

MINISTRY OF SCIENCE AND HIGHER EDUCATION
RUSSIAN FEDERATION
Federal State Autonomous Educational Institution of Higher Education
NORTH-CAUCASUS FEDERAL UNIVERSITY

**EDUCATIONAL AND METHODOLOGICAL COMPLEX FOR PRACTICAL
SKILLS TRAINING**

Microbiology, virology, epidemiology
(Name of the practical training)

- Specialty: 31.05.01 General Medicine

INTRODUCTION

1. The purpose and objectives of mastering the discipline

1. Aims of the Practical Skills Training

The goal of mastering the course "Microbiology, Virology, Epidemiology" is to develop in students the appropriate set of competencies for future specialists in the specialty 31.05.01 General Medicine.

The objectives of the practical training are:

- development of skills and abilities in the use of standard microbiological methods for observing and studying microorganisms in laboratory conditions, as well as familiarization with modern microbiological research methods;
- development of competencies corresponding to the bachelor's level of training for research and scientific production activities.

STRUCTURE AND CONTENT OF LABORATORY CLASSES

Laboratory work 1

Introduction. Microbiology as a Science. History of the Development of Microbiology

The purpose of this work is to study the morphology and distribution of bacterial species.

Procedure for completing the work

In their first assignment, students learn to operate a microscope, examine prepared slides of bacterial specimens, and then independently prepare slides of live bacteria and examine them.

First, students examine prepared fixed bacterial slides. Fixed slides are microorganisms suspended in an aqueous medium, dried on a microscope slide, and stained with aniline dyes. Students prepare slides of some live microorganisms themselves. To do this, a small drop of tap water is placed on a clean microscope slide.

A small amount of the microbes to be examined is added to the slide using a heated and cooled inoculation loop. The slide is thoroughly mixed and covered with a coverslip. Excess water is removed with filter paper. The slide is placed on the stage and secured with clamps. First, by looking through the eyepiece and rotating the macro screw, a sharp image of the object is achieved at low magnification with a 10x objective. Then the microscope is set to high magnification with a 40x objective. The screws must be turned carefully, as lowering them too quickly can crush the lenses.

When using a microscope, it should be kept in mind that the microscope, especially at high magnifications, does not capture the full depth of the object. Therefore, when gradually lowering the tube using the micro screw, the object is first visible from above, and then in optical section.

1. Review of fixed preparations of microorganisms.

Lactococcus lactis – the causative agent of lactic acid fermentation; spherical cells (cocci); cells are connected in chains. It is used to produce lactic acid products.

Lactobacillus acidophilum – rod-shaped bacteria; the causative agent of lactic acid fermentation. It is used to produce lactic acid products.

Acetobacter aceti – rod-shaped bacteria that oxidize ethanol to acetic acid. It is used to produce vinegar.

Streptomyces griseus – an actinomycete with cells shaped like thin, branched threads; producer of the antibiotic streptomycin.

Sacccharopolyspora erythrae – an actinomycete with cells shaped like thin, branched threads; producer of the antibiotic erythromycin.

2. Viewing live specimens

Bacillus subtilis – cells shaped like thin, motile rods; forms spores; produces enzyme preparations.

Spirulina platensis – cyanobacteria; cells shaped like a single filament, consisting of cylindrical cells tightly adjacent to each other; exhibits gliding movement. Used as a dietary supplement.

Report Contents

1. Latin name of the microorganism.
2. Cell morphology (provide a drawing indicating the microscope magnification and maintaining the cell size ratio).
3. Industrial use of the bacteria being studied.

Quiz Questions

1. Describe the structure of a microscope.
2. How do you determine the magnification of a microscope?
3. What is the shape of bacterial cells?

4. Name the morphological features of actinomycetes.
5. What types of bacteria do you know, and what is their practical significance?
6. Define microbiology and provide a comprehensive list of the major groups of microorganisms studied within this scientific discipline.
7. Who is historically credited with the first observation of microorganisms, and how did his invention of the simple microscope in the 17th century change the perception of the living world?
8. Explain the theory of *spontaneous generation* (abiogenesis) and describe the key experiments conducted by Louis Pasteur that definitively disproved this theory.
9. What are Robert Koch's postulates, and why are they considered a cornerstone in establishing the germ theory of disease and the etiology of specific infections?
10. How did Joseph Lister apply microbiological principles to the field of surgery, and what was the significance of his introduction of antiseptic techniques?

List of key literature:

1. Murray P.R., Rosenthal K.S., Tenover F.C., Tenover F.C. *Medical Microbiology*. – 10th ed. – Philadelphia: Elsevier, 2025. – 880 p. (ISBN: 978-0-323-93321-0)
2. Carroll K.C., Butel J.S., Morse S.A., et al. *Jawetz, Melnick & Adelberg's Medical Microbiology*. – 29th ed. – New York: McGraw-Hill Education, 2024. – 864 p. (ISBN: 978-1-264-93210-6)
3. Ryan K.J., Ahmad N., Alspaugh J.A., et al. *Ryan & Sherris Medical Microbiology*. – 9th ed. – New York: McGraw-Hill Education, 2024. – 992 p. (ISBN: 978-1-264-26874-0)

List of additional literature:

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6. Kumar S. *Medical Microbiology For Undergraduates*. – 2nd ed. – New Delhi: Jaypee Brothers Medical Publishers, 2024. – 560 p. (ISBN: 978-93-5465-789-2)

Laboratory work 2

Taxonomy and nomenclature of microorganisms

The aim of the work is to study the morphology of representatives of various groups of eukaryotes.

Procedure for completing the work

Students independently prepare live slides of fungi, yeasts, algae, and protozoa; examine them with a 40x objective and draw sketches indicating the microscope's magnification.

Mold Fungi

Aspergillus niger – the cells are septate mycelium; some hyphae bear unbranched conidiophores with spores. The conidiophores are single-celled, spherically inflated, with short, pin-shaped cells (sterigmata) located on the surface of the swelling, each of which produces a chain of black-colored conidia. It is used to obtain organic acids and enzymes.

Penicillium chrysogenum – the hyphae are septate; some hyphae bear branched conidiophores with spores. Conidia are formed at the ends of whorled, branched conidiophores. They are used to produce the antibiotic penicillin.

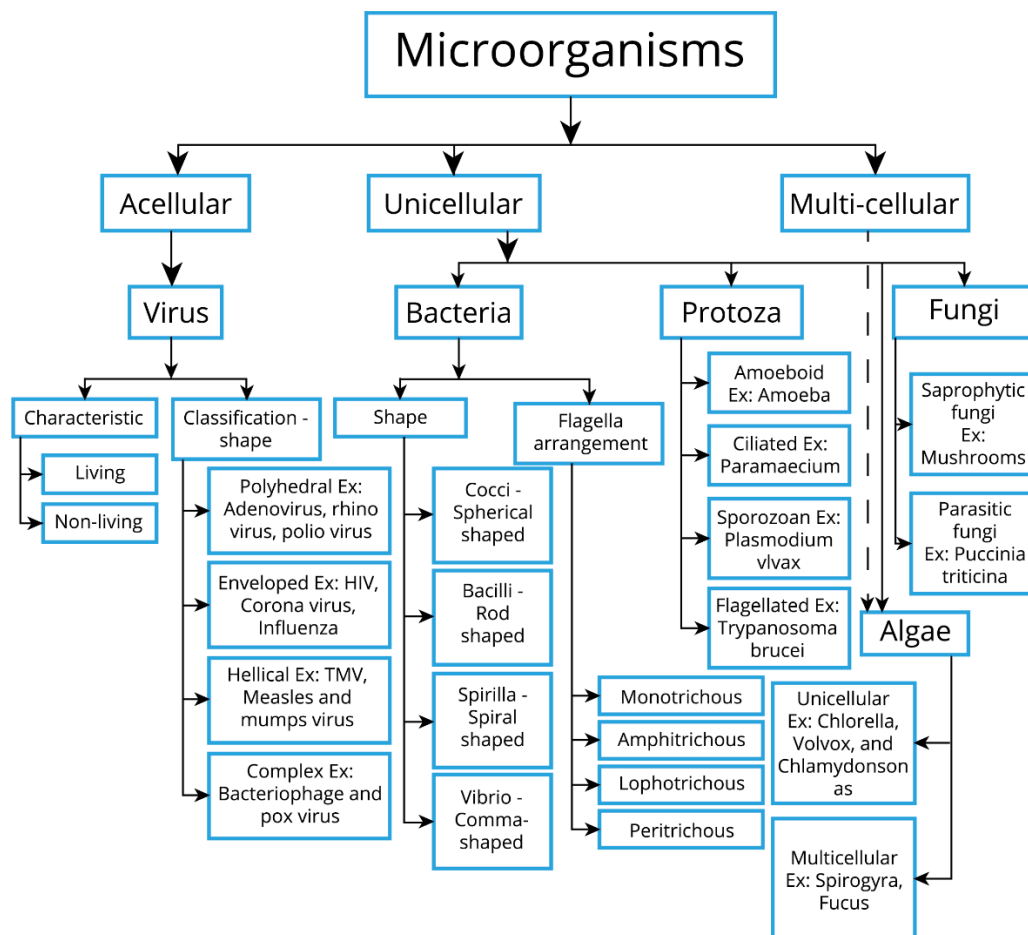
Yeast

Saccharomyces cerevisiae is a solitary yeast with oval and round cells; it reproduces by budding. It is used in brewing, baking, and alcohol production. It is found naturally on the surface of berries and other fruits.

Saccharomyces vini has oval and round cells; it reproduces by budding; after budding, it does not separate for some time, forming small "branches." It is used in winemaking.

Rhodotorula glutinis has elliptical cells; the cells are solitary; it reproduces by budding. Its orange coloration comes from the carotenoids it contains. It can grow in environments containing petroleum hydrocarbons as a carbon source. It is used as a fodder yeast.

Candida tropicalis is a yeast with oval and highly elongated cells that form a "pseudomycelium." It can grow in a mineral medium with hydrocarbons as a carbon source. It is used as a fodder yeast. Industrially, it is grown on waste from alcohol and paper production.



Algae

Chlorella vulgaris is a microscopic green alga with round cells (5-10 μm in diameter); it reproduces by autospores, which form within the mother cell, in numbers from 4 to 32, and are released after the cell wall ruptures. It can be used for mass cultivation to obtain feed protein, as well as for air regeneration in closed systems (submarines, space stations, etc.). *Scenedesmus* sp. belongs to the group of green algae; it has oval-shaped cells; The outer cells often have pointed ends; the cells are connected together in groups of four. Used to obtain dietary protein.

Protozoa

The preparation is prepared from activated sludge from an aeration tank. Study the morphology of the discovered protozoa and draw them.

Report Contents

1. Latin name of the microorganism.
2. Cell morphology (provide a drawing indicating the microscope magnification and maintaining the cell size ratio).
3. Industrial use of the bacteria being studied.

Quiz Questions

1. Define microbial taxonomy and explain its three interrelated subdisciplines: classification, nomenclature, and identification.
2. What is the purpose of the binomial system of nomenclature in microbiology, and who originally introduced this system for living organisms?

3. What are the main phenotypic criteria used for the classification of microorganisms (e.g., morphology, physiology, biochemical tests)?
4. What is a type strain, and why is it essential for the valid publication of a new prokaryotic species name?
5. What are the main differences between the classical phenetic classification and the modern phylogenetic (cladistic) classification of microorganisms?
6. Describe the Bergey's Manual of Systematic Bacteriology and explain how it serves as the authoritative reference for bacterial taxonomy.
7. Describe the cell shape of fungi and their practical uses.
8. Describe the cell shape of yeast; name the examples and describe them. Practical choice.
9. Describe the morphology of microalgae and their practical applications. Use.
10. Name the examples of protozoa and describe their use in biotechnology.

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Laboratory work 3

Morphology and ultrastructure of bacteria

The objective of this work is to master the basic methods of studying the morphology of microorganisms.

Procedure for completing the work

1. Prepare fixed preparations of lactic acid bacteria from biokefir and acidophilus milk.
2. Gram stain *Bacillus subtilis* (Gram+) and *Escherichia coli* (Gram-) cells.
3. Determine the size of *Saccharomyces cerevisiae* yeast cells.
4. Determine the number of live and dead *Saccharomyces cerevisiae* cells in the yeast suspension.

1. Loeffler's staining method for detecting volutin granules:

1. Apply 1-2 drops of a hydroalcoholic solution of methylene blue to a prepared, fixed smear. Stain for 3-5 minutes.
 2. Pour off the stain, rinse with water, and dry.
 3. Under an immersion microscope.
- Result: the smear shows blue cells with blue, almost black volutin granules (depending on the intensity of the stain).

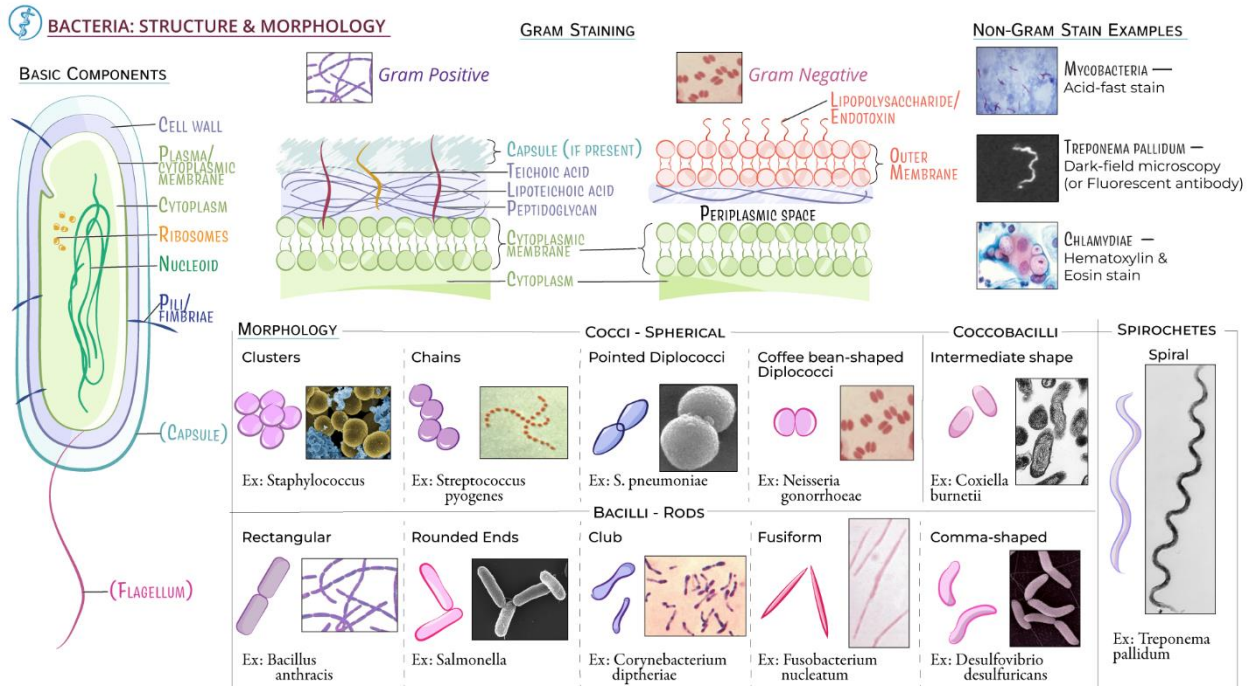
2. Staining method for detecting glycogen:

1. Apply a small drop of the microorganism suspension to a clean glass slide, and add an equal drop of an iodine solution in potassium iodide (7 g iodine and 20 g potassium iodide in 100-300 ml distilled water) for 2-3 minutes.

2. Place a coverslip on top, and remove excess liquid with filter paper.

3. Observe the specimen under oil immersion (apply a drop of oil to the coverslip) using a 40x objective.

Result: The cell cytoplasm is stained light yellow, and the glycogen granules are stained reddish-brown.



3. Granulosa staining technique:

1. Add a small drop of Lugol's solution to a small drop of butyric acid bacteria suspension.

2. Cover with a coverslip, and remove excess liquid with filter paper.

3. Apply a drop of oil to the coverslip and examine with an immersion system.

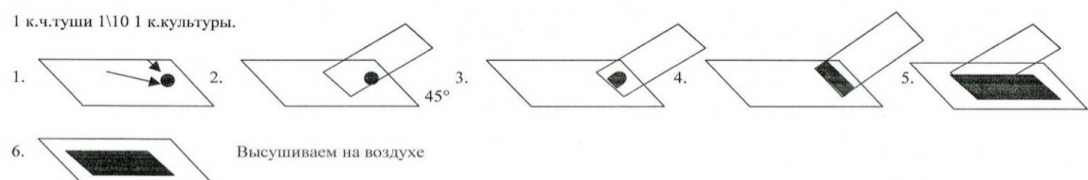
Result: Granulosa inclusions are stained dark blue.

4. Burri staining method:

1. Mix a drop of microbial suspension with a drop of India ink on a defatted slide, and prepare a smear

with the ground edge of the slide, like a blood smear; air-dry.

2. Undergo an immersion microscope.



Result: Unstained light capsules and bacterial cells are visible against the dark background of the India ink.

5. Burri-Gins staining method:

1. Mix a drop of microbial suspension with a drop of India ink on a defatted slide, and prepare a smear

with the ground edge of the slide, like a blood smear; air-dry and fix.

2. Apply an aqueous fuchsin solution for 1-2 minutes.

3. Rinse, dry, and examine the slide.

Result: Unstained light capsules containing red bacteria are visible against the dark background of the India ink.

6. Mikhin's staining method:

1. The fixed smear is stained with Loeffler's methylene blue for 2-3 minutes while warming (until vapors appear).

2. The dye is washed off with water and the smear is dried with filter paper.

It is better to stain with an old dye solution.

Result: capsules are light pink, bacteria are dark blue.

Report Contents

1. Morphology of lactic acid bacteria cells (drawings of fixed preparations of lactic acid bacteria with indicated microscope magnification).

2. Drawings of fixed preparations of gram-negative and gram-positive bacteria.

3. Determining the division value of the measuring drum of the ocular micrometer.

4. Size of the *Saccharomyces cerevisiae* yeast cell.

5. Number of live and dead cells in a yeast suspension.

Quiz Questions

1. How to prepare a fixed bacterial preparation?

2. What causes differences in bacteria in Gram staining?

3. What is the Gram staining method?

4. How to determine the size of microorganisms?

5. How to determine the number

6. List and briefly describe the three basic morphologies of bacteria, and provide the Latin-derived terms used to describe their cellular arrangements (e.g., chains, clusters, pairs).

7. Compare and contrast the ultrastructure of the cell wall in Gram-positive and Gram-negative bacteria, highlighting the differences in peptidoglycan thickness, the presence of an outer membrane, and the composition of teichoic acids.

8. Explain the structural and functional role of the bacterial cytoplasmic membrane, and describe the mechanisms by which substances cross this membrane (e.g., passive diffusion, active transport, and group translocation).

9. What is the periplasmic space, and why is it particularly significant in Gram-negative bacteria in terms of enzyme activity and antibiotic resistance

10. Describe the chemical structure of peptidoglycan (murein), including the alternating sugar subunits (N-acetylglucosamine and N-acetylmuramic acid) and the cross-linking peptide bridges.

11. Explain the acid-fast cell wall structure, using *Mycobacterium* as an example. How does this unique structure affect staining properties and environmental resistance?

12. Define the bacterial capsule and slime layer, distinguish between them, and discuss their primary virulence functions, including antiphagocytosis and adhesion.

13. Describe the ultrastructure, protein composition, and mechanism of action of bacterial flagella. How do arrangements such as monotrichous, lophotrichous, peritrichous, and amphitrichous differ?

14. What are fimbriae and pili (sex pili)? Compare their structural features and list their distinct biological roles in bacterial physiology and genetics.

15. Describe the structural layers of the bacterial endospore (from the core to the exosporium). Why are endospores considered the most resistant life form known?

16. Explain the stages of sporulation (endospore formation) and germination, and discuss the clinical significance of spore-forming genera such as *Bacillus* and *Clostridium*.

17. Define the bacterial nucleoid and distinguish it from the eukaryotic nucleus in terms of membrane enclosure, DNA structure, and the presence of associated proteins.

18. What are plasmids, and how do they differ from the bacterial chromosome? Classify them based on their functions (e.g., R-plasmids, F-plasmids, and col-plasmids).

19. Describe the structure and function of bacterial ribosomes, specifying their sedimentation coefficient (70S) and explaining how they differ from eukaryotic ribosomes as a target for antibiotics.

20. What are inclusion bodies (intracytoplasmic inclusions)? Provide examples such as poly- β -hydroxybutyrate (PHB) granules, volutin (metachromatic granules), and gas vacuoles, and state their physiological roles.

21. Explain the principle of the Gram staining procedure step-by-step, and discuss why some bacteria (e.g., *Brucella* or older cultures of Gram-positives) may show variable Gram reactions.

22. Compare the ultrastructure of mycoplasmas to that of typical bacteria. Why are mycoplasmas considered pleomorphic and resistant to antibiotics that target the cell wall?

23. What modern ultrastructural techniques (e.g., transmission electron microscopy, scanning electron microscopy, and cryo-electron tomography) are used to study bacterial morphology, and what specific structural information does each provide?

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1. Murray P.R., Rosenthal K.S., Tenover F.C. *Medical Microbiology*. – 10th ed. – Philadelphia: Elsevier, 2025. – 880 p. (ISBN: 978-0-323-93321-0)
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Laboratory work 4

Growth, development and cultivation of prokaryotes

The objective of this work is to master microbiological culture methods and acquire skills in describing the cultural characteristics of microorganisms.

Students must culture various cultures using various methods. Metal loops, pipettes, and spatulas are used for microbial culture. Loops are used to collect small amounts of culture from solid media. Pipettes are used to collect samples from liquid media. Spatulas are used to spread the biomass over the surface of the agar medium. Pipettes and spatulas are sterilized while wrapped in paper. To sterilize the loop, it is heated in a torch flame before and after culture.

Subcultures are performed near a torch flame. The necks of flasks and test tubes, as well as the pipettes and spatulas used for culture, are passed through the flame. Used pipettes and spatulas are placed in special containers with a disinfectant solution.

Procedure for completing the work

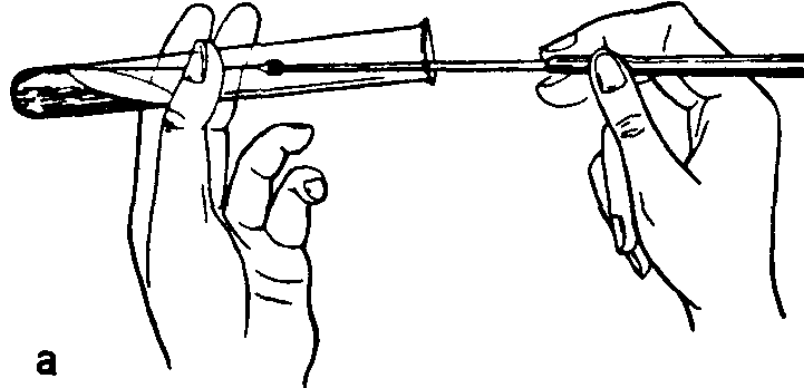
Part 1

1. Inoculation with a loop onto a test tube with a slant agar. Inoculation of one of the following yeast cultures:

Saccharomyces cerevisiae race HP

Saccharomyces vini – onto wort agar

Bacillus subtilis – onto meat-peptone agar (MPA)

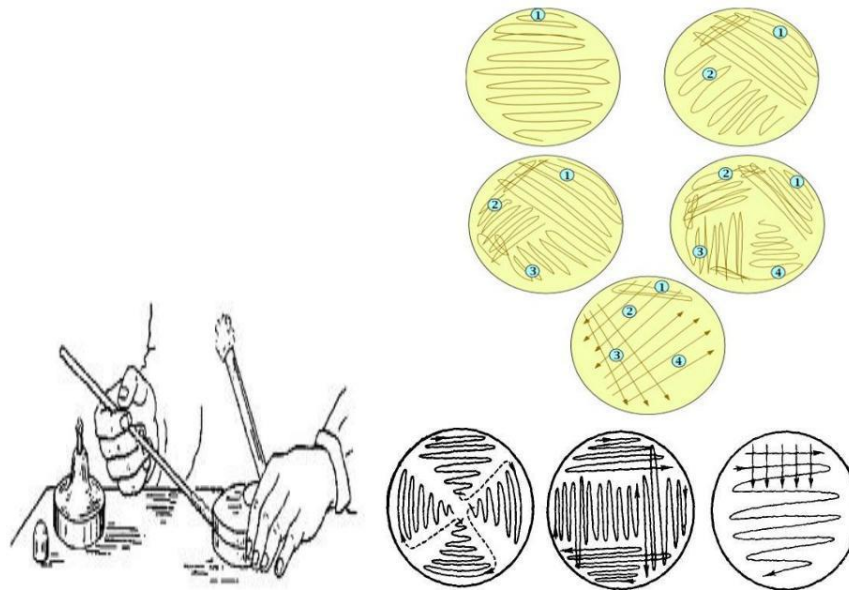


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2. Inoculation onto Petri dishes. (One of the following cultures is used):

Rhodotorula glutinis – onto wort agar

Candida tropicalis – onto wort agar



3. Inoculation into liquid medium.

Inoculation of the mold fungus *Aspergillus niger* – inoculation with a loop from a test tube onto mineral medium with sucrose in flasks.

The plates, test tubes, and flasks are labeled with the date of inoculation, the name of the culture, and the student's name. The dishes are wrapped upside down in paper. The cultures are placed in a thermostat.

A record of the cultures is made in the notebook. The recording scheme includes:

Sowing date. Sowing method. Culture name. Medium name.

A week later, the cultures are examined. Students visually and under a microscope examine the cultures cultured in the first lesson and describe the microorganism growth using the attached diagram.

Part 2: Describe the cultural characteristics of culture growth on a test tube with an agar slant.

1. Describe the cultural characteristics of culture growth on Petri dishes.
2. Describe the cultural characteristics of culture growth on a liquid medium.

Report Contents

1. Crop Name. Medium Name.
2. Sowing Method. Cultural Characteristics. Microscopic Image. Crop Age.
3. Cultivation Temperature.

Quiz Questions

1. Principles of composing nutrient media.

2. Classification of nutrient media by composition, physical state, and purpose. Provide examples of nutrient media.
3. How are microorganisms classified based on physicochemical factors such as pH, temperature, and aeration?
4. Methods for sterilizing microbiological vessels and media.
5. Basic methods for inoculating microorganisms.
6. What characteristics are used to describe microorganism growth in liquid and solid media?
7. Name the cultural characteristics of the culture studied.
8. Define prokaryotic growth in microbiological terms, and explain why it is typically measured as an increase in cell number rather than an increase in cell size.
9. Draw and describe the four phases of a typical bacterial growth curve in a closed batch culture (lag, exponential/log, stationary, and death phases). What physiological events characterize each phase?
10. Explain the reasons for the lag phase and the stationary phase in a batch culture. What factors contribute to the onset of the stationary phase (e.g., nutrient depletion, accumulation of toxic metabolites, and changes in pH)?
11. Classify culture media into defined (synthetic) and complex (undefined) media. Provide examples of each and discuss their respective uses in microbiological practice.
12. Distinguish between selective, differential, and enrichment media. Give a specific example for each type and explain the principle behind their action (e.g., MacConkey agar, Mannitol salt agar, or blood agar).
13. Describe the streak plate method and the pour plate method for obtaining pure cultures. Compare their advantages and disadvantages in isolating individual colonies.
14. Classify prokaryotes based on their optimal temperature range into psychrophiles, mesophiles, thermophiles, and hyperthermophiles. What molecular adaptations do thermophiles possess to survive at high temperatures?
15. Classify prokaryotes according to their relationship with molecular oxygen into obligate aerobes, facultative anaerobes, obligate anaerobes, aerotolerant anaerobes, and microaerophiles. How do these groups differ in their possession of enzymes such as superoxide dismutase (SOD), catalase, and peroxidase?
16. Explain the principles of anaerobic cultivation. Describe the function of reducing media, the GasPak anaerobic system, and the role of resazurin as an oxygen indicator.
17. Classify microorganisms based on their optimal pH range (acidophiles, neutrophiles, alkaliphiles) and discuss how pH changes in the medium can affect nutrient availability and enzymatic activity.
18. Define osmotolerance and halophily. Explain how moderate halophiles and extreme halophiles cope with high osmotic pressure in their environment.
19. What are the major macronutrients (e.g., carbon, nitrogen, phosphorus, sulfur) and micronutrients (trace elements) required for bacterial growth? Briefly state the biological role of each.
20. Distinguish between autotrophs and heterotrophs, and further classify them based on their energy sources (phototrophs vs. chemotrophs). Provide a bacterial example for each of the four major nutritional types (photoautotroph, chemoautotroph, photoheterotroph, chemoheterotroph).
21. What are growth factors (organic growth requirements), and why must they be supplied exogenously for certain fastidious bacteria (e.g., *Haemophilus influenzae* requiring NAD and hemin)?

