasks for independent work:

a) The list of issues that must be studied:

1. Biological properties of diphtheria agent. Classification. Biovary. Resistance.
2. Pathogenicity factors. Diphtheria toxin, the mechanism of action. Toxigenity as a result of phage conversion, molecular mechanism of action of diphtheria toxin.
3. Epidemiology and pathogenesis of diphtheria.
4. Antitoxic immunity. Bacteriocarrier.
5. Methods of microbiological diagnostics of diphtheria. Immunological and genetic methods for determining toxicity of diphtheria. Differentiation of diphtheria with other pathogenic and nonpathogenic for people corynebacterias, control of toxigenity.
6. To specific prophylaxis and treatment of diphtheria.

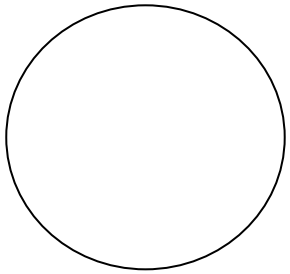
b) The list of practical skills that are necessary to master:

1. To microscope preparations in the light microscope with immersion lens.
2. Differentiation of microorganisms by morphological and tinctorial characteristics .
3. Ability to conduct consideration and evaluate the results of serological reactions (precipitation reaction in agar).

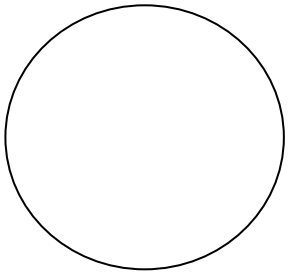
Practical lesson's Protocol
Practical tasks should be done:

Task №1. To microscope and to sketch the preparations made from the cultures of Corinebacteria diphtheria

Stained by Loeffler



Stained by Neisser

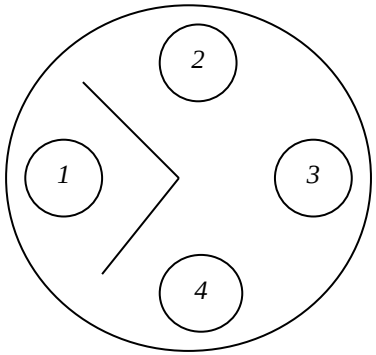


To mark morphological and tinctorial properties of microorganisms

Task №2. To conduct consideration of biochemical properties of clean cultures of corinebacteria and make a conclusion about their specific belonging.

Indexes / Type of corinebacteria	glucose	saccharose	starch	Cystinase test	Ureaza test	renewal of nitrates to nitrites	Conclusion
Corynebacterium diphtheriae							The explored culture №___
Corynebacterium pseudodiphtheriticum							The explored culture №___

Task №3. To define the toxigenity of the corinebacteria cultures by the reaction of precipitation in agar. To do a conclusion.



1. Specific immune precipitated serum (antidiphtheria).
2. The known antigen (toxigenic culture of Corynebacterium diphtheriae).
3. Normal serum.
4. The unknown antigen (the explored culture of Corynebacterium diphtheriae).

Consideration:_____
_____

Conclusion: the explored culture of Corynebacterium diphtheriae _____(toxigenic, nontoxigenic).

Task №4. To describe immunological preparations for a specific prophylaxis and medical treatment of diphtheria

Preparations	Type	Purpose of application	Immunity
For active immunization			
For passive immunization			

Signature of teacher_____

Date:_____

Practical lesson № 34

Topic: Microbiological diagnostics of diseases, caused by Bordetella.

Genus: *Bordetella*

Species*: Bordetella pertussis, Bordetella parapertussis, Bordetella bronchoseptica.*

Tasks for independent work:

a) The list of issues that must be studied:

1. Biological properties of Bordetella, classification. Pathogenic representatives: Bordetella pertussis, Bordetella parapertussis, Bordetella bronchoseptica.
2. Epidemiology, pathogenesis and immunity at whooping-cough.
3. Microbiological diagnostics of whooping-cough.
4. Specific prevention of whooping-cough.
3. Principles of ethiothropical therapy of whooping-cough.
6. Differentiation of whooping-cough, parawhooping-cough and bronhosepticosis pathogenes.

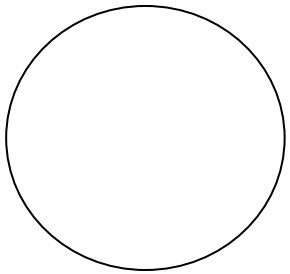
b) The list of practical skills that are necessary to master:

1. To microscope preparations in the light microscope with immersion lens.
2. Differentiation of microorganisms by morphological and tinctorial characteristics.
3. Ability to conduct consideration and evaluate the results of serological reactions (agglutination tests).

Practical lesson's Protocol

Practical tasks should be done:

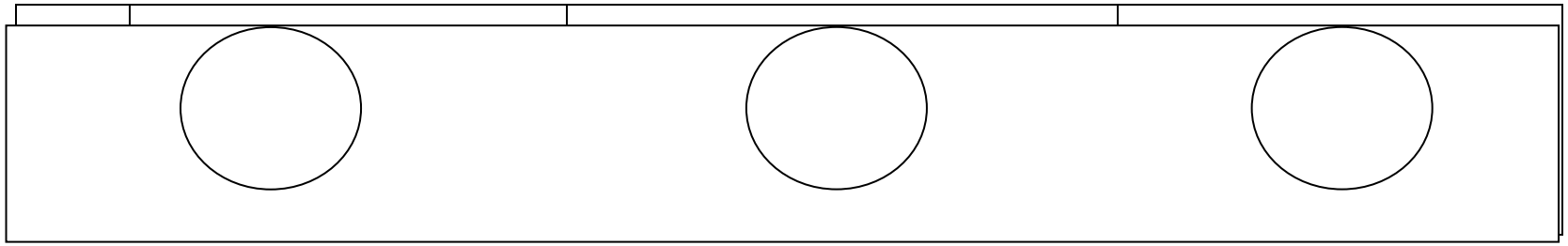
Task№1. Microscope and sketch the preparation of whooping-cough pathogen stained by Gram.



To mark morphological and tinctorial signs of the microorganisms

Task№2. To conduct the results of the slide agglutination reaction with the bacteria of the explored colonies and whooping-cough and parapertussis serums (solubilization 1:10). To do a conclusion. Results were got to sketch.

Control Whooping-cough serum The Parapertussis serum



Conclusion:_____
_____

Task №3. To conduct and estimate the results of indirect hemagglutination reaction (IHAR) with the serums of sick child and erithrocyte whooping-cough diagnosticum.

	№ p/p welles in the plate	1	2	3	4	5	Control of serum	Control of diagnosticum
Consider	Serum №1							
	Serum №2							
Solubilization of serum		1:4	1:8	1:16	1:32	1:64	-	-

Conclusion:_____
_____

Task №4. To describe immunobiological preparations for a specific prophylaxis and treatment of whooping-cough.

Preparations	Type	Purpose of application	Immunity
For active immunization			
For passive immunization			

Signature of teacher_____

Date:_____

Practical lesson № 35

Topic: Microbiological diagnostics of wounds anaerobic infections.

Family*: Bacillaceae*
Genus: *Clostridium*
Species: *C. perfringens, C. histolyticum, C. sordeli, C. novyi, C. septicum*

Tasks for independent work:

a) The list of issues that must be studied:

1. Classification of clostridia. Ecology, properties. Resistance to environmental factors.
2. Toxigenity of clostridia.
3. Clostridium - anaerobic pathogens infection of wounds. Speciecs.
4. Biological properties of pathogens of wounds anaerobic infection. Pathogenicity factors, toxins.
5. Epidemiology, pathogenesis, main clinical manifestations of wounds anaerobic infection. Antitoxic immunity.
6. Methods of microbiological diagnosis of wounds anaerobic infections.
7. Prophylaxis and treatment of anaerobic infections of wounds.

b) The list of practical skills that are necessary to master:

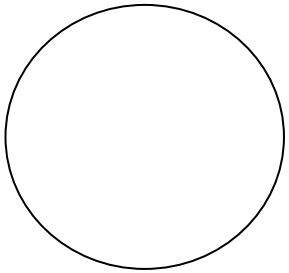
1. Make preparations for microscopic research.
2. Stain preparations by sophisticated methods (by Gram)
3. Microscope preparations in the light microscope with immersion lens.
4. Differentiation of microorganisms by morphological and tinctorial characteristics .

Practical lesson's Protocol

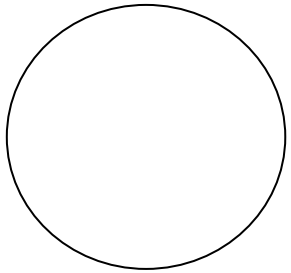
Practical tasks should be done:

Task№1. To microscope and to sketch the preparations of anaerobic infection pathogens from the wounds stained by Ojeshco, by Peshcov, by Gram.

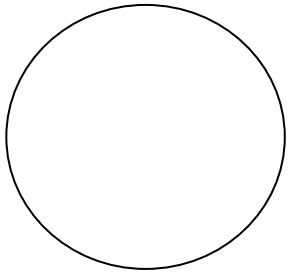
Stained by Ojeshco



Stained by Peshcov



Stained by Gram



To mark morphological and tinctorial properties of microorganisms

Task №2. To familiarize with the features of Clostridium perfringens growth on the special medias:

a) Media of Vilson – Bler_____

b) Media of Kitt-Tarozzi_____

c) Sterile fat free lacmus milk_____

Завдання № 3. To describe immunological preparations for a specific prophylaxis and treatment of anaerobis infections of wounds.

Preparations	Type	Purpose of application	Immunity
For active immunization			
For passive immunization			

Signature of teacher:_____

Date:_____

Practical lesson № 36

Topic: Microbiological diagnostics of tetanus and botulism.

Family: *Bacillaceae*
Genus: *Clostridium*
Species: *C.tetanus, C.botulinum*

Tasks for independent work:

a) The list of issues that must be studied:

1. Classification of clostridia. Ecology, properties. Resistance to environmental factors.
2. Biological properties of pathogens clostridia tetanus and botulism. Pathogenicity factors. Toxins.
3. Epidemiology, pathogenesis, main clinical manifestations of tetanus and botulism. Immunity.
4. Methods of microbiological diagnosis of wounds anaerobic infections, tetanus and botulism.
5. Prophylaxis and treatment of tetanus and botulism.

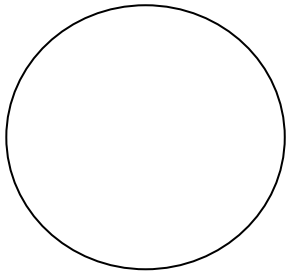
b) The list of practical skills that are necessary to master:

1. Make preparations for microscopic research.
2. Stain preparations by sophisticated methods (by Gram)
3. Microscope preparations in the light microscope with immersion lens.
4. Differentiation of microorganisms by morphological and tinctorial characteristics .

Practical lesson's Protocol

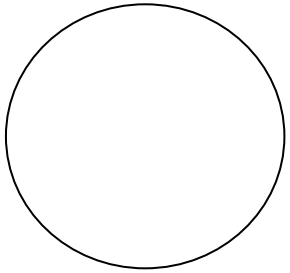
Practical tasks should be done:

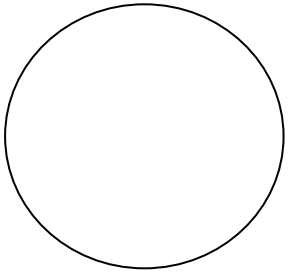
Task №1. To prepare the preparations from the culture of anaerobic bacterias grew in Kitt-Tarozzi media, to stain by Gram, to microscope and to sketch.



To mark morphological and tinctorial properties of microorganisms

Task № 2. To microscope and to sketch the preparations of tetanus and botulism clostridias stained by Gram





To mark morphological and tinctorial properties of microorganisms

Завдання № 3. To describe immunological preparations for a specific prophylaxis and treatment of tetanus and botulism.

Preparations	Type	Purpose of application	Immunity
For active immunization			
For passive immunization			

Signature of teacher:_____

Date:_____

Practical lesson № 37

Topic: Microbiological diagnostics of Syphilis.

Family: *Spirohaetaceae*
Genus: *Treponema*
Species*: T. pallidum*

Tasks for independent work:

a) The list of issues that must be studied:

1. General characteristics of spirochaetes. Classification.
2. The causative agent of syphilis. Biological properties. Treponema.
3. Epidemiology, pathogenesis and immunogenesis of syphilis.
4. Methods of microbiological diagnostics of syphilis.
5. Prophylaxis and treatment of syphilis.

