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

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

VITEBSK 2004
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Methodics instructions for practical training in General Microbiology and Immunology for students of medical faculty are prepared according to basic educational plan and program, proved 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 these methodics instructions.

The instructions are worked out for students of medical faculties of higher medical educational institutions.

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Practical classes №1

The topic: acquaintance with the equipment of microbiological laboratory. The rules of the work in the laboratory. Systematics and nomenclature of microorganisms. The morphology of bacteria. Microscopic method of examination. Simple methods of staining

The main aim and the tasks of the work:
1. To make acquaintance with rules of the work in microbiological laboratory.
2. To know the work of the light biological microscope.
3. To make acquaintance with rules of microbe culture manipulations.
4. To learn how to prepare the smear, to stain it with simple method, how to perform microscopy.

The questions to the lesson:

1. The subject and main tasks of microbiology.
2. Basic historical stages in microbiology development.
3. International classification and taxonomy of microorganisms.
4. Basic morphological forms of bacteria.
5. The methods of study of bacterial morphology and structure.
6. The stages of smear preparation from agar and broth microbial cultures.
7. Simple methods of staining.
8. The rules of microscope usage.
9. Immersion objective, its advantages.

THE LITERATURE:
2. Laboratory training course in microbiology. L.B.Borisov, 1984, p. 3-8, 10-11, 18-21, 24.

Rules of the work in microbiology laboratory:
1. Work in microbiology laboratory is performed with contagious material that requires discipline in the work.
2. The students must be in laboratory in white gowns and caps.
3. The person on duty takes all necessary material from laboratory assistant stuff before the beginning of the lesson.
4. At the end of lesson the whole material must be returned to the table of the teacher. The student on duty delivers it to laboratory assistant.
5. While performing the practical work the students have not have any incidental thing on their tables.
6. The students are obliged to execute carefully all instructions of the teacher.
7. Each student gets for his work a microscope. He is responsible for it during all course of training. The students have to work carefully with microscope to stay it in working order.
8. If student break accidentally the test tube with contagious material, he is obliged to report about the matter to his teacher and to desinfect the working place together with him.
9. It is forbidden to have a meal and to drink in the lab.
10. At the end of the lesson any student is obliged to set in order his working place; then to deliver the whole material and preparations back to the student on duty; to wash his hands; to present the album with the protocols and drawings to teacher to sign it.

**Personal work of students:**

1. The preparation of smear of broth culture of staphylococci. Methylene blue staining.
2. The preparation of the smear of agar culture of Escherichia coli. Staining with fuchsin.
3. Demonstration microscopy of streptococci smears.

**The basic stages of smear preparation**
The preparation of the smear of agar culture:
1. Put a drop of saline by sterile microbiology loop on the surface of defatted glass.
2. Take the material from the agar and disperse it by the sterile loop in the drop of the saline.
3. Thoroughly distribute the culture on the glass in the circle with diameter about 2-2,5 cm.
4. Sterilize the loop in flame.
5. Dry the smear under room temperature or with the help of spirit lamp.
6. Fix the smear in the middle part of the flame three times.
7. Stain the smear with necessary staining.
8. Wash it with water.
9. Dry the smear.
10. Drop on the smear the immersion oil.
11. Begin the microscopy (objective – 90, ocular – 7-10).
Practical classes №2

The topic: The morphology and ultrastructure of prokaryots. Differential methods of staining

The main aim and the tasks of the work:
1. To master the theoretical knowledge of the topic.
2. To know the mechanisms and usage of differential methods of staining: Gram stain, Neisser stain, Ozheshko stain, Ziehl-Neelsen stain.

The questions to the lesson:
1. Structure of a bacterial cell (obligate and facultative structural components).
2. Methods for study of microbial cell structure.
3. Bacterial envelope, its composition and function of different layers.
4. Bacterial cell wall, its role and structure.
5. Bacterial capsule, its structure and function.
7. Flagella, methods of bacteria motility detection
7. Differential methods of staining (Gram stain, Neisser stain, Ozheshko spore stain, Ziehl-Neelsen acid-fast stain).

