Ministry of Health Care, Republic of Belarus

Vitebsk State Medical University

Department of Clinical Microbiology

INSTRUCTIONS

FOR LABORATORY TRAINING

in General Microbiology & Immunology
for Students of Faculty of Dentistry

VITEBSK 2014

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Generalov I.I.
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Instructions for laboratory training in General Microbiology and Immunology for students of Dentistry Faculty are prepared according to basic educational plan and program, approved by Ministry of Health Care of Republic of Belarus. The plan, schedule of practical training and basic practical skills in general microbiology and immunology are presented in this workbook.

The instructions are worked out for students of dentistry faculties of medical universities.

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CONTENTS

Laboratory classes №1

The topic: ***General acquaintance with microbiology laboratory. Safety rules of the work with pathogenic microbial agents (biological safety). Systematics and nomenclature of microorganisms. The morphology of bacteria. Principles of bright field microscopy. Simple methods of stain***

The main aim and the tasks of the work:

1. To make acquaintance with the safety rules of the work in microbiology laboratory.
2. To get skills of primary manipulations with bacterial cultures.
3. To get skills of making the slide with bacterial culture, and to stain it with simple staining method.
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.

THE LITERATURE:

1. I.I. Generalov. General Microbiology & Essential Immunology: Lecture Course. Vitebsk, 2006, p. 4-19, 28-29.
2. "Laboratory work in medical microbiology". A.Pavlovich, 1993, p. 4-12, 19-20.

SAFETY RULES OF THE WORK IN MICROBIOLOGY LABORATORY:

1. Practical training in microbiology 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 takes all necessary materials, reagents and microbial cultures from laboratory assistant before the beginning of the classes.
7. At the end of classes 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 classes 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. Microscopy of slides with streptococci (demonstration).

The basic steps of slide preparing for microscopy
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 classes №2

The topic: ***The morphology and ultra-structure of prokaryotes. 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, Neisser stain, Gins 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 the 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. Differential methods of stain (Gram stain, Gins capsule stain)
10. Flagella, methods for detection of bacteria motility. Pili and fimbria. Injectisome, its structure and function.

THE LITERATURE:
1. I.I. Generalov. General Microbiology & Essential Immunology: Lecture Course. Vitebsk, 2006, p. 19-26, 28-31.
2. "Laboratory work in medical microbiology". A.Pavlovich, 1993, p. 13-19.

Personal work of students:
1. Preparation of slides with bacterial mixture of *Sarcina flava* and *Escherichia coli*, Gram stain.
2. Microscopy of slides with broth culture of *Corinebacterium diphtheria* for detection of volutin granules, Neisser stain (demonstration).
3. Microscopy of slides with capsular bacteria (demonstration).
4. Laser scanning confocal fluorescent microscopy for detection of nucleoid in bacterial cells, auramine-propidium iodide stain (demonstration).
5. Drawing of slides.

Basic methods of differential stain to determine the tinctorial properties of bacteria

Gram stain
1. Prepare the slide with bacterial culture grown at liquid or solid medium as described in the topic of laboratory classes N1.
2. Put the filter paper impregnated with ***gentian violet*** (crystal violet, methyl violet) upon the fixed slide and thoroughly soak it with distilled water. Incubation with dye for 2 minutes
3. After incubation end remove the paper with gentian violet and add Lugol's iodine solution for 1 minute.
4. Add ethanol to cover the slide strictly for 30 seconds.
5. Wash the slide.
6. Counterstain with fuchsin solution for 1 minute.
7. Wash thoroughly and dry the slide.

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

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

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

Neisser stain for volutin granules

1. Prepare and fix the slide made from liquid or solid medium with microbial culture.
2. Stain the slide with Neisser methylene blue stain for 3-5 minutes.
3. After incubation add Lugol's iodine solution for 10-30 seconds.
4. Wash the slide.
5. Counterstain with chrysoidin or vesuvine (Bismarck brown) dye solution for 1 minute.
6. Wash thoroughly and dry the slide.

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

Ziehl-Neelsen stain to detect acid-fast bacteria

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

Acid-fast bacteria retain the red stain whereas all other bacteria are stained blue.

Ozheshko method for spore stain

1. Prepare the slide of spore-containing bacilli culture.
2. Before fixing put 0.5% solution of hydrochloric acid upon the slide and heat the slide on burner for 3-5 minutes.
3. After incubation wash the slide thoroughly with tap water.
4. Fix the slide.
5. Stain the slide with Ziehl-Neelsen method.
6. Wash thoroughly and dry the slide

Spores will stain ***red***, the ***vegetative part*** of microbial cell will be ***blue***.

Laboratory classes №3

The topic: ***The morphology and ultra-structure of prokaryotes and eukaryotes (continuation). Differential methods of stain. Bacteriological method of examination (isolation of microbial culture, 1st day)***

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 know the mechanisms and practical applications of differential methods of stain: Ozheshko stain, Ziehl-Neelsen stain, Romanowsky-Giemsa stain.
4. To get skills of microbial agar plating for isolating of pure bacterial culture.