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Laboratory work 5

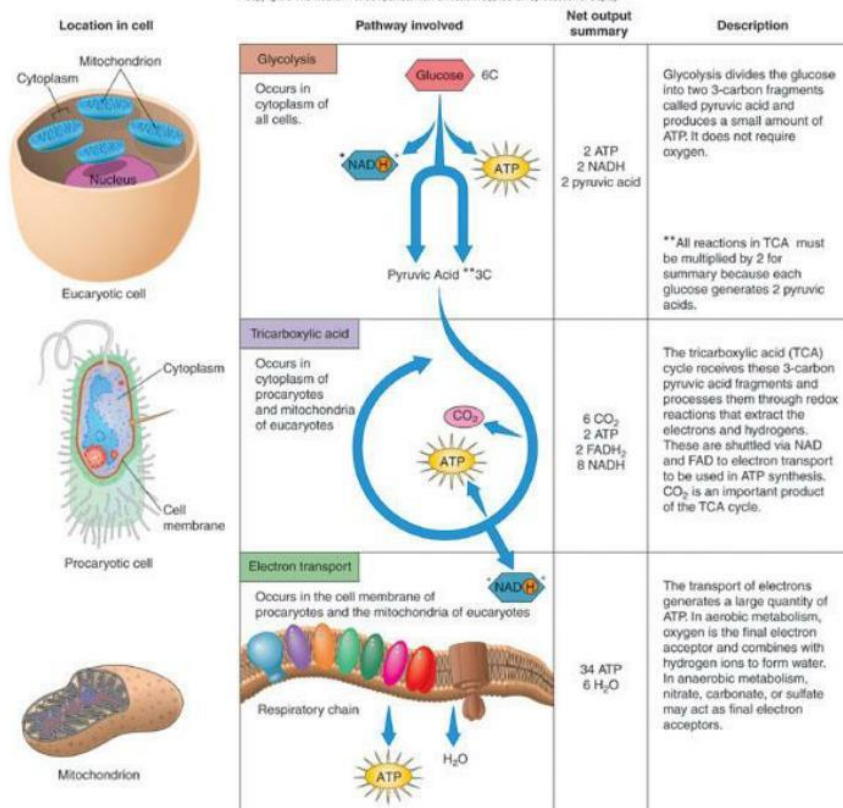
Main types of prokaryotic metabolism

Growth, development and cultivation of prokaryotes

The objective of this work is to master the technique of preparing butyric acid bacteria cultures and become familiar with the main representatives of this group.

Butyric acid fermentation is a complex process of converting carbohydrates into butyric acid and other products, carried out by a group of anaerobic spore-forming bacteria. Butyric acid bacteria possess various active enzymes capable of breaking down complex carbohydrates. The resulting butyric acid, in low concentrations, is a substance that stimulates the growth of higher plants.

Three Stages of Metabolism



*Note that the NADH⁺ transfers H⁺ and e⁻ from the first 2 pathways to the 3rd.

Materials and equipment: large test tubes, rubber stoppers, potato tubers, tweezers, scalpels, crushed chalk, a water bath, Lugol's solution, and a 5% ferric chloride solution.

Procedure for completing the work

1. Cut unpeeled potatoes into slices that fit easily into the test tube.
2. Fill a test tube 1/3 full, add a pinch of chalk, and fill almost to the top with water.
3. Place the test tubes in a water bath at 80°C for 10-15 minutes.
4. Then cap the test tubes and place them in a thermostat set at 35°C.

Under these conditions, butyric acid fermentation bacteria are detected in the liquid within 2-3 days.

The culture is elective. Anaerobic conditions are created to promote their preferential development; sporeless forms are killed by preheating; the addition of chalk neutralizes the resulting acids and promotes bacterial growth.

Examine the culture microscopically; it primarily reveals *Clostridium pasteurianum* - motile rods with rounded ends, solitary and paired. In older cultures, a spore is found at one end of the cell. A drop of Lugol's iodine solution can be added to a drop of culture liquid for microscopic examination. Butyric acid bacteria contain granules in their cells, which stain blue with Lugol's solution.

A qualitative butyric acid reaction is performed on the culture fluid. To do this, add 2 ml of 5% ferric chloride to 5 ml of the fluid. Upon heating, brown ferric butyrate is formed. Record the results in your lab notebook. Draw a diagram.

Report Contents

1. Record the characteristics of butyric acid bacteria.
2. Cell morphology (provide a drawing indicating the microscope magnification and maintaining the cell size ratio).
3. Industrial uses of the bacteria studied.

Quiz Questions

1. What function do butyric acid bacteria perform in the ecosystem?
2. Where do they live in nature?
3. What reserve product do bacteria in this group synthesize?
4. Name the main representatives of the butyric acid bacteria group.
5. What type of metabolism do these bacteria perform?
6. What environmental conditions do they prefer?
7. Define the four major nutritional types of prokaryotes based on their carbon and energy sources (photoautotrophs, chemoautotrophs, photoheterotrophs, and chemoheterotrophs), and provide a representative prokaryotic genus for each category.
8. Distinguish between chemolithotrophy and chemoorganotrophy. What types of inorganic compounds serve as electron donors for chemolithotrophs, and what is the ecological significance of these organisms?
9. What is anaerobic respiration? List at least three alternative terminal electron acceptors (other than O₂) used by prokaryotes, and name specific genera that carry out each type (e.g., nitrate reduction, sulfate reduction, or methanogenesis).
10. Explain the process of fermentation. How does it differ fundamentally from respiration in terms of ATP generation (substrate-level phosphorylation), the regeneration of NAD⁺, and the final metabolic end products?
11. Compare and contrast oxygenic photosynthesis (e.g., in cyanobacteria) with anoxygenic photosynthesis (e.g., in purple and green sulfur bacteria). What are the respective electron donors, and what are the final oxidized products?
12. What is biological nitrogen fixation, and why is it a vital process for the biosphere? Name the prokaryotic genera capable of this process (both free-living and symbiotic) and the oxygen-labile enzyme complex responsible for reducing atmospheric N₂ to ammonia.

13. Explain the two-step process of nitrification carried out by chemolithoautotrophic prokaryotes. Which genera oxidize ammonia to nitrite, and which oxidize nitrite to nitrate? What type of energy metabolism do they employ?
14. Define denitrification and describe its role in the global nitrogen cycle. Why is this process of environmental concern in agriculture (e.g., loss of soil fertility) and atmospheric science (e.g., production of greenhouse gases)?
15. Compare substrate-level phosphorylation with oxidative phosphorylation and photophosphorylation. In which specific metabolic pathways (fermentation, respiration, and photosynthesis) does each type of ATP synthesis predominantly occur?
16. How do the metabolic capabilities of obligate aerobes, facultative anaerobes, obligate anaerobes, and microaerophiles differ? Explain the roles of detoxifying enzymes such as catalase, peroxidase, and superoxide dismutase in these groups.
17. Describe the metabolic pathways of sulfate-reducing bacteria (e.g., *Desulfovibrio*). Where are these organisms typically found, what is their terminal electron acceptor, and what is the final reduced product of their anaerobic respiration?
18. What are the major end products of homolactic fermentation (e.g., in *Lactobacillus*) versus heterolactic fermentation? How do these distinct pathways affect the organoleptic properties of fermented food products such as yogurt, cheese, and sauerkraut?

List of key literature:

1. Murray P.R., Rosenthal K.S., Tenover F.C. *Medical Microbiology*. – 10th ed. – Philadelphia: Elsevier, 2025. – 880 p. (ISBN: 978-0-323-93321-0)
2. Carroll K.C., Tenover F.C., Tenover F.C., et al. *Jawetz, Melnick & Adelberg's Medical Microbiology*. – 29th ed. – New York: McGraw-Hill Education, 2024. – 864 p. (ISBN: 978-1-264-93210-6)
3. Ryan K.J., Tenover F.C., Tenover F.C., et al. *Ryan & Sherris Medical Microbiology*. – 9th ed. – New York: McGraw-Hill Education, 2024. – 992 p. (ISBN: 978-1-264-26874-0)

List of additional literature:

4. Goering R.V., Dockrell H.M., Zuckerman M., Chiodini P.L. *Mims' Medical Microbiology and Immunology*. – 7th ed. – Philadelphia: Elsevier, 2025. – 600 p. (ISBN: 978-0-323-93324-1)
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6. Kumar S. *Medical Microbiology For Undergraduates*. – 2nd ed. – New Delhi: Jaypee Brothers Medical Publishers, 2024. – 560 p. (ISBN: 978-93-5465-789-2)

Laboratory work 6

Genetics of microorganisms

The purpose of this work is to study the morphology and distribution of bacterial species.

Procedure for completing the work

1. The effect of mutagens on microorganisms (see experimental setup diagram).

Experimental setup diagram.

0.5 ml suspension 0.1 0.5 0.5
 2.5 ml H₂O + 9.9 4.5 4.5
 Control 1:100 1:1000 1:10000
 0.1 ml 0.1 ml
 inoculation per dish
 0.5 ml suspension 0.1 0.5

8 ml initial 2.5 ml H₂O₂ + 9.9 4.5
 suspension in a test tube - Shaker 30 min 1:100 1:1000
 0.1 ml 0.1 ml inoculation per dish
 4 ml per dish 0.1 0.5 0.5
 experiment with UV light 9.9 4.5 4.5
 (1300 erg/mm² min) 1:100 1:1000 1:10000
 0.1 ml 0.1 ml inoculation

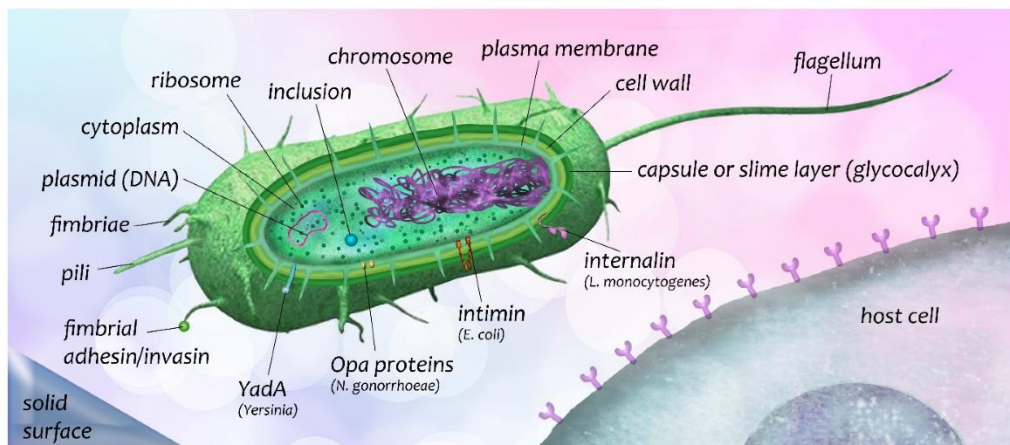
1. Prepare an 8 ml cell suspension of one of the following clonal yeast cultures: *Saccharomyces cerevisiae*, *Rhodotorulas sp.*, or *Aureobasidium pullulans*, using a 500 million standard. Add 0.5 ml of the suspension to a test tube containing 2.5 ml of the mutagen solution (0.5% H₂O₂ solution). Shake the tube for 30 minutes at 25°C. After 30 minutes, take samples, make dilutions of 1:100 and 1:1000, and inoculate 0.1 ml of each dilution onto agar medium using a spatula.

2. Pour the prepared yeast suspension into a flat-bottomed Petri dish, 4 ml at a time, and expose it to UV irradiation, gently rotating the dish to mix the suspension. Irradiation duration: *Saccharomyces cerevisiae* – 1 min, *Rhodotorulas sp.*, *Aureobasidium pullulans* – 2 min. Immediately after irradiation, the dishes are wrapped in black paper to prevent photoreactivation. Then, 0.1 ml of each dilution from the diluted suspension (1:1000, 1:10,000) is inoculated onto agar medium in a Petri dish containing MPA. The control suspension is not exposed to mutagens. It is diluted 1:1000 and 1:10,000, and 0.1 ml of each dilution is inoculated onto a dish.

3. Study of the mechanism of phenol action on bacterial motility.

The *Proteus vulgaris* culture is inoculated into two tubes containing MPA, one of which has been pre-inoculated with a 1% phenol solution. After incubation at 37°C (in the next lesson), determine *Proteus* motility in both tubes (crushed drop preparation).

Subculture the immobile *Proteus* culture from the phenol-containing medium onto fresh MPB and incubate at 37°C. Determine reversion of the lost trait (motility) and draw a conclusion about the modification nature of the variability.



In their first assignment, students learn to operate a microscope, examine prepared slides of bacterial specimens, and then independently prepare slides of live bacteria and examine them.

First, students examine prepared fixed bacterial slides. Fixed slides are microorganisms suspended in an aqueous medium, dried on a microscope slide, and stained with aniline dyes. Students prepare slides of some live microorganisms themselves. To do this, a small drop of tap water is placed on a clean microscope slide.

A small amount of the microbes to be examined is added to the slide using a heated and cooled inoculation loop. The slide is thoroughly mixed and covered with a coverslip. Excess water is removed with filter paper. The slide is placed on the stage and secured with clamps. First, by looking through the eyepiece and rotating the macro screw, a sharp image of the object is achieved at low magnification with a 10x objective. Then the microscope is set to high magnification with

a 40x objective. The screws must be turned carefully, as lowering them too quickly can crush the lenses.

When using a microscope, it should be kept in mind that the microscope, especially at high magnifications, does not capture the full depth of the object. Therefore, when gradually lowering the tube using the microcrew, the object is first visible from above, and then in optical section.

Table 1

Lethal effects of mutagens

Mutagen	Duration of exposure to a mutagen	Number of colonies per plate during dilution			Number of viable cells in ml	Survival rate, %
		1:100	1:1000	1:10000		
Control						
H ₂ O ₂						
Ultraviolet						

Table 2

Variability under the influence of mutagens

Mutagen	Duration of exposure to a mutagen	Total number of colonies on plates	Number of morphologically altered colonies	Variability, %
Control				
H ₂ O ₂				
Ultraviolet				

Report Contents

1. Description of the isolated colonies obtained.
2. Determination of colony purity (Gram staining). Drawing of microorganisms.
3. Complete Tables 1 and 2.

Quiz Questions

1. Distinctive features of the bacterial genotype.
2. Extrachromosomal factors of heredity.
3. What are plasmids, their functions, and roles. Types of plasmids. What are R-, F-, Col-, Tox-, and Hem-plasmids responsible for?
4. Differences between transposons and IS elements.
5. What is modification variability, and its types?
6. What underlies the adaptive responses of bacteria?
7. Characteristics of phenotypic variability in bacteria?
8. What is mutational variability, and its types?
9. Characteristics of genotypic variability in bacteria?
10. What is recombination variability, and its types?
11. What underlies transduction, transformation, and conjugation?
12. What is used to conduct transduction, transformation, and conjugation experiments?

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Laboratory work 7

Microbiological methods for studying environmental objects

Air Microflora Study

The purpose of this work is to determine the bacterial contamination of indoor air using theoretical training.

Procedure for completing the work

Part I

To determine the number of atmospheric tests using Koch's method, the following procedures are performed.

1. Each student places a Petri dish with meat-peptone agar (MPA) at a lowered height (on the floor, at a height of 75 cm, 150 cm, or 250 cm above the floor).
2. Open the dishes completely and let them sit for 5 minutes.
3. Then cover the dishes and transfer them to a thermostat set at 37°C for 24 hours.
4. Then leave them at a high temperature in the laboratory for 48 hours.

Part 2

1. Count the colonies grown in the Petri dishes.
2. Based on the number of colonies counted, determine the microbial contamination of the air as follows.

It has been experimentally established that in 5 minutes, at rest, the air in an area of 100 cm² settles to the same number of colonies as contained in 10 liters of air.

The area of the nutrient medium in the dish is limited by the significant number of colonies grown on it microbial colonies, the number of colonies contained in 1 m³ of air is calculated.

Example: Let's say 25 colonies grew in a Petri dish with a diameter of 10 cm and died. Then, the area of the nutrient medium is equal to πr^2 :

$$3.14 \times 5^2 = 78.5 \text{ cm}^2.$$

If 25 microbial cells remained in an area of 78.5 cm² after 5 minutes, then in the same time, the number of microbes that would settle in an area of 100 cm² would be:

$$78.5 \text{ cm}^2 - 25 \text{ L}$$

$$100 \text{ cm}^2 - X$$

$$X = 25 \times 100 / 78.5 = 32$$

Therefore, in 5 minutes, a total of 32 microbial cells would settle in an area of 100 cm² from 10 L of air.

To determine the number of microbes contained in 1 m³ (1000 L) of air, we create a proportion:

$$10 \text{ L} - 32 \text{ microbes} \quad 1000 \text{ L} - X$$

$$X = 32 \times 1000 / 10 = 3200$$

Therefore, 1 m³ of air contains 3200.

Based on the data from the entire group, a table called "Air Microflora in the Laboratory" is compiled.

Water Microflora Study

The objective of this study is to determine the total microbial count of tap water and open water samples collected by students from various locations.

To determine the total microbial count, students collect water samples on the day of class in sterile test tubes.

Procedure for completing the work

Part 1

1. Depending on the degree of suspected contamination, dilute the test water with sterile water. Typically, tenfold dilutions are made from 1:10 to 1:100.

2. Inoculate 1 ml of undiluted tap water or 1 ml of diluted water from a natural reservoir. To do this, 1 ml of the test water from the appropriate dilution, starting with the higher one, is added to sterile Petri dishes (at least two) and covered with 15 ml of melted meat-peptone agar, cooled to 45°C.

3. Gently move the dish across the table to mix the contents evenly and leave it on the table until the agar has solidified.

4. Then place the dishes with the solidified nutrient medium upside down in the incubator; place one dish in the incubator at 37°C for 24 hours, and leave the other at room temperature for 48 hours.

Part 2

1. After the microbes have germinated, count the colonies that have grown using a magnifying glass, marking each colony on the bottom of the dish with a marker. The number of colonies that have grown corresponds to the number of microorganisms in the inoculated volume of water with the corresponding dilution.

2. Calculate the total microbial count of the water, expressed as the arithmetic mean of the number of colonies that have grown. To do this, for each inoculation, count the number of colonies that have grown in each dish and multiply by the corresponding dilution. The results calculated for individual dishes are added together and then divided by the number of dishes inoculated. The resulting number will be the total microbial count of the water. Tap water should have a microbial count of no more than 100.

3. Using the data from the entire group, the students create a table "Microflora of Water in Stavropol."

Soil Microflora Study

The objective of this study is to determine the number of bacteria and actinomycetes in soil samples brought by students.

For inoculation, prepare the following for each soil sample:

- 1) 100 ml of sterile water in a 250 ml flask;
- 2) 10 ml test tubes with sterile water for preparing dilutions;
- 3) sterile 1 ml pipettes, keeping in mind that a different pipette is needed for each dilution;
- 4) sterile Petri dishes;
- 5) sterile nutrient media.

Procedure for completing the work

Part 1

1. Moisten a 10 g sample of soil to a paste-like consistency, rub it with a finger wearing a rubber glove, and add it to a flask containing 90 ml of sterile tap water. Shake the flask contents vigorously for 5–10 minutes until a uniform suspension is obtained.

2. Dilute the soil suspension using the serial dilution method.

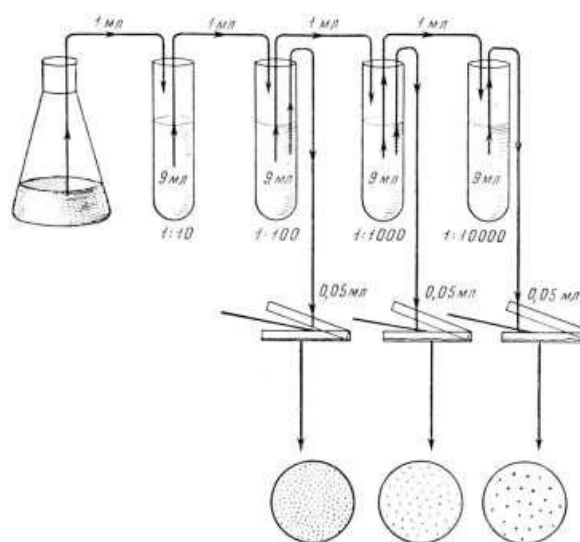


Рис. 21. Схема приготовления разведений суспензии микроорганизмов и проведения посева поверхностным способом

After allowing the prepared suspension to stand for one and a half to two minutes (1:10), remove 1 ml of the mixture with a sterile pipette and add it to a test tube containing 9 ml of sterile water.

This results in a 1:100 dilution. After stirring thoroughly for 1 minute and allowing it to stand for 30 seconds, remove 1 ml of the suspension from the test tube with a sterile pipette and transfer it to a second test tube containing 9 ml of sterile water. This results in a 1:1000 dilution. Similarly, transfer 1 ml of the suspension from the second test tube to the third, from the third to the fourth, and so on, obtaining the required tenfold dilutions. Typically, dilutions up to 1:1,000,000 are prepared. A new sterile pipette is used to prepare the next dilution.

3. Transfer 1 ml of sample from the 3rd and 4th dilutions to empty sterile Petri dishes, labeling the dilution on them first. At least two different dilutions of each soil sample should be used for inoculation, depending on the expected microbial content in the soil. Each dilution should be inoculated into at least two dishes. Before inoculation, the contents of each test tube are thoroughly mixed.

4. Pour 20 ml of melted and cooled to 45°C starch-ammonia medium or Czapek medium over the prepared dilutions.

5. Similarly, add 1 ml of the 5th and 6th dilutions to empty Petri dishes and pour 20 ml of melted and cooled to 45°C meat-peptone agar over them.

6. Gently move the dish around on the table to mix the soil suspension with the nutrient medium.

7. After the medium has solidified, place the dishes upside down in an incubator set at 30–35°C for 34–48 hours, then keep at room temperature for 1–2 days. The group number, student's name, and the dilution level of the soil suspension are written on the lid of the dish. After the medium has solidified, wrap the dishes upside down in paper, label them, and place them in the incubator. Cultivation temperature: 28–30°C. Cultivation duration: 8–10 days.

Part 2

1. Determine the number of bacteria contained in 1 g of soil based on the number of colonies grown in the dish. For counting, use dilutions that yield between 50 and 150 colonies. Dishes with fewer than 50 colonies are considered unreliable. If the number of colonies grown is large (more than 150), count the number of colonies on $\frac{1}{4}$ of the dish and then recalculate for the entire dish. The arithmetic mean is calculated from the sum of the colonies grown on the two dishes, and then recalculated to the number of bacteria contained in 1 g of soil. To do this, multiply the average number of colonies grown on the dishes of the corresponding dilution by the dilution ratio.

Example: Dishes inoculated with a soil suspension at a 1:10,000 dilution yielded an average of 60 colonies. $60 \times 10,000 = 600,000$ bacteria are contained in 1 g of soil.

It is more convenient to express such large quantities as 10^n . In our example, $600,000 = 6 \times 10^5$.

2. All colony count results and subsequent recalculations are entered into Table 1.

Table 1.

Number of bacterial colonies grown on MPA			
Breeding	Repeatability. (№ of cups)	Number of colonies per plate	Number of microorganisms in 1 g of soil
$1:10^5$	1		
	2		
$1:10^6$	1		
	2		

Colony Description. Provide descriptions and sketches of the predominant and most interesting colonies. The observation results are recorded in Table 2.

Table 2.

Characteristics of Bacterial Colonies Grown on MPA							
№ of colonies	form	diameter, mm	Color	Shine, transparency	Surface character	Profile	Drawing of the colony
1							
2							
3							

3. Prepare slides from the colonies of the predominant microorganisms and examine them under a microscope with a 40X objective. Note the shape of the cells and their combinations, motility, and the presence or absence of spores. Sketch the microscopic image. Record all observation results and drawings in Table 3.

Table 3.

Some morphological features of bacterial cells grown on MPA					
№ of colonies	Microscopic picture (drawing)	Cell shape	Cell arrangement	Motility	Presence of spores
1					
2					
3					

Report Contents

1. Indicate the location where the air sample was collected.
2. The number of microorganisms in the sample, cells/m³.
3. Table "Air Microflora in the Laboratory."

Quiz Questions

1. What characterizes air microflora?
2. How can microbial contamination of the air be determined?
3. What is the purpose of bacteriological testing of water?
4. How are water samples collected?
5. How is the total microbial count of water determined?
6. Which microorganisms serve as indicators of the sanitary condition of water and why?
7. What role do soil microorganisms play?
8. What groups of microorganisms live in soil?
9. How are soil samples collected for microbiological studies?
10. How are soil samples prepared for sowing?
11. How can the total number of bacteria in the soil be determined?

12. Describe the morphological features of actinomycetes.

List of key literature:

1. Murray P.R., Rosenthal K.S., Pfaller M.A. *Medical Microbiology*. – 10th ed. – Philadelphia: Elsevier, 2025. – 880 p. (ISBN: 978-0-323-93321-0)
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Laboratory work 8

Normal Microflora of the human body

The objective of this work is to master complex staining techniques for microscopic specimens, study the morphology of microorganisms, and become familiar with gram-negative and gram-positive bacteria.

Procedure for completing the work

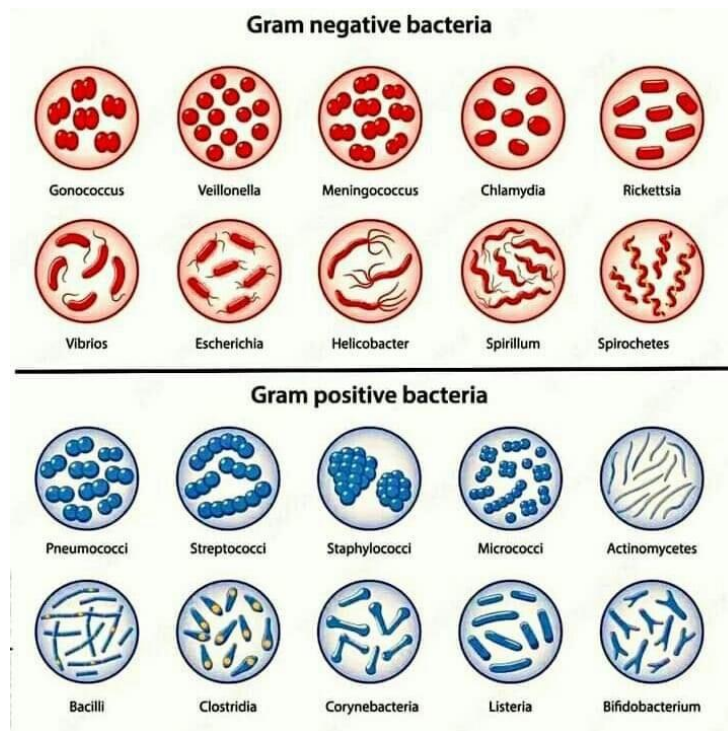
1. Prepare fixed dental plaque preparations.
2. Gram stain the smear.
3. Determine the predominant microbial morphotypes.
4. Sketch and describe the microbial landscape.
5. Sketch a diagram of the main representatives of gram-negative and gram-positive bacteria.

I. Gram stain:

1. Apply filter paper soaked in a carbolic-alcohol solution of gentian violet to the fixed smear, and add a few drops of distilled water. Leave for 2-3 minutes.
2. Remove the filter paper and, without rinsing with water, apply Lugol's solution for 1-2 minutes.
3. Discard Lugol's solution.
4. Decolorize with alcohol for 30 seconds.
5. Rinse thoroughly with distilled water.
6. Stain with an aqueous fuchsin solution for 1-2 minutes, rinse, dry, and examine the specimen under a microscope.

Result: Gram(+) bacteria stain blue-violet, Gram(-) bacteria stain pink.

- II. Diagram of the main representatives of gram-negative and gram-positive bacteria.



Report Contents

1. Cell morphology (drawings of fixed preparations of bacterial smears of dental plaque with indication of microscope magnification).
2. Diagram of the main representatives of gram-negative and gram-positive bacteria.

Quiz Questions

1. How to prepare a fixed bacterial preparation?
2. What is the reason for the difference in bacterial staining by Gram?
3. What is the Gram staining method?
4. Name the representatives of gram-negative bacteria.
5. Name the representatives of gram-positive bacteria.
6. Define the term normal microflora (microbiota) and distinguish between resident (autochthonous) and transient (allochthonous) microorganisms in the human body.
7. List the major anatomical sites of the human body that harbor a rich normal microbiota, and describe the predominant bacterial genera found on the skin, in the oral cavity, and in the gastrointestinal tract.
8. Explain how the composition of the gut microbiota changes from birth to adulthood, including the roles of delivery mode (vaginal vs. cesarean), breastfeeding, and diet.
9. What are the main beneficial functions of the normal microflora for the human host? Discuss at least three mechanisms, such as colonization resistance, immune system modulation, and vitamin synthesis.
10. Describe the concept of dysbiosis and provide examples of conditions (e.g., antibiotic-associated diarrhea, inflammatory bowel disease, obesity) that are linked to an imbalance in the normal microbiota.
11. What factors (both host-derived and environmental) influence the stability and composition of the normal microflora throughout a person's life?
12. Explain the role of the normal microflora in protecting against pathogen colonization. What is colonization resistance, and what microbial products or mechanisms contribute to it?
13. Compare the normal microflora of the stomach (e.g., *Helicobacter pylori* role) with that of the colon in terms of bacterial density, pH, oxygen availability, and predominant metabolic activities (e.g., fermentation vs. acid tolerance).

List of key literature:

1. Murray P.R., Rosenthal K.S., Pfaller M.A. *Medical Microbiology*. – 10th ed. – Philadelphia: Elsevier, 2025. – 880 p. (ISBN: 978-0-323-93321-0)
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Laboratory work 9

Antibiotics and chemotherapy

The objective of this work is to study the antibiotic activity of an actinomycete culture and to quantitatively determine the activity of the finished medicinal product.

