

**Ministry of Health Care, Republic of Belarus
Vitebsk State Medical University
Department of Clinical Microbiology**

I. I. Generalov, A. V. Frolova, N. V. Zheleznyak

**INSTRUCTIONS FOR LABORATORY TRAINING
in General Microbiology & Immunology**

for Students of Medical Faculty (English Medium)

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Reviewed by:

T.I. Dmitrachenko, MD, PhD, Dr.Sci, Professor of Infectious Disease Dpt,
Vitebsk State Medical University

Generalov, I. I.

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Instructions for laboratory training in General Microbiology and Immunology for students of the faculty of General Medicine were compiled according to basic educational plan and program, approved by Ministry of Health Care of Republic of Belarus. The plan, schedule of laboratory training and basic practical skills in general microbiology and immunology are presented in this workbook.

The instructions are prepared for medical students of higher educational establishments.

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LABORATORY CLASS №1

The topic: General acquaintance with microbiological laboratory. Safety rules of the work with pathogenic microbial agents (biological safety). Systematics and nomenclature of microorganisms. The morphology of bacteria. Basic methods for examination of microbial morphology. Simple methods of stain.

The main aim and the tasks of the work:

1. To make acquaintance with safety rules of the work in microbiological laboratory.
2. To get skills of primary manipulations with bacterial cultures.
3. To get skills of making slides of bacterial cultures with their next stain by simple staining methods.
4. To revise the principles and techniques of bright field microscopy.

The questions to the topic:

1. The subject and main tasks of medical microbiology.
2. Basic historical periods of microbiology.
3. International taxonomy of microorganisms. Principles of microbial classification.
4. The main morphological forms of bacteria.
5. The methods of study of bacterial morphology and structure.
6. Basic steps of preparing of slides from agar and broth microbial cultures. Simple methods of slide stain.
7. Bright field microscopy. The use of immersion objective lens.
8. Current advanced methods of microscopy (dark-field, phase contrast, luminescent microscopy, laser scanning confocal microscopy, atomic force microscopy, electron microscopy).

THE LITERATURE:

1. Lecture contents.
2. “Medical Microbiology, Virology & Immunology: Lecture Course”. Part 1. I. I. Generalov, Vitebsk, 2016. P. 9-26, 37-42.

Safety rules of the work in microbiological laboratory

1. Practical training in microbiological laboratory is performed with contagious material that requires strict discipline in laboratory work.
2. The students are allowed to work only with non-pathogenic or pathogenic biological agents of IV group of pathogenicity.

3. Before start the students must read and know all the details of current laboratory session.
4. The students must wear special uniform when working in the laboratory (white coats and/or aprons, clean closed shoes, caps, gloves, and safety glasses if needs).
5. When entered the laboratory room, students have to place their bags, books, mobile phones and other personal things into specified locations.
6. The student on duty should take all the necessary materials, reagents and microbial cultures from laboratory assistant before the beginning of the class.
7. At the end of class all contagious material and utensils must be put back to the separate table and placed into the special discharge container. Duty person delivers it to the laboratory assistant.
8. When making microbiological practicalities the students shouldn't keep any incidental tools or goods at their working areas.
9. The students are obliged to execute carefully all the instructions of the teacher.
10. Each student is supplied with a microscope for his laboratory personal work. It is assigned to the person for the whole course of training. The students have to work carefully with a microscope to maintain its proper working state.
11. If any student has broken accidentally the test tube or the flask with contagious material (*microbiological accident*) he is obliged to report about the matter to the teacher and make disinfection of the accident area.
12. It is completely prohibited to have a meal or drink, or chewing a gum in the laboratory rooms.
13. At the end of laboratory class all students must set into order their working areas; afterwards deliver the cultures, materials and reagents back to the student on duty; wash the hands, and present the albums and workbooks with their class notes, protocols and drawings to the teacher for sign.

Personal work of students:

1. Preparing of slides of broth culture with staphylococci (methylene blue stain).
2. Preparing of slides of agar culture with *Escherichia coli* (fuchsin stain).
3. **Demonstration:** microscopy of slides with streptococci (methylene blue stain).
4. Drawing of smears.

The basic steps of slide preparing for microscopy

The preparation of the slide of broth culture

1. Put a drop of broth culture by sterile microbiological wire loop on the surface of defatted glass slide and thoroughly spread the culture upon the glass surface in the circle with the diameter about 2.5-3 cm.
2. Sterilize the wire loop and loop holder.
3. Dry the slide at room temperature or with the help of ethanol burner.

4. Fix the slide passing it three times through the middle part of the flame.
5. Stain the slide with proper staining dye (e.g., methylene blue stain for 8-10 minutes or fuchsin stain for 1-2 minutes).
6. Wash it with tap water.
7. Dry the slide.
8. Drop the immersion oil on the slide.
9. Start bright field microscopy (immersion objective lens – 100x, eyepiece – 10x).

The preparation of the slide of agar culture

1. Put a drop of saline by sterile microbiological wire loop on the surface of defatted glass slide. Sterilize the wire loop and loop holder.
2. Take the sample from the agar and disperse it by sterile loop in the drop of saline.
3. Thoroughly spread the culture upon the glass surface in the circle with the diameter about 2.5-3 cm.
4. Sterilize the loop in the flame of burner.
5. Dry the slide at room temperature or with the help of ethanol burner.
6. Fix the slide passing it three times through the middle part of the flame.
7. Stain the slide with proper staining dye.
8. Wash it with tap water.
9. Dry the slide.
10. Drop the immersion oil on the slide.
11. Start bright field microscopy (immersion objective lens – 100x, eyepiece – 10x).

LABORATORY CLASS №2

The topic: Morphology and ultra-structure of bacteria. Differential methods of stain.

The main aim and the tasks of the work:

1. To learn the theoretical knowledge of the topic.
2. To know basic and advanced methods of microscopy.
3. To know the mechanisms and practical applications of differential staining methods: Gram stain, Gins capsule stain, Neisser stain, Ziehl-Neelsen acid-fast bacilli stain, Ozheshko spore stain.

The questions to the topic:

1. Structure of bacterial cell (obligate and non-obligate structural components).
2. Study of microbial morphology: basic and advanced methods of microscopy.
3. Nucleoid, its structure and functions, methods of detection.

4. Cytoplasm and ribosomes of bacteria. Cytoplasmic inclusions. Methods for detection of volutin granules.
5. Bacterial envelope, its composition and function of different layers. Cytoplasmic membrane, its structure and function.
6. Bacterial cell wall, its biological role. Structure of cell wall of gram-positive bacteria.
7. The cell wall of gram-negative bacteria. LPS, its functions.
8. Bacterial capsule, its structure and function.
9. Spores, stages of sporulation, methods of detection.
10. Flagella, methods for detection of bacteria motility.
11. Pili and fimbria. Injectisome, its structure and function.
12. Differential methods of stain (Gram stain, Gins capsule stain, Neisser stain, Ziehl-Neelsen acid-fast bacilli stain, Ozheshko spore stain).

THE LITERATURE:

1. Lecture contents.
2. “Medical Microbiology, Virology & Immunology: Lecture Course”. Part 1. I. I. Generalov, Vitebsk, 2016. P. 26-42.

Personal work of students:

1. Preparing of smears with mixture of *Sarcina flava* and *Escherichia coli* (Gram stain).
2. **Demonstration:**
 - 2.1. microscopy of smears with capsular bacteria (Gins capsule stain).
 - 2.2. microscopy of smears with spore-forming bacteria (Ozheshko stain).
3. Drawing of smears.