THE LITERATURE:

Personal work of students:

1. Preparation of smears of mixture of Sarcina flava et Escherichia coli, Gram stain.
2. Preparation of smears of yeasts broth culture for detection of volutin granules, Neisser stain.
3. Microscopy of smears with capsular bacteria (demonstration).
5. Drawing of smears.

Basic methods of differential stain to determine tinctorial properties of bacteria

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

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

**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 carbolic fuchsin upon the fixed smear, thoroughly soak it with distilled water and heat the slide upon spirit lamp until vapor appearance.
3. After incubation remove the paper and wash the smear with tape 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 are stained blue.

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

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

**Negative staining for capsule presence**
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 fuchsine 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.

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 iodine solution for 10-30 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.

Practical classes №3

The topic: The morphology and ultra-structure of prokaryotes (continuation). Differential methods of staining

The main aim and the tasks of the work:
1. To learn the theoretical knowledge of the topic.
2. To continue examination of the main morphology forms of bacteria (spirochetes, mycoplasmas, rickettsiae, chlamydiae, actinomycetes, fungi).

The questions to the topic:
1. Nucleoid, its structure, methods of examination.
2. Cytoplasm, ribosomes, cytoplasmatic membrane, their structure and function.
3. The morphology and structure of spirochetes.
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:
Personal work of students:

1. Preparation of smears of Sabouraud agar culture of Candida spp. yeasts, methylene blue stain.
2. The microscopy of smears with C. trachomatis infected cells, methylene blue stain.
3. The microscopy of smears with different mould fungi (Mucor mucedo, Aspergillus fumigatus, Penicillium chrysogenum) – demonstration.
4. Drawing of smears.

Practical classes №4

The topic: the final studies in the topic “Morphology and structure of prokaryotes and eukaryotes”

The main aim and the tasks of the work:
1. To learn the morphology and structure of microorganisms, methods of bacterial structure examination, simple and differential methods of staining, different methods of microscopy.

The questions to the lesson:
1. Systematics and nomenclature of microorganisms. The definition of species as the main taxonomy unit.
2. Characteristics of round (spherical) forms of bacteria.
5. Structure of a bacterial cell (obligate and facultative components).
7. Bacterial cell wall, its role and structure.
8. Bacterial capsule, its structure and function.
10. Flagella, pili, methods of bacteria motility detection.
17. Morphology and characteristics of spirochetes.
18. Morphology and characteristics of chlamydiae.
19. Morphology and characteristics of rickettsiae.
20. Morphology and characteristics of mycoplasmas.
22. Morphology and characteristics of yeasts fungi.
23. L. Pasteur, his outstanding contribution into microbiology science.
24. R. Koch, his work in microbiology.

THE LITERATURE:

Practical classes №5


The main aim and the tasks of the work:
1. To learn the theoretical knowledge of the topic.
2. To make acquaintance with equipment for microbial cultivation and for sterilization.
3. To know the classification and the composition of nutrient media.
4. To receive skills of microbial inoculation and agar plating for isolating of pure bacterial culture.

The questions to the topic:
1. Metabolism of bacteria. Classification of bacteria according to their nutrition type.
4. Classification of nutrient media.
5. Asepsis and antiseptics, their significance.
6. Classification of antiseptics. The requirements to antiseptic drugs.
7. Disinfection, its main goal. Variants of disinfection.
THE LITERATURE:

Personal work of students:

1. The acquaintance of students with apparatus for cultivation and sterilization of microorganisms.
2. The acquaintance with nutrient media and their preparation.
3. Preparation of agar slants and various media on Petri dishes.
4. Getting skills of microbial planting on Petri dish with MPA for pure culture isolation.
5. Protocol recording.