The actinomycete culture *Saccharopolyspora erythraea* utilizes the antibiotic erythromycin. Its activity is determined using the agar block method. *Saccharopolyspora erythraea* is preseeded as a continuous lawn on solid medium in Petri dishes. After the actinomycete has grown well, agar spots containing actinomycete mycelium are excised and transferred to other Petri dishes previously seeded with a test culture. *Bacillus subtilis* bacteria were used as a test culture. The dishes are placed in an incubator at 30°C. If popular antibiotics containing actinomycete inhibit the growth of *Bacillus subtilis*, then

After 20–24 hours, a growth zone of the test culture appears around the agar block. The more additional antibiotics or the more active they are, the larger the zone diameter *Bacillus subtilis* growth occurs.



As an example of determining the antibiotic activity of a finished drug, In their course, students determine penicillin activity using a microbiological-chemical method.

Penicillin is produced by the mold fungus *Penicillium chrysogenum*. *Bacillus subtilis* serves as a test culture. Several sterile paper disks (8-10 mm in diameter) soaked in a healthy penicillin solution were placed on the surface of a solid nutrient medium in a Petri dish inoculated with the test culture.

After growing *Bacillus subtilis* in a thermostat for 18-20 hours, zone. Based on the experiment results, a graph was plotted on semi-logarithmic graph paper. On the abscissa, plot the diameters of the sterile zones, and on the logarithmic ordinate, plot the logarithms of the penicillin concentrations. A calibration graph is plotted for the obtained points to determine the unknown antibiotic activity of the penicillin solution.

Procedure for completing the work

Part 1

1. Prepare a thick suspension of the test culture in sterile tap water *Bacillus subtilis*. Place 1 ml of the suspension into two sterile empty Petri dishes. Then fill the dishes with pre-melted meat-peptone agar (MPA), cooled to 50°C. Gently stir the Petri dishes in a circular motion on the table and leave until solidified.

2. Place blocks cut from the agar medium with the most intense growth of *Saccharopolyspora erythraea* on the surface of the solidified medium of the first dish. The blocks should be cut with a cork borer, burned in a blowtorch flame.

3. Place sterile disks of filter paper soaked in penicillin solution for health care on the second surface of the Petri dish with the test culture.

4. Place the dish in an incubator at 30°C.

Part 2 (to be completed in the next lesson)

1. After germination of the test culture, note the presence of hygienic zones around the agar blocks containing *Saccharopolyspora erythraea*.

2. Measure the diameters of the sterile zones of growth inhibition around the paper disks soaked in penicillin solution.

3. Plot a calibration curve to determine the penicillin content.

Report Contents

1. Date of the experiment.

2. Name of the method for determining antibiotic activity.

3. Name of the antibiotic manufacturer and test culture.

4. Presence and size of sterile zones.

5. Calibration graph for determining penicillin concentration.

Quiz Questions

1. Define the terms antibiotic and chemotherapeutic agent. What is the fundamental difference between them, and why is the concept of selective toxicity crucial in antimicrobial chemotherapy?

2. List the main characteristics of antibiotic production (environmental composition, aeration conditions, asepsis).

3. At what stage of producer growth does intensive antibiotic synthesis occur?

4. What is the essence and advantage of the microbiological method for determining antibiotic activity?

5. What properties of antibiotics are sterile zone methods based on?

6. What is a test culture?

7. In what quantities is antibiotic activity manifested?

8. List the five major mechanisms of action of antibacterial agents, providing at least one specific example of an antibiotic for each mechanism (e.g., inhibition of cell wall synthesis, protein synthesis, nucleic acid synthesis, metabolic pathways, and disruption of the cytoplasmic membrane).

9. Explain the difference between bactericidal and bacteriostatic antibiotics. In which clinical situations would a bactericidal agent be strictly preferred over a bacteriostatic one?

10. What is the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC)? Describe how these parameters are determined using the broth dilution or agar dilution methods.

11. What is the role of plasmids and transposons in the horizontal transfer of antibiotic resistance genes? Discuss the clinical significance of multidrug-resistant (MDR) strains, such as MRSA (*Staphylococcus aureus*) or carbapenem-resistant *Enterobacteriaceae* (CRE).

12. Compare and contrast the mechanisms of action of β -lactam antibiotics (e.g., penicillins, cephalosporins) with those of glycopeptides (e.g., vancomycin). Why are β -lactams generally ineffective against methicillin-resistant *S. aureus*?

13. Explain the clinical significance of antibiotic synergism and antagonism. Give an example of a synergistic antibiotic combination used in clinical practice (e.g., β -lactam with an aminoglycoside) and explain the rationale behind it.

14. What are the major adverse effects associated with antibiotic therapy? Discuss the concept of superinfection, providing specific examples of opportunistic pathogens (e.g., *Clostridioides difficile*) that may overgrow following broad-spectrum antibiotic treatment.

List of key literature:

1. Murray P.R., Rosenthal K.S., Tenover F.C., Tenover F.C. *Medical Microbiology*. – 10th ed. – Philadelphia: Elsevier, 2025. – 880 p. (ISBN: 978-0-323-93321-0)
2. Carroll K.C., Butel J.S., Morse S.A., et al. *Jawetz, Melnick & Adelberg's Medical Microbiology*. – 29th ed. – New York: McGraw-Hill Education, 2024. – 864 p. (ISBN: 978-1-264-93210-6)
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6. Kumar S. *Medical Microbiology For Undergraduates*. – 2nd ed. – New Delhi: Jaypee Brothers Medical Publishers, 2024. – 560 p. (ISBN: 978-93-5465-789-2)

Laboratory work 10

Infections and infectious diseases

The objective of this work is to elucidate the role of the macroorganism and the external environment in the infectious process. To study methods for detecting and assessing experimental infections. To acquire the skill of assessing results of biological diagnostic methods and methods for assessing natural phenomena of skin resistance and bactericidal activity.

Procedure for completing the work

On the table, on the left side of the cuvette, are placed culture media and sterile Petri dishes. On the right, a jar containing non-sterile cotton swabs and a crystallizer with hand sanitizer are placed. Biotest animals are euthanized with chloroform (ether), using a cotton swab moistened with ether and dropped into the jar. The jar is tightly covered with a lid or airtight material. All manipulations of the animal carcasses are performed only with instruments, holding them by the top. Before dissection, the animal is grasped with forceps and immersed in a 3% detergent solution for 20-30 seconds. The animal is then placed in a colander to remove excess liquid and transferred to a dissection board. The forceps are then immersed in disinfectant. The animal and forceps are transferred only over the disinfectant solution.

The biological (experimental) research method involves artificially reproducing the clinical picture of infectious diseases in laboratory animals. In practical microbiology, it is used to diagnose infectious diseases, isolate and identify pure pathogen cultures, and detect and identify

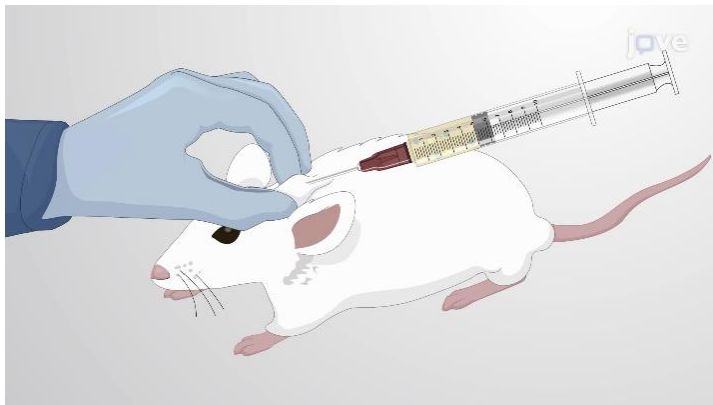
exotoxins. The method involves infecting an animal, observing it, and performing an autopsy on a dead or slaughtered animal.

The inoculation material must be collected under aseptic conditions, placed in a sterile container, homogenized, and used as quickly as possible. The inoculation route depends on the type of material, animal species, and the suspected infecting agent. The material can be injected cutaneously, subcutaneously, intradermally, intramuscularly, intraperitoneally, intravenously, through the nose, mouth, and rectum, into the heart, stomach, brain, vagina, testicle, conjunctiva, and joint cavities. Before inoculation, the surgical site must be dehaired and treated with 70% ethanol, followed by application of iodine tincture. The purpose of a bacteriological examination of a corpse is to detect the microbe contained in the injected material, determine its location in the body, and isolate a pure culture of the pathogen.

To infect white mice with the test material subcutaneously and intraperitoneally, preliminary anesthesia is necessary. To do this, place the animal in a closed glass container containing cotton wool soaked in ether or chloroform before sleep.

Subcutaneous infection of rats

For subcutaneous injection of the test material, secure the animal, wipe the injection site with a piece of cotton wool soaked in alcohol, grasp a fold of skin on the back at the base of the tail, insert the needle approximately halfway into the fold, slowly inject 0.5 ml of the material, and release the fold. Then quickly withdraw the needle, covering the injection site with cotton wool soaked in alcohol.



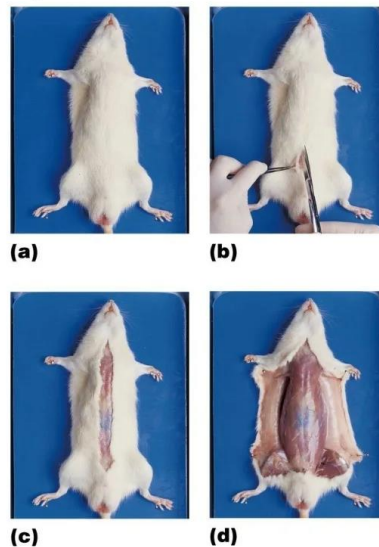
Intraperitoneal Infection

Suppress the animal head down to allow the intestines to move toward the diaphragm. After wiping the injection site in the lower left abdomen with alcohol, puncture the skin, holding the needle at an acute angle. Then, position the syringe perpendicular to the abdominal wall, thrust the needle through the peritoneum, and inject the syringe's contents. Remove the needle, covering the injection site with cotton wool soaked in alcohol.



2. Perform an autopsy on the dead white mice.

Figure 2.1 Rat dissection: Securing for dissection and the initial incision.



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The autopsy and examination of the animal's body are divided into three stages:

a) Autopsy and examination of the external integument

Fix the animal's body with the abdomen facing up, placing it on a piece of gauze soaked in a disinfectant solution. Clean the fur and skin with a cotton swab soaked in a disinfectant solution. Using sterile instruments, make an incision in the skin from the lower jaw to the pubis and lateral incisions along the extremities. Separate the skin, noting the condition of the subcutaneous tissue and lymph nodes. If any abnormal nodes are detected, culture them on nutrient media and make smears (imprints) on slides.

b) Autopsy and examination of the chest cavity

Open the chest cavity by making an incision in the diaphragm, insert the blade of a pair of scissors, cut the ribs on both sides of the sternum, fold the excised flap upward, and inspect the chest organs. If exudate is present, culture and smear it. To collect blood from the heart, cauterize an area with tweezers, puncture the area with a sterile Pasteur pipette, collect a few drops of blood, culture, and prepare a smear. Culture lung tissue and make smears (imprints).

c) Autopsy and examination of the abdominal cavity

Open the abdominal cavity, inspect its organs, and note the presence of exudate, as well as the size and color of the liver, spleen, and mesenteric lymph nodes. Culture the liver, spleen, kidneys, and lymph nodes and prepare smears—imprints of these organs. To do this, cut small pieces of organ tissue, pick them up with tweezers, and touch the agar surface in the appropriate area of the Petri dish (Fig.), then touch them to a glass slide. After the autopsy, wrap the body in gauze placed underneath.

3. Fix the prepared smears/imprints over a flame, stain with aqueous fuchsin or methylene blue, and Gram stain, and examine them microscopically.

4. Record the results of determination of aggressive enzymes (coagulase, fibrinolysin) and the hemolytic capacity of bacteria.

Report Contents

1. Title of the lab work.
2. Objective of the work.
3. Assignment.
4. Conclusions.

Quiz Questions

1. Define the terms infection and infectious disease. What is the fundamental difference between colonization, subclinical infection, and overt disease?
2. List and explain the four main stages of an infectious disease in chronological order: incubation period, prodromal period, acute (invasive) period, and convalescence. What clinical events characterize each stage?

3. Distinguish between the terms pathogenicity and virulence. How is virulence quantitatively measured (e.g., LD₅₀ and ID₅₀), and what is the practical significance of these measurements?
4. Enumerate the major portals of entry for pathogenic microorganisms into the human body (e.g., respiratory tract, gastrointestinal tract, urogenital tract, skin, parenteral route). Provide a specific pathogen example for each portal.
5. Describe the concept of infectious dose and explain why some organisms (e.g., *Shigella*) require a very low dose to cause disease, while others (e.g., *Salmonella*) require a high dose.
6. What are the main mechanisms of bacterial virulence? Include adherence factors (adhesins, pili), invaders (enzymes such as hyaluronidase and collagenase), toxins (exotoxins vs. endotoxins), and immune evasion strategies (capsule, antigenic variation, intracellular survival).
7. Compare and contrast exotoxins and endotoxins (lipopolysaccharide of Gram-negative bacteria) in terms of chemical nature, heat stability, origin, mechanism of action, and clinical effects (e.g., fever, shock, tissue damage). Give specific examples of exotoxin-producing bacteria (e.g., *Clostridium tetani*, *Corynebacterium diphtheriae*).
8. Define toxemia, bacteremia, septicemia, and sepsis. What are the clinical features of systemic inflammatory response syndrome (SIRS) and septic shock, and how are they triggered by bacterial components?
9. List the major portals of exit for infectious agents from the human body (e.g., respiratory secretions, feces, urine, blood, skin lesions, sexual secretions). Why is the portal of exit important for the transmission and epidemiology of infectious diseases?
10. Classify infectious diseases based on their epidemiological patterns: sporadic, endemic, epidemic, and pandemic. Provide a classic example of each.
11. Explain the major modes of disease transmission: direct contact (person-to-person), indirect contact (via fomites), droplet transmission, airborne transmission, vehicle-borne (water, food, blood), and vector-borne (mechanical vs. biological vectors). Give a microbial example for each mode.
12. Distinguish between exogenous and endogenous infections. Give a clinical example of an endogenous infection caused by normal flora (e.g., *Escherichia coli* urinary tract infection or *Staphylococcus epidermidis* device-related infection).
13. Describe the role of opportunistic pathogens in infectious diseases. Under what host conditions (immunodeficiency, breach of barriers, disruption of normal flora) do they typically cause disease?
14. Explain the difference between localized and systemic infections, and between acute and chronic infections. Provide specific bacterial or viral examples for each pattern (e.g., local – furuncle; systemic – typhoid fever; acute – influenza; chronic – tuberculosis).
15. What are zoonoses? Name at least three infectious diseases that are transmitted from animals to humans, and specify the typical animal reservoir for each (e.g., rabies, brucellosis, Lyme disease).
16. Compare the innate (nonspecific) immune response and the adaptive (specific) immune response in the context of infectious diseases. Which cellular and humoral components are most critical for combating bacterial versus viral infections?
17. Define carrier state (asymptomatic carrier) and incubatory carrier, and discuss their epidemiological significance using examples such as *Salmonella typhi* (chronic carrier) or *Neisseria meningitidis* (nasopharyngeal carrier).

List of key literature:

1. Murray P.R., Rosenthal K.S., Tenover F.C., Tenover P.C. *Medical Microbiology*. – 10th ed. – Philadelphia: Elsevier, 2025. – 880 p. (ISBN: 978-0-323-93321-0)

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Laboratory work 11

Viruses. History of Discovery. Structural Features

The objective of this work is to study the properties of viruses, master the principles of rapid diagnostics and methods of culturing viruses in chicken embryos.

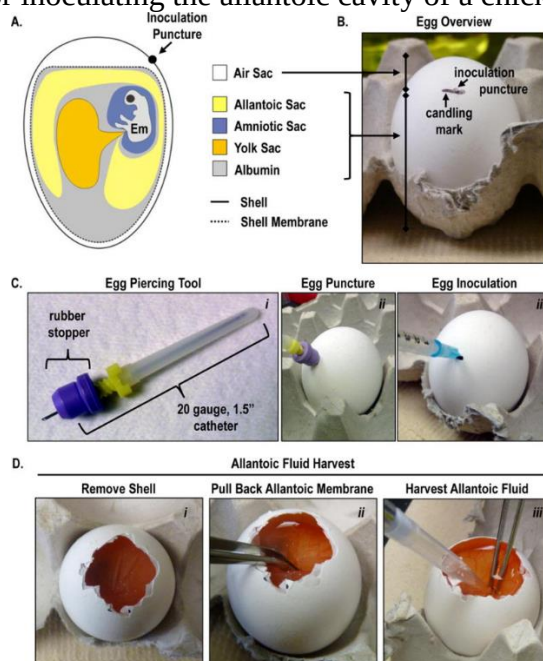
Procedure for completing the work

Equipment and materials: 10-day developing chicken embryos, scalpel, syringe, 0.9% NaCl solution for virus dilution, paraffin or wax, test tube with virus-containing material, disinfectant solution, alcohol, iodine, spirit lamp, matches, egg cup, cotton wool and gauze.

The method for inoculating chicken embryos includes the following steps:

- 1) candling the embryo (10-11 days old), marking the boundary of the air sac and vascular bundle;
- 2) treating the shell with alcohol and iodine, then burning it with an alcohol lamp;
- 3) opening the air sac (puncture) or trepanning the shell;
- 4) introducing material (nasopharyngeal swab) containing the virus into the allantoic cavity (the most common method);
- 5) sealing the opening with paraffin, incubating in a thermostat at 37°C for 48-72 hours.

Method for inoculating the allantoic cavity of a chicken embryo:



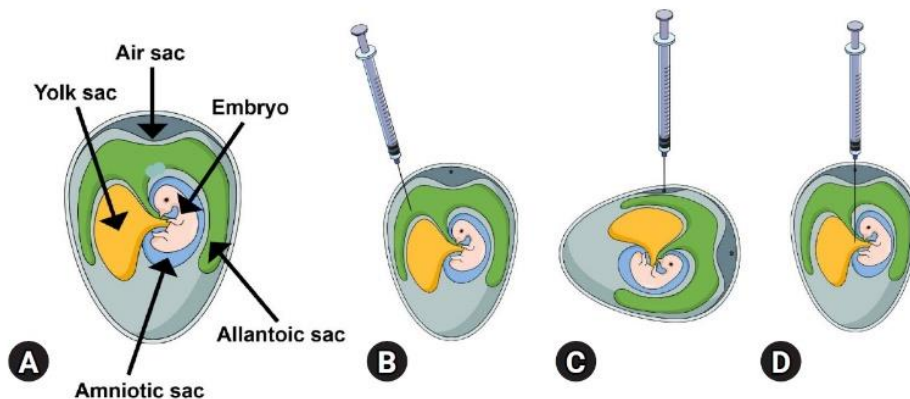
1. Place the egg on a stand (sharp end down) and treat the shell with a 70% alcohol solution, then with iodine, and then again with alcohol. 2. Make a hole in the shell with a sterile scalpel, being careful not to damage the inner shell membrane.

3. Using a syringe and needle, draw up 0.1–0.2 ml of virus and inoculate it into the allantoic cavity, inserting the needle just under the shell.

4. Seal the hole in the shell with a small amount of melted wax.

5. Incubate the infected egg in a humidified incubator with the pointed end down. Incubation time: 42–48 hours.

Method for inoculating chicken embryos into amniotic fluid:



1. Place the egg on a stand (pointed end down) and treat the shell with a 70% alcohol solution, then with iodine, and then again with alcohol.

2. Make a hole in the shell with a sterile scalpel, removing a small section of the shell to insert tweezers.

3. Using sterile tweezers, grasp the amniotic sac and inoculate it with 0.1–0.2 ml of virus using a syringe and needle. Carefully remove the needle without damaging the embryo.

4. Seal the hole in the shell with a portion of the previously removed shell and fill it with a small amount of melted wax.

5. Incubate the infected egg in a humidified incubator, pointed end down. Incubation time: 48–72 hours.

Report Contents

1. Title of the lab work.
2. Objective of the work.
3. Assignment.
4. Conclusions.

Quiz Questions

1. Define a virus in modern virological terms, and explain why viruses are considered obligate intracellular parasites rather than cellular organisms.
2. Who was Dmitri Ivanovsky, and what was his contribution to the discovery of viruses in 1892 during his studies on tobacco mosaic disease?
3. What did Martinus Beijerinck add to Ivanovsky's findings, and why is he credited with introducing the concept of a "contagium vivum fluidum" (soluble living germ)?
4. Why was the development of the electron microscope in the 1930s a turning point in virology, and who was the first scientist to visualize the tobacco mosaic virus (TMV) particles?
5. List the general properties of viruses that distinguish them from bacteria, including their size range, genomic composition, replication strategy, and lack of independent metabolism.
6. Describe the basic architecture of a viral particle (virion). What are the major components: genome, capsid, nucleocapsid, and (in some viruses) envelope?

7. What are the two primary types of capsid symmetry found in viruses?
8. Give a specific example of a virus that exhibits icosahedral symmetry and one that exhibits helical symmetry.
9. Explain the structural organization of a complex virus, using the example of bacteriophage T4. What are the head, tail sheath, base plate, and tail fibres, and what roles do they play in infection?
10. Distinguish between enveloped and non-enveloped (naked) viruses in terms of their structural composition, how they acquire their envelope, and their relative stability in the environment.
11. What is the viral envelope composed of, and what are spike proteins (peplomers)? Provide an example of a viral spike protein that mediates attachment to host receptors (e.g., hemagglutinin of influenza virus).
12. Compare and contrast DNA and RNA viruses regarding their genome structure (single-stranded vs. double-stranded, linear vs. circular, segmented vs. non-segmented). Give one example of a virus with each genome type.
13. How do viruses replicate inside a host cell? Briefly outline the six basic steps of the viral replication cycle: attachment, penetration, uncoating, synthesis, assembly, and release.
14. What are viroids and prions, and how do they differ from typical viruses in terms of structure and infectious nature? Give a disease example for each.
15. How do viral structural features (e.g., presence/absence of an envelope, size, shape, capsid symmetry) influence their stability in the environment, mode of transmission, and susceptibility to disinfectants?

List of key literature:

1. Murray P.R., Rosenthal K.S., Pfaller M.A. *Medical Microbiology*. – 10th ed. – Philadelphia: Elsevier, 2025. – 880 p. (ISBN: 978-0-323-93321-0)
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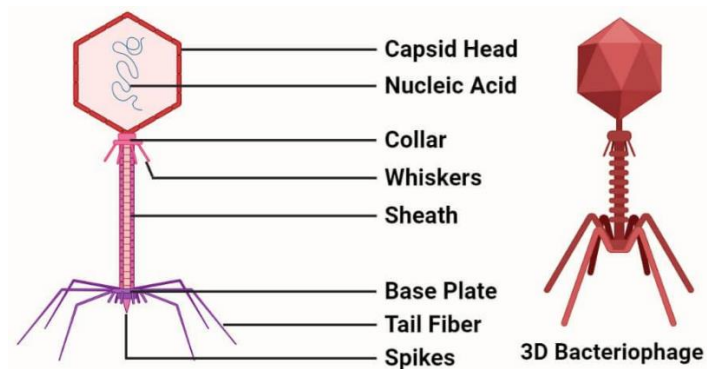
Laboratory work 12

Bacteriophages. Types of phages

The objective of this work is to study of the ability of bacteriophages to lyse bacteria.

Procedure for completing the work

Bacteriophage activity is determined by its ability to lyse a bacterial culture in liquid or solid media. Two methods are most commonly used to detect bacteriophages in laboratory practice: the negative colony method and the color test.



Negative colonies or plaques are round, transparent zones of absent growth of bacteria or cell cultures. The formation of each sterile spot can be caused by a single bacteriophage particle. The number of negative colonies can also serve as a quantitative indicator of the content of infectious phage particles in the test solution.

The color test is based on the difference in color between the medium containing a normal cell culture and the medium containing a virus-infected cell culture. During the development of a normal cell culture, acidic metabolic products accumulate, which leads to a shift in pH toward the acidic side. Since the culture medium contains an indicator, its color changes with a change in pH. Viral infection of a cell culture leads to the development of degenerative processes, including suppression of metabolism and a decrease in glycolysis. As a result, the pH of the medium remains at its initial level, and its color does not change. Thus, the presence or absence of the virus in the material added to the culture can be determined by maintaining the color of the medium.

Equipment: 24-hour *E. coli* broth culture; bacteriophage suspension; virus suspension; cell culture in 199 medium – 2 test tubes. • Sterile glassware: 2 0.1 ml pipettes, 2 1–2 ml pipettes; 1 spatula; 1 Petri dish with GRM agar.

Procedure

1. Set up an experiment to detect bacteriophage using the negative colony method.

Using a sterile pipette, add 0.1 ml of phage suspension to the *E. coli* broth culture. Then transfer 0.1 ml of this mixture to the surface of a solid nutrient medium and spread it evenly with a sterile spatula. Place the Petri dish containing the inoculum in a thermostat set at 37°C. After 24–48 hours, record the results of the experiment by counting the sterile zones on the surface of the bacterial lawn.

2. Set up an experiment to detect the virus using the color test method.

Add 0.1 ml of the virus suspension to each of the cell culture tubes. Place the tubes in a thermostat set at 37°C. After 24–28 hours, note the color of the medium in each tube.

Based on the results obtained, draw a conclusion about the effectiveness of the studied methods for detecting viruses.

Report Contents

1. Title of the lab work.
2. Objective of the work.
3. Assignment.
4. Conclusions.

Quiz Questions

1. Define bacteriophages (phages) and explain why they are described as the most abundant biological entities on Earth.
2. Who independently discovered bacteriophages in 1915 and 1917, and what was the significance of their observations for early virology?
3. Describe the typical morphology of a tailed bacteriophage (order Caudovirales). Name the main structural components, including the capsid, tail sheath, base plate, tail fibres, and the role of each in infection.
4. Compare and contrast the two main types of bacteriophage life cycles: the lytic (virulent) cycle and the lysogenic (temperate) cycle.

5. Explain, step-by-step, the events of the lytic cycle of a virulent phage, including attachment, penetration, biosynthesis of phage components, maturation (assembly), and lysis of the host cell.
6. What is a temperate phage, and what is a prophage? How does a prophage integrate into the bacterial chromosome (e.g., using the enzyme integrase)?
7. Distinguish between lysogenic conversion (phage-mediated gene transfer) and phage-mediated transduction. Give a classic example of a bacterial virulence factor carried by a temperate phage (e.g., diphtheria toxin or cholera toxin).
8. Define generalized transduction and specialized transduction. Which type of phage life cycle is involved in each, and how do they differ in the DNA they transfer between bacterial hosts?
9. What is phage typing, and how is it used in epidemiological surveillance and the identification of bacterial strains (e.g., for *Salmonella* or *Staphylococcus aureus*)?
10. Explain the concept of pseudolysogeny and describe the conditions under which it may occur in phage–host interactions.
11. What are satellite phages (e.g., phage P4) and helper phages, and how do they depend on other phages for their replication?
12. What are phage-encoded endolysins and holins, and what specific roles do these proteins play in the release of progeny virions from the host bacterium?
13. Discuss the phenomenon of phage resistance in bacteria. What mechanisms do bacteria employ to defend themselves against phage infection (e.g., restriction-modification systems, CRISPR-Cas systems, and receptor modification)?
14. Outline the principles and current challenges of phage therapy—the use of bacteriophages to treat bacterial infections. What are the advantages of phages over traditional antibiotics in the era of multidrug resistance?

List of key literature:

1. Murray P.R., Rosenthal K.S., Pfaller M.A. *Medical Microbiology*. – 10th ed. – Philadelphia: Elsevier, 2025. – 880 p. (ISBN: 978-0-323-93321-0)
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Laboratory work 13

Influenza viruses. Methodology of infection of chicken embryo

The objective of this work is to study the properties of influenza viruses, master the principles of rapid diagnostics and methods of culturing viruses in chicken embryos.

Procedure for completing the work

Family: *Orthomyxoviridae* (Greek: orthos - correct, straight; myxa - mucus).

Genus: *Influenzavirus*

Species: A, B, C.

Influenza is an acute viral infection caused by the Influenzavirus genus, characterized by upper respiratory tract infections, fever, and intoxication.

The first description of influenza symptoms is found in the writings of the ancient Greek philosopher Hippocrates.

The most devastating pandemic caused by Influenzavirus is considered to be the H1N1 Spanish flu. Swine flu circulated concurrently in 1918, but no empirical evidence was found that pigs were the intermediate hosts.

The Asian flu pandemic was recorded in 1957, and the Hong Kong flu pandemic in 1968, which were reassortants of human and avian influenza viruses. Chicken embryos are a convenient and relatively inexpensive system for culturing viruses. Almost all influenza viruses of both human and animal origin replicate to varying degrees in embryos.

The virus replicates best in 10- to 12-day-old embryos. Before use, ensure that the eggs are fertilized and the embryos are alive. To do this, simply examine the egg near a bright source in a dark room.

In standard laboratory passaging of strains, the viral inoculum typically consists of infected culture or allantoic fluid with a concentration of 10³–10⁴ plaque-forming units (PFU)/ml.

The virus is injected into the allantoic cavity, from where it can penetrate the chorioallantoic membrane. The viral progeny are released into the allantoic fluid and can be easily removed from the egg. Some strains, particularly new natural isolates, as well as type C viruses, replicate poorly in the chorioallantoic membrane, but grow well when injected directly into the amniotic cavity. This allows the virus to access a variety of embryonic tissues, and the resulting progeny are released into the amniotic fluid, which can be recovered separately.

