INSTRUCTIONS
FOR LABORATORY TRAINING
in General Microbiology & Immunology
for students of medical faculty

VITEBSK 2018
Printed according to the decision of Central Educational Council of VSMU
(protocol № 9, 25.10.2017)

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

Generalov I.I.

Instructions for laboratory training in General Microbiology and Virology for students
of the Faculty of General Medicine. / I.I. Generalov, N.V. Zheleznyak,

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.

© Generalov I.I., 2018
© VSMU Press, 2018
### CONTENTS

<table>
<thead>
<tr>
<th>Laboratory class №1</th>
<th>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</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Laboratory class №2</td>
<td>The topic: Morphology and ultra-structure of prokaryotes. Differential methods of stain</td>
<td>7</td>
</tr>
<tr>
<td>Laboratory class №3</td>
<td>The topic: Morphology and ultra-structure of prokaryotes and eukaryotes (continuation). Differential methods of stain. Bacteriological method of examination (isolation of microbial culture, 1\textsuperscript{st} day)</td>
<td>11</td>
</tr>
<tr>
<td>Laboratory class №5</td>
<td>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\textsuperscript{rd} and 4\textsuperscript{th} days of examination)</td>
<td>14</td>
</tr>
<tr>
<td>Laboratory class №6</td>
<td>The topic: Microbial genetics</td>
<td>16</td>
</tr>
<tr>
<td>Laboratory class №7</td>
<td>The topic: Microorganisms and the environment. Normal microflora of human body and its role. Sanitary microbiology. Microflora of air and water. Sanitary control of microbial pollution of air and water</td>
<td>18</td>
</tr>
<tr>
<td>Laboratory class №9</td>
<td>The topic: Immunology and immunity. Types of immunity. Structure of immune system. Immune cell receptors (CD molecules). Cytokines. Differentiation of T- and B cells</td>
<td>22</td>
</tr>
<tr>
<td>Laboratory class №10</td>
<td>The topic: Antigens. Infectious and non-infectious antigens. HLA system. Immunoglobulins and antibodies. Serological testing – the mechanisms and goals of serological reactions. Precipitation tests</td>
<td>24</td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laboratory class №15. The topic: Final control study of the section “Immunology and immunity. Immunological laboratory testing. Immunopathology. Immunoprophylaxis. Immunotherapy”</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laboratory class №17. The topic: Chemotherapy. Antibiotics. Antibiotic susceptibility testing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laboratory class №18. The topic: Causative agents of suppurative infections. Staphylococci, pseudomonads, bacteroids, and related agents</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Laboratory class №1


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.
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.

THE LITERATURE:

1. Lecture contents.

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 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 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, 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.
5. Bacterial envelope, its composition and function of different layers. Cytoplasmic membrane, its structure and function.
7. The cell wall of gram-negative bacteria. LPS, its functions.
8. Bacterial capsule, its structure and function.
11. Pili and fimbria. Injectisome, its structure and function.

**THE LITERATURE:**

1. Lecture contents.

**Personal work of students:**

1. Preparing of smears with mixture of *Sarcina flava* and *Escherichia coli* (Gram stain).

2. *Demonstration:*
   a) microscopy of smears with capsular bacteria (Gins capsule stain).
   
   b) microscopy of smears with spore-forming bacteria (Ozheshko stain).

4. 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. Ozeshko 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 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 get skills of microbial agar plating for isolation of pure bacterial culture.

The questions to the topic:
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.

Personal work of students:
1. Demonstration:
   a) microscopy of slides with C. trachomatis infected cells (methylen blue stain);
   b) microscopy of slides with various mould fungi (Mucor mucedo, Aspergillus fumigatus);
   c) microscopy of slides with of Candida fungi (methylen blue stain).
   d) laser scanning confocal fluorescent microscopy of Penicillium chrysogenum culture (auramine stain, 3D reconstruction).
2. Drawing of slides.


**Protocol №1. Isolation of pure culture of microorganisms**

<table>
<thead>
<tr>
<th>Day of examination</th>
<th>Material for examination</th>
<th>Steps of examination</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Mixture of bacteria</td>
<td>Plating of specimen on Petri dish with MPA. Incubation at 37°C for 24 h</td>
<td>—</td>
</tr>
</tbody>
</table>

**Laboratory class №4**


**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.
7. Different methods of sterilization.
8. Antisepsis, definition. The basic requirements to antiseptic drugs.
10. Disinfection, its main goal. Variants of disinfection.
11. Asepsis, definition and common principles.