Basic methods of differential stain to determine tinctorial properties of bacteria

1. Gram stain

1. Prepare the smear from liquid or solid medium as described in topic of laboratory class №1.
2. Put the filter paper impregnated with *gentian violet* (crystal violet, methyl violet) upon the fixed smear and thoroughly soak it with distilled water. Stain with dye for 1-2 minutes.
3. After incubation remove the paper with gentian violet and add Lugol's iodine solution for 1-2 minutes.
4. Add ethanol to cover the smear strictly for 30 seconds.
5. Wash the smear.
6. Counterstain with fuchsin solution for 1 minute.

7. Wash thoroughly and dry the smear.

Gram-positive bacteria stain *violet* whereas **gram-negative bacteria** stain *pink*.

2. Neisser stain for volutin granules

1. Prepare and fix the smear from liquid or solid medium.
2. Stain the smear with Neisser methylene blue stain for 3-5 minutes.
3. After incubation add Lugol's iodine solution for 30 seconds.
4. Wash the smear.
5. Counterstain with chrysoidin solution for 1 minute.
6. Wash thoroughly and dry the smear.

Volutin granules stain *blue*, **vegetative part** of bacteria stain *brown-yellow*.

3. Negative stain for capsule (*Gins capsule stain*).

1. Prepare the smear of capsule bacilli culture mixing the drop of material and the drop of Indian ink.
2. Dry and fix the smear.
3. Stain the slide with fuchsin solution for 1 minute.
4. Wash thoroughly and dry the smear.

Indian ink makes the dark background for capsular bacteria. **Capsules** are visualized as *colorless halo* around **red microbial bodies** at the dark background.

4. Ziehl-Neelsen stain to detect acid-fast bacteria

1. Prepare and fix the smear from sputum specimen.
2. Stain it with Ziehl carbolic fuchsin solution for 5 minutes, or put the filter paper impregnated with Ziehl fuchsin upon the fixed smear, thoroughly soak it with distilled water and heat the slide upon burner until vapor appearance.
3. After incubation remove the paper and wash the smear with tap water.
4. Decolorize the smear with 5% sulfuric acid for 3-5 seconds.
5. Thoroughly wash the smear.
6. Counterstain the smear with methylene blue solution for 5 minutes.
7. Wash thoroughly and dry the smear.

Acid-fast bacteria retain the red stain while all other bacteria and the background are stained blue.

5. Ozheshko method for spore stain

1. Prepare the smear of spore-containing bacilli culture.
2. Before fixing put 0.5% solution of hydrochloric acid upon the smear and heat the slide on burner for 3-5 minutes.

3. After incubation wash the smear thoroughly with tap water.
4. Fix the smear.
5. Stain the slide with Ziehl-Neelsen method.
6. Wash thoroughly and dry the smear.

Spores stain red, the vegetative parts of microbial cell are blue.

6. Romanowsky-Giemsa stain

1. Prepare the smear of leukocyte culture incubated with bacterial or protozoan agents.
2. Fix the slide.
3. Stain it with Romanowsky-Giemsa's solution for 15-30 min. Romanowsky-Giemsa's stain (mixture of azure, eosin, and methylene blue dyes) is prepared from primary stock solution adding 1-2 drops of primary stock mixture into 1 ml of distilled water.
4. After incubation wash the slide with tap water and dry it.

The bacteria stain violet-purple, cell nuclei – red, cytoplasm – blue; protozoan nuclei – red-violet, their cytoplasm – blue.

LABORATORY CLASS №3

The topic: Morphology and ultra-structure of rickettsiae, chlamydiae, mycoplasmas. Isolation of pure culture of aerobic bacteria (1st day of examination).

The main aim and the tasks of the work:

1. To learn the theoretical knowledge of the topic.
2. To continue examination of the basic morphological forms of bacteria (spirochetes, mycoplasmas, rickettsiae, chlamydiae, actinomycetes, fungi).
3. To get skills of microbial agar plating for isolation of pure bacterial culture.

The questions to the topic:

1. The morphology and structure of spirochetes. Romanowsky-Giemsa stain.
2. The morphology and structure of rickettsiae.
3. The morphology and structure of chlamydiae.
4. The morphology and structure of mycoplasmas.
5. The morphology and structure of actinomycetes.
6. Classification and structure of fungi.
7. The morphology of mould and yeast fungi.

THE LITERATURE:

1. Lecture contents.
2. “Medical Microbiology, Virology & Immunology: Lecture Course”. Part 1. I. I. Generalov, Vitebsk, 2016. P. 38-39, 43-53.

Personal work of students:

1. *Demonstration:*

- 1.1. microscopy of slides with *C. trachomatis* infected cells (methylene blue stain);
- 1.2. microscopy of slides with various mould fungi (*Mucor mucedo*, *Aspergillus fumigatus*);
- 1.3. microscopy of slides with of *Candida* fungi (methylene blue stain).
- 1.4. laser scanning confocal fluorescent microscopy of *Penicillium chrysogenum* culture (auramine stain, 3D reconstruction).

2. Drawing of slides.

3. Planting of microbial mixture on Petri dish with MPA for isolation of pure culture of microorganisms. Protocol recording.

Protocol №1. Isolation of pure culture of microorganisms

Day of examination	Material for examination	Steps of examination	Results
1	Mixture of bacteria	Plating of specimen on Petri dish with MPA. Incubation at 37°C for 24 h	—

LABORATORY CLASS №4

The topic: Nutrition of bacteria. The mechanisms of transport of nutrients into bacterial cells. Nutrient media. Growth and reproduction of microorganisms. Bacterial pigments. Isolation of pure culture of aerobic bacteria (2nd day of examination).

The main aim and the tasks of the work:

1. To learn the theoretical knowledge of the topic.
2. To know the classification of nutrient media and their composition.
3. To make acquaintance with special equipment for sterilization and disinfection.
4. To be able to describe bacterial morphological, tinctorial, and cultural properties.
5. To get skills of inoculation of microbial specimen into slant agar.

The questions to the topic:

1. Metabolism of bacteria. Classification of bacteria according to their nutrition type and energy gain.
2. The mechanisms of bacterial nutrition and transport of nutrients into bacterial cells.
3. Secretion systems for transport of proteins and other substances outside the bacterial cells.
4. Main principles of microbial culture. Various groups of nutrient media.
5. Cultural characteristics of bacteria. Microbial colonies. Bacterial pigments, their significance. Classification of pigments.
6. Optimal conditions for bacterial growth and culture. Growth and reproduction of microorganisms.
7. Microbial growth in biofilms.

THE LITERATURE:

1. Lecture contents.
2. “Medical Microbiology, Virology & Immunology: Lecture Course” Part 1. I. I. Generalov, Vitebsk, 2016, p. 54-65.

Personal work of students:

1. The acquaintance of students with laboratory equipment for disinfection and sterilization.
2. Isolation of pure bacterial culture (2nd day of examination) – continuation of test protocol started at previous laboratory class:
 - a) description of bacterial growth, characteristics of colonies according to their cultural properties.
 - b) inoculation of bacteria into slant agar for isolation of pure culture.

The continuation of protocol №1 – Isolation of pure culture of microorganisms

Day of examination	Material for examination	Steps of examination	Results
2	—	Examination of bacterial growth with characteristics of microbial colonies according to their size, shapes, color, surface, edges, consistency. Inoculation of bacteria taken from single colony onto slant agar. Incubation at 37°C for 24 h	

3. **Demonstration** of the basic steps of nutrient media making (melting, hardening, storage).

LABORATORY CLASS №5

The topic: Bacterial enzymes. Biological oxidation in bacteria: fermentation and respiration. Testing of microbial biochemical activity. Methods of anaerobic bacteria culturing. Isolation of pure culture of aerobic bacteria (3rd and 4th days of examination).