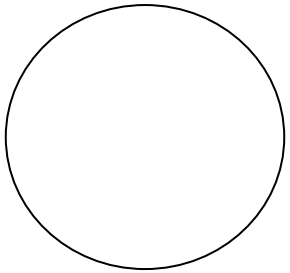
b) The list of practical skills that are necessary to master:

1. Microscope preparations in the light microscope with immersion lens.
2. Differentiation of microorganisms by morphological and tinctorial characteristics .
3. Ability to conduct consideration and evaluate the results of serological reactions (complement fixation, ELISA).

Practical lesson's Protocol

Practical tasks should be done:

Task №1. To microscope and to sketch spirochaetes in the preparation of dental raid, made by Burri.



To mark the morphological properties of spirochaetes

Task №2. To conduct and estimate the results of Wasserman reaction. To make a conclusion.

№ p\p / Ingredients	1	2	3	4
Serum of patient (inactive, 1:4, ml)	0,5	0,5	0,5	0,5
Antigen1 (specific, ml)	0,5	-	-	-
Antigen 2 (unspecific, ml)	-	0,5	-	-
Antigen 3 (unspecific, ml)	-	-	0,5	-
Complement (working dose, ml)	0,5	0,5	0,5	0,5
Solution (ml)				0,5
Haemolytic system (ml)	1,0	1,0	1,0	1,0
Consideration				

Note: Before the introduction of haemolytic system the samples are incubated at 37 º C for 45 minutes. After the introduction of haemolytic system the samples are incubated at 37 º C for 1 hour.

Conclusion: _____

_____

_____

Task №3. To conduct and estimate the results of microprecipitation reaction (MPR) with the serum of inspected and cardiolipid antigen. To make a conclusion.

Conclusion:_____

_____

_____

Task №4. To estimate the results of ELISA with serums of donors with the purpose of antibodies exposure to the antigens of pathogen of syphilis.

SANOF 1 DIAGNOSTICS PASTEUM PR 21.00

TEST NO : 50 W\L MODE : DUAL DATE : 17/10/03 *** INDICATES VALUE OUT OF RANGE
TEST NAME : SYPHO . 10 TEST FILTER : 490 nm TIME : 12:05 POS INDICATES A POSITIVE REACTION
PLATE : 0038 REF. FILTER : 620 nm OPERATOR : Neg INDICATES A NEGATIVE REACTION

QUALITY CONTROL

NCi<0,2 0,018<0,2
0,022<0,2
0,022<0,2
0,021<0,2
Valid (NC)>=3 4>=3
NCi<NCi2 0,018<0,0415
0,022<0,0415
0,022<0,0415
0,021<0,0415
Valid (NC)>=3 4>=3
PC>0,6 2,25>0,6
+ EON = (NC + 0.10) - EON = (NC + 0,10) * 0,9
= 0,121 = 0,109

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

Conclusion:_____
_____
_____
_____

Task №5. To describe immunological preparations for a specific prophylaxis and treatment of syphilis.

Preparations	Type	Purpose of application	Immunity
For active immunization			
For passive immunization			

Signature of teacher:_____

Date:_____

Practical lesson № 38

Topic: **Microbiological diagnostics of recurrent typhus and leptospirosis.**

Family: *Spirohaetaceae*
Genus: *Borrelia* Genus: *Leptospira*
Species: *B. recurrentis,B. caucasica,B.duttoni* Species: *L. interrogans*

Tasks for independent work:

a) The list of issues that must be studied:

1. Taxonomical position of spirochetes and their classification. General description of spirochetes.
2. Morphological and biological properties of recurrent typhus and leptospirosis agents.
3. Epidemiology, clinical manifestations and pathogenicity of recurrent typhus and leptospirosis.
4. Microbiological methods of diagnostics: microscopic, serological, express-diagnostics.
Immunity at recurrent typhus and leptospirosis.
5. Medical treatment and prophylaxis of recurrent typhus and leptospirosis.

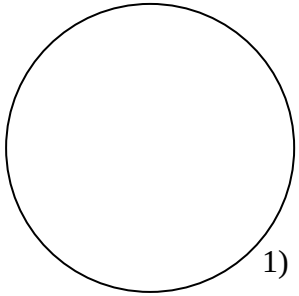
b) The list of practical skills that are necessary to master:

1. Microscope preparations in the light microscope with immersion lens.
2. Differentiation of microorganisms by morphological and tinctorial characteristics .
3. Ability to conduct consideration and evaluate the results of serological reactions (complement fixation, ELISA).

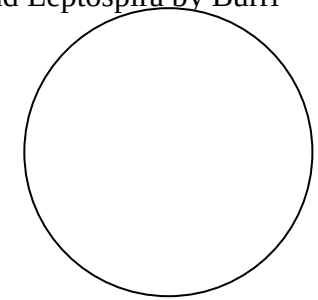
Practical lesson's Protocol

Practical tasks should be done:

Task №1. Microscope and sketch preparations of Borrelia, stained by Romanovscy-Giemza and Leptospira by Burri



1) Borrelia



2) Leptospira

To mark morphological properties of the microorganisms

Task №2. To conduct consideration and estimate the results of the complement binding reaction (CBR), with the serum of patient and leptospirosis diagnosticum. To do a conclusion.

№ test tubes	1	2	3	4	Control of serum	Control to the antigen
Solubilization of the explored serum	1:10	1:100	1:1000	1:10000		
Consideration of hemolysis						
Consideration CBR						

Conclusion: _____

_____

_____

Task №3. To describe immunobiological preparations for a specific prophylaxis and treatment of recurrent typhus and leptospirosis.

Preparations	Type	Purpose of application	Immunity
For active immunization			
For passive immunization			

Signature of teacher_____

Date:_____

Practical lesson № 39

Topic: **Microbiological diagnostics of the diseases caused by Chlamidia and Mycoplasma**

Family: *Chlamidiaceae*
Genus: *Chlamidia*
Species*: Chlamidia trachomatis,C. psittaci*

Family: *Mycoplasmaceae*
Genus: *Mycoplasma*
Species*: M. pneumoniae*

Tasks for independent work:

a) The list of issues that must be studied:

1. Taxonomical position of Chlamidia and Mycoplasma and their classification. General description of Chlamidia and Mycoplasma.
2. Morphological and biological properties of Chlamidia and Mycoplasma.
3. Epidemiology, clinical manifestation and pathogenicity of Chlamidiosis and Mycoplasmosis.
4. Immunity at Chlamidiosis and Mycoplasmosis.
5. Medical treatment and prophylaxis of Chlamidiosis and Mycoplasmosis.
6. Microbiological methods of diagnostics: microscopic, serological, biological, express-diagnostics.

b) The list of practical skills that are necessary to master:

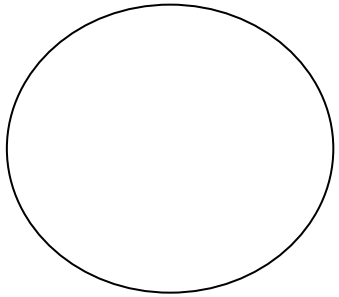
1. Microscope preparations in the light microscope with immersion lens.

2. Differentiation of microorganisms by morphological and tinctorial characteristics .
3. Ability to conduct consideration and evaluate the results of serological reactions (complement fixation, ELISA, PCR).

Practical lesson's Protocol

Practical tasks should be done:

Task №1. Microscope the material from the urethra of patient with chlamidiosis, stained by Romanovscy-Gimza.



To mark the inclusion of Chlamidia in the staggered epithelium cell

Task №2. To conduct consideration of the complement binding reaction (CBR), with the serums of patient and with Chlamidia and M.pneumoniae diagnosticums .

Solubilization of serum / **Proper diagnosticums**		1:8	1:16	1:32	1:64	1:128	Control of serum	Control of diagnosticum
Consideration of results								
	Chlamidii psittaci							
	7th day of disease							
	20th day of disease							
	Mycoplasma pneumonia							
	7th day of disease							
	20th day of disease							

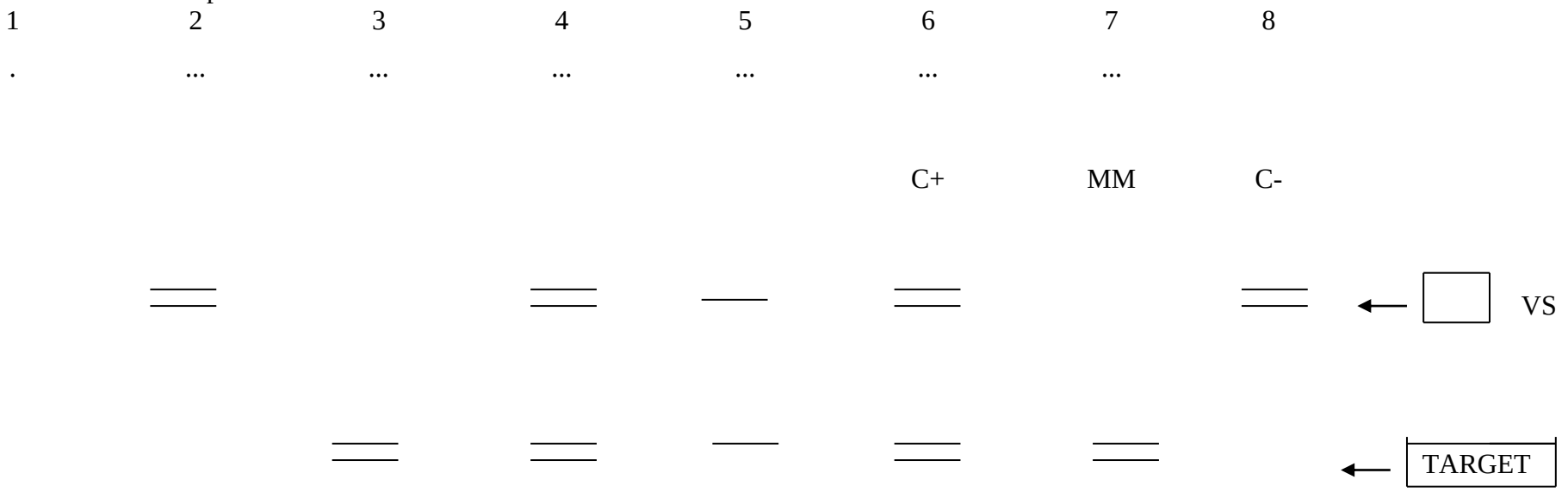
Conclusion:

_____

_____

Task №3. To conduct consideration of results of the polimerase chain reaction (PCR) for determination of presence of DNA Chlamydia trachomatis in diagnostic material from a patient with suspicion on chlamidiosis.

Results of electroforesis products:



Notes:

1. 1 – 5 – clinical standards;
2. 6 – 7 – positive control;
3. 8 – negative control;
4. ← VS - strip in a gel, that answers the size of amplicon internal standard;
5. ← TARGET is a strip in a gael, that answers the area of *Chlamidia trachomatis* DNA

Task №4. To describe immunobiological preparations for a specific prophylaxis and treatment of the diseases caused by Chlamidia and Mycopasma.

Preparations	Type	Purpose of application	Immunity
For active immunizations			
For passive immunizations			

Signature of teacher_____

Date:_____

Practical lesson № 40

Topic: Microbiological diagnostics of Rickettsiosises.

Family: *Rickettsiaceae*
Genus: *Rickettsia, Coxiella*
Species*: Coxiella burnetti, R. prowazekii, R.typhi*

Tasks for independent work:

a) The list of issues that must be studied:

1. Rickettsii. Classification. Biological properties.
2. Rickettsii are agents of the epidemic spotted fever and Brill – Zinsser desease, endemic spotted fever. Ecology of agents. Antigens structure, toxineforming.
3. Epidemiology, pathogenesis and immunity at spotted fevers.
4. Pathogenesis of Q-fever. Ecology. Antigens structure, toxineforming.
5. Epidemiology, pathogenesis, immunity of Q-fever.
6. Microbiological diagnostics of ricketsiosises.
7. Specific prophylaxis and treatment of ricketsiosises.

b) The list of practical skills that are necessary to master:

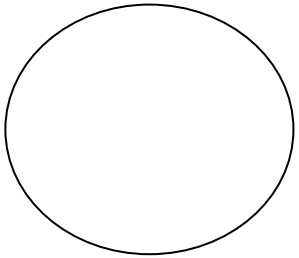
1. Make preparations for microscopic research.
2. Stain preparations by sophisticated methods (by Zdrodovscy, by Gimsa)
3. Microscope preparations in the light microscope with immersion lens.