Practical Part

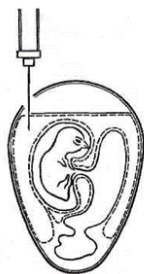
Equipment and Materials: 10-day-old developing chicken embryos, scalpel, syringe, 0.9% NaCl solution for virus dilution, paraffin or wax, test tube with virus-containing material, disinfectant solution, alcohol, iodine, spirit lamp, matches, egg cup, cotton wool, and gauze.

The method for inoculating chicken embryos includes the following steps:

- 1) candling the embryo (10-11 days old), marking the boundary of the air sac and vascular bundle;
- 2) treating the shell with alcohol and iodine, then burning it with an alcohol lamp;
- 3) opening the air sac (puncture) or trepanning the shell;
- 4) introducing material (nasopharyngeal swab) containing the virus into the allantoic cavity (the most common method);
- 5) sealing the opening with paraffin, incubating in a thermostat at 37°C for 48-72 hours.

Method for inoculating the allantoic cavity of a chicken embryo:

1. Place the egg on a stand (sharp end down) and treat the shell with a 70% alcohol solution, then with iodine, and then again with alcohol.
2. Make a hole in the shell with a sterile scalpel, being careful not to damage the inner membrane of the shell.
3. Using a syringe and needle, draw up 0.1–0.2 ml of virus and inoculate it into the allantoic cavity, inserting the needle just under the shell.
4. Seal the hole in the shell with a small amount of melted wax.
5. Incubate the infected egg in a humidified incubator, pointed end down. Incubation time: 42–48 hours.



Method for inoculating chicken embryos into amniotic fluid:

1. Place the egg on a stand (pointed end down) and treat the shell with a 70% alcohol solution, then with iodine, and then again with alcohol.



2. Make a hole in the shell with a sterile scalpel, remove a small section of the shell to insert tweezers.
3. Using sterile tweezers, grasp the amniotic sac and inoculate 0.1–0.2 ml of virus using a syringe and needle. Carefully remove the needle without damaging the embryo.
4. Seal the hole in the shell with a portion of the previously removed shell and pour a small amount of melted wax over it.
5. Incubate the infected egg in a thermostat in a humid atmosphere pointed end down. Incubation time: 48-72 hours.

RHA (hemagglutination assay):

Purpose: To detect the virus in the allantoic fluid of an infected embryo.

Procedure: Place a drop of saline (control) and a drop of allantoic fluid on a glass slide, then add a drop of a 1% chicken red blood cell suspension. If the virus is present in the test drop, agglutination (clumping) of the red blood cells is observed.

	Components	Interaction	Microtiter test results	Hemagglutination Assay • Requirements • Principle • Types • Procedure • Results • Applications • Advantages • Limitations
A	RBC		No reaction	
B	Virus RBC		Hemagglutination	
C	Virus Antibody RBC		Hemagglutination inhibition	

Report Contents

1. Title of the lab work.
2. Objective of the work.
3. Assignment.
4. Conclusions.

Quiz Questions

1. History of the disease. Influenza pandemics.
 2. Taxonomic position of influenza viruses.
 3. General characteristics of orthomyxoviruses.
 4. Structure of the influenza virus virion.
 5. Classification of orthomyxoviruses. Influenza virus types: A, B, C.
 6. Characteristics of the antigenic structure (neuraminidase NA and hemagglutinin HA).
- Concept of antigenic drift and shift.
7. The influenza virus reproduction cycle.
 8. Epidemiology of influenza: sources of infection, routes of transmission, population susceptibility.
 9. Pathogenesis of influenza infection. Damage to the epithelium of the upper respiratory tract, viremia. Immunity (local secretory IgA and systemic).
 10. Clinical presentation of influenza.
 11. Specific prevention and treatment: live and inactivated vaccines, influenza immunoglobulin, interferon preparations, chemotherapeutic agents (oseltamivir, rimantadine).
 12. Laboratory diagnosis of influenza. Material for influenza testing (nasopharyngeal swab, print swabs, blood). Laboratory diagnostic methods:

13. Rapid diagnostics (IFA, PCR).
14. Serological method (HI, ELISA - testing of paired sera).
15. Virological method (infection of chicken embryos).

List of key literature:

1. Murray P.R., Rosenthal K.S., Tenover F.C., Tenover F.C. *Medical Microbiology*. – 10th ed. – Philadelphia: Elsevier, 2025. – 880 p. (ISBN: 978-0-323-93321-0)
2. Carroll K.C., Butel J.S., Morse S.A., et al. *Jawetz, Melnick & Adelberg's Medical Microbiology*. – 29th ed. – New York: McGraw-Hill Education, 2024. – 864 p. (ISBN: 978-1-264-93210-6)
3. Ryan K.J., Ahmad N., Alspaugh J.A., et al. *Ryan & Sherris Medical Microbiology*. – 9th ed. – New York: McGraw-Hill Education, 2024. – 992 p. (ISBN: 978-1-264-26874-0)

List of additional literature:

4. Goering R.V., Dockrell H.M., Zuckerman M., Chiodini P.L. *Mims' Medical Microbiology and Immunology*. – 7th ed. – Philadelphia: Elsevier, 2025. – 600 p. (ISBN: 978-0-323-93324-1)
5. Melter O., Castelhana R. (eds.). *The MicroBook: Clinical Microbiology for Medical Students*. – 1st ed. – Prague: Charles University Press, 2023. – 320 p. (ISBN: 978-80-246-5123-4)
6. Kumar S. *Medical Microbiology For Undergraduates*. – 2nd ed. – New Delhi: Jaypee Brothers Medical Publishers, 2024. – 560 p. (ISBN: 978-93-5465-789-2)

Laboratory work 14

Enterovirus infections. Poliomyelitis viruses. Coxsackie A, B. Echo. Virological method of infection of cell culture

The objective of this work is to study the properties of enteroviruses and methods of their quantitative analysis.

Procedure for completing the work

Family: *Picornaviridae* (pico - very small, rna - RNA).

Genus: *Enterovirus*

Subtype: *Poliovirus*

Serotypes: *PV1, PV2, and PV3*

Subtype: *Coxsackie A virus, Coxsackie B virus, ECHO viruses*.

Enterovirus infections are a group of acute infectious diseases of the gastrointestinal tract caused by various serotypes of the genus *Enterovirus*, characterized by fever and a polymorphic clinical picture.

Poliovirus infection has been known since the time of Hippocrates, who described it as an epidemic leading to paralysis with high mortality rates.

Historical evidence of the presence of this disease around 1500 BC is a slab from a temple in Memphis depicting a priest of Rumu with a deformed (shortened) lower limb. A more detailed study of spinal paralysis in children is associated with the German physician J. Heine, who in 1840 described the clinical presentation, pathogenesis, and recovery from poliomyelitis.

Another serious enteroviral disease that causes herpangina and rash is the Coxsackie virus. Enteroviral infection caused by Coxsackie A virus was first studied by Gilbert Dalldorf and Grace Skiles in 1948 in a small town in upstate New York. They isolated Coxsackie A virus from the feces of a child paralyzed by polio. In 1949, Dr. D. Melnick discovered Coxsackie B virus while infecting mice with material from children with serous meningitis. In 1951, in the United States, Charles Andrews isolated a new type of virus from human feces with a cytopathogenic effect on cells, which he named ECHO (Enteric Cytopathogenic Human Orphan) virus.

1. PRIMARY TRYPSINIZED - chicken embryo fibroblasts (FEF), human embryonic fibroblasts (HEF), human embryonic kidney cells, etc.

2. SEMI-TRANSPLANTABLE - obtained from human embryonic lung or muscle tissue.

3. TRANSPLANTABLE - HeLa (cervical carcinoma), HEp-2 (human laryngeal carcinoma), BHK-21 (hamster kidney), etc.

Equipment and materials: syringe, 0.9% NaCl solution for virus dilution, disinfectant solution, alcohol, iodine, alcohol lamp, matches, cotton wool and gauze, cell culture (e.g., Vero, MRC-5, RD, HeLa) in a monolayer (in test tubes or 96-well plates), virus-containing fluid (culture, nasopharyngeal swabs, etc.), diagnostic immune serum, control serum (normal serum): animal serum that does not contain antibodies to the given virus, cell culture medium (e.g., RPMI-1640, DMEM) with the addition of 2% serum and antibiotics, reference virus: type virus strain to control serum activity, phosphate-buffered saline (PBS), neutral red solution (for staining living cells) or crystal violet.

Safety instructions: work with cell culture and virus-containing material is carried out in a laminar flow Class II cabinet. Dispose of contaminated vials and culture media (autoclaving). Disinfection of work surfaces.

Working with cell cultures

Procedure: To obtain primary cells from a healthy animal, no later than 2-3 hours after slaughter, the relevant organs or tissues are taken, minced into pieces (1-4 mm), and treated with enzymes: trypsin, pancreatin, collagenase, and others. The enzymes break down the intercellular substances, and the resulting individual cells are suspended in a nutrient medium and cultured on the inner surface of test tubes or mattresses in an incubator at 37°C.

As the cells multiply, they settle on the glass surface and, when completely covered in a single layer, contact each other and stop dividing (contact inhibition). A layer one cell thick forms on the glass (therefore, these cell cultures are called single-layer or monolayer).

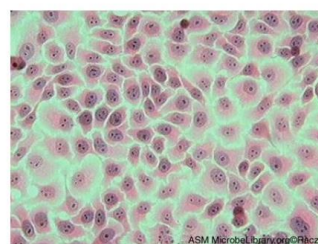
Typically, a monolayer forms within 3-5 days. The rate of its formation depends on the type of tissue, age, and cell type. Animal, nutrient medium quality, cell inoculation concentration, and other factors.

Virus detection in tissue culture cells:

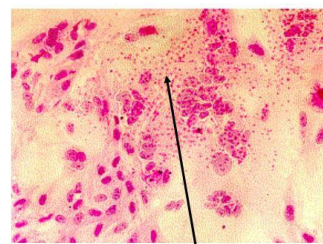
- Hemadsorption – adsorption of red blood cells added to tissue culture culture infected with parainfluenza virus (demonstration);

Hemadsorption (Hads)

- Virus growth in cell cultures is detected by testing for hemadsorption: red cells are added to the culture and adhere to virus budding from infected cells.
- If the culture tests positive, hemadsorption inhibition test with specific antisera is used to identify the virus.



cell culture



positive Hads

- Suppression of cell metabolism by poliovirus (color test – demonstration). Tissue culture cells produce acidic metabolic products during cultivation, causing the color of the medium, to which methyl red indicator is added, to change from red to yellow. Virus-infected cells are destroyed, and the color of the medium remains unchanged, remaining red;

- Viral interference – absence of virus reproduction in tissue culture infected with another virus that does not have a cytopathic effect (demonstration).

Report Contents

1. Title of the lab work.

2. Objective of the work.
3. Assignment.
4. Conclusions.

Quiz Questions

- 1/ General characteristics of the Picornaviridae family. Genus Enterovirus.
2. Classification of human enteroviruses.
3. Morphology and antigenic properties of the poliovirus.
4. Epidemiology of polio. Mechanism of transmission. Portals of entry. Clinical manifestations of polio, complications.
5. Pathogenesis of polio: stages (intestinal, lymphogenous, viremia, neural). Damage to motor neurons of the anterior horn of the spinal cord.
6. Treatment of polio and specific prevention.
7. Methods of specific and non-specific prevention. Specific prevention: Salk vaccine (inactivated IPV) and Sebin vaccine (live OPV). Differences, vaccination schedule.
8. Morphology and antigenic properties of Coxsackieviruses and ECHO viruses.
9. Clinical course variants of diseases caused by Coxsackie and ECHO viruses.
10. Laboratory diagnostic methods for diseases caused by Coxsackie viruses.
11. Features of laboratory diagnostics of diseases caused by ECHO viruses.
12. Clinical forms: abortive, meningeal, paralytic (spinal, bulbar).
13. Laboratory diagnostics: virus isolation from feces and cerebrospinal fluid in cell cultures (HeLa, Vero, primary fibroblasts), identification by CPE. Serotyping in RN (neutralization tests).
14. Stability in the environment.
15. Epidemiology of enterovirus infections (fecal-oral and airborne transmission). Seasonality.
16. Diseases caused by non-polomyelitic enteroviruses (herpangina, pleurodynia, myocarditis, aseptic meningitis, exanthema).
17. Pathogenesis: portal of entry (gastrointestinal and upper respiratory tract mucosa), replication in lymphoid tissue, viremia, CNS and visceral organ damage.
18. Laboratory diagnosis of enteroviruses.
19. Virological method.
20. Serological method (complex-base balance, neutralization reaction).

List of key literature:

1. Murray P.R., Rosenthal K.S., Pfaller M.A. *Medical Microbiology*. – 10th ed. – Philadelphia: Elsevier, 2025. – 880 p. (ISBN: 978-0-323-93321-0)
2. Carroll K.C., Butel J.S., Morse S.A., et al. *Jawetz, Melnick & Adelberg's Medical Microbiology*. – 29th ed. – New York: McGraw-Hill Education, 2024. – 864 p. (ISBN: 978-1-264-93210-6)
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Laboratory work 15

Viral hepatitis. Study of phenomena that allow the detection of viruses

The objective of this work is to study the properties of hepatitis viruses, methods of their indication and identification.

Procedure for completing the work

Family: *Picornaviridae* (pico - very small, rna - RNA).

Genus: *Hepatovirus*

Hepatitis A (HAV)

Family: *Hepadnaviridae* (hepa - liver, dna - DNA).

Genus: *Orthohepadnavirus*

Hepatitis B (HBV)

Family: *Flaviviridae* (flavus - yellow).

Genus: *Hepacivirus*

Hepatitis C (HCV)

Family: *Unspecified* (viroid-like)

Genus: *Deltavirus*

Hepatitis D (HDV)

Family: *Hepeviridae*

Genus: *Orthohepevirus*

Hepatitis E (HEV)

Family: *Flaviviridae*

Genus: *Pegivirus*

Hepatitis HGV/GBV-C (G)

Family: *Anelloviridae* (anello - ring, dna - DNA).

Genus: *Alphatorquevirus*

Hepatitis TTV

Viral hepatitis is a group of infectious diseases that primarily affect liver hepatocytes. The pathogens belong to different families and have different mechanisms of infection.

The first descriptions of a contagious form of jaundice date back to the 5th century BC in the writings of Hippocrates.

In 1849, the German pathologist Rudolf Virchow called the disease "catarrhal jaundice," associating the pathology with bile duct obstruction. However, 39 years later, Russian clinician Sergei Petrovich Botkin refuted this theory, attributing the disease's infectious nature to its underlying cause. Subsequently, Alexander Andreevich Kisel, the founder of the Russian pediatric school, proposed calling infectious hepatitis "Botkin's disease."

In the mid-20th century, experimental studies on volunteers allowed British virologist Frederick Ogden McCallum to divide hepatitis into two forms: hepatitis A (short incubation period, fecal-oral transmission) and hepatitis B (prolonged incubation period, parenteral transmission).

In 1965, American geneticist Baruch Blumberg discovered surface HBsAg of hepatitis B in the blood of an Australian Aborigine, and in 1970, British virologist David Surrey Dane described the complete virion under an electron microscope.

That same year, American virologist Harvey Alter documented cases of post-transfusion hepatitis, which was later discovered by Michael Hought and identified as the hepatitis C virus.

In 1973, American virologist Stephen Feinstone isolated and described the hepatitis A virus from the feces of patients in the acute phase of the disease. In 1977, while studying liver biopsies from hepatitis B patients, Italian scientist Mario Rizzetto discovered an unknown antigen in the nucleus of hepatocytes, which turned out to be the antigenic subunit of the hepatitis D virus.

In 1978, a new, large-scale hepatitis epidemic broke out in Kashmir, resulting in death and most often affecting pregnant women. In 1983, Russian virologist Mikhail Surenovich Balayan

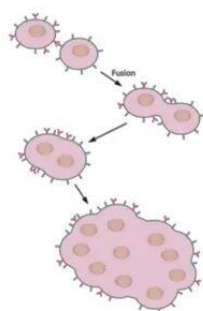
investigated the new hepatitis E virus by heroically infecting himself by drinking an extract of a patient's feces. Practical Part

Equipment and Materials: Syringe, 0.9% NaCl solution for virus dilution, disinfectant solution, alcohol, iodine, alcohol lamp, matches, cotton wool and gauze, cell culture (e.g., Vero, MRC-5, RD, HeLa) in a monolayer (in test tubes or 96-well plates), virus-containing fluid (culture, nasopharyngeal swabs, etc.), diagnostic immune serum, control serum (normal serum): animal serum that does not contain antibodies to the given virus, cell culture medium (e.g., RPMI-1640, DMEM) supplemented with 2% serum and antibiotics, reference virus: type virus strain to control serum activity, phosphate-buffered saline (PBS), neutral red solution (for staining living cells) or crystal violet.

Safety Instructions: Comply with biosafety regulations when working with Diagnostic systems, reagents, and potentially infectious material (serum, plasma) for ELISA and PCR. Rules for collecting, storing, and transporting samples for diagnosing viral hepatitis.

Cytopathic effect (CPE) of the virus.

The purpose of the method is to identify the virus or determine the level of neutralizing antibodies by suppressing the cytopathic effect (CPE) in cell culture with specific immune serum.



Procedure: 1) virus preparation; 2) neutralization reaction; 3) cell infection.

Step 1: Preparation of the working dose of virus

Virus titration: Before the experiment, the virus titer is determined (usually in TCID₅₀/ml – tissue cytopathic dose). A standardized dose of 100 TCID₅₀/0.2 ml is used in the reaction.

Preparation of dilutions: The stock virus is diluted with the maintenance medium so that 0.2 ml contains 100 TCID₅₀.

Step 2: Setting up the neutralization reaction

The experimental design requires several controls. The experiment is conducted under sterile conditions (a laminar flow hood).

Mixing: In sterile test tubes or a plate, mix equal volumes (e.g., 0.2 ml each) of the working dilution of the virus and the diagnostic serum.

Controls are prepared simultaneously: virus + normal serum; virus + medium (virus control); medium + cells (cell control).

Incubation (Neutralization): The mixtures are incubated at room temperature for 2 hours (or 1 hour at 37°C) to allow the antigen-antibody complex to form.

Step 3: Cell Inoculation: The growth medium is drained from all cell culture tubes. Add 0.2 ml of each prepared mixture (virus + serum) to 2-4 tubes (wells) containing a cell monolayer. For better virus adsorption, the plates are incubated for 1-2 hours at 37°C in a CO₂ incubator.

After adsorption, add 0.8-1.0 ml of maintenance medium to each tube (well) to cover the monolayer.

Incubation: The cultures are incubated at 36-37°C until signs of CPE appear. The incubation period depends on the virus (3 to 14 days). For some viruses (e.g., coronaviruses), the optimal temperature may be 33°C.

The results are analyzed microscopically, comparing the condition of the cells in the experiment and controls. Validity criteria: The reaction is only considered if: The control cell monolayer (uninfected) is intact, with no signs of degeneration. The virus control (virus without

serum) shows pronounced CPE (cell destruction). The control of normal serum containing the virus shows the presence of CPE (i.e., does not protect the cells).

Result evaluation: Positive result (virus neutralized): The cells in the wells containing the test serum look the same as those in the control cell monolayer. This means that the serum contains specific antibodies that have bound the virus and prevented its entry into the cells.

Negative result (virus not neutralized): CPE is observed (cell rounding, detachment from the glass, destruction), similar to the virus control.

Fixation and staining methods (for quantitative counting): In the micromethod (96-well plates), neutral red staining is used for accurate counting. Living cells absorb the dye. Three to four days after infection, the dye is added, incubated for two hours, then the dye is extracted (with an alcohol buffer), and the optical density is measured using a spectrophotometer. The higher the density (stain intensity), the better the cell survival (i.e., the higher the neutralization).

Crystal violet: Cells are fixed in formalin and stained. Live cells are stained, while dead cells are washed away.

1. Virus type determination:

The isolated virus type is determined by the serum that completely prevented cell degeneration (i.e., gave a positive result).

Plaque assay (cytopathic effect counting method).

Method: Quantitative counting of the virus in a cell culture under an agar overlay. After infection, a monolayer of cells is covered with a layer of semi-solid agar, which contains a nutrient medium and a neutral red dye. In areas of the monolayer where virus replication occurs, groups of degenerated cells form that do not stain and appear as transparent spots (plaques) against a red background.



Procedure: 1) A monolayer of cells in Petri dishes is infected with tenfold dilutions of virus-containing material;

2) The cells are covered with a thin layer of agar containing a nutrient medium and neutral red (indicator);

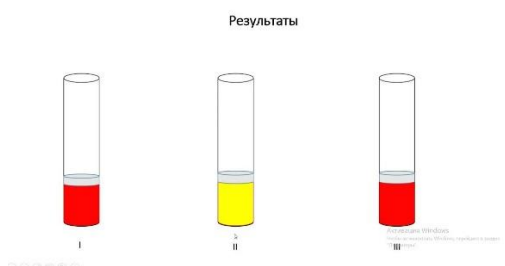
3) Incubated;

4) Living cells absorb the dye and stain red. Virus-infected cells (lysis zones, or "plaques") do not stain and appear as light spots against a red background.

Result: Plaque count and viral titer recalculation (PFU – plaque-forming units).

Salk color test.

Principle of the method: cell culture growth in a nutrient medium is accompanied by the accumulation of acidic metabolic products and a change in pH, which is visually manifested by a change in the color of the indicator. During the replication of some viruses, cells die, and no accumulation of metabolic products or color change of the indicator occurs.



Hemadsorption Phenomenon.

Principle of the method: contact of virus-infected cells with erythrocytes leads to adsorption of the latter on the cell surface, which can be detected under low magnification. Uninfected cells do not adsorb erythrocytes. The hemadsorption reaction is used for the early detection of viruses in cell culture.

Interference Phenomenon.

Principle of the method: The interference phenomenon is used to detect viruses that do not produce a distinct CPE in cell culture. The cell culture is infected with the test material and then an indicator virus (VSV - vesicular stomatitis virus) is added. If the test material contains the virus, the CPE of the indicator virus will be absent (the cell is "occupied" by the target virus), which can be assessed visually under low magnification.

Report Contents

1. Title of the lab work.
2. Objective of the work.
3. Assignment.
4. Conclusions.

Quiz Questions

1. Taxonomy of hepatitis viruses.
2. Comparative characteristics of hepatitis viruses (A, B, C, D, E).
3. Nucleocapsid structure of viral hepatitis representatives.
4. Features of the antigenic structure of hepatoviruses.
5. Resistance of hepatitis viruses to environmental factors.
6. Epidemiological features, transmission mechanisms, and sources of infection.
7. Pathogenesis: the role of the immune response in hepatocyte damage (cytolysis, cholestasis).
8. Clinical presentation: syndromes (cytolytic, cholestatic, asthenovegetative).
9. Specific laboratory diagnostics.
10. Serological markers (ELISA): anti-HAV IgM, HBsAg, anti-HBc IgM, anti-HCV, anti-HEV IgM.
11. PCR diagnostics (qualitative and quantitative) to determine viral activity (HBV-DNA, HCV-RNA).
12. Therapy principles: interferons, direct-acting antiviral drugs (for hepatitis C).
13. Prevention: vaccination against hepatitis A and B, non-specific prophylaxis (blood testing, sterile instruments).

List of key literature:

1. Murray P.R., Rosenthal K.S., Tenover F.C., Tenover F.C. *Medical Microbiology*. – 10th ed. – Philadelphia: Elsevier, 2025. – 880 p. (ISBN: 978-0-323-93321-0)
2. Carroll K.C., Butel J.S., Morse S.A., et al. *Jawetz, Melnick & Adelberg's Medical Microbiology*. – 29th ed. – New York: McGraw-Hill Education, 2024. – 864 p. (ISBN: 978-1-264-93210-6)
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Laboratory work 16

Causative agents of herpes virus infections. Serodiagnosis of viral infections

The objective of this work is to study the properties of herpes viruses, methods of their indication and identification.

Procedure for completing the work

Family: *Herpesviridae*

Genus: *Simplexvirus*

Species: *Human alphaherpesvirus 1 (HSV-1)*, *Human alphaherpesvirus 2 (HSV-2)*

Genus: *Varicellovirus*

Species: *Human alphaherpesvirus 3 (VZV)*

Genus: *Cytomegalovirus*

Species: *Human betaherpesvirus 5 (HCMV)*

Genus: *Roseolovirus*

Species: *Human betaherpesvirus 6 (HHV-6)*

Genus: *Lymphocryptovirus*

Species: *Human gammaherpesvirus 4 (EBV)*

Genus: *Rhadinovirus*

Species: *Human gammaherpesvirus 8 (HHV-8)*

Diseases caused by herpesviruses have been known to mankind since ancient times. The term "herpes" (from the Greek ἕρπης, meaning "creeping" or "spreading") was coined by Hippocrates (5th century BC), who described the vesicular skin rashes characteristic of this infection. This image of a rash "creeping" across the skin accurately reflected the clinical picture of the disease.

Besides Hippocrates, similar skin manifestations are mentioned in the works of Celsus, Herodotus, Galen, and Avicenna. Historically, distinguishing between genital herpes and syphilis was an important step. This task was undertaken by John Astruch, court physician to King Louis XIV, who studied the diseases of French women.

In the 19th century, scientists moved from simple description to experimentation. It was demonstrated that fluid from herpes blisters could transmit the disease from person to person, demonstrating its infectious nature. A crucial stage in the study of herpes began in the 20th century, when its viral nature was proven. In 1912, the German physician and virologist W. Grüter first demonstrated that the contents of herpes vesicles could cause characteristic lesions in experimental animals, providing some of the first convincing evidence of the infectious nature of the disease. Later, the herpes simplex virus was isolated and characterized as an independent pathogen.

Cytomegalovirus has its own distinct history of study. As early as the 19th century, the German pathologist Hugo Ribbert described large cells with enlarged nuclei, which were later found to be associated with cytomegalovirus infection. In the mid-20th century, with the development of cell cultures and electron microscopy, cytomegalovirus was isolated and finally recognized as a distinct herpesvirus. The rapid development of virology in the 1950s–1970s allowed for the isolation and study of the major human herpesviruses, their ability to form latent infections and reactivate, and the clarification of the clinical significance of each type. Later, using molecular genetic methods, the modern taxonomy of the Herpesviridae family was established, divided into the subfamilies Alphaherpesvirinae, Betaherpesvirinae, and Gammaherpesvirinae. An important practical step was the development of antiherpes drugs, primarily acyclovir, which significantly improved the prognosis for severe and generalized forms of infection.

Human herpesviruses cause a wide range of diseases, with clinical manifestations depending on the virus type. Herpes simplex virus type 1 most commonly affects the oral mucosa, lips, facial skin, eyes, and central nervous system, causing labial herpes, herpetic stomatitis, keratitis, and herpes encephalitis. Herpes simplex virus type 2 is primarily associated with genital herpes, neonatal herpes, and can also cause meningitis and disseminated infection. Varicella-zoster virus causes chickenpox in children and herpes zoster in adults, while Epstein-Barr virus is the

causative agent of infectious mononucleosis and is associated with a number of lymphoproliferative diseases and cancers. Cytomegalovirus can cause mononucleosis-like syndrome, congenital cytomegalovirus infection, hepatitis, retinitis, pneumonia, and colitis, especially in debilitated patients. Herpesvirus type 6 most often causes sudden exanthema in young children, and herpesvirus type 7 is also associated with roseola. Herpesvirus type 8 does not cause typical acute infection but plays a key role in the development of Kaposi's sarcoma and some lymphoproliferative diseases.

Practical Part

Equipment and Materials: Sample preparations of herpes vesicle swabs, mucosal scrapings, and paired blood sera; fluorescent antibodies to HSV-1, HSV-2, and CMV antigens; ELISA test system for detecting antibodies to herpes viruses; microscope slides, coverslips; wash buffers, TMB substrate, and H₂SO₄; fluorescence or light microscope; ELISA plate, spectrophotometer; sterile pipettes and spatulas; disinfectant solution, 70% alcohol, iodine, cotton wool, gauze, gloves, and an alcohol lamp.

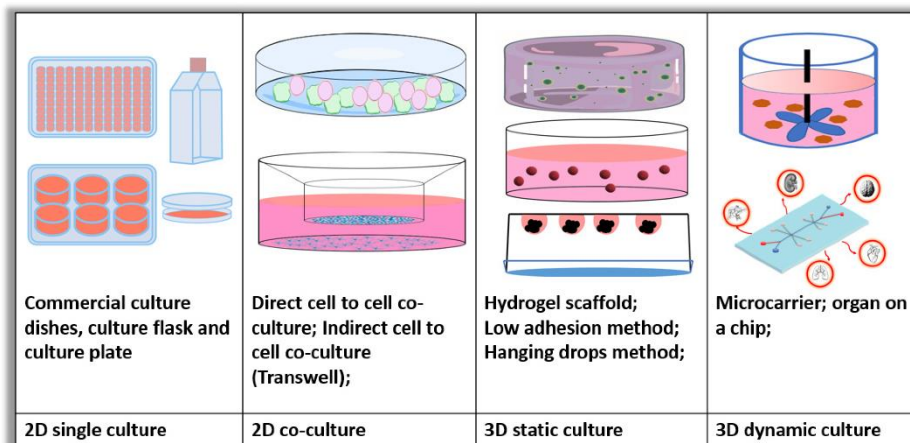
Safety Instructions: When handling sample preparations containing biological material, strictly observe aseptic and antiseptic precautions. Wear gloves and handle all manipulations near an alcohol lamp flame. After work, treat the work area with a disinfectant solution and dispose of consumables in a disinfectant container. The study specimens are safe, but are considered potentially infectious.