Protocol N1. 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 material on Petri dish with MPA</td>
<td>—</td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Practical classes №6


The main aim and the tasks of the work:
1. To learn the theoretical knowledge of the topic.
2. To get skills of evaluation of bacterial morphologic and cultural properties.
3. To get skills of inoculation of bacterial culture into the slant agar.

The questions to the topic:
2. Classification of bacteria according to the types of respiration.
5. Growth and reproduction of bacteria.
6. Isolation of pure culture of bacteria: examination of morphologic and cultural properties.

THE LITERATURE:

Personal work of students:

1. Isolation of pure bacterial culture (2\textsuperscript{nd} day of examination). Continuation of protocol from the previous lesson. Description of bacterial growth, characteristics of colonies according to size, form, colour, surface, edges, consistency (\textit{cultural properties}). Preparation of smears, fuchsin staining, microscopy (\textit{microbial morphology examination}). Inoculation of bacteria into slant agar.
2. Demonstration material: bacterial pigments, methods of anaerobes cultivation.

<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 material on Petri dish with MPA</td>
<td>—</td>
</tr>
<tr>
<td>2.</td>
<td>Examination of bacterial growth, characteristics of colonies according to their cultural properties. Preparation of smear of the colony, fuchsin staining, microscopy for microbial morphology examination. Inoculation of bacteria from separate colony onto slant agar for pure culture isolation</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Practical classes №7


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 skills how to identify isolated culture according to determined microbial properties.
4. To get skills how to perform hand wash for bacteriological examination.

The questions to the topic:
1. Bacterial enzymes, their properties and classification.
2. Role of enzymes and their significance.
5. Normal microflora of human body, its role in physiological processes and in pathology.
6. Dysbacteriosis, etiology, pathogenesis, symptoms, treatment and prophylaxis.

THE LITERATURE:

Personal work of students:

1. Isolation of pure bacterial culture (3rd day of examination). Continuation of protocol started at lesson N5.
   a) Examination of microbial growth on slant agar, evaluation of culture purity (preliminary visual examination, preparing of smears, Gram stain, microscopy for tinctorial properties evaluation).
   b) Biochemical properties evaluation: inoculation of material into Hiss media for saccharolytic enzymes estimation and into MPB for proteolytic activity determination. Catalase activity determination with hydrogen peroxide.
   c) Registration of bacterial biochemical activity using demonstration material. Finish the protocol and make conclusion about the species of isolated bacteria.
Registration of bacterial biochemical activity

<table>
<thead>
<tr>
<th>Glucose</th>
<th>Maltose</th>
<th>Saccharose</th>
<th>Lactose</th>
<th>Mannitol</th>
<th>MPB</th>
<th>H₂S</th>
<th>Indole</th>
</tr>
</thead>
</table>

2. Taking of hand wash performed with sterile napkin soaked with saline. Seeding into Kessler medium for *Escherichia coli* examination.

### Protocol N2. Microbiological examination 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>Taking of hand wash performed with sterile napkin soaked with saline. Inoculating into Kessler medium for <em>Escherichia coli</em> examination.</td>
<td>Thermostat for 24 h at 44°C.</td>
</tr>
</tbody>
</table>

*Note:* Composition of Kessler medium: MPB, lactose, bile salts, gentian violet and float. Estimation of *E. coli* growth is performed by gas accumulation in the float.

### Practical classes №8

**The topic:** *Microorganisms and the environment. Microflora of water and air. Sanitary examination of water and air*

**The main aim and the tasks of the work:**
1. To learn the theoretical knowledge of the topic.
2. To know the standards of sanitary state of water and air.
3. To get skills of sanitary testing of water and air quality.
4. To know the composition of Kessler and Endo media.