THE LITERATURE:

1. Lecture contents.

Personal work of students:

1. The acquaintance of students with laboratory equipment for disinfection and sterilization.

2. Isolation of pure bacterial culture (2\textsuperscript{nd} 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.

   \emph{Protocol №1. Isolation of pure culture of microorganisms}

<table>
<thead>
<tr>
<th>Day of examination</th>
<th>Material for examination</th>
<th>Steps of examination</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.</td>
<td>—</td>
<td>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</td>
<td></td>
</tr>
</tbody>
</table>

3. \textbf{Demonstration} of the basic steps of nutrient media making (melting, hardening, storage).

4. Individual preparation of MPA slants and MPA on Petri dishes.
Laboratory class №5


**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:**

2. Microbial growth in biofilms.
3. Enzymes of bacteria, their properties and classification. The role of enzymes in bacterial metabolism and pathogenicity.
5. Respiration in bacteria. Classification of bacteria according to their types of respiration.

**THE LITERATURE:**

1. Lecture contents.
Personal work of students:

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

2. Isolation of pure bacterial culture (3\textsuperscript{rd} day of examination) – continuation of test protocol started at previous classes:

   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 (4\textsuperscript{th} 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.

**Protocol №1. Isolation of pure culture of microorganisms**

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

**Conclusion:**

<table>
<thead>
<tr>
<th>Glucose</th>
<th>Lactose</th>
<th>Mannitol</th>
<th>Sucrose</th>
<th>Maltose</th>
<th>MPB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Indole</td>
</tr>
</tbody>
</table>
Laboratory class №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. Regulation of gene expression. Operon, its structure.
2. Plasmids and episomes, their structure and function.
5. Phenotypic bacterial variations. Modifications, their characteristics.
7. Mutations: classification, mechanisms and biological significance.

THE LITERATURE:

1. Lecture contents.
Personal work of students:

**Protocol №1. Transformation test of B. subtilis culture**

<table>
<thead>
<tr>
<th>Day of examination</th>
<th>Material for examination</th>
<th>Steps of examination</th>
<th>Results</th>
</tr>
</thead>
</table>
| 1.                 | 1. DNA of auxo-autotrophic strain, capable of tryptophan synthesis.  
2. Recipient culture of auxo-heterotrophic *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 the end of incubation the samples from both test tubes are plated onto MPA without tryptophan. | Incubation at 37°C for 24 h |

**Conclusion:**

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

<table>
<thead>
<tr>
<th>Day of examination</th>
<th>Material for examination</th>
<th>Steps of examination</th>
<th>Results</th>
</tr>
</thead>
</table>
| 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 onto Endo medium | Incubation at 37°C for 24 h |

**Conclusion:**

2. Evaluation of transduction results.
### Protocol №3. Examination of modification test of P. vulgaris culture

<table>
<thead>
<tr>
<th>Day of examination</th>
<th>Material for examination</th>
<th>Steps of examination</th>
<th>Results</th>
</tr>
</thead>
</table>
| 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** | 

**Conclusion:**

---

**Laboratory class №7**

**The topic:** *Microorganisms and the environment. Normal microflora of human body and its role. Sanitary microbiology. Microflora of air, soil 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.  
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:**

2. Antagonistic microbial relationships, their mechanisms. Types of microbial antagonism.  
6. Sanitary indicator microorganisms, their characteristics.
8. Sanitary indicator microorganisms for water. Laboratory testing of water sanitary state. Identification of total coliform and thermotolerant bacteria.
10. Microflora of soil.

THE LITERATURE:

1. Lecture contents.

Personal work of students:

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

   Protocol №1. Bacteriological sanitary testing of hand wash

<table>
<thead>
<tr>
<th>Day of examination</th>
<th>Material for examination</th>
<th>Steps of examination</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Hand wash</td>
<td>Sampling of hand wash by sterile swab soaked with saline.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inoculation of specimen into Kessler’s medium for <em>E. coli</em> determination.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Incubation at 44°C for 24 h</em></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td>Assessment of microbial growth in Kessler’s medium.</td>
<td></td>
</tr>
</tbody>
</table>

*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.
Laboratory class №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:

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:

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.
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.
24. Secretion systems for transport of proteins and other substances outside the bacterial cells.
27. Classification of bacteria according to their types of respiration and their characteristics.
29. Bacterial enzymes, their properties and classification. The role of enzymes in bacterial metabolism and pathogenicity.
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.
35. Microflora of oral cavity, gut, respiratory, and urogenital tract; role in physiology and pathology.
37. Sanitary indicator microorganisms, their common properties.
39. Sanitary indicator microorganisms for water. Laboratory testing of water sanitary state. Identification of total coliform and thermotolerant bacteria.
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.
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.
50. Mutations: classification, mechanisms and biological significance.
52. Transduction in bacteria.
53. Bacterial conjugation.
54. Genetic organization of adaptive immunity in bacteria – CRISPR/Cas system.
56. Polymerase chain reaction.

THE LITERATURE:

1. Lecture contents.

Laboratory class №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, 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.
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.