Cell Culture Infection Method (Demonstration)

Objective: Demonstrate the cytopathic effect of herpesviruses in cell culture.

Procedure:

1. Prepare a monolayer of susceptible cells (e.g., Vero or HEp-2) in test tubes or on glass slides.



From 1877 Till Now

2. Add the sample viral material (0.1–0.2 ml) to the cell culture and incubate at 37°C in 5% CO₂ for 24–48 hours.

3. Observe the development of the cytopathic effect: cell rounding, syncytia formation, monolayer breakdown, and intranuclear inclusions.

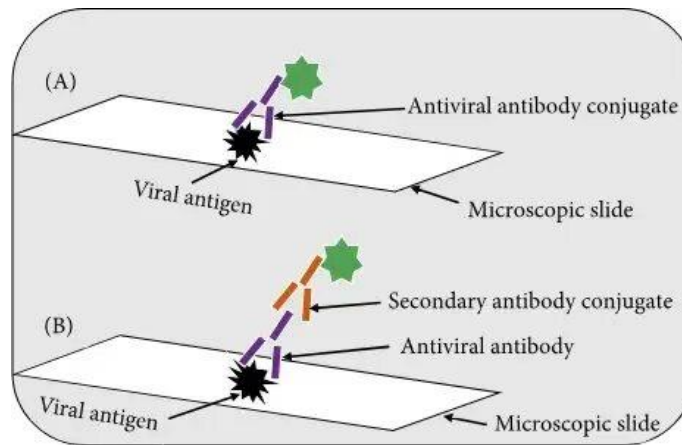
4. Confirm the presence of the virus using an immunofluorescence assay on the cells.

Immunofluorescence Assay (IFA)

Objective: Detect herpesvirus antigens in clinical specimens.

Procedure:

1. Place a drop of the sample (vesicle contents or scraping) on a glass slide, spread it into a thin layer, and air-dry.
2. Fix the specimen in a 1:1 acetone-ethanol mixture for 10 minutes at room temperature.
3. Add 1 drop of fluorescent antibodies to HSV-1, HSV-2, or CMV and incubate for 30 minutes at 37°C in a humidified chamber.



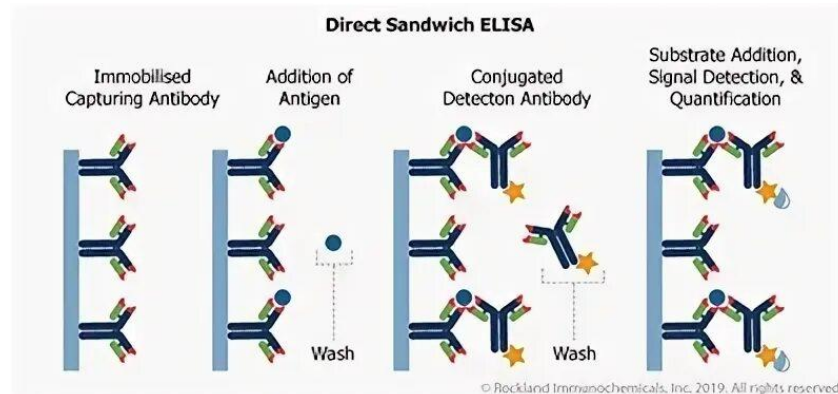
4. Wash with buffer (phosphate-saline, pH 7.2) three times for 5 minutes.
5. Mount in glycerol and examine under fluorescent light (FITC filter). The presence of antigen is indicated by a specific green fluorescence of the affected cells.

ELISA Procedure

Purpose: Detection of specific antibodies to herpes viruses in serum.

Procedure:

1. Add HSV-1/2 or CMV antigen (100 μ l per well) to the wells of the plate and incubate for 1 hour at 37°C.



2. Wash three times with Tween-20 buffer.
3. Add patient serum (diluted 1:100, 100 μ l) and incubate for 1 hour at 37°C.
4. Wash five times, add conjugate (HRP-labeled anti-human IgG, diluted 1:5000), and incubate for 1 hour.
5. Wash five times, add TMB substrate (100 μ l), and incubate for 15 minutes in the dark.
6. Stop the reaction with 2N H₂SO₄ (50 μ l) and read the optical density at 450 nm.

Cytological examination technique

Objective: To identify cytopathic changes in herpesvirus infection.

Procedure:

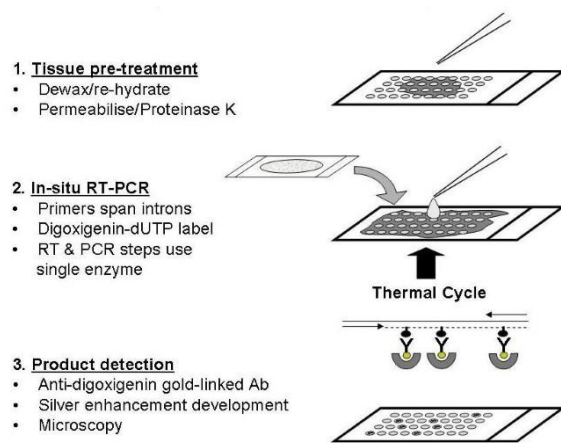
1. Apply a drop of the sample to a glass slide, spread it into a thin layer, dry, and fix with methanol.
 2. Stain using the Romanovsky-Giemsa method: soak in the solution for 10 minutes, rinse with water, and dry.
 3. Microscope at $\times 1000$ magnification.
- Herpesvirus infection is characterized by multinucleated giant cells, intranuclear inclusions, and balloon-shaped cells.

PCR diagnostics (demonstration)

Objective: To detect herpesvirus DNA in clinical specimens.

Procedure:

1. Extract DNA from the specimen (lysis, centrifugation, sedimentation).
2. Amplify in a thermal cycler with primers for HSV, CMV, or EBV (35 cycles).



- Analyze the products by electrophoresis in a 2% agarose gel.
- Visualize under UV light: a positive result is an amplicon band (HSV - 228 bp, CMV - 170 bp).

Report Contents

- Title of the lab work.
- Objective of the work.
- Assignment.
- Conclusions.

Quiz Questions

- Taxonomy of herpes viruses.
 - The structure of the nucleocapsid of herpes virus representatives.
 - Features of the antigenic structure of herpes viruses.
 - Herpes viruses. Classification.
 - Characteristics of herpes simplex viruses.
 - Resistance of herpes viruses to environmental conditions.
 - Epidemiological features, transmission mechanisms, and sources of infection.
 - What are the sources and routes of transmission of herpes simplex virus types 1 and 2?
- What are the epidemiological differences between these two types?
- Explain why the same virus (varicella-zoster virus) causes two different clinical syndromes: chickenpox in children and herpes zoster in the elderly and immunocompromised individuals.
 - Which herpesviruses have oncogenic potential, and with what malignancies are they associated? Give examples of Burkitt's lymphoma, nasopharyngeal carcinoma, and Kaposi's sarcoma.
 - What type of immunity develops after a herpesvirus infection—sterile or nonsterile? What is the role of cellular and humoral immunity in controlling latent infection and preventing reactivation?
 - List all groups of laboratory diagnostic methods for herpesvirus infections: virological, serological, and molecular genetic. What are the advantages and limitations of each group of methods?
 - What is the virological method (gold standard) for diagnosing herpesvirus infections? What cell cultures are used to cultivate various herpesviruses? Describe the cytopathic effect of herpes simplex virus, cytomegalovirus, and varicella-zoster virus.
 - What is the principle of the cytological method for diagnosing herpes infection? What clinical specimens are examined? Describe the microscopic appearance (Cowdry bodies, multinucleated giant cells, ballooning degeneration). What are the sensitivity and specificity of this method?
 - Explain the principle of enzyme-linked immunosorbent assay (ELISA) in diagnosing herpesvirus infections. What is a solid-phase "sandwich" ELISA? What components are required for the reaction?

16. What does the detection of IgM antibodies to herpes viruses in the serum mean? What does the detection of IgG antibodies mean? What is the dynamics of the appearance of these antibodies during primary infection and reactivation?

17. Define the concept of "antibody avidity." What is the clinical significance of determining IgG avidity? Under what pathological conditions is low avidity determined? What are the numerical criteria for interpreting the avidity index? 18. What is the linear immunoblot method? What are its advantages over the classical enzyme-linked immunosorbent assay? What antigens are used in the Line-Blot HHV Profile diagnostic kit?

19. In what clinical situations is the polymerase chain reaction method preferred for diagnosing herpesvirus infections? What is the difference between qualitative and quantitative polymerase chain reaction?

20. What are the features of serological diagnosis of infection caused by the Epstein-Barr virus? What are the VCA antigen, EA antigen, and EBNA antigen? What antibody profile is characteristic of acute infectious mononucleosis?

21. Conduct a comparative analysis of serological profiles in primary infection with the herpes simplex virus type 2, reactivation of latent infection, and latent carriage without clinical manifestations.

22. Clinical presentation

23. Varicella-zoster virus.

24. Cytomegalovirus. Characteristics. Significance in pregnancy and fetal pathology.

25. Principles of herpes infection therapy.

26. Prevention: herpes vaccination, non-specific prophylaxis.

List of key literature:

1. Murray P.R., Rosenthal K.S., Tenover F.C., Tenover F.C. *Medical Microbiology*. – 10th ed. – Philadelphia: Elsevier, 2025. – 880 p. (ISBN: 978-0-323-93321-0)
2. Carroll K.C., Butel J.S., Morse S.A., et al. *Jawetz, Melnick & Adelberg's Medical Microbiology*. – 29th ed. – New York: McGraw-Hill Education, 2024. – 864 p. (ISBN: 978-1-264-93210-6)
3. Ryan K.J., Ahmad N., Alspaugh J.A., et al. *Ryan & Sherris Medical Microbiology*. – 9th ed. – New York: McGraw-Hill Education, 2024. – 992 p. (ISBN: 978-1-264-26874-0)

List of additional literature:

4. Goering R.V., Dockrell H.M., Zuckerman M., Chiodini P.L. *Mims' Medical Microbiology and Immunology*. – 7th ed. – Philadelphia: Elsevier, 2025. – 600 p. (ISBN: 978-0-323-93324-1)
5. Melter O., Castelhana R. (eds.). *The MicroBook: Clinical Microbiology for Medical Students*. – 1st ed. – Prague: Charles University Press, 2023. – 320 p. (ISBN: 978-80-246-5123-4)
6. Kumar S. *Medical Microbiology For Undergraduates*. – 2nd ed. – New Delhi: Jaypee Brothers Medical Publishers, 2024. – 560 p. (ISBN: 978-93-5465-789-2)

Laboratory work 17

Gram-positive cocci, pathogens of purulent-inflammatory infections. Methods of identification of the pathogen

The objective of this work is to study the morphological, cultural and biochemical properties of staphylococci, streptococci, pneumococci and enterococci, to master the methods of microbiological diagnosis of purulent-inflammatory diseases, to learn to differentiate pathogenic and opportunistic cocci.

Procedure for completing the work

Family: Staphylococcaceae

Genus: Staphylococcus (from the Greek "staphylos" - bunch of grapes, "coccus" - berry, grain)

Species: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, etc.

Family: Streptococcaceae

Genus: Streptococcus (from the Greek "streptos" - bunch of grapes, "coccus" - berry, grain)

Species: *Streptococcus pyogenes* (group A), *Streptococcus agalactiae* (group B), *Streptococcus pneumoniae*, *Streptococcus viridans*, etc.

Purulent-inflammatory infections caused by staphylococci and streptococci are a group of infectious diseases characterized by a local or systemic inflammatory process with the formation of pus, caused by The invasion and proliferation of these microorganisms in body tissues, possibly leading to sepsis.

Staphylococci were first described by Louis Pasteur in 1880, and in 1881, Scottish surgeon Alexander Ogston discovered them in abscess pus and gave them the generic name *Staphylococcus* due to the characteristic cluster-like arrangement of the cells in smears. Robert Koch also studied these microorganisms. In 1884, Friedrich Rosenbach first isolated a pure culture and divided staphylococci into species based on colony color: *S. aureus* (golden), *S. albus* (white), and *S. citreus* (lemon-yellow).

Streptococci were first observed by Theodor Billroth in 1874 in wound infections. In 1883, Friedrich Feleisen described them in detail and proposed the name *Streptococcus*. In 1879, Louis Pasteur isolated streptococci from the blood of women with postpartum sepsis. In 1903, Schottmüller divided streptococci into hemolytic types based on their growth pattern on blood agar.

Practical Work

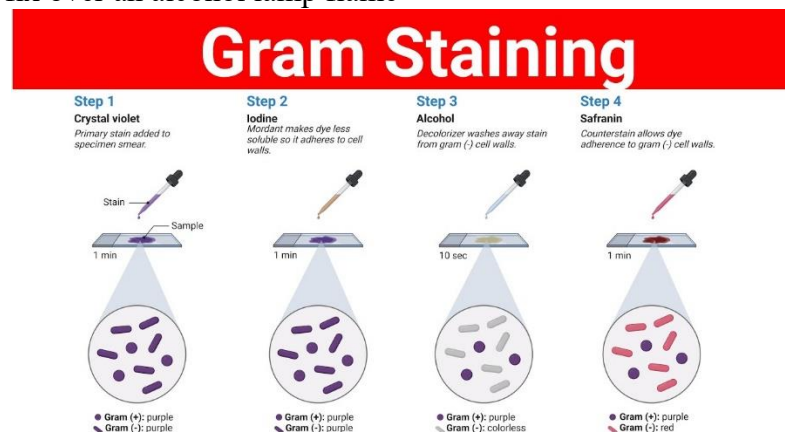
Equipment and Materials:

- Immersion microscopes
- Slides and coverslips
- Gram stain kit
- Bacteriological loops, alcohol lamps
- Nutrient media: yolk-salt agar (YSA), blood agar, MPA, MPB
- 24-hour staphylococcal cultures (museum strains)
- Purulent discharge (model or educational material)
- Coagulase activity test kit (rabbit plasma)
- Antibiotic discs
- Racks, test tubes, pipettes

Procedure:

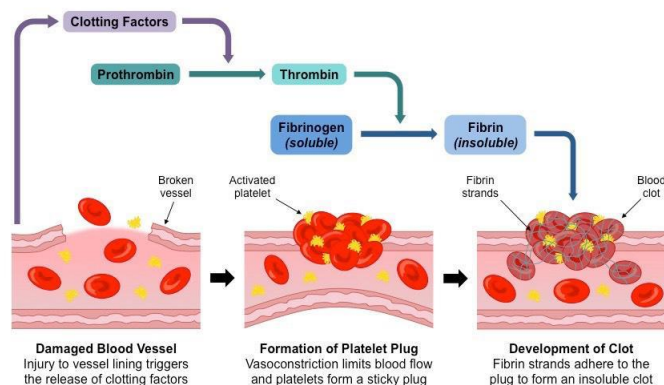
1. Preparing a smear and Gram staining

- Apply a thin layer of the sample (pus, culture) to a defatted glass slide
- Air dry, fix over an alcohol lamp flame

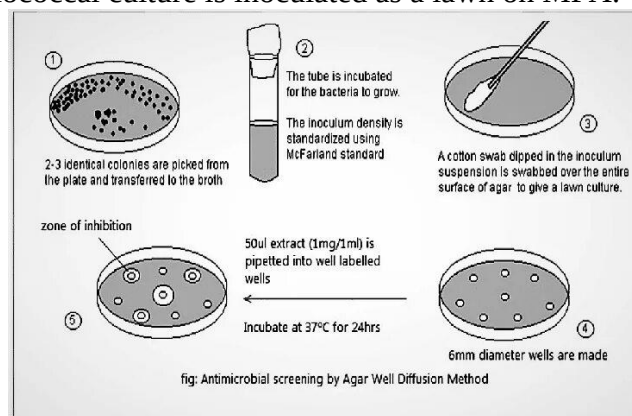


- Gram stain: gentian violet (1 min) → Lugol's solution (1 min) → rinse → alcohol (30 sec) → fuchsin (1 min)
- Dry, examine with an immersion system

- Result: Gram-positive cocci arranged in grape-like clusters (staphylococci) or chains (streptococci)
- 2. Inoculating onto nutrient media
 - Use a sterile loop to collect the sample
 - Inoculate sectors onto a Petri dish with YSA and blood agar
 - Incubate the cultures in a thermostat at 37°C for 24-48 hours
 - Results:
 - o On FSA: lecithinase activity (formation of a rainbow halo around the colonies) is a sign of staphylococcal pathogenicity
 - o On blood agar: zone of hemolysis around the colonies (β -hemolysis in pathogenic streptococci)
 - o Pigment: golden, white, lemon-yellow
- 3. Determination of plasmacoagulase activity
 - A loopful of a 24-hour staphylococcal culture is added to a test tube with 0.5 ml of rabbit plasma.



- Incubate in a thermostat for 2-4 hours and then after 24 hours.
- Recording: The appearance of a clot (plasma coagulation) indicates the presence of coagulase, a sign of *S. aureus* pathogenicity.
- 4. Determination of antibiotic susceptibility (disc diffusion method)
 - A 24-hour staphylococcal culture is inoculated as a lawn on MPA.



- Disks with Antibiotics
- Incubate for 24 hours at 37°C
- Recording: Measure the diameter of growth inhibition zones and compare with table values

Conclusions: During the practical lesson, methods for microscopic identification of gram-positive cocci were mastered, the cultural properties of staphylococci in differential diagnostic media were studied, pathogenicity factors (lecithinase, plasmacoagulase) and antibiotic sensitivity were determined. It was established that the presence of lecithinase and plasmacoagulase activity is characteristic of pathogenic *S. aureus*.

Quiz Questions

1. Taxonomy of the family. Genus *Staphylococcus*
2. Species composition of staphylococci, clinically significant species
3. Morphology of staphylococci, tinctorial properties
4. Cultural properties of staphylococci on simple and differential media
5. Enzymatic activity of staphylococci
6. Pathogenicity factors: adhesins, capsule, aggressive enzymes
7. Staphylococcal toxins: hemolysins, leukocidin, exfoliatin, toxic shock syndrome toxin
8. Coagulase-positive and coagulase-negative staphylococci
9. The role of *S. aureus* in human pathology
10. The role of *S. epidermidis* and *S. saprophyticus* as opportunistic pathogens
11. Pathogenesis of staphylococcal infections
12. Streptococci: classification by type of hemolysis (α , β , γ)
13. Antigenic structure of streptococci (Lancefield serogroups)
14. Factors of streptococcal pathogenicity
15. Rheumatism and post-streptococcal glomerulonephritis as immunopathological complications
16. Epidemiology of streptococcal infections
17. Material for research in various forms of purulent-inflammatory diseases
18. Methods of microbiological diagnosis of staphylococcal infections
19. Antibiotic resistance: methicillin-resistant staphylococci (MRSA)
20. Specific prevention and treatment of staphylococcal infections
21. The concept of staphylococcal carriage
22. The sanitary and indicative significance of staphylococci

Gram-negative pathogenic cocci, the causative agents of gonorrhea and meningococcal infection. Bacterioscopic diagnosis

The objective of this work is to study the biological properties of *Neisseria*, the causative agents of gonorrhea and meningococcal infection, to master laboratory diagnostic methods, and to learn to differentiate pathogenic *Neisseria* from saprophytic species.

Procedure for completing the work

Family: *Neisseriaceae*

Genus: *Neisseria*

Species: *Neisseria gonorrhoeae* (*gonococcus*), *Neisseria meningitidis* (*meningococcus*)

Gonorrhea is an anthroponotic sexually transmitted disease caused by *Neisseria gonorrhoeae*, characterized by purulent inflammation of the mucous membranes of the genitourinary organs, as well as possible damage to the eyes, rectum, and pharynx.

Meningococcal infection is an anthroponotic disease caused by *Neisseria meningitidis*, occurring as nasopharyngitis, purulent meningitis, or meningococemia (sepsis), with the possible development of toxic shock syndrome. The genus *Neisseria* is named after the German dermatovenerologist and bacteriologist Albert Ludwig Neisser (1855-1916), who discovered the causative agent of gonorrhea, *Neisseria gonorrhoeae*, in 1879. He discovered intracellular diplococci in leukocytes from the purulent discharge of patients. In 1882, Leonhard Letzer first isolated a pure culture of gonococci. In 1885, Ernst von Boon introduced the diagnostic method of staining smears with methylene blue.

Meningococcus (*Neisseria meningitidis*) was discovered in 1887 by the Austrian bacteriologist Anton Weichselbaum, who isolated it from the cerebrospinal fluid of patients with meningitis. In 1896, Kilton described the meningococcal carrier state. In 1906, Simon Flexner developed the first therapeutic serum against meningococcal infection.

Practical Work

Equipment and Materials:

- Microscopes with immersion systems
- Slides, stains: methylene blue, Gram stain kit

- Sterile loops, swabs, alcohol lamps
- Culture media: serum agar, ascites agar, chocolate agar
- Oxidase test kit
- Model smears with gonococci and meningococci
- Sugars for biochemical identification (glucose, maltose, sucrose, lactose)

Procedure:

1. Microscopy of urethral/cervical discharge
 - Prepare a smear from the material to be examined
 - Dry and fix over a flame
 - Stain with methylene blue (1-2 min) — indicative
 - Rinse, dry, examine microscopically
 - Result: Detection of typical bean-shaped diplococci, intracellularly (in the cytoplasm of leukocytes) — a diagnostic sign of gonorrhea
2. Gram staining (confirmatory method)
 - Prepare a parallel smear, stain with Gram
 - Result: Gonococci — gram-negative diplococci, pale pink, intracellular
3. Microscopy of cerebrospinal fluid (meningococcal infection)
 - Centrifuge the cerebrospinal fluid
 - Prepare smears from the sediment, stain with Gram and methylene blue
 - Result: Gram-negative diplococci, located intra- and extracellularly, often in a "coffee bean" shape
4. Oxidase test
 - A drop of 1% tetramethyl-p-phenylenediamine solution is applied to the grown colonies of *Neisseria*
 - Result: The appearance of a violet color within 30-60 seconds is a positive oxidase test (characteristic of *Neisseria*)



(gonorrhea)

method

located
sign of



5. Biochemical differentiation
 - Inoculation of a pure culture in media with carbohydrates (glucose, maltose, sucrose, lactose)
 - Incubation for 24 hours at 37°C
 - Fermentation assessment:
 - o *N. gonorrhoeae*: only glucose
 - o *N. meningitidis*: glucose, maltose
 - o *N. lactamica*: glucose, maltose, lactose
 - o Saprophytic *Neisseria* ferment several Sugars

Conclusions: Methods for microscopic diagnosis of gonorrhea have been developed, including the intracellular location of diplococci as a key diagnostic feature. The oxidase test and biochemical differentiation of pathogenic *Neisseria* from saprophytes were studied. It was

determined that the key characteristic of *N. meningitidis* is the fermentation of glucose and maltose, in contrast to *N. gonorrhoeae* (only glucose).

Report Contents

1. Title of the lab work.
2. Objective of the work.
3. Assignment.
4. Conclusions.

Quiz Questions

1. General characteristics of the Neisseriaceae family
2. Taxonomy of the genus *Neisseria*: pathogenic and saprophytic species
3. Morphology of gonococci, tinctorial properties
4. Cultural characteristics of gonococci: requirements for media, cultivation conditions
5. Antigenic structure of gonococci
6. Factors of gonococcal pathogenicity: pili, outer membrane proteins, IgA proteases
7. Pathogenesis of gonorrhea in men and women
8. Immunity in gonorrhea, the concept of reinfection
9. Clinical forms of gonorrhea: fresh, chronic, gonorrheal arthritis, ophthalmia
10. Gonorrhea in newborns: prevention of gonoblenorrhea
11. Laboratory diagnosis of gonorrhea: bacterioscopic, bacteriological methods
12. Serological diagnosis of gonorrhea (reaction Bordet-Gengou)
13. Meningococcus: morphology, cultural properties
14. Antigenic structure of meningococci, serogroups (A, B, C, Y, W135)
15. Factors of meningococcal pathogenicity: capsule, endotoxin
16. Epidemiology of meningococcal infection: carriage, transmission routes
17. Pathogenesis of meningococcal infection: nasopharyngitis, meningitis, meningococcemia
18. Clinical picture of meningococcal sepsis (Waterhouse-Friderichsen syndrome)
19. Laboratory diagnosis of meningococcal infection: examination of cerebrospinal fluid, blood, and nasopharyngeal mucus
20. Rapid diagnostics: latex agglutination, PCR
21. Principles of treatment of gonorrhea and meningococcal infection, the problem of antibiotic resistance
22. Specific prophylaxis of meningococcal infection (vaccines)

List of key literature:

1. Murray P.R., Rosenthal K.S., Tenover F.C., Tenover F.C. *Medical Microbiology*. – 10th ed. – Philadelphia: Elsevier, 2025. – 880 p. (ISBN: 978-0-323-93321-0)
2. Carroll K.C., Butel J.S., Morse S.A., et al. *Jawetz, Melnick & Adelberg's Medical Microbiology*. – 29th ed. – New York: McGraw-Hill Education, 2024. – 864 p. (ISBN: 978-1-264-93210-6)
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Laboratory work 18

Tuberculosis and leprosy agents. Ziehl-nielsen staining. Allergy research method

The objective of this work is to study the biological properties of *Mycobacterium tuberculosis* and leprosy, to master the methods of microbiological diagnosis of tuberculosis, including bacterioscopic, bacteriological and allergological methods.

Procedure for completing the work

Family: *Mycobacteriaceae*

Genus: *Mycobacterium*

Species: *Mycobacterium tuberculosis* (from the Latin "tuberculum" - tubercle), *Mycobacterium leprae*, also *Mycobacterium bovis*, *Mycobacterium africanum*

Tuberculosis is a chronic infectious disease caused by *Mycobacterium tuberculosis*, characterized by the formation of specific granulomas (tubercles) in various organs (most often in the lungs), with the possible development of caseous necrosis and fibrosis.

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae*, primarily affecting the skin, mucous membranes, peripheral nervous system, and internal organs.

The causative agent of tuberculosis, *Mycobacterium tuberculosis*, was discovered by Robert Koch on March 24, 1882. In his report to the Berlin Physiological Society, Koch demonstrated that this bacterium was the cause of tuberculosis, and received the Nobel Prize for this discovery in 1905. He also developed "tuberculin," an allergen for diagnostics, which later became used for tuberculin tests. In 1890, Koch published a report on the creation of tuberculin; however, its therapeutic efficacy was not confirmed, although its diagnostic value remains to this day.

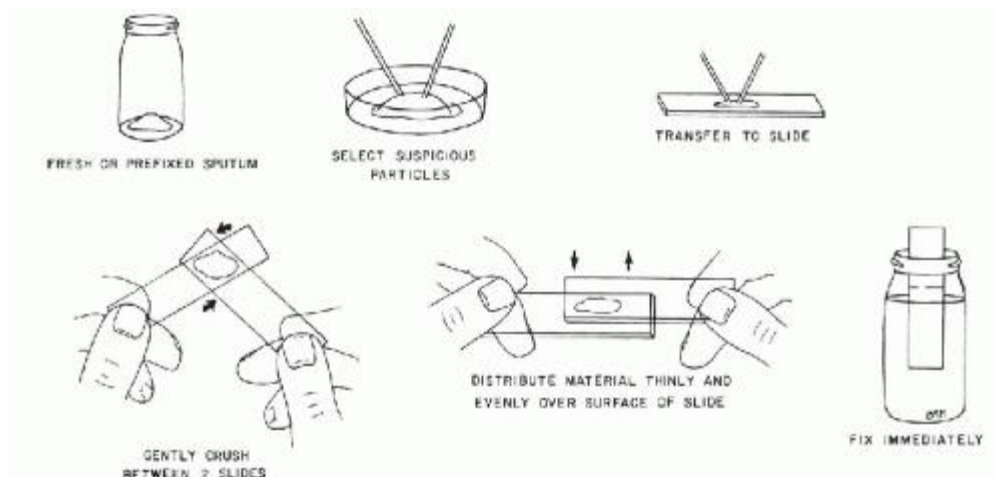
In 1885, Léon Calmette and Camille Guérin began years of work on developing a tuberculosis vaccine. In 1919, they obtained a stable, attenuated strain of bovine mycobacterium, named BCG (Bacillus Calmette-Guérin, BCG), which began to be used for human vaccination in 1921. The causative agent of leprosy, *Mycobacterium leprae*, was discovered by Norwegian physician Gerhard Hansen in 1873, marking the first reported case of a bacterial pathogen causing the disease in humans. This discovery occurred nine years before the discovery of the tuberculosis bacillus, but due to the impossibility of culturing the pathogen in vitro, confirmation of Koch's postulates was delayed for decades. Practical Section

Equipment and Materials:

- Microscopes with immersion systems
- Slides, alcohol lamps, loops
- Ziehl-Neelsen staining kit: Ziehl's carbol fuchsin, sulfuric acid (20%), alcohol, methylene blue
- Sputum smears (models) with acid-fast bacteria
- Filter paper, tweezers
- Culture media: Lowenstein-Jensen, Finn, Mordovsky
- Tuberculin (PPD) for the Mantoux test (demonstration)

Procedure:

1. Preparing a sputum smear

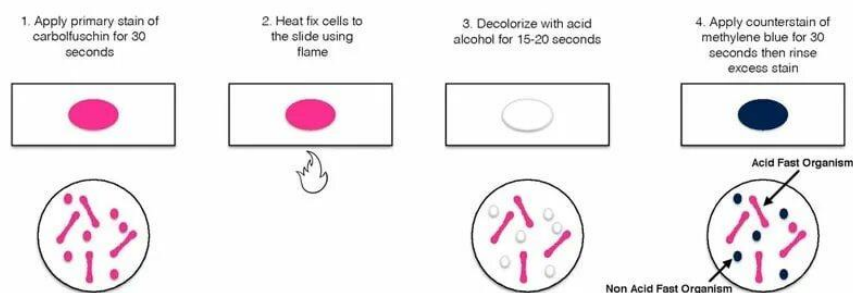


- Remove purulent clumps from the sputum using a sterile loop or Pasteur pipette
- Place the material on a glass slide and grind to obtain a uniform smear
- Dry at room temperature (under a fume hood!), fix over a flame (carefully, do not overheat)

2. Ziehl-Neelsen staining

- Place a strip of filter paper on the fixed smear

Procedure for Ziehl-Neelsen Staining



- Pour Ziehl's carbol fuchsin onto the smear and heat until vapor appears (3-5 minutes, do not boil)
- Remove the paper and rinse with water
- Decolorize with 20% sulfuric acid (immerse 2-3 times until decolorized)
- Rinse thoroughly with water
- Finish staining with methylene blue (1-2 minutes)
- Rinse, dry, and examine with immersion microscopy

3. Microscopy and inventory Results

- View at least 100 fields of view
- Result: Acid-fast mycobacteria tuberculosis stain bright ruby red, appear singly or in groups, and have the shape of thin, slightly curved rods. The smear background and non-acid-fast microflora are blue.

Conclusions: The primary method for laboratory diagnosis of tuberculosis—bacterioscopy with Ziehl-Neelsen staining—has been mastered. It has been established that the acid-fast nature of mycobacteria is due to the high content of lipids and mycolic acids in their cell walls, which allows them to be differentiated from other microorganisms. Quantitative assessment of AFB is important for determining the degree of bacterial excretion and the epidemiological risk of a patient.

Report Contents

1. Title of the lab work.

2. Objective of the work.
3. Assignment.
4. Conclusions.

Quiz Questions

1. Taxonomy of the Mycobacteriaceae family
2. Mycobacterium tuberculosis complex: species causing tuberculosis in humans
3. Morphological properties of mycobacteria: acid resistance, polymorphism
4. Chemical composition of the cell wall: the role of lipids and mycolic acids
5. Cultural properties: media requirements, growth rate, colony types
6. Antigenic structure. Pathogenicity factors: cord factor, sulfolipids
7. Pathogenesis of tuberculosis: primary and secondary tuberculosis
8. Immunity in tuberculosis: the role of the T-cell component, delayed-type hypersensitivity
9. Epidemiology of tuberculosis: sources, routes of transmission, risk groups
10. Clinical forms of tuberculosis
11. Laboratory diagnostics: bacterioscopy, fluorescence microscopy
12. Bacteriological method: culture on media, accelerated cultivation methods (BASTEC)
13. Molecular genetic methods: PCR, determination of drug resistance. Serological diagnostics
14. Tuberculin diagnostics: Mantoux test, Diaskintest
15. Tuberculosis chemotherapy: first- and second-line drugs
16. Specific prophylaxis: BCG vaccine, vaccination schedule
17. Leprosy pathogen: history of discovery (Hansen), cultivation features
18. Clinical forms of leprosy, laboratory diagnostics

List of key literature:

1. Murray P.R., Rosenthal K.S., Tenover F.C., Tenover F.C. *Medical Microbiology*. – 10th ed. – Philadelphia: Elsevier, 2025. – 880 p. (ISBN: 978-0-323-93321-0)
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Laboratory work 19

Pathogens of particularly dangerous zoonotic infections (plague, tularemia, anthrax, brucellosis). Biological method

The objective of this work is to study the properties of enteroviruses and methods of their quantitative analysis. study the biological properties of pathogens of particularly dangerous infections, to master laboratory diagnostic methods and the rules for working with material suspected of being infected with pathogens of pathogenicity groups I-II.

Procedure for completing the work

Family: *Enterobacteriaceae*

Genus: *Yersinia*

Species: *Yersinia pestis*

Family: *Francisellaceae*

Genus: *Francisella*

Species: *Francisella tularensis*

Family: *Bacillaceae*

Genus: *Bacillus*

Species: *Bacillus anthracis*

Family: *Brucellaceae*

Genus: *Brucella*

Species: *Brucella melitensis*, *B. abortus*, *B. suis*, *B. canis*

Plague is an acute natural focal transmissible disease caused by *Yersinia pestis*, characterized by fever, severe intoxication, serous-hemorrhagic inflammation of the lymph nodes, lungs, and other organs, with a high mortality rate. Tularemia is an acute zoonotic infectious disease caused by *Francisella tularensis*, characterized by fever, intoxication, and lesions of the lymph nodes, skin, eyes, pharynx, and lungs.

Anthrax is an acute, highly dangerous infection caused by *Bacillus anthracis*, which can manifest as cutaneous (carbuncular), pulmonary, intestinal, and septicemic forms.

Brucellosis is a zoonotic infectious and allergic disease caused by bacteria of the genus *Brucella*, characterized by prolonged fever, lesions of the musculoskeletal, nervous, and reproductive systems, and a tendency toward chronicity.

In 1894, during the third plague pandemic, two researchers were working in Hong Kong: Shibasaburo Kitazato of Japan and Alexandre Yersin of France. Yersin isolated a bacterium from the corpses of dead and dying rats, which he believed to be the causative agent, and demonstrated the disease's connection with rodents. Initially, the priority of the discovery was disputed, but it was later established that Kitazato had isolated the accompanying microflora. In Yersin's honor, the causative agent was named *Yersinia pestis* in 1970. In 1911, the Russian scientist D.K. Zabolotny demonstrated the natural focality of plague in Manchuria by isolating the pathogen from a sick marmot and formulating the formula: "epizooty among rodents—humans—epidemic."

This is the first bacterial infection for which a microbial etiology was proven. In 1849, Pollender described rods in the blood of sick animals. In 1855, Pollender isolated bacilli. In 1876, Robert Koch first isolated a pure culture of *Bacillus anthracis*, described its morphology and spore formation in detail, and demonstrated its ability to cause disease by inoculating the culture into laboratory animals. Louis Pasteur created the first anthrax vaccine in 1881. In Russia, S.S. Andrievsky demonstrated the identity of the disease in animals and humans through autoinfection between 1786 and 1789.

The pathogen was first isolated by David Bruce in 1887 from the spleen of a patient who died of Malta fever on the island of Malta. In 1897, Bang and Striebold described *Brucella abortus* as the cause of infectious abortion in cows. A third species, *Brucella suis*, was isolated from pigs by Traum in 1914. In 1918, Evans established the similarity of all three species, and the genus was named *Brucella* in Bruce's honor. The pathogen was discovered by McCoy and Chapin in 1912 while studying a "plague-like" disease in ground squirrels in Tulare County, California. They named it *Bacterium tularense*. The microbe was studied in detail by E. Francis, after whom it was given its second name, *Francisella tularensis*. Practical Part

Equipment and Materials:

- Nutrient media: MPA, MPB, blood agar
- Glass slides for organ smears
- Fixative
- Sterile syringes
- Filter papers soaked in sodium thiolate (1:1000) for collecting blood from the heart (for serological testing)
- Sterile wooden sticks for organ culture

- Dissection board
- Alcohol lamp on a stable stand
- Two beakers with instruments (surgical tweezers, scalpel, and scissors in one, dissecting tweezers and scissors in the other) immersed in 96% alcohol (1/3 of the beaker's volume)
- Mortar or Petri dish for preparing organ suspensions
- Glass funnel
- A stand on which to place the slides.
- Test tubes with saline solution and sterile sand for grinding the organs of the animal being dissected in a mortar
- Inoculation loop
- Pasteur pipettes and a pencil.

Procedure:

On the table, arrange culture media and sterile Petri dishes on the left side of the cuvette. On the right, place a jar containing non-sterile cotton swabs and a crystallizer with hand sanitizer. Biospecimen animals are euthanized with chloroform (ether), soaking a cotton swab in it and dropping it into the jar. Cover the jar tightly with a lid or airtight material. All manipulations of the animal carcasses are performed using instruments only, holding them by the top.



Before dissection, grasp the animal with forceps and immerse it in a 3% detergent solution for 20-30 seconds. Then, place it in a colander to remove excess liquid and transfer it to the dissection board. Then, immerse the forceps in the disinfectant. Transfer the animal and forceps only over the disinfectant solution. The biological (experimental) research method involves artificially reproducing the clinical picture of infectious diseases in laboratory animals. In practical microbiology, it is used to diagnose infectious diseases, isolate and identify pure pathogen cultures, and detect and identify exotoxins. This method involves infecting an animal, observing it, and performing an autopsy on a dead or slaughtered animal.



The sample for infection must be collected under aseptic conditions, placed in a sterile container, homogenized, and used as quickly as possible. The method of infection depends on the type of sample, the animal species, and the suspected infecting agent. The sample can be injected cutaneously, subcutaneously, intradermally, intramuscularly, intraperitoneally, intravenously, through the nose, mouth, and rectum, into the heart, stomach, brain, vagina, testicle, conjunctiva, or joint cavities. Before inoculation, the surgical site must be dehaired and treated with 70% ethanol, followed by application of iodine tincture. The purpose of a bacteriological examination of a corpse is to detect the microbe contained in the injected material, determine its location in the body, and isolate a pure culture of the pathogen.

To infect white mice with the test material subcutaneously and intraperitoneally, preliminary anesthesia is necessary. To do this, place the animal in a closed glass container containing cotton wool soaked in ether or chloroform before sleep.

Subcutaneous Infection

For subcutaneous injection of the test material, secure the animal, wipe the injection site with a piece of cotton wool soaked in alcohol, grasp a fold of skin on the back at the base of the tail, insert the needle approximately halfway into the fold, slowly inject 0.5 ml of the material, and release the fold. Then quickly withdraw the needle, covering the injection site with cotton wool soaked in alcohol.

Intraperitoneal Infection

Suppress the animal head down to allow the intestines to move toward the diaphragm. After wiping the injection site in the lower left abdomen with alcohol, puncture the skin, holding the needle at an acute angle. Then, position the syringe perpendicular to the abdominal wall, thrust the needle through the peritoneum, and inject the syringe's contents. Remove the needle, covering the injection site with cotton wool soaked in alcohol.

2. Perform an autopsy on the dead white mice. The autopsy and examination of the animal's body are divided into three stages:

a) Autopsy and examination of the external integument

Fix the animal's body with the abdomen facing up, placing it on a piece of gauze soaked in a disinfectant solution. Clean the fur and skin with a cotton swab soaked in a disinfectant solution. Using sterile instruments, make an incision in the skin from the lower jaw to the pubis and lateral incisions along the extremities. Separate the skin, noting the condition of the subcutaneous tissue and lymph nodes. If any abnormal nodes are detected, culture them on nutrient media and make smears (imprints) on slides.

b) Autopsy and examination of the chest cavity

Open the chest cavity by making an incision in the diaphragm, insert the blade of a pair of scissors, cut the ribs on both sides of the sternum, fold the excised flap upward, and inspect the chest organs. If exudate is present, culture and smear it. To collect blood from the heart, cauterize an area with tweezers, puncture the area with a sterile Pasteur pipette, collect a few drops of blood, culture, and prepare a smear. Culture lung tissue and make smears (imprints).

c) Autopsy and examination of the abdominal cavity

Open the abdominal cavity, inspect its organs, and note the presence of exudate, as well as the size and color of the liver, spleen, and mesenteric lymph nodes. Culture the liver, spleen, kidneys, and lymph nodes and prepare smears—imprints of these organs. To do this, cut small pieces of organ tissue, pick them up with tweezers, and touch the agar surface in the appropriate area of the Petri dish (Fig.), then touch them to a glass slide. After the autopsy, wrap the body in gauze placed underneath.

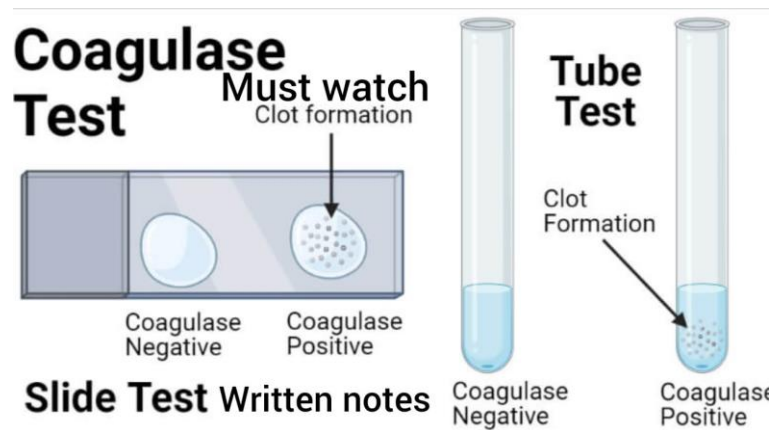
3. Fix the prepared smears (prints) over a flame, stain with aqueous fuchsin or methylene blue, and Gram stain, and examine them microscopically.

4. Record the results of determination of aggressive enzymes (coagulase, fibrinolysin) and the hemolytic capacity of bacteria.

Determination of cellular coagulase

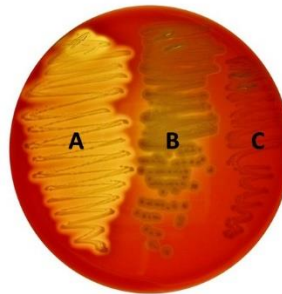
In a drop of the thick suspension of the test culture on a glass slide, add a drop of whole rabbit or human citrated plasma. If positive, a clot will form within a few seconds. Simultaneously install a known positive and a known negative control.

Test tube reaction for the determination of free coagulase



In 1 ml of rabbit or human citrated plasma, whole or diluted 2- and 4-fold with saline, add a loopful of 18- to 24-hour agar culture. Incubate the mixture, along with the positive and negative controls, at 37°C and monitor for clot formation after 2-4-18 hours. Further incubation of the clotted plasma may result in clot melting, indicating fibrinolytic activity of the culture being tested.

Determination of hemolytic activity



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Prepare blood agar by adding 5-10% warmed sterile sheep, rabbit, or human blood to 2% MPA melted and cooled to 45°C. Mix and pour into Petri dishes. Incubate for 24 hours in an incubator to prevent bacterial contamination. Inoculate the culture being tested as streaks or plaques. Hemolysis appears as clear zones around colonies or plaques of growth.

Safety precautions: Work with pathogens causing particularly dangerous infections is strictly carried out in specialized, secure laboratories. Inactivated cultures and demonstration preparations are used for educational purposes. Working with live cultures of pathogenicity groups I and II is prohibited in student laboratories.

Conclusions: Key differential characteristics of the pathogens causing anthrax, plague, tularemia, and brucellosis were studied.

Report Contents

1. Title of the lab work.
2. Objective of the work.
3. Assignment.
4. Conclusions.

Quiz Questions

1. General characteristics of especially dangerous infections (EDI), classification
2. Legal basis for working with microorganisms of pathogenicity groups I-II
3. Plague: taxonomy, morphology, cultural properties of *Yersinia pestis*
4. Pathogenicity factors of the plague microbe
5. Epidemiology of plague: natural foci, main carriers, routes of transmission
6. Pathogenesis of bubonic, pneumonic, and septicemic forms of plague
7. Clinical picture of plague
8. Laboratory diagnostics of plague: microscopy, culture, bioassay
9. Specific prevention and treatment of plague
10. Anthrax: taxonomy, morphology, spore formation
11. Pathogenicity factors of the *Bacillus anthrax*: capsule, exotoxin

12. Epidemiology of anthrax: occupational nature of the disease
13. Clinical forms of anthrax Human ulcers (cutaneous, pulmonary, intestinal, septic)
14. Differential diagnosis of anthrax carbuncle
15. Laboratory diagnosis of anthrax
16. Anthrax vaccines (Pasteur, Tsenkovsky, STI)
17. Treatment of anthrax: etiologic and pathogenetic therapy
18. Tularemia: properties of the pathogen, epidemiology, clinical features
19. Brucellosis: types, cultivation features, epidemiology
20. Laboratory diagnosis of brucellosis (Wright reaction, Heddleson reaction, Burne test)
21. Brucellosis prevention: vaccination of animals and humans
22. Organization of anti-epidemic measures in hotbeds of infectious diseases

List of key literature:

1. Murray P.R., Rosenthal K.S., Tenover F.C. *Medical Microbiology*. – 10th ed. – Philadelphia: Elsevier, 2025. – 880 p. (ISBN: 978-0-323-93321-0)
2. Carroll K.C., Tenover F.C., Tenover S.A., et al. *Jawetz, Melnick & Adelberg's Medical Microbiology*. – 29th ed. – New York: McGraw-Hill Education, 2024. – 864 p. (ISBN: 978-1-264-93210-6)
3. Ryan K.J., Tenover F.C., Tenover S.A., et al. *Ryan & Sherris Medical Microbiology*. – 9th ed. – New York: McGraw-Hill Education, 2024. – 992 p. (ISBN: 978-1-264-26874-0)

List of additional literature:

4. Goering R.V., Dockrell H.M., Zuckerman M., Chiodini P.L. *Mims' Medical Microbiology and Immunology*. – 7th ed. – Philadelphia: Elsevier, 2025. – 600 p. (ISBN: 978-0-323-93324-1)
5. Melter O., Castelano R. (eds.). *The MicroBook: Clinical Microbiology for Medical Students*. – 1st ed. – Prague: Charles University Press, 2023. – 320 p. (ISBN: 978-80-246-5123-4)
6. Kumar S. *Medical Microbiology For Undergraduates*. – 2nd ed. – New Delhi: Jaypee Brothers Medical Publishers, 2024. – 560 p. (ISBN: 978-93-5465-789-2)

Laboratory work 20

Pathogens of Anaerobic Infections (Diphtheria, Tetanus, Gas Gangrene, Botulinum). Microbiological Diagnosis of Anaerobic Infection

The objective of this work is to study the biological properties of pathogens causing anaerobic infections, master methods of microbiological diagnostics, and understand the role of exotoxins in the pathogenesis of diseases.

Procedure for completing the work

Family: Corynebacteriaceae
 Genus: Corynebacterium
 Species: Corynebacterium diphtheriae
 Family: Bacillaceae
 Genus: Clostridium
 Species: Clostridium tetani
 Family: Bacillaceae
 Genus: Clostridium
 Species: C. perfringens, C. novyi, C. septicum, C. histolyticum, C. bifermentans, C. ramosum, C. sporogenes, C. fallax, C. sordelli
 Family: Bacillaceae
 Genus: Clostridium
 Species: Clostridium botulinum

Diphtheria. The causative agent, Corynebacterium diphtheriae, was discovered in 1883-1884 by Edwin Klebs (who discovered the bacteria in pseudomembrane sections) and Friedrich

Loeffler (who isolated a pure culture). The genus name comes from the Greek coryne, meaning club, due to the characteristic thickenings at the ends. Bacteriophage-mediated toxigenicity: Loeffler noted that not all strains cause disease, but the nature of this phenomenon was discovered later. In 1890, Emil Behring and Kitasato Shibasaburo discovered the antitoxin and created the first serum. In 1913, Behring introduced active immunization with a toxin-antitoxin mixture. In 1923, Gaston Ramond proposed anatoxin.

Gas gangrene. The pathogens were discovered sequentially: in 1861, Pasteur described *Clostridium septicum*, and in 1892, Welch and Nettle isolated *Clostridium perfringens*.

Tetanus. In 1884, Nicolaier injected mice with material from a tetanus patient and developed the disease. In 1889, Kitazato isolated a pure culture of *Clostridium tetani* and demonstrated that the toxin was produced in the culture. He also demonstrated the neutralizing effect of a specific serum. In 1890, Behring and Kitazato developed the principles of serotherapy.

Botulism. In 1895, van Ermengem isolated the pathogen from ham that caused an outbreak of botulism in Belgium and named it *Bacillus botulinus* (from the Latin *botulus*, meaning sausage). In 1910, Leichmann isolated a second type of toxin.

Practical Section

Equipment and Materials:

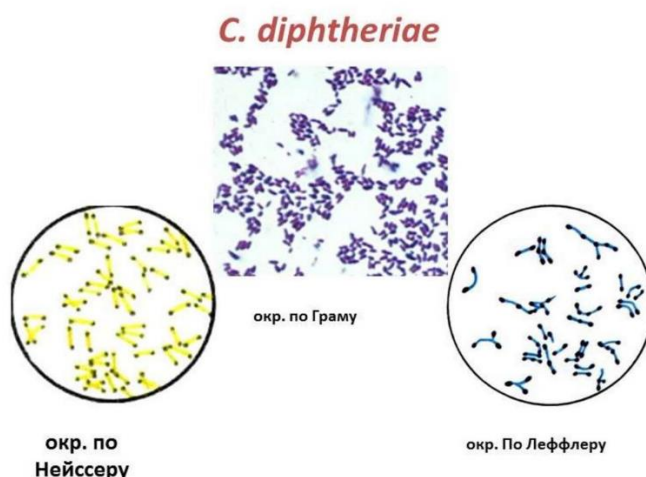
- Microscopes with immersion systems
- Slides, alcohol lamps, loops
- Dyes: methylene blue, Neisser staining kit, Gram staining kit
- Nutrient media: Clauberg's medium (tellurite agar), blood tellurite agar, Loeffler's serum medium

medium

- Cultures of *C. diphtheriae* biovars *gravis* and *mitis* (training)
- Antidiphtheria serum (demonstration)
- Toxigenicity discs

Procedure:

1. Bacterioscopy of smears from pure cultures
 - Prepare smears from *C. diphtheriae* colonies
 - Methylene blue staining (3-5 min): polymorphic rods, often with thickened ends (club-shaped), are arranged at an angle in the form of a V, Y, or spread fingers
 - Neisser staining (detection of volutin grains): Solution 1 (methylene blue acetate) — 2-3 min. Rinse. Solution 2 (chrysoidine) — 30-60 sec. Result: Cytoplasm is yellow-brown, volutin grains are dark blue or purple



2. Inoculation of material on differential media

- Clauberg's medium (tellurite agar): black colonies (tellurite reduction)
- Characteristics of biovars:
 - o *gravis*: large, rough colonies (R-form), black with radial striations, film in liquid medium
 - o *mitis*: smooth, shiny colonies (S-form), black, convex, diffuse turbidity in liquid medium

- o intermedius: small colonies (intermediate)
3. Cystinase test (Pisa test)
- Prick inoculation into a column of medium with cystine and lead acetate
 - Result: Blackening of the medium along the prick (*C. diphtheriae* breaks down cystine, producing hydrogen sulfide, which binds to lead)
4. Determination of Toxigenicity (Gel Immunodiffusion)
- A strip of filter paper soaked in antitoxic serum is applied to a Petri dish with nutrient agar.
 - The test cultures and control cultures (toxigenic and non-toxigenic) are inoculated on either side of the strip.

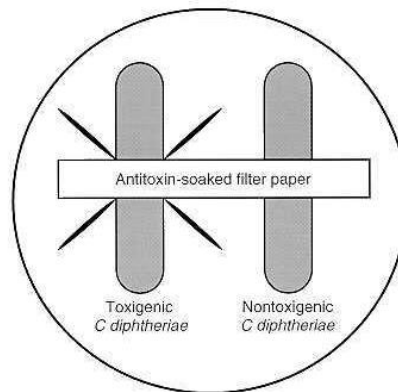
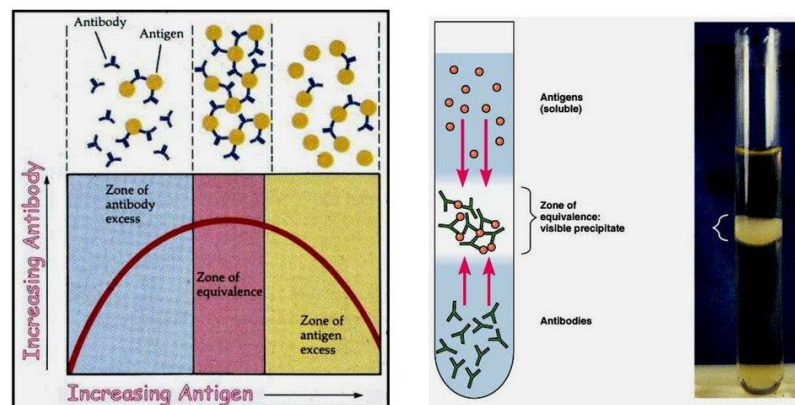


Figure 32-6 Elek immunodiffusion test

- Result: Appearance of precipitation lines (arrows) opposite the toxigenic cultures.
5. Demonstration: Ascoli Precipitation Reaction
- Extract from organs or cultures is boiled and filtered.
 - Layered on specific serum.
 - Result: Formation of a precipitate ring at the interface between the liquids.

Precipitation in solution. Ring precipitation test



The precise "Zone of Equivalence"

Conclusions: The morphological features of diphtheria corynebacteria were studied: polymorphic rods with club-shaped thickenings containing metachromatic volutin grains, a characteristic "splayed fingers" arrangement. Cultivation methods on Claiberg medium have been developed, allowing for differentiation of biovars. Determining toxigenicity is a crucial step, as the pathogenicity of *C. diphtheriae* is associated with exotoxin production.

[Report Contents](#)

1. Title of the lab work.
2. Objective of the work.
3. Assignment.
4. Conclusions.

Quiz Questions

1. General characteristics of anaerobic infections, classification
2. Diphtheria: taxonomy, morphology of *C. diphtheriae*
3. Biological properties of corynebacteria: volutin grains (Babesch-Ernst)
4. Cultural properties, biovars gravis, mitis, intermedius
5. Pathogenicity factors: diphtheria exotoxin (mechanism of action)
6. Toxigenic and nontoxigenic strains, the role of bacteriophages in toxin formation
7. Pathogenesis of diphtheria: local changes and general intoxication
8. Clinical picture of diphtheria: localized and toxic forms
9. Immunity in diphtheria (antitoxic and antibacterial)
10. Schick reaction (determination of antitoxic immunity)
11. Laboratory diagnosis of diphtheria
12. Rapid diagnostics Diphtheria (direct RIF)
13. Specific therapy: diphtheria antitoxin
14. Active immunization: diphtheria toxoid, DPT composition
15. Tetanus: properties of *C. tetani*, morphology (tennis racket)
16. Tetanus exotoxin (tetanospasmin), mechanism of action
17. Pathogenesis and clinical presentation of tetanus
18. Laboratory diagnosis of tetanus, bioassay
19. Emergency prevention and treatment of tetanus
20. Botulism: *C. botulinum*, types of toxins
21. Pathogenesis of botulism, clinical picture
22. Gas gangrene: pathogens, pathogenicity factors, diagnosis

List of key literature:

1. Murray P.R., Rosenthal K.S., Tenover F.C., Tenover F.C. *Medical Microbiology*. – 10th ed. – Philadelphia: Elsevier, 2025. – 880 p. (ISBN: 978-0-323-93321-0)
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Laboratory work 21

***E. coli* as a sanitary indicator microorganism and a pathogen of intestinal infections. Differentiation of *E. coli* in an Endo medium, taking into account its biochemical activity.**
The objective of this work is to study the biological properties of *Escherichia coli*, master methods for differentiating pathogenic and non-pathogenic *Escherichia coli*, and learn to evaluate the sanitary and indicative value of *E. coli*.

Procedure for completing the work

Family: *Enterobacteriaceae*

Genus: *Escherichia*

Species: *E. coli*

Escherichia coli was first described by the German pediatrician Theodor Escherich in 1885. He isolated this bacterium from the feces of healthy children and initially named it *Bacterium coli commune*. Escherich proposed that this microorganism was a normal inhabitant of the intestine and played a role in digestion. Later, in 1919, Castellani and Chalmers renamed the genus *Escherichia* in honor of its discoverer.

In 1893, Theobald Smith first pointed out the sanitary significance of *E. coli* as an indicator of fecal contamination of water. In the 1940s, the heterogeneity of *E. coli* was established, and pathogenic serotypes causing diarrhea were identified. By the end of the 20th century, various pathogens had been described: enterotoxigenic (ETEC), enteroinvasive (EIEC), enteropathogenic (EPEC), enterohemorrhagic (EHEC, including serotype O157:H7), and enteroaggregative (EAEC).

Practical Part

Equipment and Materials:

- Nutrient media: Endo medium, Levin medium (with eosin and methylene blue), Ploskirev medium, Kligler medium (three-sugar agar)
- Reagents for the oxidase test
- Slides, Gram stains
- Bacteriological loops, alcohol lamps
- Petri dishes with grown colonies (training material)
- Test systems for the biochemical identification of enterobacteria
- Polyvalent and monosera for serotyping (demonstration)

Safety instructions: When working with enterobacteria cultures, observe the rules for working with microorganisms of pathogenicity groups III-IV. After work, treat the table surface with a disinfectant solution and wash your hands with an antiseptic. Disinfect the cultures by autoclaving. Procedure:

1. Study of *E. coli* growth on differential media

- Endo medium: colonies are crimson-red with a metallic sheen (lactose-positive) or lusterless (lactose-negative variants)
- Levine medium: colonies are black-violet (lactose-positive)
- Ploskirev medium: colonies are pink or red



Nutrient Agar (NA)



Blood Agar (BA)



MacConkey Agar



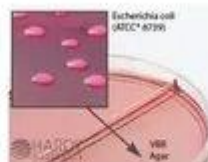
Mueller Hinton Agar



Methylene Blue Agar



m-ENDO Agar



Violet Red Bile Agar



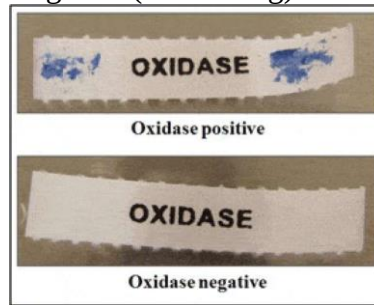
CLED Agar

2. Microscopy (Gram staining)

- Prepare a smear from the colony and stain it with Gram stain
- Result: Gram-negative rods of medium size, located singly or randomly

3. Oxidase test

- Apply a drop of oxidase reagent to the colony
- Result: E. coli is oxidase-negative (no staining)



• Note: This test differentiates enterobacteria (oxidase-negative) from pseudomonads and other oxidase-positive gram-negative bacteria

4. Sowing On Kligler medium

- Inoculate by pricking the column and streaking along the slant surface
- Incubation for 24 hours at 37°C
- Recording results:
 - o Glucose: yellow column (glucose fermentation)
 - o Lactose: yellow slant (lactose fermentation)
 - o Gas: bubbles or rupture of the medium
 - o Hydrogen sulfide (H₂S): blackening of the medium
 - o Typical E. coli: A/A (yellow/yellow), gas +, H₂S -

5. Identification using test systems

- Preparation of a bacterial suspension using a turbidity standard
 - Addition of test system (glucose, lactose, mannitol, maltose, indole, citrate, urea, etc.) to the wells
 - Incubation for 18-24 hours, recording using a color scale
- ### 6. Serological identification (demonstration)

Types of agglutination reactions

I- Direct agglutination:

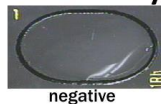
A-Slide agglutination test:

On a clean slide, place a suspension of unknown culture of an organism, then add to it a drop of known antiserum(AB), and mix well→

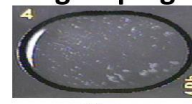
clumping(Agglutination) → **Positive**

→No clumping → **Negative**

This test is commonly used in blood grouping.



negative



positive

- Reaction Agglutination on glass with polyvalent and group O-sera
- Result: Formation of agglutinate with a matching antigen

Conclusions: Methods for culturing and identifying E. coli have been developed. The main distinguishing features of E. coli have been established: growth on Endo medium as crimson colonies with a metallic sheen, an oxidase-negative reaction, fermentation of glucose and lactose with gas formation, absence of hydrogen sulfide production, and a positive reaction to indole. The sanitary significance of E. coli is based on its constant presence in the intestines of humans and animals and its detection in the environment as an indicator of fecal contamination.

Report Contents

1. Title of the lab work.
2. Objective of the work.
3. Assignment.
4. Conclusions.

Quiz Questions

1. Taxonomy of the *Enterobacteriaceae* family
2. General characteristics of *Escherichia coli*: morphology, cultural properties
3. Biochemical activity of *E. coli*
4. Antigenic structure of *Escherichia coli*: O-, H-, K-antigens
5. Concept of serogroups and serovars
6. Pathogenic and nonpathogenic *Escherichia coli*
7. Pathogenicity factors of enteropathogenic *E. coli*
8. Classification of diarrheagenic *E. coli* (ETEC, EPEC, EIEC, EHEC, EAEC)
9. Enterotoxins (heat-labile LT and heat-stable ST)
10. Shiga toxin-producing *E. coli* (STEC), serotype O157:H7
11. Pathogenesis of coli infections
12. Clinical manifestations of *Escherichia coli* infections
13. Epidemiology of coli infections
14. Laboratory Diagnosis of *Escherichia Coli*
15. The Sanitary Value of Coliform Bacteria (*Escherichia Coli*)
16. Methods of Sanitary and Microbiological Analysis of Water, Soil, and Food Products
17. Determination of Coli Titer and Coli Index
18. *E. coli* as a Causal Agent of Extraintestinal Infections (UTIs, Sepsis, Meningitis)
19. The Problem of Antibiotic Resistance in *Enterobacteriaceae*
20. The Role of *E. coli* in Intestinal Dysbacteriosis
21. Probiotics and Their Use for Microflora Correction
22. Genetically Engineered Use of *E. coli*

List of key literature:

1. Murray P.R., Rosenthal K.S., Tenover F.C., Tenover F.C. *Medical Microbiology*. – 10th ed. – Philadelphia: Elsevier, 2025. – 880 p. (ISBN: 978-0-323-93321-0)
2. Carroll K.C., Butel J.S., Morse S.A., et al. *Jawetz, Melnick & Adelberg's Medical Microbiology*. – 29th ed. – New York: McGraw-Hill Education, 2024. – 864 p. (ISBN: 978-1-264-93210-6)
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Laboratory work 22

Pathogens of Typhoid Fever, Paratyphoid Fever, and Dysentery. Serological Diagnosis of Typhoid Fever (Vidal Reaction)

The objective of this work is to study the biological properties of salmonella and shigella, master the methods of microbiological diagnosis of typhoid fever and dysentery, and learn to interpret serological reactions.

Procedure for completing the work

Family: *Enterobacteriaceae*

Genus: *Salmonella*

Species: *Salmonella typhi*, *Salmonella paratyphi* A, *Salmonella paratyphi* B

Family: *Enterobacteriaceae*

Genus: *Shigella*

Species: *S. dysenteriae*, *S. flexneri*, *S. boydii*, *S. sonnei*.

The pathogen was first observed in 1850 by William Budd, who also established an epidemiological link with contaminated water. In 1880, Karl Ebert discovered bacteria in sections of the spleen and Peyer's patches of patients who died of typhoid fever. In 1884, Georg Gaffky isolated a pure culture and described in detail the microbe, later named *Salmonella typhi*. In 1896, Fernand Vidal proposed an agglutination test for serological diagnosis (the Widal test), which is still used in a modified form. The causative agent of paratyphoid fever A (*Salmonella paratyphi* A) was discovered by Gwin in 1902, paratyphoid fever B (*Salmonella paratyphi* B) by Schottmüller in 1900, and paratyphoid fever C by Hirschfeld in 1919.

The pathogen was first described by the Japanese bacteriologist Kiyoshi Shiga in 1898, isolating it from the feces of dysentery patients. Independently, in 1900-1901, Flexner and Strong isolated similar bacteria but with different biochemical properties. The genus was subsequently named *Shigella* in honor of Shiga. By the 1940s, all the main species of *Shigella* had been described: *S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei*.

Practical Part

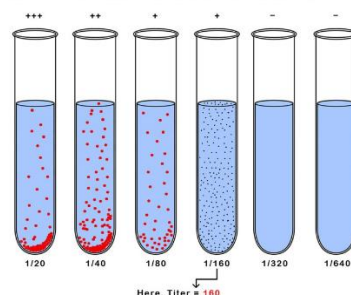
Equipment and Materials:

- Patient serum (training) — dilutions 1:100, 1:200, 1:400, 1:800
- *Salmonella* diagnostics (O- and H-antigens): *Salmonella typhi*, *S. paratyphi* A, *S. paratyphi* B
- Agglutination test tubes, racks
- Saline solution (0.9% NaCl)
- Graduated pipettes for 1 and 5 ml
- Thermostat, water bath
- Nutrient media: bismuth sulfite agar, Ploskirev medium, Rappoport medium

Safety instructions: When handling blood serum, observe biological safety regulations and wear gloves. Disinfect all materials that come into contact with blood. Avoid aerosol formation when performing serological tests. Procedure:

1. Perform the Widal agglutination test (volume method)
 - Prepare serum dilutions: from 1:100 to 1:800
 - Add 1 ml of each dilution to test tubes
 - Add 1-2 drops of the appropriate type of diagnosticum (O- or H-)
 - Shake the test tubes, place in a thermostat (37°C) for 2 hours, then leave at room temperature overnight

WIDAL TEST INTERPRETATION



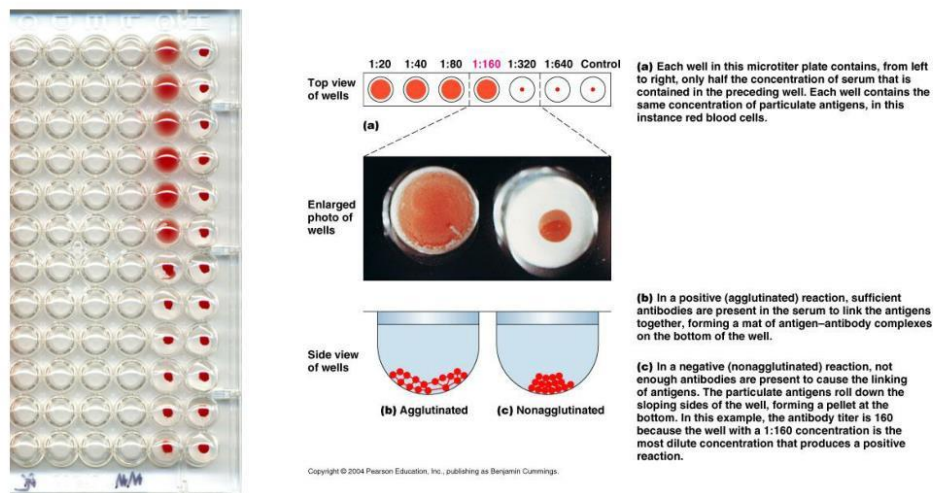
2. Record the results

- Assess the nature of agglutination (fine-grained, coarse-grained)
- Determine the serum titer - the last dilution where agglutination is still observed

- Diagnostic value: titer of 1:200 or higher for H-antigen, 1:100 or higher for O-antigen
- Interpretation:
 - o O-antibodies appear in the first week and disappear after recovery
 - o H-antibodies appear later and persist for a long time (in those who have recovered from the disease and those vaccinated)
- 3. Performing an erythrocyte antigen assay with erythrocyte diagnosticums (demonstration)
 - Serum dilutions are prepared in the wells of the plate
 - Add the erythrocyte antigen diagnosticum

Hemagglutination

- Quantitation of antigens / antibodies by immunoprecipitation / hemagglutination.
- U or V shaped wells on microplates: RBC (or latex) pellet into small dots if not precipitated. Immune reaction causes large spots of Ab-Ag networks



- Result: A positive reaction is an erythrocyte sediment in the form of an "umbrella" (agglutination), a negative reaction is a sediment in the form of a "dot"

4. Bacteriological examination of blood (hemoculture)

- Blood culture (10 ml) in 100 ml of Rappoport medium (bile broth)
- Principle: Bile inhibits the growth of other bacteria, but not salmonella
- Incubation for 24 hours, then subculture onto differential media

5. Bacteriological examination of stool (coproculture)

- Culture on Ploskirev media, bismuth sulfite agar
- On bismuth sulfite agar: *S. typhi* - black colonies with Metallic luster
- Identification by biochemical properties and in RA with diagnostic sera

Conclusions: The Widal reaction, the primary serological method for diagnosing typhoid fever, has been developed. It has been established that the increasing antibody titer in paired sera and the detection of O-antibodies in a diagnostic titer (1:100 or higher) during acute illness are of diagnostic value. The bacteriological method (blood culture) is an early and reliable diagnostic method. *Salmonella* are differentiated by biochemical properties and antigenic structure.

Report Contents

1. Title of the lab work.
2. Objective of the work.
3. Assignment.
4. Conclusions.

Quiz Questions

1. General characteristics of *Salmonella*: morphology, cultural properties
2. Antigenic structure of *Salmonella* (Kauffman-White scheme)

3. Classification of *Salmonella* by antigenic structure
4. *S. typhi*: biological features, pathogenicity factors
5. Pathogenesis of typhoid fever: phases of the infectious process
6. Clinical picture of typhoid fever
7. Epidemiology of typhoid fever: sources, routes of transmission
8. Bacterial carriage in typhoid fever
9. Laboratory diagnosis of typhoid fever depending on the duration of the disease
10. Blood culture: timing, sowing technique, media
11. Stool culture and urine culture
12. Serological diagnostics: Widal reaction, RNGA
13. Interpretation of serological test results (paired sera)
14. Paratyphoid fever A and B: causative agents, characteristics
15. *Shigella*: General Characteristics of the Genus
16. *Shigella* Species: *S. dysenteriae*, *S. flexneri*, *S. boydii*, *S. sonnei*
17. *Shigella* Pathogenicity Factors (Invasiveness, Toxin Production)
18. Shiga Toxin (Verotoxin) and Its Role
19. Pathogenesis of Dysentery: Colon Damage
20. Clinical Forms of Dysentery
21. Laboratory Diagnosis of Dysentery: Bacteriological Method
22. Serological Diagnosis of Dysentery (RNGA)
23. Differential Diagnosis of Salmonellosis and Shigellosis
24. Treatment and Prevention of Typhoid Fever and Dysentery

List of key literature:

1. Murray P.R., Rosenthal K.S., Tennant M.A. *Medical Microbiology*. – 10th ed. – Philadelphia: Elsevier, 2025. – 880 p. (ISBN: 978-0-323-93321-0)
2. Carroll K.C., Tenover J.C., Tenover S.A., et al. *Jawetz, Melnick & Adelberg's Medical Microbiology*. – 29th ed. – New York: McGraw-Hill Education, 2024. – 864 p. (ISBN: 978-1-264-93210-6)
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List of additional literature:

4. Goering R.V., Dockrell H.M., Zuckerman M., Chiodini P.L. *Mims' Medical Microbiology and Immunology*. – 7th ed. – Philadelphia: Elsevier, 2025. – 600 p. (ISBN: 978-0-323-93324-1)
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6. Kumar S. *Medical Microbiology For Undergraduates*. – 2nd ed. – New Delhi: Jaypee Brothers Medical Publishers, 2024. – 560 p. (ISBN: 978-93-5465-789-2)

Laboratory work 23

Microbial etiology of food poisoning. Dysbacteriosis.

The objective of this work is to study the causative agents of bacterial food poisoning, master methods for diagnosing dysbacteriosis and candidal infections, and learn to differentiate foodborne toxic infections and intoxications.

Procedure for completing the work

Family: *Enterobacteriaceae*
 Genus: *Salmonella*
 Species: *Salmonella enteritidis*
 Family: *Enterobacteriaceae*
 Genus: *Proteus*

Species: *Proteus vulgaris*, *Proteus mirabilis*

Family: *Enterobacteriaceae*

Genus: *Klebsiella*

Species: *Klebsiella pneumonia*, *Klebsiella oxytoca*, *Klebsiella variicola*

Family: *Enterobacteriaceae*

Genus: *Citrobacter*

Species: *Citrobacter freundii*, *Citrobacter koseri*, *Citrobacter braakii*, *Citrobacter youngae*

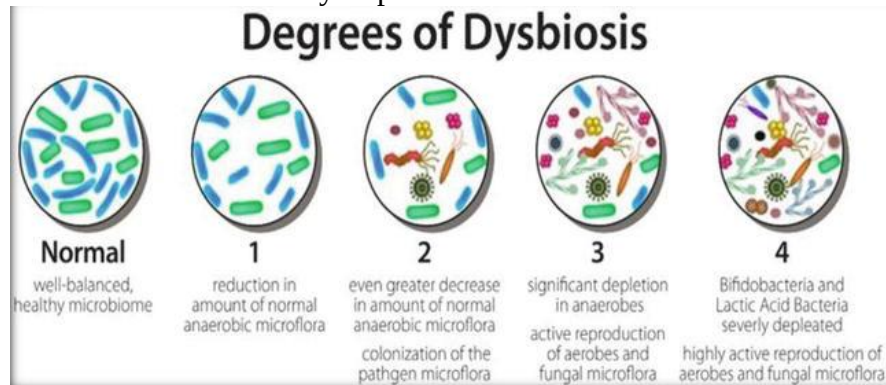
Family: *Enterobacteriaceae*

Genus: *Enterobacter*

Species: *Enterobacter cloacae*, *Enterobacter aerogenes*, *Enterobacter hormaechei*

The study of food poisoning began with the work of Robert Koch, who in 1881 isolated *Salmonella* from meat that caused poisoning. In 1888, Gaertner isolated *Salmonella enteritidis* from the meat of a cow that had caused mass poisoning. In 1896, van Ermengem discovered the causative agent of botulism. In the 1920s and 1930s, staphylococcal food poisoning was described (Duck, Barber).

The term "dysbacteriosis" was introduced by A. Nissle in 1916, while studying changes in the intestinal microflora during illness. I.I. Mechnikov's work on the role of lactic acid bacteria in longevity laid the foundations for the study of probiotics.



Practical Part

Equipment and Materials:

- Nutrient media: bismuth sulfite agar, Ploskirev medium, Endo medium, YSA, Sabouraud medium
- Saline solution, sugar broth
- Slides, Gram stains
- Bacteriological loops, alcohol lamps
- Teaching materials: gastric lavage, food residues (models)
- Polyvalent *Salmonella* sera
- Oxidase test kit

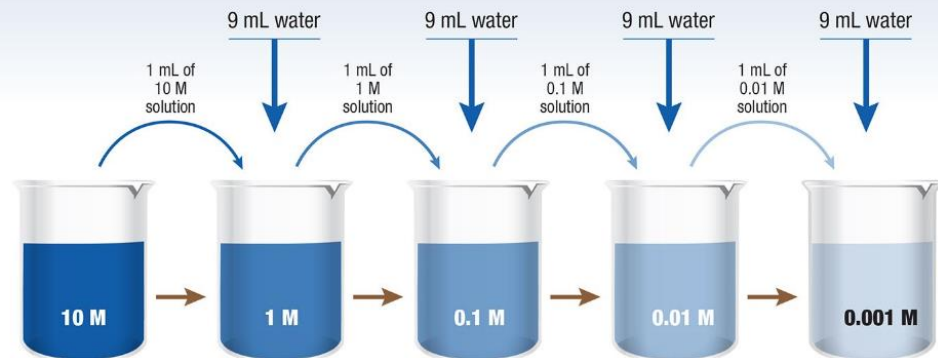
Safety instructions: The test material (vomit, lavage, feces) may contain pathogenic microorganisms. Wear gloves when handling, and disinfect surfaces after use. Incubate the cultures in a thermostat at 37°C.

Lab Basics: How to Perform Serial Dilutions

Serial Dilution of Chemicals

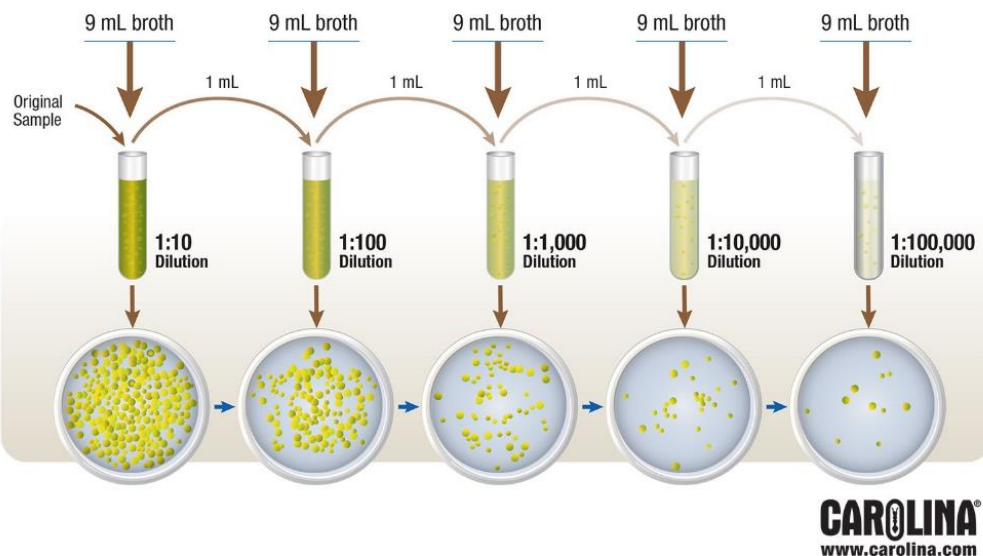
A serial dilution is a simple technique for reducing the concentration of a solution in a systematic way.

In a dose-dependent study (e.g., an LC50 experiment), you can use the technique to produce experimental concentrations of dilution factors. Start by taking 1 mL of the stock concentration of your chemical, transferring it into 9 mL of water, and mixing. Use this dilution factor throughout the series to produce experimental concentrations as shown in the illustration below.



Serial Dilution of Living Materials

The technique also works with living organisms such as bacteria. It allows you to reduce the number of bacterial cells on a plate to make counting colonies more manageable. Simply use the dilution factor in conjunction with the bacterial count on the plate to determine the number of bacteria in the original sample.



Procedure:

1. Bacterioscopy of the starting material
 - Prepare smears from washings and food residues
 - Gram stain
 - Result: Detection of abundant microflora (gram-positive cocci, gram-negative rods)
2. Sowing on differential media
 - Sowing the material in sectors on:
 - o Bismuth sulfite agar (Salmonella)
 - o Ploskirev's medium (Shigella, Salmonella)
 - o YSA (Staphylococci)
 - o Sabouraud's medium (Candida)
 - Incubation for 24-48 hours at 37°C (Sabouraud's at 28°C)
3. Identification of the isolated cultures
 - Counting colonies of different types
 - Preparation of smears from suspicious colonies

- Performing an orienting agglutination reaction on glass with polyvalent sera
- Subculture on Slant agar for pure culture accumulation
- 4. Determining the quantitative ratio of microorganisms (dysbacteriosis)
 - When culturing stool to diagnose dysbacteriosis, dilutions of 10⁻³ to 10⁻⁹ are used.
 - Culture on media: Endo (Escherichia), JSA (Staphylococci), Sabouraud (Candida), enterococci, bifidobacteria, and lactobacilli

- Measurement: Counting CFU/g, assessing the ratio of the main groups of microorganisms

Conclusions: Microbiological testing methods for food poisoning were studied. To diagnose foodborne infections, culturing material on a wide range of media is necessary, allowing for the isolation of various etiologic agents (Salmonella, opportunistic enterobacteria, staphylococci). If staphylococcal intoxication is suspected, assessing hemolytic activity is important. Diagnosis of dysbacteriosis requires a quantitative assessment of microflora and an assessment of the ratio of the main groups of microorganisms.

Report Contents

1. Title of the lab work.
2. Objective of the work.
3. Assignment.
4. Conclusions.

Quiz Questions

1. Classification of Bacterial Food Poisoning
2. Foodborne Toxic Infections: Definition, Etiology
3. Main Pathogens of Foodborne Toxic Infections (*Salmonella*, *Opportunistic Bacteria*)
4. Opportunistic Microorganisms as a Cause of Food Poisoning (*Proteus*, *Klebsiella*, *Citrobacter*, *Enterobacter*)
5. Pathogenesis of Foodborne Toxic Infections
6. Clinical Presentation of Foodborne Toxic Infections
7. Food Intoxications (Toxicoses): Definition, Etiology
8. Staphylococcal Intoxication: Properties of the Pathogen, Enterotoxin
9. Botulism as Foodborne Intoxication (Discussed in Topic 5)
10. Mycotoxicoses: Pathogens, Toxins, Clinical Manifestations
11. Laboratory Diagnosis of Food Poisoning
12. Principles of Investigating Outbreaks of Food Poisoning
13. Prevention Food poisoning
14. Dysbacteriosis: definition, causes
15. Normal intestinal microflora: composition, functions
16. Classification of dysbacteriosis by severity
17. Laboratory diagnosis of dysbacteriosis
18. Principles of dysbacteriosis correction: probiotics, prebiotics, synbiotics

List of key literature:

1. Murray P.R., Rosenthal K.S., Tenover F.C., Tenover F.C. *Medical Microbiology*. – 10th ed. – Philadelphia: Elsevier, 2025. – 880 p. (ISBN: 978-0-323-93321-0)
2. Carroll K.C., Butel J.S., Morse S.A., et al. *Jawetz, Melnick & Adelberg's Medical Microbiology*. – 29th ed. – New York: McGraw-Hill Education, 2024. – 864 p. (ISBN: 978-1-264-93210-6)
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Laboratory work 24

The Causal Agent of Cholera. Bacteriological Diagnosis

The objective of this work is to study the biological properties of the cholera vibrio, master methods of microbiological diagnosis of cholera, rules for collecting material and working with particularly dangerous infections.

Procedure for completing the work

Family: *Vibrionaceae*

Genus: *Vibrio*

Species: *Vibrio cholerae*

Biovars (biotypes): *Vibrio cholerae* biovar *cholerae*, *Vibrio cholerae* biovar *elton*

Serogroups and serovars: *Vibrio cholerae* serogroup O1, *Vibrio cholerae* serogroup O139

Cholera has been known since ancient times. The first descriptions of the disease appear in the writings of Hippocrates. The first cholera pandemic began in 1817, spreading throughout Asia. Cholera reached Europe in the 1830s. Major cholera pandemics are associated with the spread of the classic biovar.

The cholera pathogen was first discovered in 1854 by the Italian anatomist Filippo Pacini in the feces of patients, but his discovery went unnoticed. Rediscovered by Robert Koch in 1883 during an expedition to Egypt and India, Koch isolated a pure culture, described its morphology (Koch's comma), and demonstrated its role in the etiology of the disease.

In 1906, Gottschlich isolated a microorganism similar to the cholera vibrio but with distinct hemolytic properties from the stool of a pilgrim at the El Tor quarantine station. It was named *Vibrio elton*. It was long considered nonpathogenic, but between 1937 and 1960, its ability to cause cholera was demonstrated. The seventh cholera pandemic (1961 to present) was caused by the El Tor biovar.

In 1992, a new serovar, O139 (Bengal), was isolated, causing an epidemic in South Asia.

Practical Part:

Equipment and Materials:

- Immersion microscopes, phase-contrast microscopes
- Slides and coverslips
- Stains: Ziehl's carbol fuchsin (1:10), Gram stain kit
- Culture media: 1% peptone water (pH 8.0-8.5), alkaline agar, TCBS (thiosulfate-citrate-bile-salt-sucrose agar)
- Cholera O-serum (O1 and O139), diagnostic kits
- Sterile test tubes, pipettes, Petri dishes
- *Vibrio cholerae* training slides (models)

Safety instructions: Cholera is a particularly dangerous infection (pathogenicity groups I-II). Samples are collected in sterile jars with ground-glass stoppers, placed in metal tubes. Work with the samples is carried out in secure laboratories. Inactivated cultures are used for educational purposes.

Procedure (demonstration):

1. Collection of specimen (demonstration)
 - Stool (liquid, without urine admixture) is collected in sterile jars
 - Vomitus is collected in sterile containers
 - If rapid delivery is not possible, a preservative (1% peptone water, pH 8.0-8.5) is used
2. Microscopy of native specimen (phase contrast)

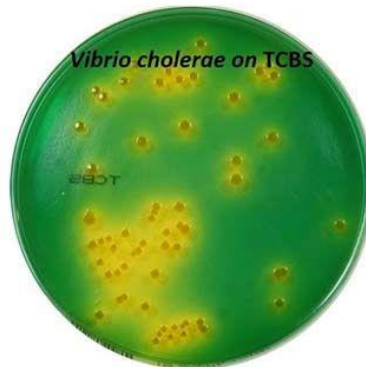
- Prepare a "crushed drop" preparation
- View under a phase-contrast or dark-field microscope
- Result: Cholera vibrios are motile, comma-shaped, and actively move (in a straight line, "falling leaves")

3. Microscopy of stained specimens

- Fixation with alcohol or Nikiforov's mixture (carefully, do not fix with heat)
- Staining with diluted carbol fuchsin (1:10) for 5-7 min
- Result: Gram-negative curved rods, comma-shaped, located singly or in pairs

4. Inoculation on nutrient media

- Inoculation on 1% peptone water (enrichment)
- Incubation for 6-8 hours at 37°C
- Subculture from enrichment medium onto alkaline agar or TCBS agar



Vibrio cholerae on TCBS Agar



Vibrio parahaemolyticus on TCBS Agar

- On TCBS agar: *V. cholerae* forms yellow colonies (sucrose fermentation)

5. Culture identification

- Agglutination test on glass with cholera O serum (O1)
- If the reaction is positive, determine the biovar (agglutination with Inaba and Ogawa serum)

- Sensitivity to vibriostatic 0/129
- Biochemical tests (oxidase positive, gelatin liquefaction, carbohydrate fermentation)

6. Vibrio immobilization test (rapid diagnostics)

- On a subject A drop of feces is applied to the glass.
- A drop of cholera serum (diluted 1:100) is added.
- The loss of motility (immobilization) of the vibrios is observed under a phase-contrast microscope.

Conclusions: The main properties of the cholera vibrio were studied: morphology (curved rods, intense motility), cultural characteristics (halophilicity, growth in alkaline media, formation of yellow colonies on TCBS agar). Rapid diagnostics include an immobilization reaction with cholera serum. Definitive identification is based on agglutination with O1 serum and biochemical tests.

Report Contents

1. Title of the lab work.
2. Objective of the work.
3. Assignment.
4. Conclusions.

Quiz Questions

1. Systematics of the genus *Vibrio*, species pathogenic to humans
2. Morphology and tinctorial properties of *Vibrio cholerae*
3. Cultural properties: halophilicity, alkaline environments
4. Biochemical activity

- O139
5. Antigenic structure: O- and H-antigens, serovars O1 (Inaba, Ogawa, Hikoshima) and
 6. Classic and El Tor biovars: differential characteristics
 7. Pathogenicity factors of *Vibrio cholerae*: exotoxin (cholero-gen), mechanism of action
 8. Toxin-coregulated pili, neuraminidase
 9. Pathogenesis of cholera: small intestinal damage, fluid loss
 10. Clinical picture: degrees of dehydration
 11. Epidemiology of cholera: source, routes of transmission (water, food, contact)
 12. The role of carriage of *Vibrio cholerae* in the spread of infection
 13. Cholera pandemics: History, current status
 14. Laboratory diagnosis of cholera: specimen collection, delivery procedures
 15. Rapid diagnostics: fluorescence microscopy, immobilization, PCR
 16. Bacteriological method: culture on enrichment media, isolation of pure culture
 17. Identification of *Vibrio cholerae* (serological, biochemical)
 18. Treatment of cholera: rehydration, antibiotics
 19. Cholera prevention: vaccines (oral, killed)
 20. Anti-epidemic measures in cholera outbreaks
 21. *V. parahaemolyticus* as a causative agent of food poisoning

List of key literature:

1. Murray P.R., Rosenthal K.S., Pfaller M.A. *Medical Microbiology*. – 10th ed. – Philadelphia: Elsevier, 2025. – 880 p. (ISBN: 978-0-323-93321-0)
2. Carroll K.C., Butel J.S., Morse S.A., et al. *Jawetz, Melnick & Adelberg's Medical Microbiology*. – 29th ed. – New York: McGraw-Hill Education, 2024. – 864 p. (ISBN: 978-1-264-93210-6)
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Laboratory work 25

Causative agents of spirochetosis (borrelia, leptospiraceae, treponema). Serological diagnosis of syphilis (wassermann reaction and RPHA)

The objective of this work is to study the morphological and biological properties of pathogenic spirochetes, to master methods of laboratory diagnostics of syphilis, leptospirosis, tick-borne borreliosis (Lyme disease).

Procedure for completing the work

Family: *Spirochaetaceae*

Genus: *Borrelia*

Species: *Borrelia burgdorferi sensu stricto*, *Borrelia afzelii*, *Borrelia garinii*, *Borrelia recurrentis*, *Borrelia hermsii*

Genus: *Leptospira*

Species: *Leptospira interrogans*, *Leptospira borgpetersenii*, *Leptospira kirschneri*, *Leptospira mayottensis*, *Leptospira noguchii*, *Leptospira santarosai*, *Leptospira weilii*

Genus: *Treponema*

Species: *Treponema pallidum*, *Treponema carateum*, *Treponema denticola*, *Treponema vincentii*

The origin of syphilis is controversial (American and European theories). The causative agent, *Treponema pallidum*, was discovered in 1905 by German scientists Fritz Schaudin and Erich Hoffmann. They discovered *Treponema pallidum* in syphilitic papules and demonstrated its etiological role. In 1906, August von Wassermann developed the complement fixation test (Wassermann reaction) for serological diagnosis.

The causative agent was described in 1914 by the Japanese scientist Inado, who isolated it from the blood of a patient with "Akiyama disease." In 1915, independently, Hübner and Reiter, as well as Uhlenhuth and Fromme, isolated leptospira from patients and rats. In 1917, Nogushi created the genus *Leptospira*.

The causative agent of relapsing fever (*Borrelia recurrentis*) was discovered by Otto Obermeyer in 1873 in the blood of a patient. The causative agent of Lyme disease (*Borrelia burgdorferi*) was isolated by Willi Burgdorfer in 1981 from Ixodid ticks. It is named after its discoverer.

Practical Part

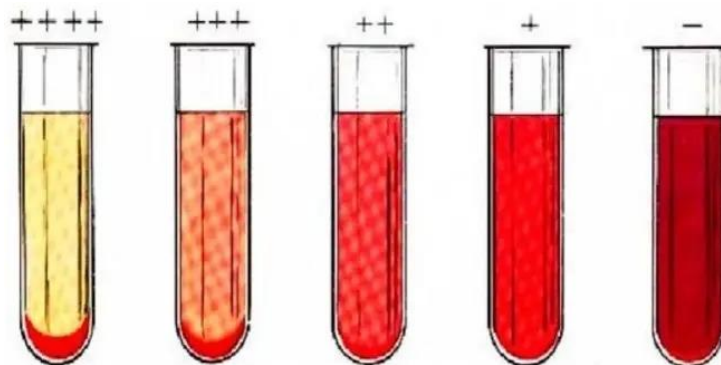
Equipment and Materials:

- Patient's blood serum (training)
- Cardiolipin antigen, ultrasound treponemal antigen (specific)
- Complement fixation system (hemolytic serum, complement, sheep red blood cells)
- Agglutination tubes, racks, pipettes
- Saline solution
- RPHA (RNHA) plates
- Erythrocyte diagnosticums (treponemal)
- Thermostat, refrigerator, water bath

Safety instructions: Blood and serum may be contaminated; wear gloves when handling. The used material is decontaminated by autoclaving. After use, glassware is soaked in a disinfectant solution.

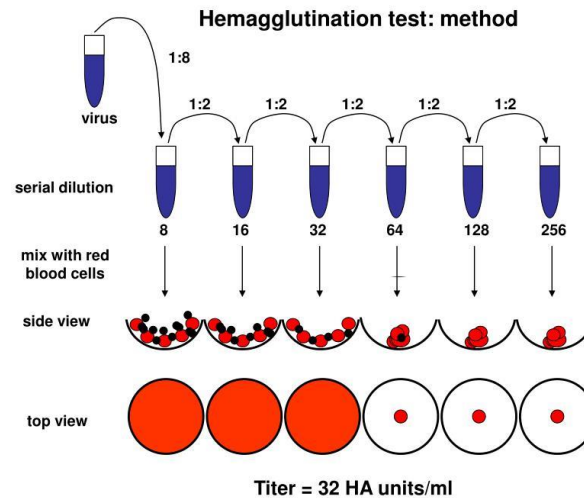
Procedure:

1. Wassermann reaction (RW) — demonstration of the procedure
 - Serum inactivation (56°C, 30 min) to destroy the patient's own complement
 - Preparation of serum dilutions 1:5, 1:10, 1:20
 - Addition of components: test serum + antigen + complement
 - Incubation for 30 min at 37°C for complement fixation
 - Addition of the hemolytic system (ram red blood cells + hemolytic serum)
 - Incubation for 30-60 min at 37°C
2. Taking into account the RW results



- Complete hemolysis inhibition (++++): The reaction is strongly positive
- Significant inhibition (+++): Positive
- Partial inhibition (++): Weakly positive
- Weak inhibition (+): Doubtful
- Complete hemolysis (-): Negative

3. Performing the RPHA (passive hemolysis reaction) Hemagglutination)
 - Serum dilutions are added to the wells of the plate
 - Erythrocyte treponemal diagnosticum is added
 - Incubation for 2-3 hours at room temperature
4. TPHA (Regular Hemoglobinase) Analysis



- Positive: "Umbrella" of agglutinated erythrocytes at the bottom of the well
 - Negative: Compact ring or "dot" at the bottom of the well
 - Titer: Last dilution yielding a positive result
5. Treponema pallidum Microscopy (Demonstration)
 - Darkfield microscopy (crushed drop method)
 - Result: Spiral-shaped bacteria with 8-12 uniform whorls, corkscrew-like movements

Conclusions: The main methods of serological diagnosis of syphilis have been studied. The Wassermann reaction (non-treponemal test) is used for screening and assessing treatment effectiveness, but can produce false-positive results. Treponemal tests (TPHA) and ELISA (treponemal assays) are more specific and are used to confirm the diagnosis. Darkfield microscopy of chancroid discharge is used for early diagnosis of primary syphilis.

Report Contents

1. Title of the lab work.
2. Objective of the work.
3. Assignment.
4. Conclusions.

Quiz Questions

1. General characteristics of spirochetes
2. Taxonomy of the family: *Spirochaetaceae* (*Treponema*, *Borrelia*) and *Leptospiraceae* (*Leptospira*)
3. Morphology of treponemas: structure, nature of movements
4. *Treponema pallidum*: cultural features (not cultivated on artificial media)
5. Pathogenesis of syphilis: stages of the disease
6. Clinical manifestations of primary, secondary, and tertiary syphilis
7. Congenital syphilis
8. Laboratory diagnosis of syphilis: dark-field microscopy
9. Serological diagnosis: non-treponemal tests (RW, RPR, VDRL)
10. Treponemal tests (IFA, RPHA, ELISA, Western blot)
11. *Treponema pallidum* immobilization reaction (TPIR)
12. False-positive serological reactions
13. *Leptospira*: Morphology, Cultural Properties

14. Epidemiology of Leptospirosis: Natural Foci
15. Pathogenesis and Clinical Features of Leptospirosis
16. Laboratory Diagnosis of Leptospirosis (Microscopy, Culture, Serology, Bioassay)
17. *Borrelia*: Morphology, Cultivation
18. Epidemiology of Relapsing Fever (Louse- and Tick-Borne)
19. Lyme Disease (Tick-Borne Lyme Disease): Pathogens, Disease Stages
20. Clinical Picture of Lyme Disease (Erythema Migrans, Neurological Manifestations, Arthritis)
21. Laboratory Diagnosis of Borreliosis
22. Treatment and Prevention of Spirochetosis

List of key literature:

1. Murray P.R., Rosenthal K.S., Tenover F.C., Tenover F.C. *Medical Microbiology*. – 10th ed. – Philadelphia: Elsevier, 2025. – 880 p. (ISBN: 978-0-323-93321-0)
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Laboratory work 26

Chlamydia, rickettsia and mycoplasma microscopic and serological diagnosis of chlamydiasis

The objective of this work is to study the biological properties of chlamydia, rickettsia and mycoplasma as intracellular parasites, to master the methods of laboratory diagnostics of ornithosis, typhus, and respiratory mycoplasmosis.

Procedure for completing the work

Family: *Rickettsiaceae*

Genus: *Rickettsia*

Species: *Rickettsia prowazekii*, *Rickettsia typhi*, *Rickettsia rickettsii*, *Rickettsia conorii*, *Rickettsia akari*, *Rickettsia sibirica*

Family: *Chlamydiaceae*

Genus: *Chlamydia*

Species: *Chlamydia trachomatis*, *Chlamydia pneumoniae*, *Chlamydia psittaci*, *Chlamydia abortus*, *Chlamydia suis*

Family: *Mycoplasmataceae*

Genus: *Mycoplasma*

Species: *Mycoplasma pneumoniae*, *Mycoplasma genitalium*, *Mycoplasma hominis*

Genus: *Ureaplasma*

Species: *Ureaplasma urealyticum*, *Ureaplasma parvum*

Named after the American pathologist Howard Taylor Ricketts, who studied the pathogen from 1906 to 1909 Rocky Mountain spotted fever and died of typhus in 1910. Independently, the Czech scientist Stanislav Provaček studied typhus and also died from the infection. In 1916, the

Brazilian scientist Henrique da Rocha Lima proposed naming the genus *Rickettsia* in honor of Ricketts and the species *proWazekii* in honor of Prowaček. Ricketts and Prowaček observed the typhus pathogen in preparations, but were unable to isolate a pure culture.

The causative agent of trachoma was described in 1907 by Halberstadter and Prowaček, who discovered intracellular inclusions in the conjunctival epithelium. The causative agent of ornithosis (psittacosis) was isolated from parrots by Beduson in 1930. In the 1960s, the life cycle of chlamydia was discovered. In 1966, the organisms were classified as a separate order, Chlamydiales. First isolated in 1898 by Nocard and Roux from the pleural fluid of cattle with bovine pleuropneumonia. Human mycoplasmas (*M. pneumoniae*) were discovered in 1944 by Eaton and described as the agent of primary atypical pneumonia. Pure cultures were obtained in 1962-1963. Practical Part

Equipment and Materials:

- Fluorescent and immersion microscopes
- Slides with fixed smears (for teaching)
- Luminescent sera (species-specific)
- Patient sera for complete stem cell assay (CSA), ELISA
- Chlamydial antigens (complement-fixing)
- Complete stem cell assay (as in the Wassermann reaction)
- ELISA plates
- Cell cultures (demonstration of infection)

Safety instructions: Chlamydia and rickettsia are pathogenicity group II microorganisms; working with them requires special precautions. Inactivated preparations are used for teaching purposes. Procedure (demonstration):

1. Microscopic examination (detection of Prowazek bodies)

- For typhus - smears from skin scrapings or organ sections
- Zdrodowski staining (fuchsin + methylene blue)
- Result: *Rickettsiae* are small coccoid or rod-shaped red formations (fuchsinophilic), located intracellularly

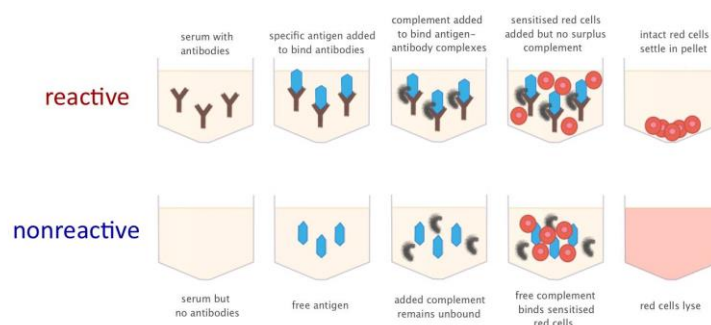
2. Fluorescence microscopy (chlamydia)

- Smears from scrapings of the urethra, cervical canal, and conjunctiva
- Treatment with luminescent serum against chlamydia
- Result: Bright green fluorescence of intracellular inclusions (Halberstadter-Provacek bodies)

3. Complement fixation test (CFT)

- For the diagnosis of ornithosis and typhus
- Paired sera are used (for 1st and 3rd weeks)
- Principle: Complement fixation by the antigen-antibody complex
- Note: Delayed hemolysis indicates a positive reaction

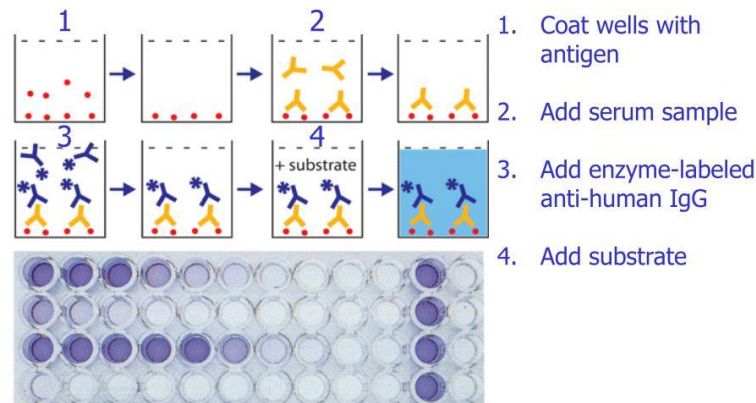
complement fixation test



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4. Enzyme-linked immunosorbent assay (ELISA) for diagnosing chlamydia
 - Serum is added to the wells of a plate containing the adsorbed antigen
 - Conjugate (enzyme-labeled antibodies against Ig) is added
 - Substrate is added and the color change is assessed

Enzyme-linked immunosorbent assay (ELISA)



H. HogenEsch, 2009

- Result: Color intensity is proportional to the amount of antibodies
5. PCR diagnostics (demonstration)
 - DNA extraction from clinical material
 - Amplification with species-specific primers
 - Electrophoretic detection of amplification products

Conclusions: The biological features of obligate intracellular parasites, rickettsia and chlamydia, were studied. The main diagnostic methods include microscopy (Zdrodovsky staining, fluorescence microscopy) and serological tests (complete blood count, enzyme immunoassay). The most sensitive method is PCR, which can detect pathogen DNA even at low levels. Bacteriological (culture on special media) and serological methods are used to diagnose mycoplasmosis.

Report Contents

1. Title of the lab work.
2. Objective of the work.
3. Assignment.
4. Conclusions.

Quiz Questions

1. General characteristics of rickettsia: taxonomy, morphology
2. Biological properties: intracellular parasitism, cultivation
3. Antigenic structure of rickettsia
4. *Rickettsia prowazekii* - the causative agent of epidemic typhus
5. Epidemiology of typhus: source (patient), vector (louse)
6. Pathogenesis of typhus: vasculitis, thrombovasculitis
7. Clinical picture of typhus
8. Laboratory diagnostics of typhus (CSF, RIF, RNGA)
9. Weigl's reaction (biological test)
10. Brill's disease (relapse of typhus)
11. Other rickettsioses (spotted fever, Volyn fever)
12. Prevention of typhus (sanitary treatment, vaccine)
13. *Chlamydia*: taxonomy, species (*C. trachomatis*, *C. pneumoniae*, *C. psittaci*)

14. *Chlamydia* life cycle: elementary and reticular bodies
15. Pathogenesis of chlamydial infections
16. Trachoma: clinical features, epidemiology, diagnostics
17. Urogenital chlamydia
18. Ornithosis (psittacosis): source, transmission routes, clinical features
19. Laboratory diagnostics of chlamydia (microscopy, PCR, ELISA)
20. *Mycoplasmas*: general characteristics, absence of a cell wall
21. Human mycoplasmas (*M. pneumoniae*, *M. hominis*, *Ureaplasma urealyticum*)
22. Respiratory mycoplasmosis: pathogenesis, clinical features, diagnostics
23. Urogenital Mycoplasmosis
24. Methods for diagnosing mycoplasmosis (culture, serology, PCR)
25. Principles of treatment for chlamydia and mycoplasmosis

List of key literature:

1. Murray P.R., Rosenthal K.S., Tenover F.C., Tenover F.C. *Medical Microbiology*. – 10th ed. – Philadelphia: Elsevier, 2025. – 880 p. (ISBN: 978-0-323-93321-0)
2. Carroll K.C., Butel J.S., Morse S.A., et al. *Jawetz, Melnick & Adelberg's Medical Microbiology*. – 29th ed. – New York: McGraw-Hill Education, 2024. – 864 p. (ISBN: 978-1-264-93210-6)
3. Ryan K.J., Ahmad N., Alspaugh J.A., et al. *Ryan & Sherris Medical Microbiology*. – 9th ed. – New York: McGraw-Hill Education, 2024. – 992 p. (ISBN: 978-1-264-26874-0)

List of additional literature:

4. Goering R.V., Dockrell H.M., Zuckerman M., Chiodini P.L. *Mims' Medical Microbiology and Immunology*. – 7th ed. – Philadelphia: Elsevier, 2025. – 600 p. (ISBN: 978-0-323-93324-1)
5. Melter O., Castelhana R. (eds.). *The MicroBook: Clinical Microbiology for Medical Students*. – 1st ed. – Prague: Charles University Press, 2023. – 320 p. (ISBN: 978-80-246-5123-4)
6. Kumar S. *Medical Microbiology For Undergraduates*. – 2nd ed. – New Delhi: Jaypee Brothers Medical Publishers, 2024. – 560 p. (ISBN: 978-93-5465-789-2)

Laboratory work 27

Actinomycetes. Method of isolation and identification from soil

The objective of this work is to study the properties of actinomycetes, to master the principles of isolation, cultivation and identification of actinomycetes from natural sources (soil) in laboratory conditions.

Procedure for completing the work

Domain: Bacteria

Phylum: Actinomycetota (formerly Actinobacteria)

Class: Actinomycetia

Order: Actinomycetales

Family: Actinomycetaceae

Genus: Actinomyces

Actinomycetes are Gram-positive, chemoorganotrophic, predominantly aerobic (some facultative anaerobes) bacteria. They are capable of forming well-developed branching mycelium, which superficially resembles true mushrooms (hence the old name "ray fungi"). The hyphae diameter is 0.4-1.5 μm . Colonies on nutrient media are usually dry, mealy or leathery, often with aerial mycelium. They can be colored (white, gray, pink, yellow, etc.), and have a characteristic "earthy" odor due to the production of geosmin. Pathogenic actinomycetes (primarily *Actinomyces israelii* and *A. bovis*) cause actinomycosis—a chronic purulent-granulomatous inflammation with the formation of dense infiltrates, abscesses, fistulas, and drusen (grains) in the pus. The disease affects the maxillofacial region, lungs, intestines, and skin. Pathogenesis is associated with

penetration through damaged tissue (often due to trauma or dental caries), resulting in the formation of granulomas and fistulas.

Actinomycetes (ray fungi) attracted the attention of scientists in the late 19th and early 20th centuries due to their unusual morphology: they form a branching mycelium reminiscent of fungi, but are actually prokaryotes (bacteria). One of the first researchers to describe these microorganisms was the Italian scientist Gasperini in the late 19th century. In 1915-1916, American microbiologist Selman Abraham Waksman (1888-1973), born in Ukraine (then the Kiev Governorate of the Russian Empire), began a systematic study of soil microorganisms, including actinomycetes, while working at Rutgers University (USA).

Waksman devoted many years to studying the role of actinomycetes in soil: their distribution, taxonomy, participation in the decomposition of organic matter, and antagonistic relationships with other microbes. As early as 1923, together with his student Robert Starkey, he noted that actinomycetes inhibit the growth of many bacteria in the soil. Beginning in the late 1930s, Waksman organized targeted screening of soil microorganisms for their ability to produce substances that inhibit the growth of pathogenic bacteria. In 1940, his group isolated actinomycin from *Streptomyces antibioticus* (then called *Actinomyces*), but this drug proved too toxic to animals. In 1943, Waksman's graduate student, Albert Schatz, isolated a substance called streptomycin from a strain of *Streptomyces griseus* (reisolated from soil). It was the first effective antibiotic against tuberculosis (*Mycobacterium tuberculosis*), and was also active against many gram-negative bacteria. In 1942, Waksman himself coined the term "antibiotic"—a substance produced by microorganisms that, in low concentrations, inhibits the growth or kills other microbes.

In 1952, Selman Waksman was awarded the Nobel Prize in Physiology or Medicine "for the discovery of streptomycin, the first antibiotic effective in the treatment of tuberculosis." His systematic approach to the search for antibiotics among actinomycetes laid the foundation for the modern antibiotic industry. Thanks to the work of Waksman and his school, actinomycetes have become the primary source of natural antibiotics: more than 70% of the antibacterial substances known today are produced by this type of bacteria.

Practical Part

Equipment and Materials: Soil sample, sterile Petri dishes, Gause No. 1 nutrient medium (or starch-ammonia medium), sterile saline solution (0.9% NaCl), sterile pipettes, Drygalski spatulas, thermostat (28-30°C), microscope, microscope slides, Gram stains, alcohol, iodine, alcohol lamp, cotton wool, gauze, and tweezers.

Safety Instructions: When working with actinomycetes, aseptic and biological safety precautions must be observed (pathogenicity groups II-III for pathogenic *Actinomyces* species). Wear a gown, gloves, and a mask. After work, disinfect the work area with 70% alcohol or 3% chloramine.

The method for isolating actinomycetes from soil includes the following steps:

1. Prepare a soil suspension: add 1 g of soil to 9 ml of sterile saline solution and shake vigorously for 10-15 minutes.
2. Prepare serial tenfold dilutions (10^{-1} - 10^{-5}).



3. For each dilution, take 0.1 ml of the suspension and spread it evenly over the surface of Gauze medium No. 1 in a Petri dish with a sterile spatula.
 4. Invert the dishes and incubate in a thermostat at 28-30°C for 7-14 days.
 5. After incubation, examine the growth pattern of the colonies: dry, mealy, often with white, gray, or colored aerial mycelium, with an "earthy" odor.
- The microscopic examination of actinomycetes involves the following steps:
1. Prepare a smear from a suspect colony on a glass slide.

Actinomycetes - Morphology, Classification, Uses



2. Air-dry and fix over a flame.
3. Gram stain.
4. Microscope at $\times 400$ and $\times 1000$ magnification (immersion).

Report Contents

1. Title of the lab work.
2. Objective of the work.
3. Assignment.
4. Conclusions.

Quiz Questions

1. History of the study of actinomycetes. The role of S. Waksman in the discovery of antibiotics.
 2. Taxonomic position of actinomycetes.
 3. General characteristics of actinomycetes.
 4. Cell structure of actinomycetes (mycelium, spores, cell wall).
 5. Classification of actinomycetes. Main genera: Streptomyces, Actinomyces, Nocardia.
 6. Characteristics of biological activity (production of antibiotics and enzymes). Concept of antibiotic drift.
 7. The developmental cycle of actinomycetes (vegetative mycelium $\rightarrow$ aerial mycelium $\rightarrow$ spores).
 8. Ecology of actinomycetes: natural sources, role in soil.
 9. Pathogenesis of actinomycosis. Tissue damage, formation of granulomas and fistulas.
- Immunity.
10. Clinical presentation of actinomycosis.
 11. Specific prevention and treatment: antibiotics, surgical treatment.
 12. Laboratory diagnosis of actinomycosis. Samples for examination (pus, tissue).
 13. Rapid diagnostics (microscopy, PCR).
 14. Bacteriological method (isolation on nutrient media, identification by morphology and biochemical tests).

List of key literature:

1. Murray P.R., Rosenthal K.S., Pfaller M.A. *Medical Microbiology*. – 10th ed. – Philadelphia: Elsevier, 2025. – 880 p. (ISBN: 978-0-323-93321-0)
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Laboratory work 28

The causative agent of candida infection

The objective of this work is to study the biological properties of pathogens candida infections, master methods of microbiological diagnostics, and understand the role of exotoxins in the pathogenesis of diseases.

Procedure for completing the work

Family: *Saccharomycetaceae*

Genus: *Candida*

Species: *Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis*, *Candida krusei*

Candidiasis has been known since ancient times, but the pathogen was first described by Langnek in 1839 in patients with thrush. The genus *Candida* was created by K. Berkhold in 1923.

Practical Section
Materials and Equipment:

Pure 24-hour culture of *Candida albicans* on Sabouraud agar

Saline solution

Slides and coverslips

Stains: methylene blue solution, Gram stain kit

Alcohol lamp, inoculation loop

Microscope with immersion system (×90 or ×100 objective, ×10 eyepiece)

Procedure and Results

Step 1. Preparation of a native preparation ("crushed drop")

Apply a small drop of saline solution to a clean slide.

Using an inoculation loop, remove a small amount of pure *Candida albicans* culture from the surface of the Sabouraud agar.



Carefully emulsify the culture in the drop of saline solution until a uniform suspension is obtained.

Cover the preparation with a coverslip, avoiding the formation of air bubbles. Place the specimen on the microscope stage and examine it first at low magnification ($\times 40$), then at high magnification ($\times 400$), and then with immersion ($\times 900$ – 1000).

Native mount microscopy results:

Native mount microscopy of *Candida albicans* reveals the following:

Cell shape: round or oval, 2–8 μm in diameter.

Budding cells (blastospores): cells with daughter buds of varying sizes are visible, from small to nearly as large as the mother cell.

Pseudomycelium: chains of elongated cells connected to each other at constrictions (resembling "sausages" with characteristic constrictions at the junctions).

Chlamydospores: When examining a culture grown on special media (cornmeal agar with Tween-80), microscopic examination reveals large, thick-walled, rounded cells located singly or at the ends of pseudohyphae. Chlamydospores are a characteristic diagnostic feature of *C. albicans*.

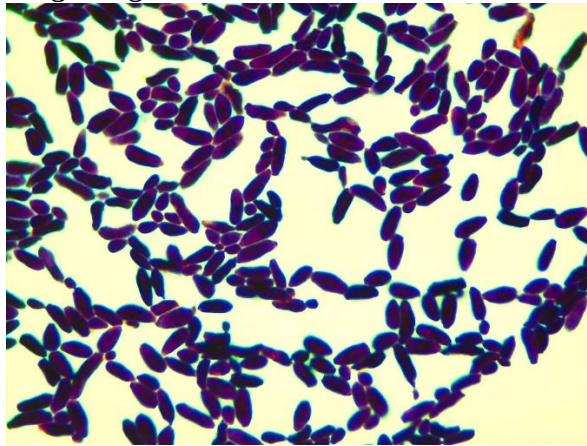
Step 2. Preparing and staining a fixed smear using Gram stain

Apply a thin, uniform smear from the pure *Candida albicans* culture to a clean, defatted glass slide (as when preparing a smear from a bacterial culture).

Air-dry the smear at room temperature.

Fix the smear over an alcohol lamp flame (pass the slide, smear upward, through the flame 3-4 times).

Perform Gram staining using the standard method:



Microscopy results of the Gram-stained specimen:

Candida cells stain dark purple (Gram-positive).

Oval or round Gram-positive yeast cells are clearly visible against the pink background of the specimen.

Budding cells and pseudohyphae are clearly visible.

Chlamydospores stain blue-black and have a thick wall.

Cells are located singly, in pairs, or in chains.

Report Contents

1. Title of the lab work.
2. Objective of the work.
3. Assignment.
4. Conclusions.

Quiz Questions

1. Candidiasis: Pathogens and Morphology of *Candida* spp.
2. Pathogenicity Factors of *Candida*
3. Clinical Forms of Candidiasis (Superficial, Visceral)
4. Oral Candidiasis (Thrush): Diagnosis and Treatment
5. Genital Candidiasis

6. Immunity in Candidiasis, the Role of Immunodeficiencies
7. Laboratory Diagnosis of Candidiasis
8. Antifungal Drugs: Groups, Mechanism of Action
9. What are the main morphological features of *Candida* fungi observed microscopically?
10. How does *Candida albicans* differ from other *Candida* species in its cultural properties?
11. What is the diagnostic value of the germ tube test?
12. What is the Gold quantitative culture method?
13. What material is examined if mucosal candidiasis is suspected?
14. What are the criteria for the etiologic significance of *Candida* fungi in quantitative culture?
15. Name the main antifungal drugs and their mechanisms of action.
16. What serological methods are used to diagnose visceral candidiasis?
17. What factors predispose to the development of candidal infection?
18. How does candidiasis, an opportunistic mycosis, differ from other fungal infections?

List of key literature:

1. Murray P.R., Rosenthal K.S., Tenover F.C., Tenover F.C. *Medical Microbiology*. – 10th ed. – Philadelphia: Elsevier, 2025. – 880 p. (ISBN: 978-0-323-93321-0)
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