**The questions to the topic:**
1. Model sanitary microorganisms, their characteristics.
2. Microorganisms, inhabiting the environment. Microbial ecology, microbial communities, ecosystem, ecological variants.
3. Symbiosis, its variants.
4. Antagonistic microbial relationships, their mechanisms. Variants of antagonism.
8. Methods for determination of air sanitary state.

THE LITERATURE:

Personal work of students:

2. Completion of hand wash examination: evaluation of microbial growth on Kessler medium, plating on Endo medium, estimation of red colonies growth on Endo medium (demonstration), preparation of smear of red lactose-positive colony with Gram stain, oxydase testing.

Practical classes №9

The topic: the final studies in the topic “Physiology of bacteria. Sanitary microbiology”

The main aim and the tasks of the work:
1. To learn main physiological processes in bacterial cell (nutrition, respiration), methods of bacterial cultivation, principles of asepsis, antiseptics, disinfection, sterilization, methods for environmental sanitary state evaluation.

The questions to the lesson:
1. Metabolism of bacteria. Classification of bacteria according to their nutrition type.
5. Classification of bacteria according to the types of respiration.
13. Model sanitary microorganisms, their characteristics.
17. Dysbacteriosis, etiology, pathogenesis, symptoms, treatment and prophylaxis.

**Demonstration material:** Hiss media, MPB with indicators for proteolytic activity determination, anaerostat, Kitt-Tarozzi medium, ferric sulphite agar.

**THE LITERATURE:**

**Practical classes №10**

**The topic:** Immunology and immunity. Types of immunity. Structure of immune system. Development and differentiation of T- and B-cells. Mononuclear phagocytes system. Dendritic 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-, B-cells and macrophages development and differentiation.
3. To get skills of estimation of T-cells blood quantity with sheep red blood cells rosette formation.
4. To get skills of evaluation of smears with complete and incomplete phagocytosis.

The questions to the lesson:

1. Immunology and immunity. Innate, acquired, artificial, natural immunity.
2. Anti-infectious immunity, its forms.
3. Types of non-infectious immunity.
4. Immune system and its sub-systems. Central and peripheral immune organs.
5. CD-antigens, their significance.
6. Cytokines, their classification.
7. Interleukins, their biological role and functions.
8. Interferons and tumor necrosis factor cytokines.
9. T-cells, their development and differentiation.
10. T-cells subpopulations, their role.
11. B-cells, their development and differentiation.
13. Phagocytosis, its stages.
14. Antigen presentation and monokine secretion by phagocytes

Personal work of students:

1. Microscopy and drawing of demonstration smears.

Smears for demonstration:
1. Rosette-formation test of T-cells with sheep red blood cells.
2. Complete phagocytosis of *Escherichia coli*.
3. Incomplete phagocytosis of gonococci.

All smears are stained with Romanowsky-Giemsa stain.

THE LITERATURE:
Practical classes №11


The main aim and the tasks of the work:
1. To learn the theoretical knowledge of the topic.
2. To be able to perform tentative slide agglutination reaction, extended agglutination reaction with evaluation of reaction results.
3. To get skills of indirect hemagglutination reaction for serologic diagnosis (detection of antibodies in patient serum).
4. To know the usage and methods of obtaining of immunobiologic preparations: bacterial diagnosticums, erythrocytes diagnosticums, agglutination serum.

The questions to the topic:
1. Antigens, their properties.
2. Main bacterial and viral antigens.
3. Protective antigens, superantigens, antigenic mimicry.
5. Alloantigens. Human blood group antigen systems. HLA-antigens.
6. Reactions of immunity (serologic reactions), their purposes. Mechanism and conditions for serologic reactions.
7. Indirect hemagglutination tests, their goal. Reagents for indirect hemagglutination.

THE LITERATURE:

Personal work of students:

1. Tentative slide agglutination test for microbial species estimation.
Reagents: 1) unknown microbial culture
   2) agglutination sera for E. coli var. paracoli
   3) saline.
2. Demonstration of extended agglutination reaction for microbial species determination.
3. Hemagglutination reaction for antibody titer evaluation in patients' serum (serologic diagnosis).