**Personal work of students:**

1. **Demonstration:**
   
   a) microscopy of slides with T cell rosettes with sheep red blood cells (Romanowsky-Giemsa stain);

   b) microscopy of slides with immunofluorescence assay for B cell identification (laser scanning confocal microscopy)

2. Drawing of slides.

3. Analysis of flow cytometry histogram for quantitation of immune cells.
Laboratory class №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’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:

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. Classes of immunoglobulins, their characteristics.
9. Genetic control of specificity and diversity of antibodies and T cell receptors (TCRs).
11. Precipitation tests, reagents and main goal.

THE LITERATURE:

1. Lecture contents.

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.
   5. Drawing of the results and making the conclusion.

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

Laboratory class №11


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.

**The questions to the topic:**

1. Humoral factors of non-specific (innate) immune response.
2. Complement system. Classical pathway of activation.
3. Alternative and lectin pathways of complement activation.
5. Mononuclear phagocyte system, general characteristics and functions.
7. Granulocytes, their role in immune response.
8. Macrophage and granulocyte system assessment. Phagocytic number, phagocytic index. NBT test, its main goal.

**THE LITERATURE:**

1. Lecture contents.

**Personal work of students:**

1. **Complement fixation test for determination of specific antibodies in patient’s serum**

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Serum dilutions</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1:10</td>
<td>1:20</td>
</tr>
<tr>
<td>Saline</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Patient serum</td>
<td>0,05</td>
<td>0,05</td>
</tr>
<tr>
<td>Antigen in working dose</td>
<td>0,05</td>
<td>0,05</td>
</tr>
<tr>
<td>Complement in working dose</td>
<td>0,05</td>
<td>0,05</td>
</tr>
</tbody>
</table>

**Incubation**

- Hemolytic system: Incubation at 37°C 1 h, or at 4°C 18-20 h

<table>
<thead>
<tr>
<th>Results**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

**Conclusion:**
**Results are tested after control probes evaluation (test tubes №№ 8, 9, 10). Hemolysis absence means positive complement fixation test result.**

2. **Demonstration:**
   a) microscopy of slides with complete phagocytosis of *Escherichia coli* (Romanowsky-Giemsa stain);
   b) microscopy of slides with incomplete phagocytosis of *Neisseria gonorrhoeae* (methylene blue stain).

3. Drawing of demonstration slides.

Laboratory class №12


**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 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; indirect hemagglutination assay.

**The questions to the topic:**

1. Pathogen-binding receptors and pattern-based recognition. Toll-like receptors in humans, their functions.
4. Inductive phase: activation and differentiation of T helper cells.
7. Primary and secondary immune response, their characteristics.

**THE LITERATURE:**

1. Lecture contents.

**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

   Drowing the results
   ![Image of agglutination results](image)

   ![Image of agglutination results](image)

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)

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Serum dilutions</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1:20</td>
<td>1:40</td>
</tr>
<tr>
<td>Saline</td>
<td>0,1</td>
<td>0,1</td>
</tr>
<tr>
<td>Patient`s serum diluted 1:10</td>
<td>0,1</td>
<td>0,1</td>
</tr>
<tr>
<td>Erythrocyte diagnosticum</td>
<td>0,1</td>
<td>0,1</td>
</tr>
</tbody>
</table>

   **Incubation at 37°C, 1 h**

   **Results:**

   **Conclusion:**
Laboratory class №13


The main aim and the tasks of the work:

1. To learn the theoretical knowledge of the topic.
2. To know biological products 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 specific antibodies by enzyme-linked immunosorbent assay and data analysis by 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.
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.
THE LITERATURE:

1. Lecture contents.

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

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>K₁(–)</td>
<td>K₂(–)</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>K₁(+)</td>
<td>K₂(+)</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Conclusion:**

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 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.

2. Demonstration: the assessment of lymphocyte blast transformation test (orcein stain).
Laboratory class №14


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.
7. Stimulatory and blocking hypersensitivity. Autoimmune diseases, developed by these reactions.

THE LITERATURE:

1. Lecture contents.

**Personal work of students:**

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

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>K₁(−)</td>
<td>K₂(−)</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>K₁(+)</td>
<td>K₂(+)</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Conclusion:**

Reading of results is performed by microplate colorimetric reader at $\lambda=492$ nm.