4. Differentiation of microorganisms by morphological and tinctorial characteristic .To examine with microscope the slides with ricketsies stained and to define morphological properties, to sketch.
4.To estimate the results of the indirect hemagglutination reaction, make a conclusion.
5. Describe immunobiological specimens for a specific prophylaxis and medical treatment of rickettsiosises.

Practical lesson's Protocol

Practical tasks should be done:

Task №1. Microscope and sketch the preparation of rickettsia, stained by Zdrodovscy.



To mark morphological and tinctorial properties of microorganisms

Task №2. To conduct consideration of reaction of indirect hemagglutination (RIHA), put with the patient`s serums and *Coxiella burnetti* diagnosticum . To do a conclusion.

	№ p/p	1	2	3	4	5	Control of serum	Control of diagnosticum
Consider	Serum №1							
	Serum №2							
Solubilization of serum		1:4	1:8	1:16	1:32	1:64		

Conclusion: _____

_____

Task №3. To describe immunobiological preparations for a specific prophylaxis and treatment of rickettsiosises.

Preparations	Type	Purpose of application	Immunity
For active immunizations			
For passive immunizations			

Signature of teacher_____

Date:_____

Practical lesson № 41

Topic: **Elements of medical Mycology. Microbiological diagnostics of candidosis, aspergillosis, penicillosis.**

Genus*: Candida*
Species*: Candida albicans, C.tropicales, C.krusei, C.guillermondii, C.lusitaniae*
Genus*: Aspergillus*
Species*: Aspergillus fumigatus, A.niger, A.flavus, A.nidulans*
Genus*: Penicillium*
Species*: Penicillium crustosum, P.notatum, P.glaucum*

Tasks for independent work:

a) The list of issues that must be studied:

1. Pathogenic fungi. Classification.
2. Biological properties of pathogenic fungi, pathogenicity factors, toxins. Resistance. Sensitiveness to antibiotics.
3. Fungi of the genus Candida. Biological properties. Pathogenicity for humans. Factors that cause the occurrence of candidosis.
4. Methods of microbiological diagnostics of candidosis.
5. Pathogens aspergillosis, penicillosis, dermatomycosis. Biological properties. Pathogenicity for humans.
6. Methods of microbiological diagnosis of aspergillosis, penicillosis.

7. Prophylaxis and treatment of candidiasis, aspergillosis, penicillosis.

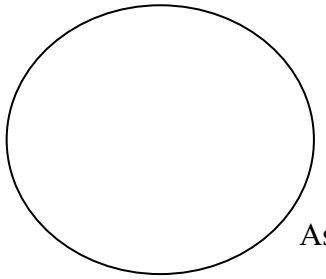
b) The list of practical skills that are necessary to master:

1. Make preparations for microscopic research.
2. Stain preparations by sophisticated methods (by Gram)
3. Microscope preparations in the light microscope with immersion lens.
4. Differentiation of microorganisms by morphological and tinctorial characteristics.

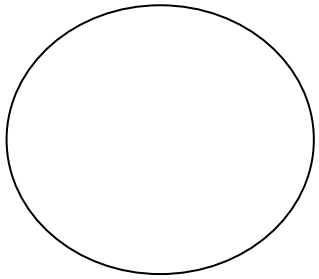
Practical lesson's Protocol

Practical tasks should be done:

Task №1. To microscope and to sketch the preparations of Aspergillus, Penicillium.



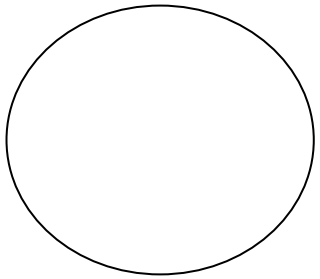
Aspergillus



Penicillium

To mark the morphological properties of Fungi

Task №2. To prepare the preparations from pathological material of patient with candidosis, to stain by Gram, to microscope and to sketch..



To mark morphological and tinctorial properties of microorganisms

Task №3. Inoculate pathological material on Sabouraud medium to obtain isolated colonies of yeasts.

Task №4. To describe immunological preparations for a specific prophylaxis and treatment of mycosis.

Preparations	Type	Purpose of application	Immunity
For active immunization			
For passive immunization			

Signature of teacher:_____

Date:_____

Practical lesson № 42

Topic: Microbiological diagnostics of dermatomycosis and system mycosises

Genus: *Microsporum, Epidermophyton, Trichophyton*.
Species*: Microsporum canis, Epidermophyton floccosum, T. schoenleinii, T. mentagrophytes, T. verrucosum*

Tasks for independent work:

a) The list of issues that must be studied:

1. Pathogenic fungi. Classification.
2. Biological properties of pathogenic fungi, pathogenicity factors, toxins. Resistance. Sensitiveness to antibiotics.
3. Fungi of the genus *Microsporum, Epidermophyton, Trichophyton*.
4. Pathogens of dermatomycosis. Biological properties. Pathogenicity for humans.
5. Methods of microbiological diagnosis of dermatomycosis.
6. Prophylaxis and treatment of dermatomycosis.
7. Pneumocystis. Pneumocystis pneumonia in AIDS patients.
8. Methods of microbiological diagnosis of systemic mycosis.

b) The list of practical skills that are necessary to master:

1. Make preparations for microscopic research.
2. Stain preparations by sophisticated methods (by Gram)
3. Microscope preparations in the light microscope with immersion lens.
4. Differentiation of microorganisms by morphological and tinctorial characteristics.

Practical lesson's Protocol

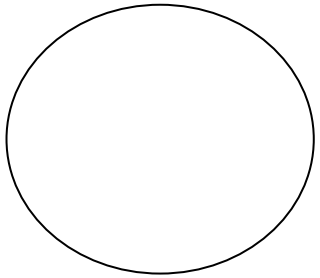
Practical tasks should be done:

Task №1. To define the macro- and microscopic properties of the isolated colonies on Sabouraud media.

Cultural properties	Sabouraud media
Size (diameter)	
Form of outlines	

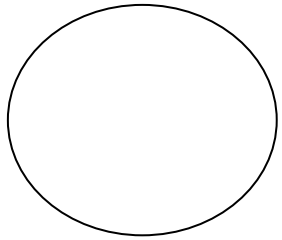
Degree of transparency	
Color of colony	
Character of surface	
Position on media	
Microscopic research	
Character of edge	
Structure	
Other properties	
Consistency	

Task №2. To prepare the preparations from colony, to stain by Gram, to microscope and to sketch.

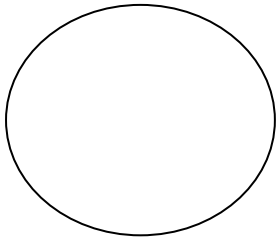


To mark morphological and tinctorial properties of microorganisms

Task №3. To microscope and to sketch the preparations of dermatophytes Microsporum and Trichophyton.



Microsporum



Trichophyton

To mark the morphological properties of the Fungi

Task №4. To describe immunological preparations for a specific prophylaxis and treatment of mycosis.

Preparations	Type	Purpose of application	Immunity
For active immunization			
For passive immunization			

Signature of teacher:_____

Date:_____

Practical lesson № 43

Topic: Final examination Modul II control

Questions for theory control

1. General characteristic of coccal bacteria group.
2. Classification. Biological properties of staphylococci. Pathogenicity factors of staphylococci.
3. The role of staphylococcus in human pathology, epidemiology and pathogenesis of infection posed by them.
4. The role of staphylococcus in the progress of hospital infections.
5. Immunity and its features in staphylococcal diseases.
6. Methods of microbiological diagnosis of staphylococcal diseases.
7. Prophylaxis and treatment of staphylococcal infections. Preparations for specific prevention and therapy.
8. Biological properties of streptococci. Classification. Serological group of streptococci that inhabit the mouth's cavity.Characteristics of factors streptococcal pathogenicity.
9. The role of streptococcus in human pathology; epidemiology and pathogenesis of disease that are caused by them.
10. Etiological and pathogenetic role of streptococci group A under conditions of erysipelas, scarlet fever and rheumatism. Scarlet fever stomatitis.
11. Inflammatory processes in the mouth caused by streptococci without group antigen.
12. Immunity and its features with streptococcal infections.
13. Methods of microbiological diagnosis of streptococcal diseases
14. Prevention and treatment of streptococcal infections
15. Biological properties of Neisseria. Classification.
16. Biological properties of meningococci, their classification. Factors of pathogenicity of meningococci.
17. Epidemiology and pathogenesis of meningococcal disease. Bacteriocarrier.
18. Immunity at meningococcal disease.
19. Methods of microbiological diagnosis of meningococcal disease and bacteriocarrier state.
20. Differentiation of meningococcal and gramnegative diplococcus of nasopharynx.
21. Prophylaxis and therapy of meningococcal infections.
22. Biological properties of gonococcus, their variability.
23. Pathogenicity for humans. Epidemiology and pathogenesis of gonorrhea. Acute and chronic gonorrhea.
24. Immunity at gonorrhea.
25. Methods of microbiological diagnosis of gonorrhea.
26. Prophylaxis and therapy of gonorrhea and gonoblenorrhea

26. Classification and general characteristics of the family Enterobacteriaceae.
27. Biological properties of the genus Escherichia. Classification.
28. Antigenic structure of pathogenicity factors of colon bacilla.
29. Epidemiology and pathogenesis of diseases caused by Escherichia coli. Immunity.
30. Role of E. coli in the etiology of purulent-inflammatory diseases.
31. Role of intestinal rod in causing hospital infections.
32. Methods of microbiological diagnostics of esherihiosis infections.
33. Prophylaxis and treatment of esherihiosis.
34. General characteristics of the genus Salmonella. Classification of the genus Salmonella bacteria by biochemical and antigenic properties of the structure (Kauffman – White table).
35. Biological properties of the causative agents of typhoid and paratyphoid A and B. Antigenic structure factors of pathogenicity.
36. Epidemiology and pathogenesis of typhoid and paratyphoid A and B. Phase of the pathogenesis.
37. Immunity at typhoid and paratyphoid A and B. The dynamics of accumulation of O-, H-, Vi-antibodies in the serum of the patient.
38. Methods for microbiological diagnosis of typhoid and paratyphoid A and B on the 1st and 2nd week of illness.
40. Pathogenesis of typhoid and paratyphoid A and B (3rd and 4th week of the disease) .
41. Methods of microbiological diagnosis of typhoid and paratyphoid A and B on the 3rd and 4th week of the disease.
42. Microbiological diagnosis of bacteria carring.
43. Salmonella are pathogens of acute enterocolitis . Features of the epidemiology, pathogenesis.
44. Salmonella are pathogens of nosocomial salmonellosis . Features of the nosocomial strains.
45. Methods for microbiological diagnosis of salmonellosis .
46. Prevention and treatment of typhoid, paratyphoid A and B and salmonellosises.
47. Biological properties of the genus Shigella. Classification.
48. Shigella virulence factors.
49. Epidemiology, pathogenesis, clinical manifestations of shigellosis.
50. Immunity at shigellosis.
51. Methods for microbiological diagnosis of shigellosis.
52. Prevention and treatment of shigellosis. The problem of specific prophylaxis. Specific therapy.
53. General characteristics of vibrios. Classification, mechanism of action.
54. Cholera vibrios (Vibrio cholerae). Biovary (classical and El Tor), their differentiation.
55. Classification of vibrios by Heyberg. Antigenic structure, biovary. Factors of cholera vibrios virulency. Cholerogen, mechanism of action.
56. The spread of cholera. Epidemiology, pathogenesis, main clinical manifestations of cholera. Immunity.