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Serum dilutions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1:20</td>
</tr>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td><em>Saline</em></td>
<td>0,4</td>
</tr>
<tr>
<td><em>Patients' serum diluted 1:10</em></td>
<td>0,4</td>
</tr>
<tr>
<td><em>Erythrocyte diagnosticum</em></td>
<td>0,4</td>
</tr>
</tbody>
</table>

Thermostat 37°C, 1 h

Results:

Conclusion:

Practical classes №12

**The topic:** Immunity. Immunoglobulins and antibodies. Determination of antibodies in serologic reactions. Precipitation and neutralization reactions

The main aim and the tasks of the work:
1. To learn the theoretical knowledge of the topic.
2. To get skills how to perform ring precipitation reaction for protein species determination.
3. To know the main goal and technique of Mancini radial immunodiffusion test, double immunodiffusion test and immune electrophoresis.
4. To know the main goal and technique of bacterial toxigenicity determination.
5. To know how to obtain anti-toxic and precipitin sera.

The questions to the topic:
1. Immunoglobulins, their characteristics. Immunoglobulin classes.
4. Monoclonal antibodies, preparation steps, main medical and biological applications. 5. Precipitation reaction, reagents and main goal.
5. Variants of precipitation reactions (ring precipitation, immune diffusion, immune electrophoresis).
6. Reaction of toxin neutralization, different variants of reaction. Reagents and main goal.
8. Methods of precipitin and anti-toxic sera production, their usage.

THE LITERATURE:

Personal work of students:

1. Ring precipitation reaction for protein species determination.
   Reagents: 1) blood spot extract
   2) serum for precipitation of hen proteins
   3) serum for precipitation of human proteins.
   Reaction steps:
   o Put 1 ml of serum for precipitation of human proteins on the bottom of test tube N1.
   o 1 ml of blood extract is laid very carefully on the serum.
   o The same manipulation is made for the test tube N2, where serum for hen proteins precipitation is used.
   o Incubation for about 5 min at room temperature. Ring of precipitation is to be formed.
   o Draw the results and make the conclusion.

2. Demonstration of double and radial immune diffusion tests, immune electrophoresis evaluation

Practical classes №13


The main aim and the tasks of the work:
1. To learn the theoretical knowledge of the topic.
2. To get skills of complement fixation test management.
3. To know different immune fluorescent assay applications.
4. To know the use of enzyme-linked immunosorbent assay (ELISA) with result evaluation.
5. To know the main purposes of radioimmune assay method.
6. To learn principles and technique of western blotting.

The questions to the topic:
1. Primary and secondary immune response, their characteristics.
2. Complement system. Classic pathway of activation.
3. Alternative pathway of complement system activation.
4. Immune lysis reaction. Hemolysis reaction.
5. Complement fixation test, its technique and purposes.
6. Immune fluorescent assay, its variants and main applications.
7. Enzyme-linked immunosorbent assay (ELISA), its reagents, stages and applications in immune diagnostics.
9. Western blotting analysis.

THE LITERATURE:

Personal work of students:

1. Complement fixation test for serum antibodies determination

<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>Patient serum</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Antigen in working dose</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Complement in working dose</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Saline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incubation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemolytic system</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Incubation</td>
<td></td>
<td></td>
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<tr>
<td>Results:</td>
<td></td>
<td></td>
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<tr>
<td>Conclusion:</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Results are investigated after control tubes evaluation (test tubes NN 8, 9, 10). *Hemolysis absence* means positive complement fixation test result.

Practical classes №14


The main aim and the tasks of the work:
1. To learn the theoretical knowledge of the topic.
2. To know different methods of immune status evaluation.
3. To get skills of evaluation of blast transformation reaction.
4. To be able to evaluate the results of enzyme-linked immunosorbent assay (ELISA) for IgE allergen-specific antibodies determination.
5. To be able to evaluate the results of NBT-test.
6. To know the preparations for immunoprophylaxis and immunotherapy.