The main aim and the tasks of the work:

1. To learn the theoretical knowledge of the topic.
2. To know biochemical properties of bacteria.
3. To get acquaintance with the methods of anaerobic bacteria isolation.
4. To be able to identify isolated bacterial culture.

The questions to the topic:

3. Enzymes of bacteria, their properties and classification. The role of enzymes in bacterial metabolism and pathogenicity.
4. Biological oxidation in bacteria. Fermentation.
5. Respiration in bacteria. Classification of bacteria according to their types of respiration.
6. Methods of anaerobic bacteria culturing. Enzymatic differences between anaerobic and aerobic bacteria.
7. Practical use of enzymatic properties of bacterial cultures. Assessment of microbial carbohydrate hydrolysis. Hiss media.
8. Tests for detection of bacterial proteolytic activity. Determination of bacterial catalase, oxidase and urease activities.

THE LITERATURE:

1. Lecture contents.
2. “Medical Microbiology, Virology & Immunology: Lecture Course”. Part 1. I. I. Generalov, Vitebsk, 2016. P. 65-79.

Personal work of students:

1. *Demonstration:*

- 1.1. bacterial pigments
- 1.2. methods for anaerobic bacteria culturing.
2. Isolation of pure bacterial culture (3nd day of examination) – continuation of test protocol started at previous class:
 - a) examination of microbial growth on slant agar, assessment of culture purity (preliminary visual examination, preparing of slides with Gram stain and bright field immersion microscopy for determination of *morphological* and *tinctorial* properties of isolated culture).

- b) determination of catalase activity in reaction with hydrogen peroxide.
- c) testing of microbial *biochemical* activity: inoculation of bacteria into Hiss media and meat-peptone broth (MPB) for detection of carbohydrate hydrolysis and proteolytic activity.
3. Isolation of pure bacterial culture (4th day of examination):
- a) registering of bacterial biochemical activity according to the demonstration of plate biochemical tests.
- b) completion of bacteriological testing with final conclusion about the species of isolated microbial culture.

The continuation of protocol №1 – Isolation of pure culture of microorganisms

Day of examination	Material for examination	Steps of examination	Results
3	—	Assessment of microbial growth on slant agar. Preparing the slide from slant culture with Gram stain. Determination of bacterial catalase activity. Inoculation of bacteria into Hiss media and MPB.	
4	—	Registration of bacterial biochemical activity	

Glucose	Lactose	Mannitol	Sucrose	Maltose	MPB	
					Indole	H ₂ S

Conclusion:

LABORATORY CLASS №6

The topic: Microbial genetics. Methods of molecular genetic analysis.

The main aim and the tasks of the work:

1. To learn the theoretical knowledge of the topic.
2. To get skills of genetic transformation and transduction tests.
3. To be able to assess phenotypic variation of *Proteus* culture.
4. To be able to determine R- and S-forms of microbial colonies.

The questions to the topic:

1. Bacterial genotype and phenotype, their characteristics. Organization of bacterial genome. Regulation of gene expression. Operon, its structure.

2. Plasmids and episomes, their structure and function.
3. Mobile genetic elements of bacterial genome. Transposons, IS-elements.
4. Genetic organization of adaptive immunity in bacteria – CRISPR/Cas system.
5. Phenotypic bacterial variations. Modifications, their characteristics.
6. Genotypic variations in bacteria, their classification. Bacterial dissociation.
7. Mutations: classification, mechanisms and biological significance.
8. Recombinations in bacteria, classification. Molecular mechanisms of recombinations. Bacterial transformation.
9. Transduction in bacteria.
10. Bacterial conjugation.
11. Methods of molecular genetic analysis. Molecular hybridization of nucleic acids.
12. Polymerase chain reaction. Nucleic acid sequencing.
13. Principles of genetic engineering. Practical applications of recombinant technologies in biology and medicine.

THE LITERATURE:

1. Lecture contents.
2. “Medical Microbiology, Virology & Immunology: Lecture Course”. Part 1. I. I. Generalov, Vitebsk, 2016 P. 80-108.

Personal work of students:

Protocol №1. Transformation test of B. subtilis culture

Day of examination	Material for examination	Steps of examination	Results
1	<p>1. DNA of auxo-autotrophic strain, capable of tryptophan synthesis.</p> <p>2. Recipient culture of auxo-heterotrophic <i>B. subtilis</i> strain.</p>	<p>Bacterial suspension of slant culture of <i>B. subtilis</i> is prepared by saline wash.</p> <p>0.5 ml of bacterial suspension is added into 2 test tubes. First one is supplemented with 0.5 ml of DNA solution, second – with 0.5 ml of saline (control test).</p> <p>Test tubes are placed for incubation at 37°C for 30 min.</p> <p>After the end of incubation the samples from both test tubes are plated onto MPA without tryptophan.</p>	—
<i>Incubation at 37°C for 24 h</i>			

2		Assessment of transformation results.	
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Conclusion:

Protocol №2. Transduction test of *E. coli* strain (biovar *Paracoli*)

Day of examination	Material for examination	Steps of examination	Results
1	1. Bacteriophage, able to transfer genes for lactose fermentation. 2. Recipient non-fermenting culture of <i>E. coli</i> biovar <i>Paracoli</i>	Bacterial suspension of <i>E. paracoli</i> strain is prepared by slant saline wash. 0.5 ml of bacterial suspension is added into 2 test tubes. First is supplemented with 0.5 ml of phage culture, second – with 0.5 ml of saline (control test). Test tubes are placed for incubation at 37°C for 30 min. After incubation inoculation of samples from both test tubes is made onto Endo medium.	—
Incubation at 37°C for 24 h			
2		Evaluation of transduction results.	

Conclusion:

Protocol №3. Examination of modification test of *P. vulgaris* culture

Day of examination	Material for examination	Steps of examination	Results
1	1. Broth culture of <i>P. vulgaris</i> . 2. Petri dish with MPA. 3. Petri dish with MPA supplemented with phenol.	One-streak plating of <i>P. vulgaris</i> culture is made upon Petri dish with phenol-supplemented MPA and control medium (non-modified MPA).	—

<i>Incubation at 37°C for 24 h</i>			
2		Assessment of “swarming” microbial growth in experimental and control Petri dishes.	

Conclusion:

LABORATORY CLASS №7

The topic: Sanitary microbiology.

Anti-microbial measures: sterilization, antisepsis, disinfection, asepsis.

Normal microflora of human body and its role.

The main aim and the tasks of the work:

1. To learn the theoretical knowledge of the topic.
2. To know the composition of normal human microbiota.
3. To know the standards of sanitary state of water and air.
4. To get skills of sanitary testing of water and air quality.
5. To know the composition of Kessler's and Endo media.

The questions to the topic:

1. Microbial Distribution in Nature. Symbiosis, its forms.
2. Antagonistic microbial relationships, their mechanisms. Types of microbial antagonism.
3. Sanitary indicator microorganisms, their characteristics.
4. Microflora of water, sources of water pollution. Water quality characteristics. Total microbial count of water.
5. Sanitary indicator microorganisms for water. Laboratory testing of water sanitary state. Identification of total coliform and thermotolerant bacteria.
6. Microflora of air. Microbial standards for air quality. Methods for determination of air sanitary state.
7. Microflora of soil.
8. Sterilization, its purposes. Sterilizing factors.
9. Different methods of sterilization.
10. Antisepsis, definition. The basic requirements to antiseptic drugs.
11. Groups of antiseptics, mechanisms of action.
12. Disinfection, its main goal. Variants of disinfection.
13. Asepsis, definition and common principles.
14. Normal microflora of human body, its role in human physiology and pathology.