57. Methods of microbiological diagnostics of cholera.
48. Prophylaxis and treatment of cholera.
58. Ecology of anthrax pathogens.
59. Biological properties of anthrax pathogens. Classification. Resistance. Pathogenicity factors. Pathogenicity for humans and animals.
60. Epidemiology and pathogenesis. The main clinical manifestations of anthrax in humans.
61. Immunity at anthrax.
62. Biological properties of brucellosis pathogens. Virulence factors. Classification.
63. Epidemiology, pathogenesis and clinical forms of brucellosis.
64. Immunity at brucellosis.
65. Methods of microbiological diagnosis of anthrax and brucellosis.
66. Principles of prophylaxis and treatment of anthrax and brucellosis. Specific prophylaxis and treatment.
67. Biological properties of plague pathogens. Virulence factors. Classification.
68. Epidemiology, pathogenesis and clinical forms of plague.
69. Immunity under the plague.
70. Ecology of tularemia pathogen.
71. Biological properties of tularemia pathogen. Classification. Resistance. Pathogenicity factors. Pathogenicity for humans and animals.
72. Epidemiology and pathogenesis. The main clinical manifestations of tularemia in humans.
73. Immunity at tularemia.
74. Methods of microbiological diagnosis of tularemia and plague.
75. Principles of prophylaxis and treatment of tularemia and plague. Specific prophylaxis and treatment.
76. Pathogenic and saprophytic mycobacteria.
77. Biological properties of the agents of tuberculosis.
78. Variability of tuberculosis bacteria, pathogenicity factors. Tuberculin.
79. Epidemiology and pathogenesis of tuberculosis.
80. Patterns of immunity, the role of cellular mechanisms under conditions of tuberculosis.
81. Pathogens of mycobacterioses. Classification, properties. Role in human pathology. Mycobacterioses as a manifestation of HIV infection.
82. General characteristics of the genus of actinomycetes.
83. Pathogens of actinomycosis. Ecology. Resistance. Properties.
84. Epidemiology and pathogenesis of actinomycosis. Immunity.
85. Methods of microbiological diagnosis of tuberculosis and actinomycosis. Material for research.
86. Prophylaxis and treatment of tuberculosis and actinomycosis.

87. Biological properties of diphtheria agent. Classification. Biovary. Resistance.
88. Pathogenicity factors. Diphtheria toxin, the mechanism of action. Toxigenity as a result of phage conversion, molecular mechanism of action of diphtheria toxin.
89. Epidemiology and pathogenesis of diphtheria.
90. Antitoxic immunity. Bacteriocarrier.
91. Methods of microbiological diagnostics of diphtheria. Immunological and genetic methods for determining toxicity of diphtheria. Differentiation of diphtheria with other pathogenic and nonpathogenic for people corynebacterias, control of toxigenity.
92. To specific prophylaxis and treatment of diphtheria.
93. Biological properties of Bordetella, classification. Pathogenic representatives: Bordetella pertussis, Bordetella
94. parapertussis, Bordetella bronchoseptica. Epidemiology, pathogenesis and immunity at whooping-cough.
96. Microbiological diagnostics of whooping-cough.
95. Specific prevention of whooping-cough.
96. Principles of ethiothropical therapy of whooping-cough.
97. Differentiation of whooping-cough, parawhooping-cough and bronhosepticosis pathogenes.
98. Classification of clostridia. Ecology, properties. Resistance to environmental factors.
99. Toxigenity of clostridia.
100. Clostridium - anaerobic pathogens infection of wounds. Speciecs.
101. Biological properties of pathogens of wounds anaerobic infection. Pathogenicity factors, toxins.
102. Epidemiology, pathogenesis, main clinical manifestations of wounds anaerobic infection. Antitoxic
103. immunity.
104. Methods of microbiological diagnosis of wounds anaerobic infections.
105. Prophylaxis and treatment of anaerobic infections of wounds.
106. Biological properties of pathogens clostridia tetanus and botulism. Pathogenicity factors. Toxins.
107. Epidemiology, pathogenesis, main clinical manifestations of tetanus and botulism. Immunity.
108. Methods of microbiological diagnosis of wounds anaerobic infections, tetanus and botulism.
109. Prophylaxis and treatment of tetanus and botulism.
110. Taxonomical position of spirochetes and their classification. General description of spirochetes.
111. The causative agent of syphilis. Biological properties. Treponema.
112. Epidemiology, pathogenesis and immunogenesis of syphilis.
113. Methods of microbiological diagnostics of syphilis.
114. Prophylaxis and treatment of syphilis.
115. Morphological and biological properties of recurrent typhus and leptospirosis agents.

116. Epidemiology, clinical manifestations and pathogenicity of recurrent typhus and leptospirosis.
117. Microbiological methods of diagnostics: microscopic, serological, express-diagnostics.
118. Immunity at recurrent typhus and leptospirosis.
119. Medical treatment and prophylaxis of recurrent typhus and leptospirosis.
120. Taxonomical position of Chlamidia and Mycoplasma and their classification. General description of
121. Chlamidia and Mycoplasma.
122. Morphological and biological properties of Chlamidia and Mycoplasma.
123. Epidemiology, clinical manifestation and pathogenicity of Chlamidiosis and Mycoplasmosis.
124. Immunity at Chlamidiosis and Mycoplasmosis.
125. Medical treatment and prophylaxis of Chlamidiosis and Mycoplasmosis.
126. Microbiological methods of diagnostics: microscopic, serological, biological, express-diagnostics.
127. Rickettsii. Classification. Biological properties.
128. Rickettsii are agents of the epidemic spotted fever and Brill – Zinsser desease, endemic spotted fever. Ecology of agents. Antigens structure, toxineforming.
129. Epidemiology, pathogenesis and immunity at spotted fevers.
130. Pathogenesis of Q-fever. Ecology. Antigens structure, toxineforming.
131. Epidemiology, pathogenesis, immunity of Q-fever.
132. Microbiological diagnostics of ricketsiosises.
133. Specific prophylaxis and treatment of ricketsiosises.
134. Pathogenic fungi. Classification.
135. Biological properties of pathogenic fungi, pathogenicity factors, toxins. Resistance. Sensitiveness to antibiotics.
136. Fungi of the genus Candida. Biological properties. Pathogenicity for humans. Factors that cause the occurrence of candidosis.
137. Methods of microbiological diagnostics of candidosis.
138. Pathogens aspergillosis, penicillosis, dermatomycosis. Biological properties. Pathogenicity for humans.
139. Methods of microbiological diagnosis of aspergillosis, penicillosis.
140. Prophylaxis and treatment of candidiasis, aspergillosis, penicillosis.
141. Fungi of the genus *Microsporum*, *Epidermophyton*, *Trichophyton*.
142. Pathogens of dermatomycosis. Biological properties. Pathogenicity for humans.
143. Methods of microbiological diagnosis of dermatomycosis.
144. Prophylaxis and treatment of dermatomycosis.
145. Pneumocystis. Pneumocystis pneumonia in AIDS patients.
146. Methods of microbiological diagnosis of systemic mycosis.

Question for practical skills examination

1. Microscope preparation, to define morphology and tinctorial properties of bacteria.
2. To prepare preparation from the culture of bacteria, to stain it by Gram. Microscope preparation, to define morphology and tinctorial properties of bacteria.
3. To prepare preparation from the culture of bacteria, to stain it by a simple method, to microscope it, to define morphology.
4. Composition and mechanism of action of Endo media. Application.
5. Composition and mechanism of action of Levin media. Application.
6. Composition and mechanism of action of Ploscirev media. Application.
7. Media Roux and Loeffler. Composition. Practical application.
8. Citt-Tarocci media, composition. Application.
9. To do consideration of biochemical properties of the isolated bacteria pure culture, to define the genus.
10. To define the sensitiveness of Staphylococcus culture to the antibiotics by the diagnostic disks method. To do conclusion.
11. To define the sensitiveness of Staphylococcus culture to penicillin by the serial solubilisation method. To do conclusion.
12. To make the reaction of precipitation by Ascoli. To do conclusion.
13. To apply the Vidal reaction (with the patient sera and typhoid O-diagnosticum). To do conclusion.
14. To apply the slide agglutination test with an unknown culture and typhoid diagnostic sera. To do conclusion.
15. To apply CBT with the patient sera and gonococcal diagnosticum, to do conclusion.
16. To define a bacteriophage title.
17. To apply HAIR. To do a conclusion.
18. To apply ELISA. To do a conclusion.

Date: _____

Practical lesson №44

Topic: Methods of cultivation, indication and identification of viruses.

Tasks for independent work:

a) The list of issues that must be studied:

1. General characteristics of viruses. Classification.
2. Reproduction of viruses during their interaction with cells. The main stages of the interaction of viruses with cells for productive infection.
4. Integrative and abortive types of viruses interact with host cells. Persistence of the virus in cells. Interference ща virusб defective interfering particles. Viruses satellites.
5. Methods of culturing viruses in cell cultures in chicken embryos, in the body of laboratory animals. Classification of cell cultures used in virology, their characteristics.
6. Methods of detection (indication) of viral reproduction by cytopathogenic action , reactions of hemagglutination (RHA) hemadsorbtion (RHAds), viral inclusions.
7. Identification of viruses by the antigenic properties (HAR, RHHA, RHHAds, CBT, IF, RIA, ELISA).
8. Genetic methods for determining the viruses and their nucleic acid components.

b) The list of practical skills that are necessary to master:

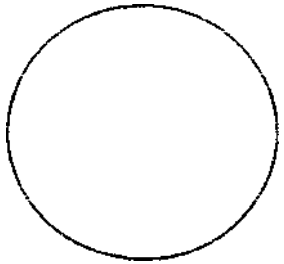
1. Microscope preparations in the light microscope with immersion lens..
2. Ability to identify the virus in chicken embryos for hemagglutination reaction in cell culture by cytopathic action
3. Ability to set, conduct consideration and evaluate the results of serological tests used in virology (hemagglutination reaction).

Practical lesson's Protocol

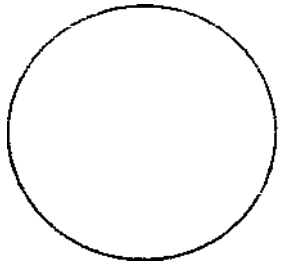
Practical tasks should be done:

Task № 1. To sketch the structure of chicken embryo. Mark the ways of its infection.

Task № 2. To identify in the single-layer cell culture the action of viruses



Intact cell culture



Infected cell culture

Task № 3. To conduct consideration and estimate the results of hemagglutination reaction (HAR) for virus presence determination in a chicken embryo. To make a conclusion.

Solubilization / Ingredients	1:10	1:20	1:40	1:80	1:160	1:320	Control of red corpuscles
Alantois liquid (ml)	0,1	0,5	0,5	0,5	0,5		-
Ph.solution (ml)	0,5	0,5	0,5	0,5	0,5	0,5	-
1% red corpuscles (ml)	0,5	0,5	0,5	0,5	0,5	0,5	0,5
Incubation 30 minutes at a room temperature							
Consideration							

Conclusion:

_____
_____
_____
_____

Signature of teacher _____

Date: _____

Practical lesson №45

Topic: Bacteriophages.

Tasks for independent work:

a) The list of issues that must be studied:

4. General characteristics of viruses. Classification.
5. Reproduction of viruses during their interaction with cells. The main stages of the interaction of viruses with bacterial cells for productive infection.
6. Morphology, structure and chemical composition of bacteriophages.

7. Virulent and moderate bacteriophages. Stages of productive interaction of bbacteriophages type of bacterial cells.
8. Lizogenecity and Phage conversion.
9. The specificity of bacteriophages.
10. Practical use of bacteriophages in microbiology and medicine to identify bacteria.
11. Prophylaxis and treatment of infectious diseases, assessment of microbial contamination.

b) The list of practical skills that are necessary to master:

1. Titrate phages
2. Read the phagotype bacteria.

Practical lesson's Protocol

Practical tasks should be done:

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

Task № 2. Write down the essence of each of these types of interaction of phages with bacteria

1. Productive interaction type : _____
_____
_____
2. Integrative type of interaction : _____
_____
_____

3. Abortive type of interaction :

_____

_____

_____

Task № 3. Mark table possible types of interaction with these phage sensitive bacteria.

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

Task № 4. Conduct accounting results of phage identification of culture isolated from a patient with suspected typhoid.

Specific diagnostic phage	Typhoid Bacteriophage			paratyphoid A bacteriophage		paratyphoid B bacteriophage	
Haemoculture	Control cultures	Examined culture	Bacteriophage Control	Examined culture	Bacteriophage Control	Examined culture	Bacteriophage Control

Task № 5. To conduct the consideration of titration of the results of intestinal bacteriophage in water of open reservoirs by the Appelman's method.

№ p/p / Ingredients (ml)	1	2	3	4	5	6	7	8	9	10	11 Control of phage	12 Control of culture
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
Solubilization	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	10^{-1}	–
Consideration												

0,5ml

" + " – presence of lysis "-" – absence of lysis.

Conclusion:_____

_____

Task № 6: To conduct the results of phagetyping of clean culture of staphylococus. The results were got bring to table.

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

" + " – presence of lysis "-" – absence of lysis.

Conclusion: _____

Signature of teacher _____

Date:_____

Practical lesson №46

Topic: Laboratory diagnostics of Orthomyxoviral, Paramixoviral and Rhabdoviral infections.