The questions to the topic:
1. Spores, stages of sporulation, methods of detection. Ozheshko spore stain.
2. The morphology and structure of actinomycetes. Ziehl-Neelsen acid-fast stain.
3. The morphology and structure of spirochetes. Romanowsky-Giemsa stain.
4. The morphology and structure of rickettsiae.
5. The morphology and structure of chlamydiae.
6. The morphology and structure of mycoplasmas.
7. Classification and structure of fungi.
8. The morphology of mould and yeast fungi.

THE LITERATURE:
1. I.I. Generalov. General Microbiology & Essential Immunology: Lecture Course. Vitebsk, 2006, p. 26-30, 32-38.
2. "Laboratory work in medical microbiology". A.Pavlovich, 1993, p. 13-14, 20-23.

Personal work of students:
1. Preparation of slides with Sabouraud agar culture of *Candida* fungi, methylene blue stain.
2. Microscopy of slides with bacilli culture for detection of spores, Ozheshko stain (demonstration).
3. Microscopy of slides with *C. trachomatis* infected cells, methylene blue stain.
4. Microscopy of slides with various mould fungi (*Mucor mucedo, Aspergillus fumigatus*) – demonstration.
5. Laser scanning confocal fluorescent microscopy of *Penicillium chrysogenum* culture; auramine stain, 3D reconstruction (demonstration).

6. Drawing of slides.

7. Planting of microbial mixture on Petri dish with MPA for isolation of pure culture of bacteria. 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	—
2.			

Laboratory classes №4

The topic: ***Physiology of microorganisms. Nutrition of bacteria. Principles of bacterial culturing. Classification of nutrient media. Anti-microbial measures: sterilization, antisepsis, disinfection, asepsis. Isolation of pure culture of bacteria (2^{nd} 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. Mechanisms of bacterial trans-membrane transportation.
3. Methods of bacterial cultivation. Requirements to nutrient media.
4. Classification of nutrient media and their characteristics.
5. Isolation of bacterial pure culture: examination of morphological, tinctorial and cultural properties.
6 Sterilization, its purposes. Sterilizing factors.
7. Different methods of sterilization.
8. Antisepsis, definition. The basic requirements to antiseptic drugs.
9. Groups of antiseptics, mechanisms of action.

10. Disinfection, its main goal. Variants of disinfection.
11. Asepsis – definition and common principles.

THE LITERATURE:

1. I.I. Generalov. General Microbiology & Essential Immunology: Lecture Course. Vitebsk, 2006, p. 39-44, 69-72.
2. "Laboratory work in medical microbiology". A.Pavlovich, 1993, p. 29-41.

Personal work of students:

1. The acquaintance of students with laboratory equipment for disinfection and sterilization.
2. Demonstration of the basic steps of nutrient media making (melting, hardening, storage).
3. Individual preparation of MPA slants and MPA on Petri dishes.

4. Isolation of pure bacterial culture (2^{nd} day of examination).

Continuation of test protocol started at previous classes.

a) Description of bacterial growth, characteristics of colonies according to size, shapes, colour, surface, edges, consistency (*cultural properties*).

b) Inoculation of bacteria into slant agar.

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	—
2.		Examination of bacterial growth, characteristics of microbial colonies according to their cultural properties. Inoculation of bacteria taken from single colony onto slant agar for isolation of pure culture.	

Laboratory classes №5

The topic: ***Physiology of microorganisms. Growth and reproduction of bacteria. Bacterial pigments. Bacterial enzymes. Energy metabolism. Biological oxidation in bacteria: fermentation and respiration. Isolation of pure culture of bacteria (3^{rd} and 4^{th} days of examination)***

The main aim and the tasks of the work:
1. To learn the theoretical knowledge of the topic.
4. To know biochemical properties of bacteria.
5. To get acquaintance with the methods of anaerobic bacteria isolation.
6. To be able to identify isolated bacterial culture.

The questions to the topic:
1. Growth and reproduction of bacteria.
2. Bacterial pigments, their significance. Classification of pigments.
3. Enzymes of bacteria, their properties and classification. The role of enzymes in bacterial metabolism and pathogenicity.
4. Pathways of biological oxidation in bacteria. Fermentation.
5. Respiration in bacteria. Classification of bacteria according to the types of respiration.
6. Methods of anaerobic bacteria culturing.
7. Tests for bacterial carbohydrate hydrolysis and proteolytic activity. Determination of bacterial catalase and urease.

THE LITERATURE:
1. I.I. Generalov. General Microbiology & Essential Immunology: Lecture Course. Vitebsk, 2006, p. 44-55.
2. "Laboratory work in medical microbiology". A.Pavlovich, 1993, p. 41-44.