15. Microflora of skin and respiratory tract.
16. Microflora of oral cavity, general characteristics. Biological role of normal oral microbiota.
17. Microflora of human gut and urogenital tract, its role.
18. Dysbiosis (dysbacteriosis), predisposing factors and progression. Prophylaxis and treatment of dysbiosis.

THE LITERATURE:

1. Lecture contents.
2. “Medical Microbiology, Virology & Immunology: Lecture Course”. Part 1. I. I. Generalov, Vitebsk, 2016. P. 109-117, 129-146.

Personal work of students:

1. Microscopy of specimen from dental plaque (Gram stain).
 - a) Put a drop of saline by sterile microbiological wire loop on the surface of defatted glass slide. Sterilize the wire loop and loop holder.
 - b) Take the specimen from dental plaque and disperse it by sterile wooden stick in the drop of saline.
 - c) Thoroughly spread the culture upon the glass surface in the circle with the diameter about 2.5-3 cm.
 - d) Dry the slide at room temperature or with the help of an ethanol burner.
 - e) Fix the slide passing it three times through the middle part of the flame.
 - f) Stain by Gram.
 - g) Drawing of slides.
2. Bacteriological sanitary testing of hand wash.

Protocol №1. Bacteriological sanitary testing of hand wash

Day of examination	Material for examination	Steps of examination	Results
1	Hand wash	Sampling of hand wash by sterile swab soaked with saline. Inoculation of specimen into Kessler's medium for <i>E. coli</i> determination.	—
<i>Incubation at 44 C for 24 h</i>			
2	—	Assessment of microbial growth in Kessler's medium (gas accumulation within the float)*. Planting onto Endo medium.	
3	—	Assessment of the growth of red colonies onto Endo medium.	

		Preparing of slides with Gram stain, microscopy.	
		Oxidase test.	

Conclusion:

NOTE: Composition of Kessler's medium: MPB, lactose, bile salts, gentian violet and tube for gas accumulation (float).

**E. coli* growth is indicated by gas accumulation within the float.

3. Analysis of membrane filtration and titration methods using material for demonstration.

Protocol №2. The determination of the number of air microflora using the sedimentation test

Day of examination	Material for examination	Steps of examination	Results
1	The air of the classroom	Planting air onto MPA by the sedimentation during 10 minutes. Incubation at 37°C for 24 hours.	—
2	—	The grown colonies are counted and total microbial quantity is calculated (number of colonies x 60).	

Conclusion:

LABORATORY CLASS №8

The topic: Chemotherapy. Antibiotics. Antibiotic susceptibility testing

The main aim and the tasks of the work:

1. To learn the theoretical knowledge of the topic.
2. To get skills of disc diffusion test for assessment of bacterial susceptibility to antibiotics.
3. To be able to determine end-point (minimum inhibitory concentration or MIC of antibiotic) in broth dilution susceptibility testing.
4. To make acquaintance with agar dilution susceptibility test.

The questions to the topic:

1. Antimicrobial chemotherapy and chemoprophylaxis of infectious diseases. Therapeutic ratio.
2. Antibiotics. Requirements to antibiotic drugs.

3. Classification of antibiotics according to their origin, their antibacterial effects, spectrum of action and molecular mechanisms of their antibacterial activity.
4. Antimicrobial action by inhibition of cell wall synthesis: beta-lactam antibiotics (cephalosporins, penicillins, carbapenems), vancomycin, linezolid, bacitracin.
5. Antimicrobial action by inhibition of cell membrane function: amphotericin B, polyenes, polymyxins.
6. Antimicrobial action by inhibition of protein synthesis: chloramphenicol, macrolides and azalides, lincomycins, tetracyclines, aminoglycosides.
7. Antimicrobial action by inhibition of nucleic acid synthesis: quinolones, rifampicin, sulfonamides, trimethoprim.
8. Side effects of antibiotics.
9. Microbial drug resistance, mechanisms. Non-genetic and genetic (chromosomal and extrachromosomal) resistance. Prevention of drug resistance.
10. Measurements of antimicrobial activity: disc diffusion test for determination of bacterial susceptibility to antibiotics.
11. Broth and agar dilution susceptibility tests, their evaluation. E-test.

THE LITERATURE:

1. Lecture contents.
2. “Medical Microbiology, Virology & Immunology: Lecture Course”. Part 1. I. I. Generalov, Vitebsk, 2016. P. 147-159.

Personal work of students:

1. *Demonstration:*

- 1.1. *agar dilution test* for assessment of microbial resistance to antibiotics
- 1.2. *E-test*
- 1.3. *test-systems* for determination of bacterial susceptibility to antibiotics

2. *Protocol № 1. Disc diffusion test for determination of S. aureus resistance to antibiotics*

Day of examination	Material for examination	Steps of examination	Results
1	<i>Staphylococcus aureus</i> (1·10 ⁹ cells/ml)	Plating of material on Petri dish with MPA. Placement of disks with antibiotics on Petri dish.	—
<i>Incubation at 37° C for 24 h</i>			
2	—	Assessment of microbial susceptibility testing: measurement of diameters of growth inhibition zones.	<i>Antibiotics:</i> 1. _____ mm 2. _____ mm

			3. _____ mm
			4. _____ mm

Conclusion: Isolate of *Staphylococcus aureus* is susceptible to...

NOTE: the degree of sensitivity of *S. aureus* to antibiotics is based on the following criteria:

- the diameter of growth inhibition zone to 10 mm – *the culture is resistant to antibiotics*
- 11-15 mm – *the culture is not very sensitive to the antibiotic*
- 16-25 mm – *the culture is sensitive to the antibiotic*
- more than 25 mm – *the culture is highly sensitive to the antibiotic*

Protocol № 2. Measurement of microbial resistance to antibiotic with broth dilution test

Reagents	Number of test tubes						
	1	2	3	4	5	6	7
	Final antibiotic concentration in test tubes, mkg/ml						
	128	64	32	16	8	4	control
Meat-peptone broth	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Antibiotic (initial concentration – 64 mkg/ml)	0.1	→ 0.1	→ 0.1	→ 0.1	→ 0.1	→ 0.1	–
Microbial culture	One loop of material is inoculated in all test tubes						
Incubation at 37°C for 24 h							
Results:							

↓
to disinfection

Conclusion:

LABORATORY CLASS №9

The topic: Final control study of the section “Morphology of bacteria. Physiology of bacteria. Microbial genetics. Sanitary microbiology. Chemotherapy. Antibiotics.”

The main aim and the tasks of the work:

To consolidate the basic knowledge of bacterial structure and metabolism (nutrition, respiration, growth and reproduction, genetic alterations); methods of microbial isolation, identification and molecular genetic analysis; principles and methods of sanitary microbiology, asepsis, antisepsis, disinfection, and sterilization.