Family: *Orthomyxoviridae*
Genus: *Influenzavirus A, B*
Members: *Influenza viruses*
Family: *Paramyxovirus*
Genus: *Respirovirus, Rubulavirus, Pneumovirus*
Members: *Parainfluenza viruses, measles, mumps, respiratore syncytial flu*
Family: *Rhabdoviridae*
Genus:*Lyssavirus, Vesiculovirus*
Members: *Rabies virus, Vesicular stomatitis virus*

Tasks for independent work:

a) The list of issues that must be studied:

1. General characteristics and classification ortomyxsovirus.
2. Human influenza virus. Structure of the virion. Features of the genome. Cultivation. Sensitiveness to physical and chemical factors.
3. Characteristics of antigens of human influenza virus. Hemagglutinin, neuraminidase, functional activity. Classification of human influenza virus. Types of antigenic variability and its mechanisms.
4. Epidemiology and pathogenesis of influenza. The role of virus persistence in humans and animals in the preservation of important epidemic strains. Immunity.
5. Methods of laboratory diagnostics of influenza.
6. Specific prophylaxis and treatment of influenza.
7. General characteristics and classification of paramyxovirus and rhabdovirus.
8. Paramyxovirus (parainfluenza viruses, measles, mumps, respiratory syncytial flu) and rhabdovirus (rabies virus). Structure of virions. Antigens.
9. Epidemiology and pathogenesis at paramyxovirus and rhabdovirus infections.
10. Immunity under the paramxovirus infections. Persistence paramyxovirus..
11. Methods of laboratory diagnostics and paramyxovirus and rhabdovirus infections.
12. Specific prophylaxis and treatment of paramyxovirus and rhabdovirus infections.

b) The list of practical skills that are necessary to master:

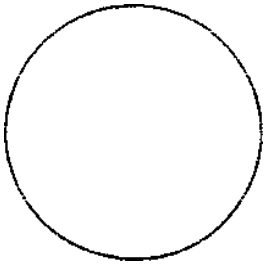
1 Microscope preparations in the light microscope with immersion lens..

2. Ability to identify the virus in chicken embryos for hemagglutination reaction in cell culture by cytopathogen action
3. Set, conduct consideration and evaluate the results of serological tests used in virology (hemagglutination inhibition reaction).

Practical lesson's Protocol

Practical tasks should be done:

Task № 1. To Microscope and to sketch the influenza virus inclusion in infected cell culture of fibroblasts, stained by the Romanovsky –Giemza



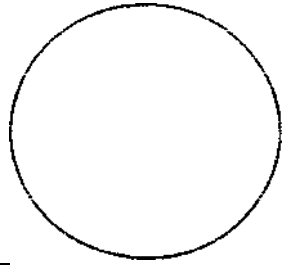
To mark the inclusion

Task № 2. To conduct consideration and estimate the results of the hemagglutination inhibition reaction, with the pair examined serums and standard parotitis diagnosticum. To make a conclusion.

№ test tubes / Ingredients		1	2	3	4	5	6	7	Контроль сироватки	Контроль еритроцитів
Solubilization of patient serum (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	-
Standard parotitis diagnosticum (ml)		0,25	0,25	0,25	0,25	0,25	0,25	0,25	-	-
Ph.solution (ml)		-		-			—	—	0,25	0,5
Incubation 30 minutes at a room temperature										
1% red corpuscles (мл)		0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5
Incubation 30 minutes at a room temperature										
Consi	Serum № 1									
deratio	Serum № 2									

Conclusion: _____
_____

Task № 3. To microscope and to sketch inclusion (Babes-Negri cells) in cells of Amon horn under rabies, stained by Turevych.



To mark the inclusion _____

Task № 4. To describe immunobiological preparations for a specific prophylaxis and treatment of Orthomyxoviral, Paramyxoviral and Rhabdoviral infections.

Preparations	Type	Purpose of application	Immunity
For active immunization			
For passive immunization			

Signature of teacher_____

Date:_____

Practical lesson №47

Topic: Laboratory diagnostics of HIV - infection.

Family**:** *Retroviridae*

Genus: *Lentivirus*

Members: *HIV-1, HIV-2*

Tasks for independent work:

a) The list of issues that must be studied:

1. General characteristics of retroviruses. Classification. Representatives.
2. Human immunodeficiency virus (HIV). The structure and chemical composition.
3. Features of the HIV genome. Variability and its mechanisms. Types of HIV. Origin and Evolution.
4. Cultivation, stage of HIV interaction with sensitive cells.
5. The sensitiveness of HIV to the physical and chemical factors.
6. Epidemiology and pathogenesis of HIV infection. Target cells in humans, characteristics of surface receptors.
7. Mechanisms of HIV and AIDS - associated infections.
8. Methods of laboratory diagnostics of HIV infection. PCR in the diagnosis of HIV infection and vesternblot (immunoblott) - test.
9. Treatment (causal, immunomodulating) of HIV. Prospects for a specific HIV prevention.

b) The list of practical skills that are necessary to master:

1. Ability to conduct consideration and evaluate the results of serological tests used in virology (ELISA).
2. PCR result estimation.

Practical lesson's Protocol

Practical tasks should be done:

Task № 1. To sketch the scheme of structure of human immunodeficiency virus.

Task № 2. To estimate the results of ELISA with the examined serums to detect antibodies to HIV antigens (anti gr 120). To make a conclusion.

	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
ɲeg INDICATES A NEGATIVE REACTION
??? INDICATES EQUAL TO OR BETWEEN LIMITS
31. INDICATES VALUE OUT OF RANGE
# INDICATES COMBINED DATA

Conclusion:_____
_____
_____

Task № 3**.** To estimate the results of chain polymerase reaction (CPR). To make a conclusion:_____
_____
_____

Task № 4. To describe immunobiological preparations for a specific prophylaxis and treatment of HIV – infection.

Preparations	Type	Purpose of application	Immunity
For active immunization			
For passive immunization			

Signature of teacher:_____

Date: _____

Practical lesson №48

Topic: Laboratory diagnostics of Enteroviral, Flaviviral and Coronaviral infections.

Family: *Picornaviridae*
Genus: *Enterovirus*
Members*: polio, Coxsackie, ECHO*
Genus: *Aphthovirus*
Family*: Coronaviridae*
Genus: *Coronavirus*

Tasks for independent work:

a) The list of issues that must be studied:

1. General characteristics and classification of family picornavirus. The division of families.
2. General characteristics of enterovirusus. Classification: poliomyelitis, Coxsackie, ECHO.
3. The role of enteroviruses in human pathology. Epidemiology, pathogenesis of poliomyelitis and other enteroviral infections. Immunity.
4. esion of oral mucosa with angina caused by Coxsackie virus group A.
5. Methods of laboratory diagnostics of enteroviral infections.
6. Specific prophylaxis and treatment of enteroviral infections.
7. Overview of flaviviruses (tick-borne encephalitis virus , Japanese encephalitis, Omsk hemorrhagic fever, yellow fever , dengue fever). Classification . Antigens . Cultivation . Sensitivity to physical and chemical factors.
8. Main pathogenic to humans flaviviral representatives - virus encephalitis, Japanese encephalitis, Omsk hemorrhagic fever, yellow fever , dengue fever . Features of pathogenesis. Epidemiology and pathogenesis of encephalitis. Immunity.
9. Laboratory diagnostics of flaviviral infections.Specific prophylaxis and treatment.
10. General characteristics of coronaviruses. Role in human pathology. Laboratory diagnostics.

b) The list of practical skills that are necessary to master:

1. Microscope preparations in the light microscope with immersion lens
2. Ability to conduct consideration and evaluate the results of serological tests used in virology (hemagglutination inhibition test, neutralization reaction).

Practical lesson's Protocol

Practical tasks should be done:

Task № 1. To conduct consideration and estimate the results of neutralization reaction (NR) – the coloured test with examined serums and diagnosticum of poliomyelitis virus antigens of 1 type. To make a conclusion.

№ test tubes / Ingredients		1	2	3	4	5	6	7	Control	
									virus	sera
Solubilization of serum (ml) Quantity of serum (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
Nourishing media (ml)		-	-	-	-	-	-	-	0,25	0,25
Virus of the 1st type 100 CPA 50 (ml)		0.25	0,25	0,25	0,25	0,25	0,25	0,25	0,25	-
Cell culture 300000 – 4000000 (ml)		0,25	0,25	0 , 2 5	0,25	0,25	0,25	0,25	0,25	0,25
Incubation at the temperature 37°C during 4-7 days										
Consider	Serum № 1									
	Serum №2									

Note: (+) - presence of cell culture (color of media is yellow);
(-) - absence of culture (color is raspberry).

Conclusion:_____
_____
_____

Task № 2. To conduct consideration and estimate the results of hemagglutination inhibition test (HAI), with examined serums and diagnosticum - antigens of respiratory coronaviruses. To make a conclusion.

№ test tubes / Ingredients		1	2	3	4	5	Контроль сироватки	Контроль діагностикуму
Solubilization of serum		1 :1 0	1:20	1:40	1:80	1:160	1:10	
Diagnosticum (“+”) - bringing		+	+	+	+	+	-	+
Incubation at a room temperature during 1 hour								
1% red corpuscles (“+”)		+	+	+	+	+	+	+
Incubation 45 minutes at a room temperature								
Consid eration	Serum № 1							
	Serum № 2							

Conclusion:_____
_____
_____
_____
_____

Task № 3. To describe immunobiological preparations for a specific prophylaxis and treatment of enteroviral and flaviviral infections.

Preparations	Type	Purpose of application	Immunity
For active immunization			
For passive immunization			

Signature of teacher:_____

Date: _____

Practical lesson № 49

Topic: Laboratory diagnostics of hepatitis A, B, C, D, E.

Family: *Picornaviridae*
Genus: *Hepatovirus*
Members: *HAV*
Family: *Hepadnaviridae*
Genus: *Orthohepadnavirus*
Members: *HBV*

Family: *Flaviviridae*
Genus: *Hepacivirus*
Members: *HCV*

Genus: *Deltavirus*
Members*: HDV*
Genus: *Hepevirus*
Members: *HEV*

Tasks for independent work:

a) The list of issues that must be studied:

1. Hepatitis B virus . The structure of the virion. Sensitiveness to physical and chemical factors.
2. Antigens: NVs - surface antigen particles of Dane. Internal antigens: SiS, NVe, their characteristics.
3. Epidemiology and pathogenesis of hepatitis B. Persistence. Immunity.
4. Laboratory diagnostics of hepatitis B. Methods of detection and diagnostic value of markers of hepatitis B (antigens, antibodies, nucleic acids).
5. Specific prophylaxis and treatment of hepatitis B.
6. The virus of hepatitis A. The structure of the virion. Sensitiveness to physical and chemical factors.
7. Epidemiology and pathogenesis of hepatitis A. Immunity. Approaches to the specific prophylaxis.
8. Other causative agents of hepatitis (C, D, E, F, G), their taxonomic position, properties.
9. The role of viruses, hepatitis C, D, E, F, G in human pathology.
10. Methods of laboratory diagnostics of hepatitis caused by virus A, C, D, E, F, G.

b) The list of practical skills that are necessary to master:

1 .Ability to conduct consideration and evaluate the results of serological tests used in virology (ELISA).

Practical lesson's Protocol

Practical tasks should be done:

Task № 1. To sketch the chart of hepatitis B structure. To mark its antigen.

Task № 2. To do the analysis of different combinations of hepatitis B serological markers, detected during the research of examined serum number 1 and 2. The results of reseach and their analysis bring to table (for the analysis or the results were got).

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

Task № 3. To estimate the results of the ELISA with the serums of patient з to identify Ig M to antigens of the virus of hepatitis A.

The principle of this test. First antibody class M immunoglobulin sorb on the walls, then added examined serum of the patient. If there is an IgM class antibodies, they bind anti-M antibody, then added a specific viral antigen (hepatitis virus A), which is produced by growing cells in culture. The system is washed out, and it added antiviral antibody labeled with peroxidase. When was the interaction of all four components of the system, there is a "sandwich": 1) antiimunoglobulin M, 2) immunoglobulin M (against Hepatitis A - in the studied patient serum) and 3) viral antigen, 4) anti-virus antibodies labeled enzyme.

	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 № 4. To give comparative description of hepatitis that are caused by the viruses of hepatitis A, B, C, D, E.