Personal work of students:

1. **Demonstrations:** bacterial pigments, methods for anaerobic bacteria culturing.

2. Isolation of pure bacterial culture (3rd day of examination).
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) Testing of microbial *biochemical* activity: inoculation of bacteria into Hiss media and meat-peptone broth (MPB) for detection of carbohydrate hydrolysis and proteolytic activity.
c) Determination of catalase activity in reaction with hydrogen peroxide.

3. Isolation of pure bacterial culture (4th day of examination).
a) Registering of bacterial biochemical activity according to the demonstration of plate biochemical tests.
e) Completion of testing protocol with final conclusion about the species of isolated microbial culture.

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, Gram stain. Determination of bacterial catalase activity. Testing of microbial biochemical properties: inoculation of bacteria into Hiss media and MPB for detection of carbohydrate hydrolysis and proteolytic activity.	
4.		Registration of bacterial biochemical activity	

Glucose	Maltose	Sucrose	Lactose	Mannitol	MPB	
					H_2S	Indole

Conclusion:	

Laboratory classes №6

The topic: ***Microbial genetics***

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. Operon, its structure.
2. Plasmids and episomes, their structure and function.
3. Mobile genetic elements of bacterial genome. Transposons, IS-elements.
4. Phenotypic bacterial variations. Modifications, their characteristics.
5. Genotypic variations in bacteria, their classification. Bacterial dissociation.
6. Mutations: classification, mechanisms and biological significance.
7. Recombinations in bacteria, general characteristics. Bacterial transformation, molecular mechanisms.
8. Tranduction in bacteria.
9. Bacterial conjugation
10. Methods of molecular genetic analysis. Molecular hybridization of nucleic acids.
11. Polymerase chain reaction. DNA and RNA sequencing.
12. Principles of genetic engineering. Practical applications of recombinant technologies in biology and medicine.

THE LITERATURE:
1. I.I. Generalov. General Microbiology & Essential Immunology: Lecture Course. Vitebsk, 2006, p. 91-113.
2. "Laboratory work in medical microbiology". A.Pavlovich, 1993, p. 56-61.

Original work of students:

Protocol №1. Transformation test of B. subtilis culture

Day of examination	Material for examination	Steps of examination	Results
1.	1. DNA of auxoautotrophic strain, capable of tryptophan synthesis. 2. Recipient culture of auxoheterotrophic *B. subtilis* strain.	Bacterial suspension of slant culture of *B. subtilis* is prepared by saline wash. 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). Test tubes are placed for incubation at 37°C for 30 min. After end of incubation inoculation of samples from both test tubes is elaborated on MPA medium without tryptophan.	—
Incubation at 37°C for 24 h			
2.		Assessment of transformation results.	
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 *E. coli* biovar *paracoli*	Bacterial suspension of *E. paracoli* 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 on Endo medium	—
Incubation at 37°C for 24 h			
2.		Characteristics 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 *P. vulgaris*. 2. Petri dish with MPA. 3. Petri dish with MPA supplemented with phenol.	One-streak plating of *P. vulgaris* culture is made upon Petri dish with phenol-supplemented MPA and control medium (non-modified MPA).	—
Incubation at 37°C for 24 h			
2.		Assessment of "swarming" microbial growth on experimental and control Petri dishes.	—
Conclusion:			

Laboratory classes №7

The topic: ***Microorganisms and the environment. Normal microflora of human body and its role. Microflora of oral cavity. Sanitary microbiology. Microflora of air and water. Sanitary control of microbial pollution of air and water***

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 and microflora of oral cavity.
3. To know the standards of sanitary state of water and air.
4. To get skills of sanitary testing of water and air quality.

The questions to the topic:

1. Microorganisms, inhabiting the environment. Microbial ecology, microbial communities, ecosystem, ecological variants.
2. Symbiosis, its forms. Antagonistic microbial relationships, their mechanisms. Types of microbial antagonism.
3. Normal microflora of human body, its role in human physiology and pathology. Microflora of skin and respiratory tract.
4. Microflora of human gut and urogenital tract, its role.

5. Microflora of oral cavity, general characteristics. Ontogenesis of normal oral microflora.
6. Microflora of saliva, tongue, dental plaque, and gingival crevice. Biological role of normal oral microbiota.
7. Dysbiosis (dysbacteriosis) in oral cavity, predisposing factors and progression. Prophylaxis and treatment of oral dysbiosis.
8. Model sanitary microorganisms, their common properties.
9. Microflora of water, sources of water pollution. Water quality characteristics. Total microbial count of water.
10. Model sanitary microorganisms for water. Laboratory testing of water sanitary state. Identification of total coliform and thermotolerant bacteria.
11. Microflora of air. Microbial standards for air quality. Methods for determination of air sanitary state.