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

Cut-off values are calculated as $(\text{mean } OD_{K(-)} + 0.3 \times OD)$ 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 class №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.
10. Main bacterial and viral antigens. Protective antigens, superantigens, antigenic mimicry.
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.
15. Classes of immunoglobulins, their characteristics.
16. Structure and biological activity of secretory IgA.
19. Genetic control of specificity and diversity of antibodies and T cell receptors (TCRs)
22. Mononuclear phagocyte system, general characteristics and functions. Granulocytes, their role in immune response.
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.
28. Acquired immunity: activation and differentiation of T helper cells. Th1 and Th2 control of various types of immune reactions.
30. Primary and secondary immune response, their characteristics. Natural inhibition of immune response.
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.
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 classes.
44. Serological reactions – agglutination tests, their main goals. Mechanisms of agglutination. Slide agglutination and extended tube agglutination tests.
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.
52. Laboratory tests for quantitative analysis of immune cells. Immunofluorescence assay. Flow cytometry and automatic cell sorting.
54. Laboratory testing of phagocytosis. Phagocyte number, phagocytic index. NBT test, its main goal.

THE LITERATURE:

1. Lecture contents.

Laboratory class №16


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).
6. Mechanisms and routes of disease transmission, their characteristics.
7. Anthroponoses, zoonoses and sapronoses, their characteristics. Sporadic, epidemic, pandemic, endemic, outbreak of infectious diseases.
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.

THE LITERATURE:

1. Lecture contents.

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.


6. Conclusion about the results of experimental infection.
Protocol № 1. Septicemia in mice resulted from experimental infection after intra-peritoneal bacterial inoculation

<table>
<thead>
<tr>
<th>Day of examination</th>
<th>Material for examination</th>
<th>Steps of examination</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Klebsiella pneumoniae</em> culture</td>
<td>Intraperitoneal inoculation of mouse with bacterial culture in dose $1 \times 10^9$ cells per 0.5 ml of saline</td>
<td>—</td>
</tr>
<tr>
<td>2.</td>
<td>-</td>
<td>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.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Preparing of slides from organ specimens, fuchsin stain, microscopy.</td>
<td></td>
</tr>
</tbody>
</table>

Conclusion:

Laboratory class №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. 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.

THE LITERATURE:

1. Lecture contents.

Personal work of students:

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

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

<table>
<thead>
<tr>
<th>Day of examination</th>
<th>Material for examination</th>
<th>Steps of examination</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Staphylococcus aureus</em> (1*10^9 cells/ml)</td>
<td>Plating of material on Petri dish with MPA. Placement of disks with antibiotics on Petri dish</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Incubation at 37°C for 24 h</em></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td>Assessment of microbial susceptibility testing: measurement of diameters of growth inhibition zones.</td>
<td></td>
</tr>
</tbody>
</table>
Conclusion: Isolate of *Staphylococcus aureus* is susceptible to…

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

<table>
<thead>
<tr>
<th>Reagents</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Final antibiotic concentration, mkg/ml</strong></td>
<td>32</td>
<td>16</td>
<td>8</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>K</td>
</tr>
<tr>
<td>Meat-peptone broth</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Antibiotic (initial concentration – 64 mkg/ml)</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Microbial culture</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>One loop of material is inoculated in all test tubes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Incubation at 37ºC for 24 h</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Conclusion:

Laboratory class №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.

7. Classification of pathogenic gram-negative non-sporeforming anaerobes. Structure and properties of bacteroids, prevotellae, porphyromonads.


9. Other representatives of nonfermenting gram-negative bacteria – *Acinetobacter baumannii* and *Stenotrophomonas maltophilia*.

**THE LITERATURE:**

1. Lecture contents.


**Personal work of students:**

1. *Laboratory investigation of pus*.

<table>
<thead>
<tr>
<th>Day of examination</th>
<th>Material for examination</th>
<th>Steps of examination</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>The pus taken from patient’s abscess.</td>
<td>Microscopy of Gram-stained slide from pus sample. Plating of pus on blood agar and yolk-salt agar.</td>
<td>—</td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td>Assessment of microbial growth on blood agar and yolk-salt agar. Inoculation of material from hemolytic lecithinase-positive colony upon slant agar.</td>
<td></td>
</tr>
</tbody>
</table>

**Conclusion:**

Учебное издание

Генералов Игорь Иванович
Железняк Наталья Васильевна
Фролова Аэлита Валерьевна

INSTRUCTIONS FOR LABORATORY TRAINING
in General Microbiology & Immunology
for Students of Medical Faculty
(English Medium)

МЕТОДИЧЕСКИЕ РЕКОМЕНДАЦИИ К ЛАБОРАТОРНЫМ ЗАНЯТИЯМ
ПО ОБЩЕЙ МИКРОБИОЛОГИИ И ИММУНОЛОГИИ
Методические рекомендации

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

Редактор И.И. Генералов
Технический редактор И.А. Борисов

Подписано в печать
Формат бумаги 64х84 1/16 Бумага типографская №2.
Гарнитура ТАЙМС. Усл. печ. листов . Уч.-изд. л
Тираж экз. Заказ № .

Издатель и полиграфическое исполнение:
УО «Витебский государственный медицинский университет»
Пр. Фрунзе, 27, 210602, г. Витебск