№	Viral hepatitis agents				
	Virus of hepatitis A	Virus of hepatitis B	Virus of hepatitis D	Virus of hepatitis C	Virus of hepatitis E
1. Morphology					
2. Genome					
3. Source of infection					
4. Ways of transmission					
5. Receptive microorganizm					
6. Entrance gates					
7. Pathogenesis					
8. Material for research					
9. Laboratory diagnostics					

Appendix

Analysis of different combinatios of serologic markers during VHB (F.Deynhard, I.D.Gast, 1982)

HBsAg	HBeAg	Анти-HBc	Анти-HBe	Анти-HBs	Analysis of the results	Infectiousness of blood
+	-	-	-	-	Acute stage or chronic transmitter	++
+	+	-	-	-	Incubation period and early acute stage	++
+	+	+	-	-	Acute chronic hepatitis or chronic transmitter	++
+	-	+	+	-	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 identifying the anti-HBs, early stage of convalescence or chronic infection	+ -

Note: 1. All persons, who have HbsAg, are HBV infected.

2. All persons, who have anti-Hbs, immune to hepatitis B.

Task № 5. To describe immunological preparations for a specific prophylaxis and treatment of viral hepatitis.

Preparations	Type	Purpose of application	Immunity
For active immunization			
For passive immunization			

Signature of teacher:_____

Date: _____

Practical lesson № 50

Topic: Laboratory diagnostics of diseases caused by DNA- viruses.

Family: *Poxviridae*
Genus: *Orthopoxvirus*
Members: *Poxviruses*

Family: *Adenoviridae*
Genus: *Mastoadenovirus*

Family: Herpesviridae
Members: *Herpes simplex virus 1*
Herpes simplex virus 2
Variocella-zoster virus
Epstein-Barr virus

Tasks for independent work:

a) The list of issues that must be studied:

1. General characteristics and classification of families of DNA-containing viruses (poxviruses, herpesviruses, adenoviruses).
2. Structure of virions of poxviruses, herpesviruses, adenoviruses. Antigens, their localization and specificity.
3. Cultivation of DNA-containing viruses. Sensitiveness to physical and chemical factors.
4. Epidemiology and pathogenesis of diseases caused pox-, herpes-and adenoviruses. Immunity.
5. Persistence of herpes viruses and adenoviruses.
6. Methods of laboratory diagnostics of diseases caused by pox-, herpes-and adenoviruses.
7. Specific prophylaxis and treatment of diseases caused by DNA-containing viruses.

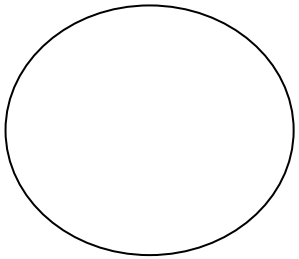
b) The list of practical skills that are necessary to master:

1. Microscope preparations in the light microscope with immersion lens
2. Ability to conduct consideration and evaluate the results of serological tests used in virology (reaction of complement fixation).
3. Reading and evaluation forms with the results of virological researches.

Practical lesson's Protocol

Practical tasks should be done:

Task № 1. To microscope and sketch the preparation of cell culture, infected by the herpes virus with cytopathic action (CPD), stained by Romanovskiy-Giemza.



To mark CPD

Task № 2. To specify methods for rapid diagnosis of simple herpes:

_____.

_____.

Task № 3. To conduct consideration and estimate the results of CBR with the examined patients sera and diagnosticum with standard specific adenoviruses antigens. To make a conclusion.

№ test tubes / Ingredients		1	2	3	4	5	Control of serum	Control to the antigen
Solubilization of serum (ml) Quantity of serum (ml)		1: 16 0,25	1:32 0,25	1: 64 0,25	1:128 0,25	1: 256 0,25	0,25	0,25
Diagnosticum ("+") - bringing		+ 0,5	+ 0,5	+ 0,5	+ 0,5	+ 0,5	+ 0,5	-
Complement ("+" – bringing)		+ 0,5	+ 0,5	+ 0,5	+ 0,5	+ 0,5	+ 0,5	+ 0,5
Ph.solution (ml) ("+" – bringing)		-	-	-	-	-	0,5	0,5
Incubation at the temperature of 4°C during 30 minutes								
Hemolytic (ml) ("+" – bringing)		+ 1,0	+ 1,0	+ 1,0	+ 1,0	+ 1,0	+ 1,0	+ 1,0
Incubation at the temperature of 37°C during 18-20 hours								
Consideration	Serum №1							
	Serum №2							

Conclusion:

_____

_____

Task № 4. To describe immunological preparations for a specific prophylaxis and treatment of DNA – viral infections.

Preparations	Type	Purpose of application	Immunity
For active immunization			
For passive immunization			

Signature of teacher_____

Date: _____

Practical lesson № 51

Topic: Sanitary-microbiological research of water, air, soil and food products

Tasks for independent work:

a) The list of issues that must be studied:

1. Values of sanitary microbiology for a doctor. Objectives and methods of microbiology.
2. Direct methods for determination of pathogenic microorganisms in the environment and indirect methods of sanitary - microbiological study. Microbial count.
3. Sanitary indicative microorganisms (SIM) of soil, water and air. The main groups of SIM: group A (indicators of fecal contamination), group B (oral contamination indicator) and group C (self-cleaning process indicators). Terms and conditions of survival of pathogenic bacteria in the

environment.
4. Methods of sanitary- bacteriological analysis of water. Determination of microbial number. Determination of the number of bacteria - indicators of fecal pollution: the coli-index and coli-titer (using membrane filters and fermentation).
5. Methods of sanitary- microbiological study of the soil. Factors that affect the qualitative and quantitative composition of soil microbes. Microbial count , coli-titer, perfrinhens-titer of soil.
6. Methods of sanitary- bacteriological study of air (sedimentation and aspiration). Assessment of health status for the overall indoor microbial contamination, the presence of SIM (staphylococci, α and β - hemolytic streptococci), which are indicators of contamination of air by microflora of human nasopharynx.
7. The role of alimentary way in the transmission of infectious agents. General principles of sanitary- bacteriological examination of food products.
8. Sanitary microbiology of milk, milk products and products from cream (total microbial count, coli-titre, the presence of pathogenic Staphylococcus aureus).
9. Sanitary and bacteriological examination of meat and sausages, canned jar, fish, beverages.
10. Sanitary and bacteriological examination of the food business, children hospitals, identifying of pathogenic microorganisms carriers.
11. Sanitary and microbiological research of bandaging and surgical material for sterility.

b) The list of practical skills that are necessary to master:
1. Sampling of water, food and air for sanitary- bacteriological studies.
2. Research swabs from hands, surfaces, utensils for sanitary- bacteriological evaluation.
3. The ability to identify and assess coli-index and coli-titer of water.
4. The ability to identify and assess the microbial number of water, soil and air.
5. Making preparations for microscopic examination of pathological material.
6. Staining of agents by complex methods.
7. Microscope preparations in the light microscope with immersion lens.
8. Differentiation of microorganisms by morphological and tinctorial characteristics.

Practical lesson's Protocol
Practical tasks should be done:

Task №1. To define drinking-water microbe number.

Conclusion:_____
_____
_____

Task №2. To define drinking-water coli-index and coli-titer. To do a conclusion.

The number of positive results from the analysis of water			ECGB- index (Coli-index)	Coli-titer
three bottles of 100 cm^2	three tubes of cm^2	three tubes of1 cm^2		
0	0	0	< 3	< 333
0	0	1	3	333
0	1	0	3	333
1	0	0	4	250
1	0	1	7	143
1	1	0	7	143
1	1	1	11	91
1	2	0	11	91
2	0	0	9	111
2	0	1	14	72
2	1	0	15	67
2	1	1	20	50
2	2	0	21	48
2	2	1	28	36
3	0	0	23	43
3	0	1	39	26
3	0	2	64	16

Conclusion:_____
_____
_____

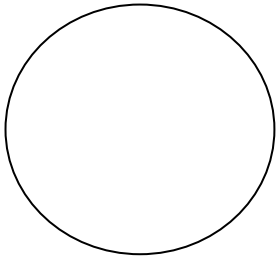
Task №3. To define drinking-water coli-index and coli-titer by the membrane filters method. To do a conclusion.
Conclusion:_____
_____
_____

Task №4. To define the soil microbe number.

Conclusion:_____
_____
_____

Task №5. To define the common microbe number of classroom air by sedimentation method.
Conclusion:_____
_____
_____

Task №6. Microscope the preparation made from yogurt. Stain by Gram



_____
To mark morphological and tinctorial properties of the microorganisms

Conclusion:_____
_____
_____

Signature of teacher_____

Date: _____

Practical lesson №52

Topic: Human normal microflora

Tasks for independent work:

a) The list of issues that must be studied:

1. Normal microflora of human body (eumicrobiocenosis). Autochtonic and allochtonic microflora in human body.
2. Microflora of skin, respiratory tracts, digestive, urinary and reproductive systems, its anti-infectious, detoxifying, immunisation and metabolic role
3. Study methods of human body normal microflora role. Gnotobiology, value of gnotobiological principles in clinic.
4. Factors which affect quantitative and qualitative composition of microflora of human body. Notion about colonization resistance and its role in infectious pathology.
5. Notion about disbacteriosis. Methods for determination.
6. Eubiotics and probiotic – preparations for renewal normal microflora of human body (bifidumbacterin, lactobacterin, colibacterin, bificol, aerococcobacterin, bioscorin, bactisubtilin and other). Action mechanism.
7. Dynamics of normal microflora formationin in ontogenesis.
8. Pathogenic role of normal microflora and pathogenic mechanisms of their acquisition properties.

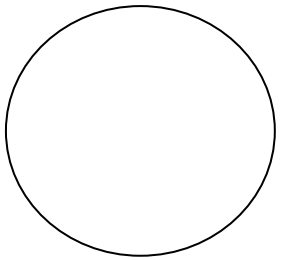
b) The list of practical skills that are necessary to master:

1. Microscope preparations in the light microscope with immersion lens.
2. Differentiation of microorganisms by morphological and tinctorial characteristics.
3. Test material inoculation by loop and pipette to solid, semi-solid and liquid culture media.

Practical lesson's Protocol

Practical tasks should be done:

Task №1. Microscope and sketch preparation of healthy human feces. Stain by Gram



To mark morphological and tinctorial properties of the microorganisms

Task №2. To describe results of patient feces bacteriological research. To do a conclusion

Result of feces bacteriological research

From «____» _____20__year
Analysis № _____
The last name, name _____
Age of patient_____
Analysis primary_____
Repeated _____
Establishment _____

№ p\p	Microflora	Norm	At a patient
1.	Common quantity of E.coli	10^6 - 4 x 10^8	
2.	E.coli with the changed enzime properties	$<10^6$	
3.	Lactosenegative E.coli	$<10^6$	
4.	Types of microorganisms, that form hemolysis	$<10^6$	
5.	Lactobacteries	$>10^6$	
6.	Bifidobacteries	$>10^7$	
7.	OM (rod and cocci of form)	$10^3 – 10^6$	
8.	Staphylococci (hemolytic, plazmocoagulative)	$<10^4$	
9.	Staphylococci (non hemolitic, epidermal)	$<10^4$ - 10^5	
10.	Candida	$<10^4$	
11.	Streptococci	$<10^5 – 10^7$	
Date of delivery _____		Doctor_____	

Conclusion:_____
_____
_____

Task №3. To inoculate the nose mucus on yolk-salt agar (YSA).

Addition to the task №2

Classification of intestinal disbacteriosis.

1th degree: latent phase of disbacteriosis. An anaerobic flora is prevails. Bifido- and lactobacteries are isolated in 10^8-$10^{7\cdot}$ One of these forms may be in solubilization 10^{10}-10^9. E.coli is present in 80% from a common quantity. The initial phase of disbacteriosis arises up as a reaction of organism practically healthy child on influencing of some unfavorable factors, in particular quality of feed. Disfunction of intestine is absent.

2th degree: starting phase of disbacteriosis. There is oppression of anaerobic bacteria, the sum of them approximately equals the quantity of aerobes. Conditional-pathogenic microbes (Staphylococci, Candida) are isolated in solubilization 10^6-10^7. Valuable E.coli are replaced by their atypical variants (lactosenegative, hemolytic).

3th degree: phase of aerobic flora aggression. Aerobic flora up to complete, absence of bifido- and lactobacteries. Especially often there are hemolytic staphylococci, hemolytic E.coli, Proteus, Klebsiella, Clostridies, Candida. A common feature of all these bacteria have multiple resistance to antibiotics.

4th degree: phase of associated disbacteriosis. It is noted the almost complete absence of bifidobacteria in the background of the number of lactic acid bacteria decrease and much aggressiveness of opportunistic microorganisms.

Depending on prevailing of opportunistic microbes staphylococcal, proteus, candidial, clostridial associated dysbiosis are shared.