THE LITERATURE:

1. I.I. Generalov. General Microbiology & Essential Immunology: Lecture Course. Vitebsk, 2006, p. 56-68.
2. I.I. Generalov. Medical Microbiology in Dentistry: Lecture Course. Vitebsk, 2014, p. 6-16.
3. "Laboratory work in medical microbiology". A.Pavlovich, 1993, p. 48-56, 193-195.

Personal work of students:

1. Microscopy of specimen from dental plaque, Gram stain.
2. Bacteriological sanitary testing of hand wash.

Protocol №1 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 *E. coli* determination.	—
Thermostat for 24 h at 44°C			
2.		Assessment of microbial growth in Kessler's medium.	—

Note: Composition of Kessler's medium: MPB, lactose, bile salts, gentian violet and float.
E. coli growth is indicated by gas accumulation within the float.

Laboratory classes №8

The topic: ***Final control study of the section "Morphology of bacteria. Physiology of bacteria. Microbial genetics. Sanitary microbiology"***

The main aim and the tasks of the work:
1. 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. Methods of bacterial culturing. Requirements to nutrient media. Classification of nutrient media and their characteristics.
25. Biological oxidation in bacteria. Pathways of fermentation and respiration.
26. Classification of bacteria according to their types of respiration.
27. Methods of anaerobic bacteria cultivation. Isolation of pure culture of anaerobes.
28. Bacterial enzymes, their properties and classification. The role of enzymes in bacterial metabolism and pathogenicity.
29. Examination of bacterial carbohydrate hydrolysis. Determination of proteolytic properties. Detection of catalase and urease activity of bacteria.
30. Growth and reproduction of bacteria.
31. Bacterial pigments, their significance. Classification of pigments.
32. Microorganisms, inhabiting the environment. Microbial ecology, microbial communities, ecosystem, ecological variants. Symbiosis, its forms. Antagonistic microbial relationships, their mechanisms. Types of microbial antagonism.
33. Normal microflora of human body, its role in human physiology and pathology. Microflora of skin, gut, respiratory, and urogenital tract, its role.
34. Microflora of oral cavity, general characteristics. Ontogenesis of normal oral microflora.
35. Microflora of saliva, tongue, dental plaque, and gingival crevice. Biological role of normal oral microbiota.
36. Dysbiosis (dysbacteriosis) in oral cavity, predisposing factors and progression. Prophylaxis and treatment of oral dysbiosis.
37. Model sanitary microorganisms, their common properties.
38. Microflora of water, sources of water pollution. Water quality characteristics. Total microbial count of water.
39. Model sanitary 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.
47. Plasmids and episomes, their structure and function.
48. Mobile genetic elements of bacterial genome. Transposons, IS-elements.
49. Phenotypic bacterial variations. Modifications, their characteristics.
50. Genotypic variations in bacteria, their classification. Bacterial dissociation.
51. Mutations: classification, mechanisms and biological significance.
52. Recombinations in bacteria, general characteristics. Bacterial transformation, molecular mechanisms.
53. Tranduction in bacteria.
54. Bacterial conjugation
55. Methods of molecular genetic analysis – molecular hybridization of nucleic acids. DNA and RNA sequencing.
56. Polymerase chain reaction.
57. Principles of genetic engineering. Practical applications of recombinant technologies in biology and medicine.

THE LITERATURE:

1. I.I. Generalov. General Microbiology & Essential Immunology: Lecture Course. Vitebsk, 2006, p. 4-72, 91-113.

Laboratory classes №9

The topic: ***Immunology and immunity. Types of immunity. Structure of immune system. Immune cell receptors (CD molecules). Cytokines. 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).
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. Immune system, its sub-systems and levels of organization. Central and peripheral immune organs.
4. CD molecules of immune cells, their significance.
5. Cytokines, the basic features and classification.
6. Interleukins, their biological role and functions.
7. Other cytokines (interferons, α-TNF, growth factors).
8. T cells, their development and differentiation. Structure of TCR, its function.
9. T cell subpopulations, their role.
10. B cells, their development and differentiation.
11. Laboratory tests for quantitative analysis of immune cells. Flow cytometry and automatic cell sorting.

THE LITERATURE:
1. I.I. Generalov. General Microbiology & Essential Immunology: Lecture Course. Vitebsk, 2006, p. 114-123, p. 150.

Personal work of students:
1. Microscopy and drawing of slides on demonstration.
   Slides for demonstration:
   a) T cell rosettes with sheep red blood cells (Romanowsky-Giemsa stain);
   b) immunofluorescence assay for B cell identification (laser scanning confocal microscopy)
2. Analysis of flow cytometry histogram for quantitation of immune cells.