The questions:

1. Systematics and nomenclature of microorganisms. Principles of microbial classification. Basic definitions in microbiology.
2. Study of bacterial morphology: modern methods of microscopy.
3. Characteristics of round (spherical) forms of bacteria.
4. Characteristics of rod-shaped forms of bacteria.
5. Characteristics of twisted (spiral) forms of bacteria.
6. Structure of bacterial cell (obligate and non-obligate components). Bacterial envelope, its composition. Cytoplasmic membrane – structure and function.
7. Bacterial cell wall, its biological role. Structure of the cell wall of gram-positive bacteria. Differential methods of staining – Gram stain.
8. The cell wall of gram-negative bacteria. LPS, its functions. Differential methods of staining – Gram stain.
9. Bacterial capsule, its structure and function. Differential methods of staining – Gins capsule stain.
10. Spores, stages of sporulation, methods of detection.
11. Flagella, pili, methods of bacteria motility detection. Injectisome, its structure and functions.
12. Cytoplasm, ribosomes, inclusions, methods of volutin granules detection.
13. Nucleoid, its structure and function. Methods of detection.
14. Morphology and characteristics of actinomycetes. Differential methods of staining – Ziehl-Neelsen acid-fast bacilli stain.
15. Morphology and characteristics of spirochetes. Differential methods of staining – Romanowsky-Giemsa stain.
16. Morphology and characteristics of chlamydiae.
17. Morphology and characteristics of rickettsiae.
18. Morphology and characteristics of mycoplasmas.
19. Classification and structure of fungi.
20. Morphology and characteristics of mould and yeast fungi.
21. L. Pasteur, his outstanding contribution into microbiological science. R. Koch, his work in microbiology.
22. Metabolism of bacteria. Classification of bacteria according to their nutrition type and energy gain.
23. Mechanisms of bacterial trans-membrane transportation.
24. Secretion systems for transport of proteins and other substances outside the bacterial cells.
25. Methods of bacterial culturing. Requirements to nutrient media. Classification of nutrient media and their characteristics.
26. Biological oxidation in bacteria. Pathways of fermentation and respiration.

27. Classification of bacteria according to their types of respiration and their characteristics.
28. Methods of anaerobic bacteria cultivation. Isolation of pure culture of anaerobes.
29. Bacterial enzymes, their properties and classification. The role of enzymes in bacterial metabolism and pathogenicity.
30. Examination of bacterial carbohydrate hydrolysis. Determination of proteolytic properties. Detection of catalase, oxidase, and urease activity of bacteria.
31. Growth and reproduction of bacteria.
32. Bacterial pigments, their significance. Classification of pigments.
33. Microorganisms, inhabiting the environment. Microbial ecology, microbial communities, ecosystem, ecological variants. Symbiosis, its forms. Antagonistic microbial relationships, their mechanisms. Types of microbial antagonism.
34. Normal microflora of human body, its role in human physiology and pathology. Ontogenesis of normal microflora. Microflora of skin, its role.
35. Microflora of oral cavity, gut, respiratory, and urogenital tract; role in physiology and pathology.
36. Dysbiosis (dysbacteriosis), predisposing factors and progression. Prophylaxis and treatment of dysbiosis.
37. Sanitary indicator microorganisms, their common properties.
38. Microflora of water, sources of water pollution. Water quality characteristics. Total microbial count of water.
39. Sanitary indicator microorganisms for water. Laboratory testing of water sanitary state. Identification of total coliform and thermotolerant bacteria.
40. Microflora of air. Microbial standards for air quality. Methods for determination of air sanitary state.
41. Asepsis and antisepsis – definitions, basic methods, and significance.
42. Classification of antiseptics. Requirements to antiseptic drugs.
43. Disinfection, its main goal. Variants of disinfection.
44. Sterilization, its purposes. Sterilizing factors. Physical methods of sterilization.
45. Methods of sterilization – mechanical and chemical sterilization. Sterilization by irradiation.
46. Bacterial genotype and phenotype, their characteristics. Organization of bacterial genome. Operon, its structure and function.
47. Plasmids and episomes, their structure and functions.
48. Mobile genetic elements of bacterial genome. Transposons, IS-elements.
49. Phenotypic bacterial variations. Modifications, their characteristics. Genotypic variations in bacteria, their classification. Bacterial dissociation.
50. Mutations: classification, mechanisms and biological significance.
51. Recombinations in bacteria, classification and general characteristics. Molecular mechanisms of recombinations. Bacterial transformation.
52. Transduction in bacteria.

53. Bacterial conjugation.
54. Genetic organization of adaptive immunity in bacteria – CRISPR/Cas system.
55. Methods of molecular genetic analysis – molecular hybridization of nucleic acids. Nucleic acid sequencing.
56. Polymerase chain reaction.
57. Principles of genetic engineering. Practical applications of recombinant technologies in biology and medicine.
58. Antimicrobial chemotherapy and chemoprophylaxis of infectious diseases. Therapeutic ratio.
59. Antibiotics. Requirements to antibiotic drugs.
60. Classification of antibiotics according to their origin, their antibacterial effects, spectrum of action and molecular mechanisms of their antibacterial activity.
61. Antimicrobial action by inhibition of cell wall synthesis: beta-lactam antibiotics (cephalosporins, penicillins, carbapenems), vancomycin, linezolid, bacitracin.
62. Antimicrobial action by inhibition of cell membrane function: amphotericin B, polyenes, polymyxins.
63. Antimicrobial action by inhibition of protein synthesis: chloramphenicol, macrolides and azalides, lincomycins, tetracyclines, aminoglycosides.
64. Antimicrobial action by inhibition of nucleic acid synthesis: quinolones, rifampicin, sulfonamides, trimethoprim.
65. Side effects of antibiotics.
66. Microbial drug resistance, mechanisms. Non-genetic and genetic (chromosomal and extrachromosomal) resistance. Prevention of drug resistance.
67. Measurements of antimicrobial activity: disc diffusion test for determination of bacterial susceptibility to antibiotics.
68. Broth and agar dilution susceptibility tests, their evaluation. E-test.

THE LITERATURE:

1. Lecture contents.
2. “Medical Microbiology, Virology & Immunology: Lecture Course”. Part 1. I. I. Generalov, Vitebsk, 2016. P. 9-149.

LABORATORY CLASS №10

The topic: Structure and functions of immune system.

Types of immunity, basic definitions. Cytokines and interleukins.

The development and differentiation of T- and B- cells.

The main aim and the tasks of the work:

1. To learn the theoretical knowledge of the topic.
2. To know basic principles of immune system structure and function, T- and B cells development and differentiation.
3. To get primary skills for laboratory testing of immune cells (assessment of peripheral T cell count by rosette formation with sheep red blood cells, immunofluorescence).
4. To get acquaintance with high-throughput methods for immunocytes quantitation (flow cytometry analysis).

The questions to the topic:

1. Immunology and immunity. Innate, acquired, artificial, natural immunity.
2. Anti-infectious immunity, its forms. Types of non-infectious immunity.
3. Structure and organization of immune system.
4. Cytokines, the basic features and classification.
5. Interleukins, their biological role and functions.
6. Interferons, TNF, growth factors.
7. CD molecules of immune cells, their significance.
8. T cells, their development and differentiation. Structure of TCR, its function.
9. Subpopulations of T lymphocytes, their role.
10. B lymphocytes, their development and functions.
11. Laboratory tests for quantitative analysis of immune cells. Flow cytometry.

THE LITERATURE:

1. Lecture contents.
2. “Medical Microbiology, Virology & Immunology: Lecture Course”. Part 1. I. I. Generalov, Vitebsk, 2016. P. 175-192.

Personal work of students:

1. Drawing of demonstration slides:
 - 1.1. microscopy of slides with T-cell rosettes with sheep red blood cells (Romanowsky-Giemsa stain);
 - 1.2. microscopy of slides with immunofluorescence assay for B-cell identification (laser scanning confocal microscopy)
2. Analysis of flow cytometry histogram for quantitation of immune cells.

LABORATORY CLASS №11

The topic: Antigens. Antibodies. Serological testing. **Precipitation tests, reactions of toxin neutralization.**

The main aim and the tasks of the work:

1. To learn the theoretical knowledge of the topic.
2. To be able to perform ring precipitation test for protein species identification.
3. To know the main goal and technique of Mancini's single radial immunodiffusion test, double immunodiffusion test and immune electrophoresis.
4. To know how to produce precipitin-containing antisera.