Signature of teacher_____

Date: _____

Practical lesson №53

Topic: Clinical microbiology. Microbiological research of respiratory organs, blood and CNS

Tasks for independent work:

a) The list of issues that must be studied:

1. Value of Clinical Microbiology for the doctor.
2. Objects of research. Pathogenic and opportunistic microorganisms. Pathogenicity. Heterogenecity and variability of microbial populations.
3. Opportunistic infection. Conditions, features: multiple organ tropism, polyetiologic, specificity of clinical manifestations, tendency to generalization.
4. Distribution of opportunistic infections. Exogenous opportunistic infections (legionellosis, pseudotuberculosis, listeriosis, serraciosis).
5. Endogenous opportunistic infections, the role of representatives of the resident microflora in their occurrence. Anaerobic nonclostridial bacteria: bacteroides, fuzobacteries and anaerobic cocci .
6. Microbiological diagnosis of opportunistic infections. Criteria for etiologic role of opportunistic bacteria isolated from pathological focus.
7. Microbiological study of the respiratory system.
8. Microbiological examination of blood .
9. Microbiological study of the central nervous system .

b) The list of practical skills, which need to master :

1. Making preparations for microscopic examination of pathological material.
2. Staining of agents by complex methods (Gram).
3. Microscopy with the light microscope with immersion lens.
4. Differentiation of microorganisms by morphological and tinctorial characteristics.

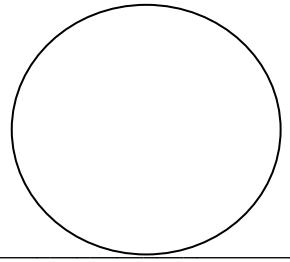
Practical lesson's Protocol

Practical tasks should be done:

Task №1. To conduct macro- and microscopic study of the isolated colonies on yolk-salt agar (YSA).

Cultural properties	Yolk-salt agar (YSA)
Size (diameter)	
Form	
Degree of transparency	
Color of colony	
Character of surface	
Position on media	
Character of margines	
Structure	
Consistency	

Task №2. To prepare slide from the colony, to stain by Gram, microscope and sketch.



To mark morphological and tinctorial properties of the microorganisms

Conclusion:_____

_____

Task №3. Microscopie microorganisms, to define their morphology and tinctorial properties. Pictures, descriptions of the microorganisms and names of media for their cultivation must be in addition 1 (columns № 6a, 6b, 6c).

Signature of teacher_____

Date: _____

Practical lesson №54

Topic: Clinical microbiology. Microbiological research of the digestive, genital and urine systems

Tasks for independent work:

a) The list of issues that must be studied:

1. Microbiota of healthy habitats body.
2. Microbiota of abnormal human habitat (in case of lesions of the digestive and urinary - genital systems).
3. Microbiological study of the digestive and urinary-genital systems.
4. Dysbacteriosis (dysmicrobiocenosis). Conditions of origin. The consequences of development.
5. Classification of dysbiosis by agent and localization .
6. Items study. Rules of capture, storage and delivery of materials to the lab.
7. Methods of diagnosis and rehabilitation of dysbiosis.

b) The list of practical skills, which need to master:

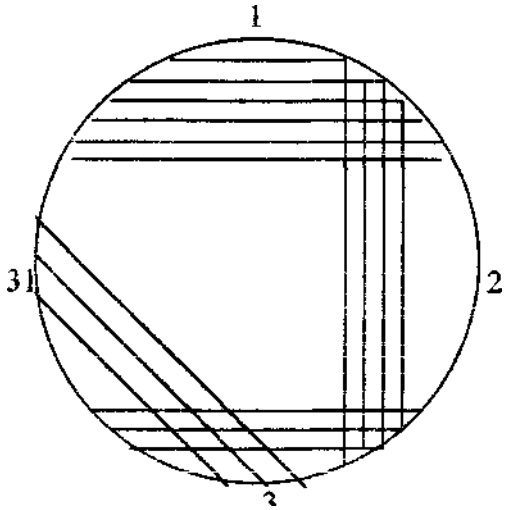
1. Compliance with rules of epidemiological regime and safety in the bacteriological laboratory.
2. Disinfection of infected material, antiseptic of hands contaminated by material or microbes culture studied.
3. Making of preparations for microscopic examination of pathological material.
4. Staining of agents by complex methods (Gram).
5. Microscopy with the light microscope with immersion lens.
6. Differentiation of microorganisms by morphological and tinctorial characteristics.
7. Production, recording and evaluation of slide agglutination.

Practical lesson's Protocol

Practical tasks should be done:

Task №1. To study urine inoculation by Gold method and define the degree of bacteriuria calculated on the table.

Chart of Gold method inoculation



Identify the main stages of the sector method. Stages of sector method

Computation table for determination of bacteria quantity in 1 ml liquid:

Quantity of colonies, that grew on a sector				Quantity of bacteria in 1 ml liquid
1Th	2Th	3Th	4Th	
1 - 6	There is no growth	There is no growth	There is no growth	<1 000
8-20	—••—	—••—	—.. —	1000
21-30	—.. —	—••—	—	5000
31-60	—••—	—••—	—.. —	10000
70-80	—••—	—••—	—•• —	50000
100-150	5-10	—••—	—	100 000
Very generous amount	20-30	—••—	-_.. —	500 000
The same	40-60	—-• —	—.- —	1 000 000
—••—	100 - 140	10-20	—-• —	5 000 000
—••—	Very generous amount	30-40	—.. —	10 000 000
—••—	Also	60 - 80	Single	50 000 000
—••—	—•• —	80 - 140	From single to 25	100 000 000

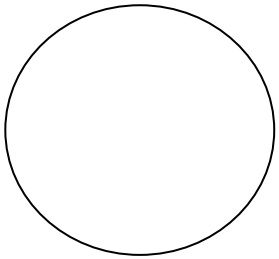
Conclusion:_____
_____
_____

Task №2. Make agglutination slide test with lactosepositive colonies (Endo media) and mixture of coli-serums (01, 08, 062, 075 + K1, K5, K13). To conduct consideration and do a conclusion. To sketch results.

Exam Control Control
(sera) (ph.solution)

Conclusion:_____
_____
_____

Task №3. Microscope and sketch a slide made from vagina excretions, to define the degree of vagina cleanness.



_____

To mark morphological and tinctorial properties of microorganisms

Conclusion:_____
_____
_____

Task №4. Microscope preparations of microorganisms, to define their morphology and tinctorial properties. Pictures, descriptions of the explored microorganisms and names of media for their cultivation must be in addition 1 (columns № 6a, 6c, 6d, 6e).

Addition
Determination of vagina cleanness degree

4 degrees of vagina cleanness are:

1st degree of cleanness – there are the pure culture of Dederlein rods and single epithelium cells in slide:
at the 2nd degree of cleanness the Dederleyn rods, gramnegative rods (Comma variabile), single leucocytes are found in preparations;
for a 3rd degree there are absence of vaginal rods, presence of pus flora, a plenty of leucocytes;
4th degree of cleanness – Dederleyn rods are absent, there is a pus flora, a lot of leucocytes.

Signature of teacher _____

Date:_____

Practical lesson №55

Topic: Hospital infections

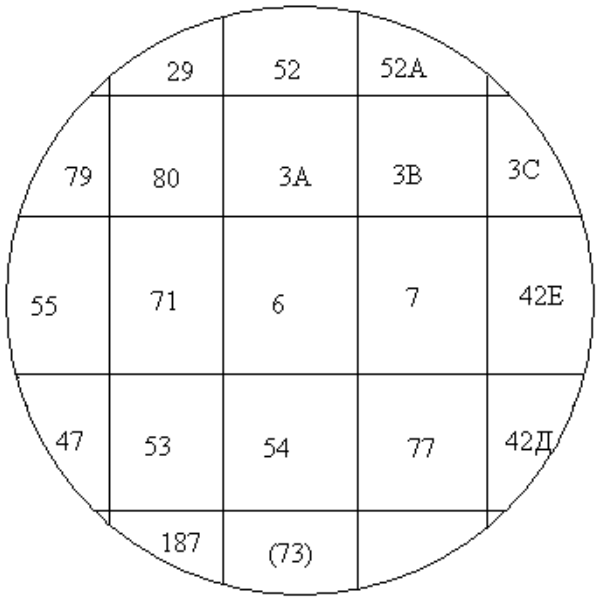
Tasks for independent work:

a) The list of issues to be studied :
1. Hospital Infection . Definitions. Classification . Conditions conducive to the emergence and widespread in hospitals institutions.
2. Etiology, pathogenesis, clinical forms of nosocomial infections caused by obligate pathogenic microbes (hepatitis B, salmonella toksykoseptychnyy nosocomial , hospital kolienteryty , adenoviral conjunctivitis , local and generalized forms of herpes and cytomegalovirus infection, mycoplasma and chlamydial urethritis, ringworm, etc.).
3. Opportunistic iatrogenic infection. Etiological structure.
4 Hospital ekovary strains and opportunistic microbes.
5 Opportunistic infections associated with medical intervention. Features immunity.
6 Microbiological basis of prevention and treatment of opportunistic infections.
7 Scientific substantiation of preventive measures in preventing nosocomial infections.

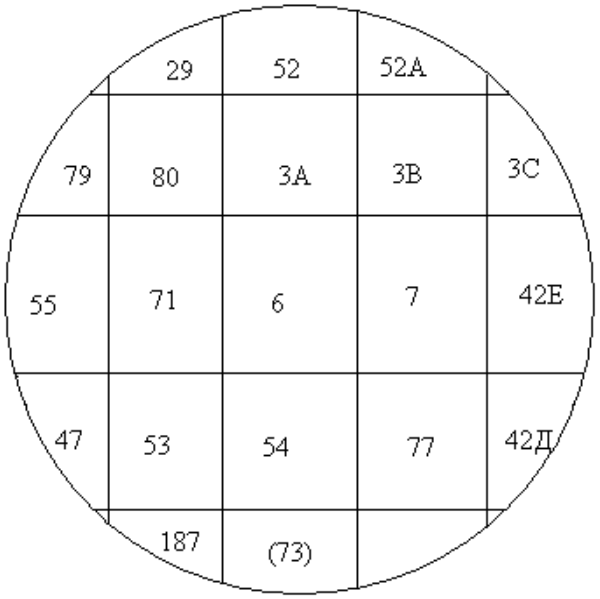
b) The list of practical skills, which need to master :
1. Be able to identify bacteria phagetype.
2. Be able to determine the sensitivity of microorganisms to antibiotics.
3. Compliance with rules epidemiological regime and safety in bacteriological laboratories .
4. inoculation loop pathological material on solid culture medium .
5. Decontamination of infected material, antiseptic hand, the investigated material or contaminated culture microbes.
6. Microscopic preparations in the light microscope with immersion lens.
7. Differentiation of organisms based on morphological characteristics and tynktorialnymy .
8. Referral form filling test material to the laboratory for microbiological examination .

Practical lesson's Protocol
Practical tasks should be done:

Task №1. Phagotype the Staphylococci cultures: 1)- from patient; 2) - medical workers of surgical department. To define phagogroupes and do a conclusion.



1



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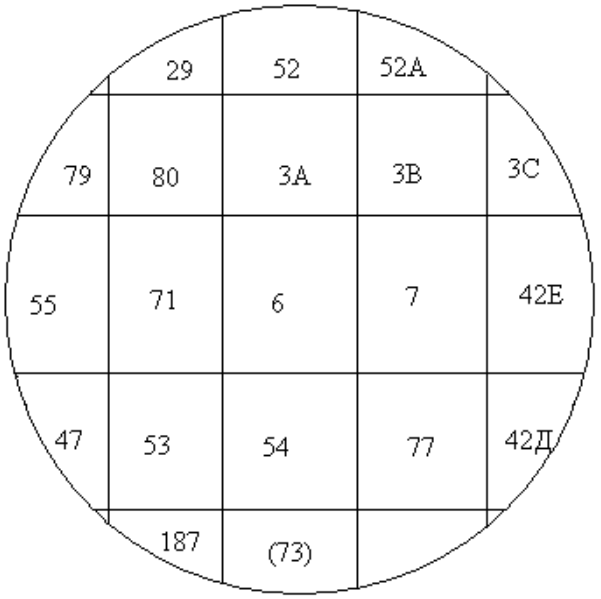


3

Note:

The first group were lysed by 29, 52, 52A, 79, 80 phages
The second group were lysed by AFTER, 3B, ZS, 55, 71phages
The third group were lysed by 6, 7, 42E, 47, 53, 54, 75, 77 phages
Fourth group were lysed by 42 D.
The group were lysed by 187 is mixed (73)

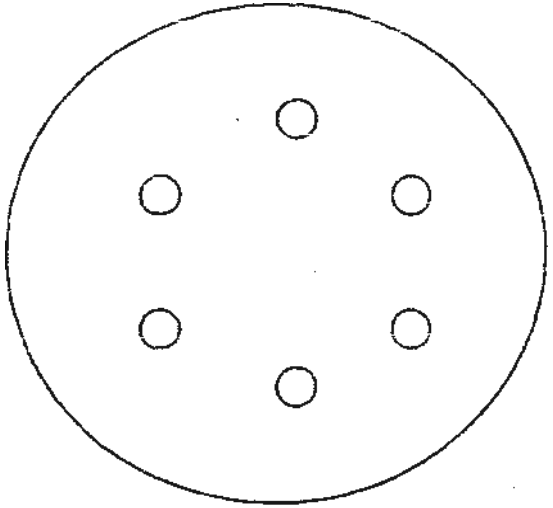
Pathogenic Staphylococci belong to the first group (at furunculosis, osteomyelitis, phlegmon).
Conditional-pathogenic Staphylococci belong to the second group (skins, chronic processes, subject to the condition quinsy, cystitis).
Staphylococci--saprophytes belong to the third group.

Conclusion:

_____

_____
_____
_____

Task №2. To conduct consideration of Staphylococci pure culture sensitiveness by the method of standard disks. . To do a conclusion



№	Antibiotic	Diameter of growth inhibition area	Sensitiveness
1.			
2.			
3.			
4.			
5.			
6.			

Conclusion:

_____
_____
_____

Task №3. Microscope microorganisms, define their morphology and tinctorial properties. Pictures, descriptions of the explored microorganisms and names of media for their cultivation to bring to addition 1 (column № 6f).

Task №4. To fill up the form of patient with suspicion on sharp gastroenteritis direction to a laboratory for microbiological research.

Direction №___
to microbiological (bacteriological, virology, parazitologic) research
«_____» _____20___p. _____ hours _____ minutes
(date and time of taking of biomateriala)

In _____ laboratory

Name, surname_____ Age _____
Medical card № _____ Establishment _____ examined _____
Address _____

Place of work, teaching (the names of child's establishment, schools)_____

Diagnosis, date :_____

Patient, reconvalestsent, bacterio-, viruso-carrier, contact, prophylactic inspection_____

Material: blood, urine, feces, saliva, sputum, spinal liquid, punctat, pus, wound, , mucus and others
_____

Purpose and name of research: _____

Position, last name, signature of person who sent material

Signature of teacher _____

Addition 1. Agents of opportunistic and hospital infections

1	2	3	4	5	6					
№	Agent	Media	Picture	Morphological and tinctorial properties	a	b	c	d	e	f
					Respiratory system	CNS	Blood	DT	Genital and urine system	Wounds
1.	S. aureus									
2.	S. eridermidis									
3.	S. pyogenes									
4.	S. pneumoniae									
5.	N. meningitidis									
6.	Pseudomonas aeruginosa									:
7.	E. coli									
8.	Salmonella									
9.	Shigella									
10.	Proteus									
11.	Prevotella									
12.	Enterobacter									
13.	Serratia									
14.	Klebsiella pneumoniae									
15.	Actinomyces									

16.	M. tuberculosis									
17.	S. septicum									
18.	S. ramosum									
19.	Bacteroides fragilis									
20.	Moraxella catarrhalis									
21	Haemophilus									
22	Chlamidia psittaci									
23.	Legionella pneumophilia									
24.	Mycoplasma pneumoniae									
25.	Pneumocystis carinii									
26.	Pasteurella multocida									
27.	Acinetobacter calcoaceticus									
28	Listeria monocytogenes									
29.	Cryptococcus neoformans									
30.	Nocardia asteroides									

Date:_____

Lesson №56 is test control

Date:_____

Practical lesson № 57

Topic: Examination on practical skills

Question for practical skills examination

1. To estimate the results of hemagglutination reaction (RHA) for virus determination in chicken embryo. To do a conclusion.
2. To conduct consideration of results of hemoculture phage identification, isolated from a patient with suspicion to typhoid.To do a conclusion.
3. To conduct consideration of results of intestinal bacteriophages titration in water by Apelman`s method.
5. To estimate the results of Hemaglutination inhibition test (IHAT) with the pair serums of inspected and standard parotitis diagnosticum. To do a conclusion.
6. To estimate the results of ELISA with inspected serums and HIV antigens (anti gp120). To do a conclusion.
7. To estimate the results of neutralization reaction (NR) - the coloured test with the pair serums of inspected and diagnosticum (cultures of 1st type poliomyelitis virus). To do a conclusion.
8. To estimate the results of complement fixation reaction (CFR) with the inspected pair serums and diagnosticum (standard specific adenoviral antigen). To do a conclusion.
9. To define the microbne number of drinking-water.
10. To define coli-index and coli-titr of drinking-water by the method of membrane filters. To estimate the results. To do a conclusion.
11. To define the common microbe number of classroom air by sedimentation method.
12. To learn urine inoculation, which is done by a sector method (by Gold) and to find the degree of microbe settling (bacteriouria) with computation table.
13. Microscope inspected vagina slides and define the degree of cleanness of vagina.
14. To conduct consideration of staphylococcal cultures phagotyping, which were isolated from: a) patient; b) and c) - medical workers of surgical department. To define phagotype and to do a conclusion.
15. To conduct consideration of sensitiveness of clean staphylococcal culture (which is isolated from a patient) to the antibiotics, defined by the method of standard disks. To do a conclusion.

Date:_____

Practical lesson № 58

Topic: Final module III control

Question for module III control theory examination

1. Conditionally-pathogenic microorganisms, biological properties, etiologic role in opportunistic infection. Characteristic of diseases caused by conditionally-pathogenic microorganisms.
2. Pseudomonas aeruginosa and Proteus vulgaris. Etiologic role at festering processes. Role in hospital infections. Microbiological diagnostics.
3. Hospital infections, terms of their origin. Properties of hospital microorganisms. Microbiological diagnostics of the infections caused by hospital cultures.
4. Normal microflora of human body.
5. Changes of microflora of human body depending on age, the state of health of man and other factors.
6. Role of human body microflora.
7. Normal microflora of intestine. Basic representatives, their role.
8. Methods of study of human body microflora role. Gnotobiology.
9. Factors, that affect quantitative and quality composition of microflora of human body.
10. Disbacteriosis. Methods of determination. Eubiotics and probiotics. Mechanism of action.
11. Dynamics of normal microflora in ontogenesis. Pathogenic role of microflora.
12. Clinical microbiology. Object, tasks, methods. Etiologic role of the conditional-pathogenic microbes.
13. Methods sanitary – bacteriological research of water.
14. Sanitary – model microorganisms which use for estimation of water quality.
15. Microflora of air, its description. Role of air in the transmission of infectious diseases.
16. Microbe number and sanitary–model microorganisms of air of the closed apartments, methods of determination, estimation of methods.
17. Sanitary microbiology. Object, tasks. Value of sanitary microbiology.
18. Sanitary – model microorganisms, requirements to them, their value for objects of external environment description.
19. Viruses are the special forms of living organization. Principles of structure of viruses. Virion and its components. Genetic methods of viruses and their nucleic components revealing.
20. Reproduction of viruses. Basic stages of viruses co-operation with cells at a productive infection. Integrative and abortive types of co-operation. Persistency of viruses.
21. Methods of cultivation of viruses. Classification of cellular cultures which are used in virology. Methods of indications of viruses.
22. Morphology and structure of viruses. Types of symmetry of viruses. Chemical composition of viruses.
23. Bacteriophages. Structure, classification by morphology. Methods of bacteriophages determinations.
24. Co-operation of bacteriphages with a bacterial cell. Virulent and temporal phages. Description of productive co-operation. Lizogenia and phage conversion.
25. Reactions of viral hemaglutination and hemadsorbtion. Mechanism, practical use. Diagnostic use.
26. Reaction of hemaglutination inhibition, its mechanism. Principles of the use, diagnostic value.
27. Reactions with the marked antigens and antibodies in virology. Reaction of immunofluorescention (RIF).
28. Polimerase chain reaction (PCR). Mechanism, practical use.
29. History of virology. Methods of viruses study , their estimation.

30. Methods of viruses cultivation and their estimation.
31. Principles of classification of viruses. Basic properties of viruses.
32. Serological reactions which use in virology. Reaction of neutralization of viruses. Mechanism, principles of the use, diagnostic value.
33. The use of cellular cultures in virology. Classification of cultures of cells. Nourishing media for cultivation of cells.
34. Types of co-operation of viruses and cells. Description of productive co-operation, stages.
35. Features of pathogenesis of viral infections. Acute and persistent viral infections.
36. Methods of viruses revealing in the culture of cells and their estimation. Cytopatic action of viruses, its kinds.
37. Antigen structure and types of antigen changeability of flu virus. Modern hypotheses which explain antigen changeability of Orthomyxoviruses.
38. Problem of specific prophylaxis and therapy of flu. Preparations.
39. Family of Orthomyxoviruses. Biological properties, antigen structure. Classification of flu viruses. The methods of laboratory diagnostics of flu.
40. Pathogenesis and immunity at flu. Role of specific and unspecific mechanisms in immunity.
41. Family of Rhabdoviruses. Virus of hydrophobia, biological properties. Pathogenesis of disease. Specific prophylaxis. Laboratory diagnostics. Differentiation of the fixed and wild viruses of hydrophobia.
42. Virus of epidemic parotitis. Pathogenesis of infection. Laboratory diagnostics, specific prophylaxis of parotitis.
43. Virus of measles, biological properties, cultivations. Pathogenesis of infection. Laboratory diagnostics, specific prophylaxis.
44. Family of Picornaviruses, general description. Biological properties. Antigens.
45. Retroviruses. Classification. Virus of human immunodeficiency (AIDS). Morphology and chemical composition.
46. Retroviruses. Features to the genome. Changeability, its mechanismes. Origin and evolution. Cultivation, stages of co-operation with sensible cells.
47. Pathogenesis of HIV. Mechanism of development of immunodeficiency. Laboratory diagnostics. The AIDS-assotiated infections. Principles of treatment and specific prophylaxis.
48. Genus of Enteroviruses, general description. Viruses Cocsaci and ECHO. Biological properties, role in human pathology. Diagnostics of enteroviral infections.
49. Viruses of poliomyelitis, description, classification. Pathogenesis and immunogenesis of infection. Laboratory diagnostics, specific prophylaxis.
50. Picornaviruses. Virus of hepatitis A, features. Pathogenesis of hepatitis A, laboratory diagnostics. Approaches to the specific prophylaxis of hepatitis.
51. Viral hepatitis, properties and classification of viruses. Pathogenesis of diseases. Laboratory diagnostics. Prospects of specific prophylaxis.
52. Virus of hepatitis B. Structure of virion, antigens. Features of pathogenesis of disease. Persistency.
53. Virus of hepatitis B. Laboratory diagnostics, methods of hepatitis B markers revealing. Specific prophylaxis.
54. Hepatitis C, D, E agents. Properties, role in human pathology, methods of laboratory diagnostics.
55. Family of Herpesviruses: classification, biological properties. Human pathology. Laboratory diagnostics of diseases.
56. Virus of smallpox. Pathogenesis of infection. Methods of diagnostics, specific prophylaxis. Virus of cowpox. Liquidation of smallpox in a whole world.
57. Priones. Properties. The animals (screpi, cows spongy encephalopathy) and human prion disease (Kuru, Creycfeld-Yacobs disease etc). Pathogenesis of prion diseases. Diagnostics.

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Мікробіологія, вірусологія та імунологія
Посібник для іноземних студентів медичного факультету

Навчальний посібник
(англійською мовою)

Підписано до друку 01.2015.
Формат Папір офсетний. Гарнітура
Ум. друк. арк.11,88. Обл.вид. арк. 12.
Наклад 300 прим. Зам. №

Видавництво
Вищого навчального закладу України
«Українська медична стоматологічна академія»

Свідоцтво суб'єкта видавничої справи, серія ДК №1691 від 17.02. 2004
36024, м. Полтава, вул. Шевченка, 23

Надруковано з оригінал-макета у редакційно-видавничому відділі
видавництва Вищого навчального закладу України
«Українська медична стоматологічна академія»
36024, м. Полтава, вул. Шевченка, 23