Laboratory classes №10

The topic: ***Antigens. Infectious and non-infectious antigens. HLA system. Immunoglobulins and antibodies. Serological testing – the mechanisms and goals of serological reactions. Precipitation tests***

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 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, their properties and general characteristics. Haptens.
2. Main bacterial and viral antigens. Protective antigens, superantigens, antigenic mimicry.
3. Non-infectious antigens, their classification. Auto-antigens and alloantigens, general characteristics. Human blood group antigens.
5. HLA system, general characteristics. HLA molecules of I class, structure and functions.
6. HLA molecules of II class, structure and functions. Biological role of HLA system.
7. Immunoglobulins, molecular structure and functions.
9. Classes of immunoglobulins, their characteristics.
8. Biological activity of secretory IgA.
9. Antibodies. Mechanisms of antibody action.
10. Serological tests, their goals, advantages and clinical value. Classification of serological tests. Molecular mechanisms and conditions for serological reactions.
11. Precipitation tests, reagents and main goal.
12. Variants of precipitation tests (ring precipitation, immune diffusion, immune electrophoresis). Single radial immunodiffusion (Mancini test) for determination of immunoglobulin concentrations.

THE LITERATURE:

1. I.I. Generalov. General Microbiology & Essential Immunology: Lecture Course. Vitebsk, 2006, p. 133-145, 151, 152-154, 159-161.
2. I.I. Generalov. Medical Microbiology in Dentistry: Lecture Course. Vitebsk, 2014, p. 20-21.
3. "Laboratory work in medical microbiology". A.Pavlovich, 1993, p. 71-72, 78-81.

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

Steps of the reaction:

- Put 1-2 ml of antiserum against human proteins on the bottom of test tube N1.

- 1 ml of blood extraction is laid very carefully upon the serum.
- The same manipulation should be made for the test tube N2, where antiserum against chicken proteins is used.
- Incubation for about 5 min at room temperature. Ring of precipitation is to be formed.
- Draw the results and make the conclusion.

2. Demonstration of double immunodiffusion test and radial immunodiffusion test; assessment of the results of immune electrophoresis

Laboratory classes №11

The topic: ***Complement system. Classical, alternative and lectin pathways of activation. Serological testing – complement fixation test. Mononuclear phagocyte system. Granulocytes. Phagocytosis, its stages. Laboratory testing of phagocytosis. Dendritic cells, their functions. Toll-like receptors and pattern-based microbial recognition. Natural killer cells***

The main aim and the tasks of the work:
1. To learn the theoretical knowledge of the topic.
2. To get skills of elaboration and results reading of complement fixation test.
3. To acquire skills of laboratory testing of phagocytosis.

The questions to the topic:
1. Complement system. Classical pathway of activation.
2. Alternative and lectin pathways of complement activation.
3. Serological tests – immune lysis reactions. Complement fixation test, principle of analysis, reagents and medical applications.
4. Mononuclear phagocyte system, general characteristics and functions.
5. Granulocytes, their role in immune response.
6. Phagocytosis, its stages. Mechanisms of microbial killing by phagocytes.
7. Laboratory testing of phagocytosis. Phagocyte number, phagocytic index. NBT test, its main goal.
8. Dendritic cells, their functions. Toll-like receptors and pattern-based microbial recognition.
9. NK cells, mechanisms of activation and microbial killing.

THE LITERATURE:

1. I.I. Generalov. General Microbiology & Essential Immunology: Lecture Course. Vitebsk, 2006, p. 124-125, 126-132, 145-148, 151-152, 162-164.
2. I.I. Generalov. Medical Microbiology in Dentistry: Lecture Course. Vitebsk, 2014, p. 21-23, 25.
3. "Laboratory work in medical microbiology". A.Pavlovich, 1993, p. 69-70, 84-88.

Personal work of students:

1. Complement fixation test for determination of serum antibodies

Reagents	*Serum dilutions*							*Controls*		
	1:10	1:20	1:40	1:80	1:160	1:320	1:640	Hemol. system	Ag	Compl.
	1	2	3	4	5	6	7	8	9	10
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
Saline								0,15	0,05	0,1
Incubation	at 37°C 1 h or at 4°C 18-20 h									
Hemolytic system	0,1	0,1	0,1	0,1	0,1	0,1	0,1	0,1	0,1	0,1
Incubation	at 37°C 1 h									
Results:										
Conclusion:										

Results are registered after the assessment of control wells (positions NN 8, 9, 10).
Hemolysis absence indicates ***positive result*** of complement fixation test.

2. Microscopy and drawing of demonstration slides.

Slides for demonstration:

1. Complete phagocytosis of *Escherichia coli* (Romanowsky-Giemsa stain).
2. Incomplete phagocytosis of *Neisseria gonorrhoeae* (methylene blue stain).