The questions to the topic:
1. Immune status evaluation. General characteristics. Humoral immunity evaluation.
6. Cytotoxic hypersensitivity, mechanisms of development. Autoimmune diseases, promoted by this reaction type.
7. Immune-complex-mediated hypersensitivity. Autoimmune diseases, evolved due to this mechanism.
9. Stimulatory and blocking hypersensitivity. Autoimmune diseases, developed by these reactions.
10. Primary immunodeficiencies. Combined immunodeficiencies T- and B-cell immunodeficiencies.
12. Active immunoprophylaxis. Vaccines, their classification and characteristics.

THE LITERATURE:


Personal work of students:

Demonstration
1. Estimation of lymphocyte blast transformation reaction.
2. Evaluation of enzyme-linked immunosorbent assay (ELISA) results for IgE allergen-specific antibodies determination.
3. NBT-test evaluation.

Practical classes №15

The topic: the final studies in the topic “Immunology and immunity. Immunodiagnostics. Immunopathology. Immunoprophylaxis. Immunotherapy”

The main aim and the tasks of the work:
To learn basic principles of immune system work in normalcy and pathology, main methods of immunodiagnostics, immunoprophylaxis and immunotherapy.

The questions to the topic:
1. Immunology and immunity. Innate, acquired, artificial, natural immunity.
2. Anti-infectious immunity, its forms. Forms of non-infectious immunity.
3. Immune system and its sub-systems. Central and peripheral immune organs.
4. CD-antigens, their significance.
5. Cytokines, their classification.
6. Interleukins, their biological role and functions.
7. Interferons and tumor necrosis factor group cytokines.
8. T-cells, their development and differentiation.
9. T-cells subpopulations, their role.
10. B-cells, their development and differentiation.
12. Phagocytosis, its stages.
18. Primary and secondary immune response, their characteristics.
22. Cell-mediated (delayed) hypersensitivity. Skin tests for infection allergy diagnostics. Stimulatory and blocking hypersensitivity. Autoimmune diseases, developed by these reactions.
23. Primary immunodeficiencies. Combined and T-cell immunodeficiencies.
25. Active immunoprophylaxis. Vaccines, their classification and characteristics.
28. Reactions of immunity (serologic reactions), their purposes. Mechanism, conditions and reagents for serologic reactions.
29. Indirect hemagglutination test, its goal. Reagents for indirect hemagglutination.
31. Precipitation reaction, reagents and main goal. Variants of precipitation reactions (ring precipitation, immune diffusion, immune electrophoresis). Reaction of toxin neutralization, different variants of reaction. Reagents and main goal.
33. Immune fluorescent assay, its variants and main applications.
34. Enzyme-linked immunosorbent assay (ELISA), its reagents, stages and usage in immune diagnostics.

THE LITERATURE:


Demonstration material: indirect hemagglutination test, enzyme-linked immunosorbent assay (ELISA) test, complement fixation test, extended microbial agglutination reaction, preparations for immunodiagnostics, immunoprophylaxis and immunotherapy.

Practical classes №16

The topic: Infection and infectious process. Epidemic process. Microbial pathogenicity and virulence. Virulence factors

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-mortem examination of infected experimental animals (mice).
The questions to the topic:

1. Infection (or infectious process), its types. Conditions for infectious process development.
2. Characteristics of infectious diseases, their periods.
3. Different forms of infections, their characteristics. Classification of infections according to their origin, localization and spreading, manifestation. Reinfection, relapse, superinfection.
4. Carrier state characteristics.
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 exotoxins, their characteristics, classification and mechanisms of action.
11. Bacterial endotoxins, their characteristics and mechanism of action.