The questions to the topic:

1. Antigens: structure and main properties. Haptens.
2. Antigenic structure of microbial cell. Protective antigens, superantigens, antigenic mimicry.
3. Non-infectious antigens, their classification. Auto-antigens and alloantigens, general characteristics. Human blood group antigens.
4. HLA system, general characteristics. HLA molecules of I class, structure and functions.
5. HLA molecules of II class, structure and functions. Biological role of HLA system.
6. Immunoglobulins, molecular structure and functions.
7. Class of immunoglobulins, their characteristics.
8. Antibodies. Mechanisms of antibody action.
9. Genetic control of specificity and diversity of antibodies and T cell receptors (TCRs).
10. Serological reactions and their practical applications: general characteristics of serological reactions. Immune reagents for serological reactions. Classification of serological reactions.
11. Precipitation tests, reagents and main goal.
12. Variants of precipitation tests (ring precipitation, immune diffusion, immune electrophoresis). Single radial immunodiffusion (Mancini's test) for determination of immunoglobulin concentrations.
13. Reactions of toxin neutralization. Reagents and main goals.

THE LITERATURE:

1. Lecture contents.
2. "Medical Microbiology, Virology & Immunology: Lecture Course". Part 1. I. I. Generalov, Vitebsk, 2016. P. 205-222, 246-248, 253-257.

Personal work of students:

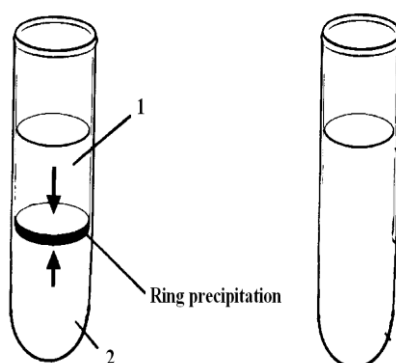
1. *Ring precipitation test for identification of species-specific proteins.*

Reagents: 1) blood spot extraction
2) serum for precipitation of human proteins
3) serum for precipitation of chicken proteins

Reaction steps:

1. Put 1-2 ml of antiserum against human proteins on the bottom of test tube N1.
2. 1 ml of blood extraction is laid very carefully upon the serum.
3. The same manipulation should be made for the test tube N2, where antiserum against chicken proteins is used.
4. Incubation for about 5 min at room temperature. Ring of precipitation is to be formed.

Drawing of the results and making the conclusion.



2. *Demonstration:*

- 2.1. double immunodiffusion test and single radial immunodiffusion test;
- 2.2. assessment of the results of immune electrophoresis.

LABORATORY CLASS №12

**The topic: Innate immunity: humoral and cellular response.
Toll-like receptors. Phagocytes and phagocytosis. Granulocytes.
Natural killer cells. Complement system. Serological testing –
agglutination tests, indirect hemagglutination test, Coombs` test.**

The main aim and the tasks of the work:

1. To learn the theoretical knowledge of the topic.

2. To know the basic mechanisms of innate immune response.
3. To get skills of elaboration and results reading of complement fixation test.
4. To acquire skills of laboratory testing of phagocytosis.
5. To get acquaintance with basic agglutination tests and their medical applications.
6. To know the reagents for agglutination tests – agglutinating sera, microbial diagnosticums, erythrocyte diagnosticums.
7. Laboratory training of slide agglutination and tube agglutination tests; indirect hemagglutination assay.

The questions to the topic:

1. Pathogen-binding receptors and pattern-based recognition. Toll-like receptors in humans, their functions.
2. Humoral factors of non-specific (innate) immune response.
3. Mononuclear phagocyte system, general characteristics and functions.
4. Phagocytosis, its stages. Mechanisms of microbial killing by phagocytes.
5. Granulocytes, their role in immune response.
6. Macrophage and granulocyte system assessment. Phagocytic number, phagocytic index. NBT test, its main goal.
7. Natural killer cells (NK cells). NKT Cells.
Complement system. Classical pathway of activation.
8. Alternative and lectin pathways of complement activation.
9. Serological tests – immune lysis reactions. Complement fixation test, principle of analysis, reagents and medical applications.
10. Agglutination tests, their main goals. Mechanisms of agglutination. Slide agglutination and extended tube agglutination tests.
11. Indirect hemagglutination test. Reagents for indirect hemagglutination.
12. Reactions with incomplete antibodies. Coombs` test, its variants. Reagents for Coombs` reaction.

THE LITERATURE:

1. Lecture contents.
2. “Medical Microbiology, Virology & Immunology: Lecture Course”. Part 1. I. I. Generalov, Vitebsk, 2016. P. 192-204, 222-234, 245-246, 249-253, 257-259.

Personal work of students:

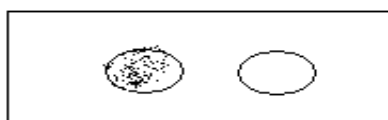
1. Tentative slide agglutination test for identification of microbial species.

Reagents: 1) unknown microbial culture

2) agglutinating serum for *E. coli* (*var. paracoli*)

3) saline

Drawing the results



Conclusion:

2. **Demonstration:** the assessment of *extended tube agglutination test* for microbial species identification.

3. **Demonstration:** the assessment of *indirect hemagglutination test* for serological diagnosis of disease (determination of specific antibodies titer in patient`s serum)

Reagents	Number of tubes				
	1	2	3	4	5
	Serum dilutions				
	1:20	1:40	1:80	1:160	Control
Saline	0,1	0,1	0,1	0,1	0,1
Patient`s serum diluted 1:10	0,1	→ 0,1	→ 0,1	→ 0,1	--
Erythrocyte diagnosticum	0,1	0,1	0,1	0,1	0,1
Incubation at 37°C for 1 h					
Results					

↓
to disinfection

Conclusion:

4. **Demonstration:** the assessment of *complement fixation test* for determination of specific antibodies in patient`s serum

Reagents	Serum dilutions							Controls		
	1:10	1:20	1:40	1:80	1:160	1:320	1:640	Hemo l. system	Ag	Compl.
	1	2	3	4	5	6	7	8	9	10
Saline	0,05	0,05	0,05	0,05	0,05	0,05	0,05	0,15	0,05	0,1
Patient serum	0,05	→ 0,05	→ 0,05	→ 0,05	→ 0,05	→ 0,05	→ 0,05			
Antigen in working dose	0,05	0,05	0,05	0,05	0,05	0,05	0,05		0,05	
Complement in working dose	0,05	0,05	0,05	0,05	0,05	0,05	0,05		0,05	0,05
Incubation										
<i>Incubation at 37°C for 1 h or at 4°C for 18-20 h</i>										
Hemolytic system	0,1	0,1	0,1	0,1	0,1	0,1	0,1	0,1	0,1	0,1
Incubation										
<i>Incubation at 37°C for 1 h</i>										
Results**										

↓
to disinfection

Conclusion:

**Results are tested after control probes evaluation (test tubes №№ 8, 9, 10).

Hemolysis absence means positive complement fixation test result.

5. Drawing of demonstration slides:

5.1. microscopy of slides with complete phagocytosis of *Escherichia coli* (Romanowsky-Giemsa stain);

5.2. microscopy of slides with incomplete phagocytosis of *Neisseria gonorrhoeae* (methylene blue stain).

LABORATORY CLASS №13

The topic: Dynamics of immune response.

Genetic control of immune response.

Serological testing – enzyme-linked immunosorbent assay, radioimmunoassay, immunofluorescence assay, immunoblotting (western blotting).

The main aim and the tasks of the work:

1. To learn the theoretical knowledge of the topic.
2. To know the mechanisms of acquired immune response.
3. To know the reagents for ELISA test and immunofluorescence assay.
4. To perform serological testing of specific antibodies by enzyme-linked immunosorbent assay and data analysis by microplate reader.