Laboratory classes №12

The topic: ***Non-immune and immune defensive mechanisms in oral cavity. Humoral and cellular reactions of innate immunity. Acquired immunity: T-dependent and T-independent immune responses. Primary and secondary immune response. Serological reactions – agglutination tests. Indirect hemagglutination assay. Coombs` test***

The main aim and the tasks of the work:

1. To learn the theoretical knowledge of the topic.
2. To know the mechanisms of innate and acquired immune response in oral cavity.
3. To get acquaintance with basic agglutination tests and their medical applications.
4. To know the reagents for agglutination tests – agglutinating sera, microbial diagnosticums, erythrocyte diagnosticums.
5. Laboratory training of slide agglutination and tube agglutination tests and indirect hemagglutination assay.

The questions to the topic:

1. Non-specific defensive factors of oral cavity. Humoral innate immunity.
2. Cellular reactions of innate immune response in protection of oral cavity.
3. Acquired immunity, general characteristics. Thymus-independent immune reactions.
4. Acquired immunity: T-dependent immune response, stages. Antigen processing and presentation.
5. Acquired immunity: activation and differentiation of T helper cells. Th1 and Th2 control of various types of immune reactions.
6. Activation of effector cells. Mechanisms of pathogen elimination.
7. Natural inhibition of immune response.
8. Primary and secondary immune response, their characteristics.
9. Serological reactions – agglutination tests, their main goals. Mechanisms of agglutination. Slide agglutination and extended tube agglutination tests.
10. Indirect hemagglutination test. Reagents for indirect hemagglutination.
11. Coombs` test, its variants. Reagents for Coombs` reaction.

THE LITERATURE:

1. I.I. Generalov. General Microbiology & Essential Immunology: Lecture Course. Vitebsk, 2006, p. 129-130, 155-159, 168.
2. I.I. Generalov. Medical Microbiology in Dentistry: Lecture Course. Vitebsk, 2014, p. 17-30.
3. "Laboratory work in medical microbiology". A.Pavlovich, 1993, p. 72-78.

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

2. Demonstration of extended tube agglutination test for microbial species identification.

3. Indirect hemagglutination test for serological diagnosis of disease *(determination of specific antibodies titer in patient's serum)*

Reagents	Serum dilutions				
	1:20	1:40	1:80	1:160	Control
	1	2	3	4	5
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, 1 h				
Results:					
Conclusion:					

Laboratory classes №13

The topic: ***Active and passive immunoprophylaxis and immunotherapy. Vaccines and toxoids. Immune antisera and immunoglobulins. Monoclonal antibodies, their practical use. Serological testing – reactions of toxin neutralization. Reactions with labeled antibodies and antigens. Immunofluorescence assay. Enzyme-linked immunosorbent assay. Radioimmunoassay. Immunoblotting (western blotting). Laboratory testing of immune status. Assessment of cellular and humoral immunity***

The main aim and the tasks of the work:
1. To learn the theoretical knowledge of the topic.

2. To know the drugs for immunoprophylaxis and immunotherapy.
3. To get acquaintance with hybridoma technology.
4. To know the basic methods for immune status assessment.
5. To get skills of interpretation of blast transformation test.
6. To know the reagents for ELISA test and immunofluorescence assay.
7. To perform serological testing of unknown antibodies by enzyme-linked immunosorbent assay and data analysis with microplate reader.

The questions to the topic:

1. Active immunoprophylaxis. Vaccines and toxoids, their classification and characteristics. Modern vaccines and toxoids for prophylaxis of infectious diseases.
2. Passive immunotherapy. Immune antisera and immunoglobulins, their clinical use.
3. Monoclonal antibodies (mAbs). Stages of hybridoma technology. Medical applications of mAbs for laboratory diagnosis and treatment of diseases. Humanized monoclonal antibodies.
4. Serological testing – reactions of toxin neutralization. Reagents and main goals.
5. Serological testing – reactions with labeled antibodies and antigens. Immunofluorescence assay, its variants and main applications.
6. Enzyme-linked immunosorbent assay (ELISA), its reagents, stages and applications in immunological diagnosis.
7. Radioimmunoassay.
8. Western blotting analysis.
9. Immune status assessment – general characteristics. Humoral immunity evaluation.
10. Methods for assessment of quantity and functional activity of T- and B cells. Blast transformation test.

THE LITERATURE:

1. I.I. Generalov. General Microbiology & Essential Immunology: Lecture Course. Vitebsk, 2006, p. 144-145, 149-151, 161-162, 164-167, 179-182.
2. "Laboratory work in medical microbiology". A.Pavlovich, 1993, p. 90-93, 101-105.

Personal work of students:

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

	1	2	3	4	5	6	7	8	9	10	11	12
A											$K_1(-)$	$K_2(-)$
B											$K_1(+)$	$K_2(+)$
C												
D												
Conclusion:												

Reading of results is performed by microplate colorimetric reader at $\lambda=450$ *nm*.
The optical density (OD) for positive results must exceed previously established cut-off values.
Cut-off values are calculated as (*mean* $OD_{K(-)}$ + *0,1 OD*) where $OD_{K(-)}$ indicates the values of optical density of negative controls (wells A11 and A12).
Mean value of optical density of positive controls (wells B11 and B12) should be 3 times more than optical density of negative controls.