THE LITERATURE:


Personal work of students:

1. Infection of mice with *K. pneumonia* culture.
2. Post-mortem examination of infected dead mice with bacteriology examination: plating of mouse inner organs material on Petri dish with MPA.
3. Preparing of smears of organ material, fuchsin stain.
5. Conclusion about the results of experimental infection.
Protocol N__. Microbial spread in mice during experimental infection after intraperitoneal 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>Klebsiella pneumoniae 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>Evaluation of the results of experimental infection.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post-mortem examination of infected dead mice with bacteriology examination: plating of mouse inner organs material on Petri dish with MPA.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Preparing of smears of organ material, fuchsin staining, microscopy.</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>Evaluation of microbial growth on Petri dish.</td>
<td></td>
</tr>
</tbody>
</table>

Conclusion:

Practical classes №17

The topic: *Chemotherapy. Antibiotics. Evaluation of microbial resistance to antibiotics*

The main aim and the tasks of the work:

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

The questions to the topic:

2. Antibiotics. Requirements to antibiotic preparations.
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), vancomycin, bacitracin.
5. Antimicrobial action by inhibition of cell membrane function: amphotericin B, polynenes, polymyxins.
6. Antimicrobial action by inhibition of protein synthesis: chloramphenicol, erythromycin group and azalides, lincomycin group, tetracyclines, aminoglycosides.
7. Antimicrobial action by inhibition in nucleic acid synthesis: quinolones, rifampicin, sulfonamides, trimethoprim.
8. Side effects of antibiotics.

THE LITERATURE:

Personal work of students:
1. **Disc diffusion test for determination of staphylococcal culture susceptibility 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>
</tbody>
</table>

**Incubation in thermostat at 37°C for 24 h**

2. Evaluation of culture susceptibility testing: measurement of growth inhibition zones diameter.

**Conclusion:** *Staphylococcus aureus* strain is susceptible to...

2. **Measurement of microbial susceptibility to antibiotics 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>
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<tr>
<td>128</td>
<td>1,0</td>
<td>1,0</td>
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<td>1,0</td>
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<td>1,0</td>
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</tr>
</tbody>
</table>

- Meat-peptone broth: 1,0 1,0 1,0 1,0 1,0 1,0 1,0
- Antibiotic (initial concentration - 256 mkg/ml): 1,0 1,0 1,0 1,0 1,0 1,0
- Microbial culture: One loop of material is inoculated in all test tubes

**Incubation in thermostat at 37°C for 24 h**

**Results:**

**Conclusion:**

3. **Demonstration of agar dilution test for measurement of microbial susceptibility to antibiotics.**

**Practical classes №18**

**The topic:** *Bacteriophages*

The main aim and the tasks of the work:
1. To learn the theoretical knowledge of the topic.
2. To get skills of bacteriophages titration in liquid nutrient medium.
3. To know the method of bacteriophages titration in solid nutrient medium.
4. To know the practical use of bacteriophages.

**The questions to the topic:**
1. Bacteriophages, their characteristics.
2. The morphology of bacteriophages.
3. Interaction of phages with bacterial cells.
5. Production of the phage culture.
7. Practical applications of phages in biology and medicine.

**THE LITERATURE:**

**Personal work of students:**
1. **Titration of bacteriophage on liquid nutrient medium**

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Bacteriophage dilutions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10^-1</td>
</tr>
<tr>
<td>Meat-peptone broth</td>
<td>4,5</td>
</tr>
<tr>
<td><em>E. coli</em> bactériophage</td>
<td>0,5</td>
</tr>
<tr>
<td><em>E. coli</em> culture</td>
<td>One loop of culture is added to every test tube</td>
</tr>
</tbody>
</table>

Incubation in thermostat at 37°C for 18-20 h

**Results:**

**Conclusion:**

2. Titration of bacteriophage on solid nutrient medium (demonstration).

3. Bacteriophage typing for bacterial identification
Educational Edition

Zheleznyak Natalya Vasilevna

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