The questions to the topic:

1. Dynamics of the immune response. Non-specific mechanisms.
2. Acquired immunity, general characteristics. T-independent immune response.
3. T-dependent immune response, stages. Antigen processing and presentation. Dendritic and other antigen-presenting cells.
4. Inductive phase: activation and differentiation of T helper cells.
5. Activation of effector cells. Mechanisms of pathogen elimination.
6. Natural inhibition of immune response.
7. Primary and secondary immune response, their characteristics.
8. Serological testing – reactions with labeled antibodies and antigens. Immunofluorescence assay, its variants and main applications.
9. Enzyme-linked immunosorbent assay (ELISA), its reagents, stages and applications in immunological diagnosis.
10. Radioimmunoassay.
11. Western blotting analysis.

THE LITERATURE:

1. Lecture contents.
2. "Medical Microbiology, Virology & Immunology: Lecture Course". Part 1. I. I. Generalov, Vitebsk, 2016. P. 227-234, 259-263.

Personal work of students:

1. *Evaluation of ELISA test for serological diagnosis of M. pneumoniae infection: identification of IgM class anti-mycoplasmal antibodies in patient's serum*

	1	2	3	4	5	6	7	8	9	10	11	12
A											C+	C+
B											C-	C-
C												
D												

Conclusion:

NOTE: The appearance of the brown color of the reaction medium is regarded as a positive result.

Reading of results is performed by microplate colorimetric reader at $\lambda=492\text{ nm}$. The optical density (OD) for positive results must exceed previously established cut-off values.

Cut-off values are calculated as $(\text{mean } OD_{K(-)} + 0,3 \text{ OD})$ where $OD_{K(-)}$ indicates the values of optical density of negative controls (wells B11 and B12).

Mean value of optical density of negative controls (wells B11 and B12) should be equal or less than 0.2 OD units.

Mean value of optical density of positive controls (wells A11 and A12) should be 3 times more than optical density of negative controls.

LABORATORY CLASS №14

The topic: Immunopathology. Allergy, autoimmune diseases.
Primary and secondary immunodeficiencies.
Immunodiagnostics. Evaluation of immune status.
Immunoprophylaxis and immunotherapy.

The main aim and the tasks of the work:

1. To learn the theoretical knowledge of the topic.
2. To know basic mechanisms of immunopathology.
3. To know the basic methods for immune status assessment.

4. To know biological products for immunoprophylaxis and immunotherapy.
5. To get acquaintance with hybridoma technology.
6. To be able to read and interpret the results of enzyme-linked immunosorbent assay (ELISA) for determination of IgE allergen-specific antibodies.
7. To be able to evaluate the results of allergic skin tests.
8. To get skills of interpretation of blast transformation test.

The questions to the topic:

1. Immunopathology, classification. Immediate and delayed types of hypersensitivity, general characteristics and mechanisms.
2. Coombs & Gell classification of hypersensitivity reactions.
3. Anaphylactic hypersensitivity. Allergy and allergic diseases, basic mechanisms and stages of development.
4. Cytotoxic hypersensitivity, mechanisms. Autoimmune diseases, triggered by this type of reactions.
5. Immune-complex-mediated hypersensitivity. Autoimmune diseases, stimulated by this type of reactions.
6. Cell-mediated (delayed) hypersensitivity. Skin tests for laboratory diagnosis of infectious allergy.
7. Stimulatory and blocking hypersensitivity. Autoimmune diseases, developed by these reactions.
8. Primary immunodeficiencies. Combined immunodeficiencies. T- and B cell immunodeficiencies.
9. Phagocyte and complement system immunodeficiencies. Secondary immunodeficiencies, their mechanisms.
10. Immune status assessment – general characteristics. Humoral immunity evaluation.
11. Methods for assessment of quantity and functional activity of T- and B-cells. Blast transformation test.
12. Active immunoprophylaxis. Vaccines and toxoids, their classification and characteristics. Modern vaccines and toxoids for prophylaxis of infectious diseases.
13. Passive immunotherapy. Immune sera and immunoglobulins, their clinical use.
14. Monoclonal antibodies (mAbs). Stages of hybridoma technology. Medical applications of mAbs for laboratory diagnosis and treatment of diseases. Humanized monoclonal antibodies.

THE LITERATURE:

1. Lecture contents.
2. “Medical Microbiology, Virology & Immunology: Lecture Course”. Part 1. I. I. Generalov, Vitebsk, 2016. P. 218-219, 243-245, 264-281.

Personal work of students:

1. Evaluation of ELISA test for determination of IgE allergen-specific antibodies

	1	2	3	4	5	6	7	8	9	10	11	12
A											C+	C+
B											C-	C-
C												
D												

Conclusion:

2. Demonstration: the assessment of lymphocyte blast transformation test (orcein stain).

2.1. the determination of the content of blast forms of lymphocytes (%) from 100 lymphocytes.

2.2. the drawing of the blast forms from blast transformation test.

LABORATORY CLASS №15

**The topic: Final control
study of the section “Medical immunology”**

The main aim and the tasks of the work:

To learn the principles of immune system function in normalcy and pathology; to consolidate the knowledge of basic methods of immunological laboratory diagnosis, immunoprophylaxis and immunotherapy.

The questions to the topic:

1. Immunology and immunity. Innate, acquired, artificial, natural immunity. Anti-infectious immunity, its forms. Types of non-infectious immunity.
2. Immune system, its sub-systems and levels of organization. Central and peripheral immune organs.
3. CD molecules of immune cells, their significance.
4. Cytokines, the basic features and classification. Groups of cytokines (interferons, TNF, growth factors, chemokines).
5. Interleukins, their biological role and functions.
6. T cells, their development and differentiation. Structure of TCR, its function.
7. T cell subpopulations, their role.
8. B cells, their development and differentiation.

9. Antigens, their properties and general characteristics. Haptens.
10. Main bacterial and viral antigens. Protective antigens, superantigens, antigenic mimicry.
11. Non-infectious antigens, their classification. Auto-antigens and alloantigens, general characteristics. Human blood group antigens.
12. HLA system, general characteristics. HLA molecules of I class, structure and functions. Biological role of HLA system.
13. HLA molecules of II class, structure and functions. Biological role of HLA system.
14. Immunoglobulins, molecular structure and functions.
15. Class of immunoglobulins, their characteristics.
16. Structure and biological activity of secretory IgA.
17. Antibodies. Mechanisms of antibody action.
18. Monoclonal antibodies (mAbs). Stages of hybridoma technology. Medical applications of mAbs for laboratory diagnosis and treatment of diseases.
19. Genetic control of specificity and diversity of antibodies and T cell receptors (TCRs)
20. Complement system. Classical pathway of activation.
21. Alternative and lectin pathways of complement activation.
22. Mononuclear phagocyte system, general characteristics and functions. Granulocytes, their role in immune response.
23. Phagocytosis, its stages. Mechanisms of microbial killing by phagocytes.
24. Toll-like receptors and pattern-based microbial recognition. Dendritic and other antigen-presenting cells (APCs), their functions.
25. NK cells, mechanisms of activation and microbial killing.
26. Innate and acquired immunity, general characteristics. Thymus-independent immune response.
27. Acquired immunity: T-dependent immune response, stages. Antigen processing and presentation.
28. Acquired immunity: activation and differentiation of T helper cells. Th1 and Th2 control of various types of immune reactions.
29. Activation of effector cells in acquired immune response. Mechanisms of pathogen elimination.
30. Primary and secondary immune response, their characteristics. Natural inhibition of immune response.
31. Active immunoprophylaxis. Vaccines and toxoids, their classification and characteristics. Modern vaccines and toxoids for prophylaxis of infectious diseases.
32. Passive immunotherapy. Immune antisera and immunoglobulins, their clinical use. Medical applications of monoclonal antibodies for laboratory diagnosis and treatment of diseases.

33. Immunopathology, classification. Immediate and delayed types of hypersensitivity, general characteristics and mechanisms.
34. Coombs & Gell classification of hypersensitivity reactions, their general characteristics.
35. Anaphylactic hypersensitivity. Allergy and allergic diseases, basic mechanisms and stages of development.
36. Cytotoxic hypersensitivity, mechanisms. Autoimmune diseases, triggered by these reactions.
37. Immune-complex-mediated hypersensitivity. Autoimmune diseases, stimulated by this type of reactions.
38. Cell-mediated (delayed) hypersensitivity. Skin tests for laboratory diagnosis of infectious allergy.
39. Stimulatory and blocking hypersensitivity. Autoimmune diseases, developed by these reactions.
40. Primary immunodeficiencies. Combined immunodeficiencies. T- and B cell immunodeficiencies.
41. Phagocyte and complement system immunodeficiencies. Secondary immunodeficiencies, their mechanisms.
42. Serological tests, their goals, advantages and clinical value. Classification of serological tests. Molecular mechanisms and conditions for serological reactions.
43. Precipitation tests, reagents and main goals. Variants of precipitation tests (ring precipitation, immune diffusion, immune electrophoresis). Single radial immunodiffusion (Mancini's test) for quantitation of immunoglobulin class.
44. Serological reactions – agglutination tests, their main goals. Mechanisms of agglutination. Slide agglutination and extended tube agglutination tests.
45. Indirect hemagglutination test. Reagents for indirect hemagglutination. Coombs` test, its variants. Reagents for Coombs` reaction.
46. Serological tests – immune lysis reactions. Complement fixation test, principle of analysis, reagents and medical applications.
47. Serological testing – reactions of toxin neutralization. Reagents and main goals.
48. Serological testing – reactions with labeled antibodies and antigens. Immunofluorescence assay, its variants and main applications.
49. Enzyme-linked immunosorbent assay (ELISA), its reagents, stages and applications in immunological diagnosis.
50. Radioimmunoassay. Western blotting analysis.
51. Immune status assessment – general characteristics. Humoral immunity evaluation.
52. Laboratory tests for quantitative analysis of immune cells. Immunofluorescence assay. Flow cytometry and automatic cell sorting.
53. Methods for assessment of functional activity of T- and B cells. Blast transformation test.

54. Laboratory testing of phagocytosis. Phagocyte number, phagocytic index. NBT test, its main goal.

THE LITERATURE:

1. Lecture contents.
2. “Medical Microbiology, Virology & Immunology: Lecture Course”. Part 1. I. I. Generalov, Vitebsk, 2016. P. 175-275.

LABORATORY CLASS №16

**The topic: Infection and infectious process. Epidemic process.
Pathogenicity and virulence.**

The main aim and the tasks of the work:

1. To learn the theoretical knowledge of the topic.
2. To know the principles of experimental infection (animal inoculation, or biological method).
3. To get skills of experimental infection on mouse model.
4. To get skills of post-mortal examination of infected experimental animals (mice).

The questions to the topic:

1. Infection (or infectious process), its types. Basic conditions for emergence of infectious process.
2. Characteristics of infectious diseases, their periods.
3. Various forms of infection, their characteristics. Classification of infections according to their origin, localization and spread, clinical manifestations. Reinfection, relapse, superinfection.
4. Microbial carrier state (microbial carriage).
5. Epidemic process. Conditions for epidemic process emergence and spread.
6. Mechanisms and routes of disease transmission, their characteristics.
7. Anthroponoses, zoonoses and sapronoses, their characteristics. Sporadic, epidemic, pandemic, endemic, outbreak of infectious diseases.
8. Pathogenicity and virulence, their characteristics. Measurement of virulence.
9. Adhesion and invasion of bacteria. Bacterial adhesins. Invasive enzymes. Injectisome, its role in bacterial invasiveness and pathogenesis of infections.
10. Bacterial endotoxins, their characteristics and molecular mechanism of action.
11. Bacterial exotoxins, their common properties. Classification of exotoxins.
12. Most active bacterial exotoxins, structure and mechanisms of action.
13. Systemic infectious process. Pathogenesis of sepsis. Laboratory diagnosis of sepsis.

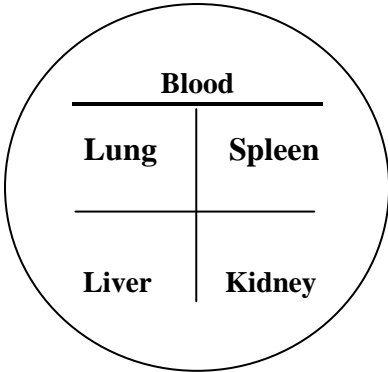
THE LITERATURE:

1. Lecture contents.
2. “Medical Microbiology, Virology & Immunology: Lecture Course”. Part 1. I. I. Generalov, Vitebsk, 2016. P. 160-173.

Personal work of students:

1. **Demonstration** of results from experimental infection of mice with *K. pneumoniae* culture:
 - 1.1. post-mortem examination of infected dead mice with bacteriological testing: plating of specimens from mouse inner organs on Petri dish with MPA.
 - 1.2. preparing of slides from animal organ specimens, fuchsin stain.
2. Assessment of microbial growth on Petri dishes.
3. Conclusion about the results of experimental infection.

Protocol № 1. Septicemia in mice resulted from experimental infection after intra-peritoneal bacterial inoculation

Day of examination	Material for examination	Steps of examination	Results
1.	<i>Klebsiella pneumoniae</i> culture	Intraperitoneal inoculation of mouse with bacterial culture in dose $1 \cdot 10^9$ cells per 0.5 ml of saline	—
2.	-	<p>Analysis of the results of experimental infection.</p> <p>Post-mortem examination of infected dead mice with isolation of bacterial culture: plating of mouse inner organ samples on Petri dish with MPA.</p> <div style="text-align: center;">  </div> <p>Preparing of slides from organ specimens, fuchsin stain, microscopy.</p>	
3.		Registering of microbial growth on Petri dish.	

Conclusion:

Themes for the reports on the lecture course

General Microbiology Section:

1. The history of the development of microbiology.
2. Modern methods of microscopic examination.
3. Methods of molecular genetic analysis.
4. Types of protein secretion systems.
5. The role of microorganisms in the development of the infectious process. Genetic control over the pathogenicity and virulence of bacteria
6. Antibiotics. The Mechanisms of Antibiotics Action.

Immunology Section:

1. Innate immunity.
2. Immunological tolerance.
3. Mechanisms of anti-infectious immunity.
4. Natural killer cells.
5. Cellular immune response.
6. Anti-tumor immunity.
7. Transplantation immunity.
8. Primary immunodeficiencies.
9. Secondary immunodeficiencies.
10. Drug allergy.
11. Immunoprophylaxis.
12. Immunotherapy.
13. Autoimmune diseases.

Учебное издание

Генералов Игорь Иванович
Фролова Ээлиа Валерьевна
Железняк Наталья Васильевна

INSTRUCTIONS FOR LABORATORY TRAINING
in General Microbiology & Immunology

for Students of Medical Faculty (English Medium)

МЕТОДИЧЕСКИЕ УКАЗАНИЯ К ЛАБОРАТОРНЫМ ЗАНЯТИЯМ
ПО ОБЩЕЙ МИКРОБИОЛОГИИ И ИММУНОЛОГИИ

для студентов лечебного факультета с английским языком обучения
высших медицинских учебных заведений

Редактор И.И. Генералов

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