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

Laboratory classes №14

The topic: ***Immunopathology. Immediate and delayed types of hypersensitivity. Coombs & Gell classification of hypersensitivity reactions. Anaphylactic hypersensitivity. Allergy and allergic diseases. Cytotoxic hypersensitivity. Immune-complex-mediated hypersensitivity. Cell-mediated (delayed) hypersensitivity. Skin tests for laboratory diagnosis of infection allergy. Stimulatory and blocking hypersensitivity. Autoimmune diseases. Primary and secondary immunodeficiencies***

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 be able to read and interpret the results of enzyme-linked immunosorbent assay (ELISA) for determination of IgE allergen-specific antibodies.

4. To be able to evaluate the results of allergic skin tests.

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 infection 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.

THE LITERATURE:

1. I.I. Generalov. General Microbiology & Essential Immunology: Lecture Course. Vitebsk, 2006, p. 169-178.
2. "Laboratory work in medical microbiology". A.Pavlovich, 1993, p. 98-100.

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											$K_1(-)$	$K_2(-)$
B											$K_1(+)$	$K_2(+)$
C												
D												
Conclusion:												

Reading of results is performed by microplate colorimetric reader at λ=*450 nm*.

The optical density (OD) for positive results must exceed previously established cut-off values.

Cut-off values are calculated as (*mean* $OD_{K(-)}$ + *0,2 OD*) where $OD_{K(-)}$ indicates the values of optical density of negative controls (wells A11 and A12).

Mean value of optical density of negative controls (wells A11 and A12) should be equal or less than 0.2 OD units.

Mean value of optical density of positive controls (wells B11 and B12) should be 3 times more than optical density of negative controls.

Laboratory classes №15

The topic: ***Final control study of the section "Immunology and immunity. Immunological laboratory testing. Immunopathology. Immunoprophylaxis. Immunotherapy"***

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. Classes 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. Complement system. Classical pathway of activation.
20. Alternative and lectin pathways of complement activation.
21. Mononuclear phagocyte system, general characteristics and functions. Granulocytes, their role in immune response.
23. Phagocytosis, its stages. Mechanisms of microbial killing by phagocytes.
24. Dendritic cells, their functions. Toll-like receptors and pattern-based microbial recognition. NK cells, mechanisms of activation and microbial killing.
25. Non-specific defensive factors of oral cavity. Humoral reactions of innate immunity.
26. Cellular reactions of innate immune response in protection of oral cavity.
27. Acquired immunity, general characteristics. Thymus-independent immune reactions. 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. Conventional vaccines and toxoids for prophylaxis of infectious diseases (examples).
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 infection 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 test) for determination of immunoglobulin concentrations.
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.
51. Western blotting analysis.
52. Immune status assessment – general characteristics. Humoral immunity evaluation.
53. Laboratory tests for quantitative analysis of immune cells. Immunofluorescence assay. Flow cytometry and automatic cell sorting.

54. Methods for assessment of functional activity of T- and B cells. Blast transformation test.
55. Laboratory testing of phagocytosis. Phagocyte number, phagocytic index. NBT test, its main goal.

THE LITERATURE:
1. I.I. Generalov. General Microbiology & Essential Immunology: Lecture Course. Vitebsk, 2006, p. 114-182.
2. I.I. Generalov. Medical Microbiology in Dentistry: Lecture Course. Vitebsk, 2014, p. 17-30.

Demonstration materials: indirect hemagglutination test, enzyme-linked immunosorbent assay (ELISA), complement fixation test, extended microbial agglutination test, reagents for immunological laboratory diagnosis, drugs for immunoprophylaxis and immunotherapy.

Laboratory classes №16

The topic: Infection and infectious process. Epidemic process. Microbial pathogenicity and virulence. Virulence factors. Systemic bacterial infection (sepsis)

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 infectious process emergence.
2. Characteristics of infectious diseases, their periods.

3. Different forms of infections, their characteristics. Classification of infections according to their origin, localization and spread, clinical manifestations. Reinfection, relapse, superinfection.
4. Carrier state characteristics.
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. Bacteremia, septicemia. Definitions for systemic inflammatory response syndrome (SIRS) and sepsis. Stages of systemic bacterial infection. Pathogenesis of odontogenic sepsis.

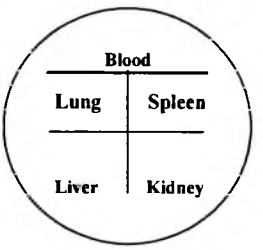
THE LITERATURE:

1. I.I. Generalov. General Microbiology & Essential Immunology: Lecture Course. Vitebsk, 2006, p. 183-195.
2. I.I. Generalov. Medical Microbiology in Dentistry: Lecture Course. Vitebsk, 2014, p. 48-51.
3. "Laboratory work in medical microbiology". A.Pavlovich, 1993, p. 63-68.

Personal work of students:

1. Experimental infection of mice with *K. pneumonia* culture.
2. Post-mortem examination of infected dead mice with bacteriological testing: plating of specimens from mouse inner organs on Petri dish with MPA.
3. Preparing of slides from animal organ specimens, fuchsin stain.
4. Assessment of microbial growth on Petri dishes.
5. Conclusion about the results of experimental infection.

Protocol № 1. Septicemia in mice resulted from experimental infection after intraperitoneal bacterial inoculation

Day of examination	Material for examination	Steps of examination	Results
1.	*Klebsiella pneumoniae* culture	Intraperitoneal inoculation of mouse with bacterial culture in dose $1*10^9$ cells per 0.5 ml of saline	–
2.	-	Analysis of the results of experimental infection. Post-mortem examination of infected dead mice with isolation of bacterial culture: plating of mouse inner organ samples on Petri dish with MPA.  Preparing of slides from organ specimens, fuchsin stain, microscopy.	
3		Registering of microbial growth on Petri dish.	
Conclusion:			

Laboratory classes №17

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. Chemotherapy. Classification of biocides. 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, lincosamide group, tetracyclines, aminoglycosides.
7. Antimicrobial action by inhibition in nucleic acid synthesis: quinolones, rifampicin, sulfonamides, trimethoprim.
8. Side effects of antibiotics.
9. Resistance to antimicrobial drugs, its mechanisms. Non-genetic and genetic (chromosomal and extrachromosomal) resistance. Prevention of drug resistance.
10. Measurement 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. I.I. Generalov. General Microbiology & Essential Immunology: Lecture Course. Vitebsk, 2006, p. 73-83.

Personal work of students:

1. Disc diffusion test for determination of S. aureus resistance to antibiotics.

Day of examination	Material for examination	Steps of examination	Results
1.	*Staphylococcus aureus* ($1*10^9$ cells/ml)	Plating of material on Petri dish with MPA. Placement of disks with antibiotics on Petri dish	—
Incubation at 37°C for 24 h			
2.		Assesment of microbial susceptibility testing: measurement of diameters of growth inhibition zones.	
Conclusion: *Staphylococcus aureus* strain is susceptible to...			

2. Measurement of microbial resistance to antibiotic with broth dilution test.

	1	2	3	4	5	6	7
Reagents	**Final antibiotic concentration, mkg/ml**						
	32	**16**	**8**	**4**	**2**	**1**	**K**
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:							
Conclusion:							

3. Demonstration of agar dilution test for assessment of microbial resistance to antibiotics.

Laboratory classes №18

The topic: ***Causative agents of suppurative infections. Staphylococci, pseudomonads, bacteroids, and related agents***

The main aim and the tasks of the work:

1. To learn the properties, and the role of various staphylococcal species in human pathology.
2. To get skills of laboratory diagnosis of staphylococcal infections.
3. To know the properties of pseudomonads and bacteroids, their role in human suppurative infections.
4. To know the principles of laboratory diagnosis of infections, caused by pseudomonads and bacteroids.

The questions to the topic:

1. Classification, structure and properties of staphylococci.
2. Virulence factors of staphylococci.
3. Pathogenesis and clinical findings in staphylococcal infections.
4. Laboratory diagnosis of staphylococcal infections, specific prophylaxis and treatment.
5. *Pseudomonas aeruginosa*: classification, structure and properties.
6. Pathogenesis and clinical findings in infections caused by *Pseudomonas aeruginosa*. Laboratory diagnosis, prophylaxis and treatment.
7. Classification of pathogenic gram-negative non-sporeforming anaerobes. Structure and properties of bacteroids, prevotellae, porphyromonads.
8. Pathogenesis and clinical findings in bacteroidal infections. Laboratory diagnosis, prophylaxis and treatment.

THE LITERATURE:

1. Lecture material.
2. "Special Microbiology & Medical Virology: Lecture Course". I. I. Generalov, 2005, p. 5-17.
3. "Laboratory work in medical microbiology". A. Pavlovich, 1993, p. 109-111.

Personal work of students:

1. Laboratory examination of pus

Day of examination	Material for examination	Steps of examination	Results
1.	The pus taken from patient's abscess.	Microscopy of Gram-stained slide from pus sample. Plating of pus on blood agar and yolk-salt agar.	—
2.		Assessment of microbial growth on blood agar and yolk-salt agar. Inoculation of material from hemolytic lecithinase-positive colony upon slant agar.	
3.		Determination of catalase and coagulase activity of isolated culture. Mannitol fermentation testing. Antibiotic susceptibility testing of isolated culture.	
4.		Assessment of mannitol fermentation test. Evaluation of the results of antibiotic susceptibility test.	1. _____mm. 2. _____mm. 3. _____mm. 4. _____mm. 5. _____mm.
Conclusion: