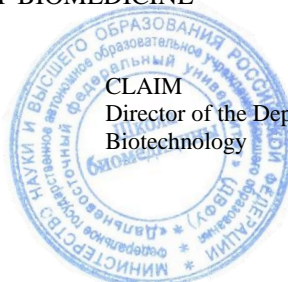




MINISTRY OF SCIENCE AND HIGHER EDUCATION OF RUSSIAN FEDERATION  
Federal State Autonomous Educational Institution of Higher Education  
**Far Eastern Federal University**  
(FEFU)  
SCHOOL OF BIOMEDICINE

AGREED  
Head of OP

(Signed) (Full name)



CLAIM

Director of the Department of Medical Biology and  
Biotechnology

(Signed) (Acting Name)  
December 06, 2022

WORK PROGRAM OF THE DISCIPLINE  
Methods of molecular and cellular diagnostics  
Direction of training 06.04.01 Biology  
(Molecular and Cell Biology)  
Form of training: full-time

Course 1 semester 2  
lectures 18 h.  
practical exercises - hour.  
lab work 6 hours  
total hours of classroom load 36 hours.  
independent work 45 hours.  
including 27 hours to prepare for the exam.  
exam 2 semester

The work program is drawn up in accordance with the requirements of the Federal State Educational Standard in the direction of training 19.03.01 Biotechnology, approved by the order of the Ministry of Education and Science of Russia dated 10.08.2021No.736.

The work program was discussed at the meeting of the Department of Medical Biology and Biotechnology Protocol dated December 06, 2022 No. 2

Director of the Department of Implementing Structural Unit of the Cand. Biol. Ph.D., Associate Professor V.V. Kumeiko

Compiled by: Assistant D.V. Lansikh

Vladivostok  
2022

Reverse side of the RPD cover page

1. The work program was revised at the meeting of the Department / department / department (implementing the discipline) and approved at the meeting of the Department / department / department (issuing structural unit), the protocol from " \_\_\_\_\_ № \_\_\_\_\_

2. The work program was revised at the meeting of the Department / department / department (implementing the discipline) and approved at the meeting of the Department / department / department (issuing structural unit), the protocol from " \_\_\_\_\_ № \_\_\_\_\_

3. The work program was revised at the meeting of the Department / Department / Department (implementing the discipline) and approved at the meeting of the Department / Department / Department (issuing structural unit), the protocol from " \_\_\_\_\_ № \_\_\_\_\_

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5. The work program was revised at the meeting of the Department / Department / Department (implementing the discipline) and approved at the meeting of the Department / Department / Department (issuing structural unit), the protocol from " \_\_\_\_\_ № \_\_\_\_\_

1. Goals and objectives of mastering the discipline:

**Purpose:** formation of students' systematic knowledge of modern methods of molecular and cellular diagnostics, mastery of the basic methods of molecular and cellular diagnostics in medical and biological research.

**Tasks:**

1) To acquaint masters with the current state of molecular and cellular diagnostics, its application in the clinic, promising developments in this area.

2) To study the technologies of conducting experiments, analyzes and tests in molecular and cellular diagnostics.

3) To train masters to work in the laboratory, to apply in practice the basics of planning research work.

Professional competencies of graduates and indicators of their achievement:

Task type	Code and name of professional competence (the result of mastery)	Code and name of the competency achievement indicator
research	PC-2 is able to apply the methodological foundations of design, perform laboratory biological, environmental research, use modern equipment and computing complexes in molecular and cell biology.	PC-2.1 Develops rules and algorithms for the design, implementation of laboratory biological and environmental research.
		PC-2.2 Performs laboratory biological, environmental research using the scientific methodological foundations of fundamental research.
		PK-2.3 Applies the methodological foundations of design, laboratory biological, environmental research, uses modern equipment and computing complexes in molecular and cellular biology.

Code and name of the competency achievement indicator	Name of the assessment indicator (the result of training in the discipline)
PC-2.1 Develops rules and algorithms for the design, implementation of laboratory biological and environmental research.	Knows: Fundamental concepts of cell and molecular biology; methodology for setting up basic laboratory tests; approaches to the analysis of the information received. Can: Determine the goals and objectives of the experiment, choose the object and methods of research; develop and optimize the conditions for setting up an experiment, perform laboratory biological research; draw logical conclusions based on the results of the experiment. Owns: Skills and rules and algorithms for designing, performing laboratory research.
PC-2.2 Performs laboratory biological, environmental research using the	Knows: Fundamentals of molecular and cellular biology, biochemistry

<p>scientific methodological foundations of fundamental research.</p>	<p>and biotechnology, fundamental principles of organization and functioning of living systems, methods and approaches to the study of the structure and properties of biological objects, organic and inorganic compounds.  Can:  Identify the problem in the analysis of scientific literature and / or the results of their own experimental work, set original goals and objectives of the scientific experiment, choose the appropriate object and method of research; perform laboratory biological research using modern equipment and computing facilities; analyze the biological data obtained during the experiment, draw logical conclusions and conclusions.  Owns:  Skills in performing laboratory research using the scientific methodological foundations of fundamental research.</p>
<p>PK-2.3 Applies the methodological foundations of design, laboratory biological, environmental research, uses modern equipment and computing complexes in molecular and cellular biology.</p>	<p>Knows:  Basic principles and stages of modern methods of analysis of the structure and properties of biological objects; with the possession of the main regulatory documents that ensure the conduct of research and production-technological biological work.  He can:  Translate in practice the knowledge of the basics of the organization and planning of research and production work using regulatory documents; analyze the data and operate with the information obtained; collect the necessary theoretical and practical material for the implementation of research work.  Owns:  For the organization and conduct of research and production-technological biological works; methods of self-analysis of available biological information; Work with library catalogues.</p>

### 1. Labor intensity of discipline and types of training sessions in the discipline

The total labor intensity of the discipline is 3 credit units (108 academic hours), (1 credit unit corresponds to 36 academic hours).

Types of training sessions and work of the student in the discipline are:

Designation	Types of training sessions and work of the student
Lek	Lecture
Lek electr.	
Lab	Labs
Lab Electr.	
WED:	Independent work of the student during the period of theoretical training
including control	Independent work of the student and contact work of the student with the teacher during the period of intermediate certification

### Structure of the discipline:

The form of training is full-time.

№	Name of the section Discipline	Se me ster	Number of hours by types of training sessions and work of the student						Intermediate attestation forms
			Lek	Lab	Av e	OK	WE D	Cont rol	
1.	Section No.1. Purpose, tasks, methods and theoretical foundations of molecular and cellular diagnostics (4 hours)	2	4	-	-	-	10	9	Oral questioning
2.	Section No2. Methods of molecular and cellular diagnostics and their application (12 hours)	2	12	18	-	-	35	18	Oral questioning
Total:		2	18	18	-	-	45	27	Exam

## **THE STRUCTURE AND CONTENT OF THE THEORETICAL PART OF THE COURSE**

Lectures (18 hours)

### **Section 1. Purpose, objectives, methods and theoretical foundations of molecular and cellular diagnostics (4 hours)**

**Topic 1.** Introduction. Purpose and objectives of methods of molecular and cellular diagnostics (2 hours. ).

Subject, tasks and methods of molecular and cellular diagnostics. Objects of research. Types of molecular biological research methods. The main directions of their application.

**Topic 2.** Theoretical foundations of molecular diagnostics (1 hour. ).

Basic concepts of molecular biology. Structure and properties of biological molecules. Realization of hereditary material, gene expression. Change in the sequence of nucleotides in DNA and RNA, effects of mutations. Methods of quantitative and qualitative analysis of nucleic acids and proteins. Genomics, transcriptomics, proteomics.

**Topic 3.** Theoretical foundations of cell diagnostics (1 hour. ).

Basic concepts of cell biology. The cell as a unit of living things, its structure and functions. Cell cycle, metabolism, cytoskeleton, mechano- and chemoreception, intracellular signaling. Review of methods of cell diagnostics, assessment of the phenotype and functional state of cells.

### **Section 2. Methods of molecular and cellular diagnostics and their application (12 hours)**

**Topic 1.** Cytogenetic research methods: karyotyping, gibrizia in situ (2 has. ).

Review of cytogenetic diagnostic methods. Basics of karyotyping: sample preparation, staining of the metaphase plate, chromosomal banding, microscopy, kariogram counting and analysis of results. Introduction to in situ hybridization technique. Overview of methods and approaches, varieties of FISH. Probes for in situ hybridization: preparation, direct and indirect tagging of probes, types of fluorochromes commercial solutions. Enzyme label features: Types of substrates for label enzymes. Theoretical basis of using different types of probes for FISH. Hybridization in situ of labeled DNA probe. Advantages and disadvantages of hybridization in solution. Hybridization on a solid carrier. Detection and analysis of hybridization results. The use of mini- and microsatellites as a probe for DNA fingerprinting, application for personal identification, in population biology, in programs related to the conservation of rare and endangered species, in medicine.

**Topic 2.** Methods based on the use of amplification Polymerase chain reaction. Ligase chain reaction (2 hours.).

Issues of safety, labor protection in the molecular genetic laboratory. Device of the PCR laboratory. Methods of preventing contamination: compliance with sterility during sample preparation, separation of zones. Equipment of the molecular genetic laboratory. Principles of PCR diagnostics. Basics of PCR: mechanism, stages. Devices, reagents and consumables for PCR. Development of pcr protocol, development of primers and necessary software. Biological material. Types and technologies of PCR: classical, allele-specific PCR, real-time PCR. Detection of amplification results. Ligase chain reaction. Ligation oligonucleotide probes. NASBA method, advantages over the traditional method, application. Examples of practical use of amplification methods.

**Topic 3.** Determination of the primary structure of nucleic acids, search for mutations (2 hours. ).

Identification of mutations. Application of methods of primary identification of mutations. Analysis of conformational polymorphism of single-stranded DNA. Method of denaturing gradient gel electrophoresis. Method of heteroduplex analysis. Method of chemical cleavage of nucleotide mismatch sites. DNA sequencing. DNA sequencing according to Cenger: principle of method, steps, components of reaction mixtures. DNA sample preparation and PCR purification of the sequencing mixture. Accounting for PCR results using Sanger tokenization. Analysis of chromatograms. Mass parallel sequencing: platforms, equipment, applications in diagnostics. Platforms for NGS, advantages and disadvantages of various techniques. NGS Data Analysis: NgS Processing and Data Interpretation Steps: Mutation Search and Gene Expression Analysis. Genomics. Transcriptomics. Methods for detecting known mutations: the method of

amplification-restriction, its reprocessing. PCR-mediated site-directed mutagenesis. A method for detecting mutations in different sites of the same gene.

**Topic 4.** Electrophoretic separation of proteins. Western blotting. Chromatographic methods of protein purification (2 hours).

The principle of the method of electrophoresis of proteins. Electrophoretic mobility. Classification of electrophoretic methods. Features of electrophoresis in polyacrylamide gel. Native and SDS-PAGE electrophoresis. Disc electrophoresis. Basics of protein immunoblotting. Methods of immunodetection. Qualitative and quantitative processing of electrophoregrams. Introduction to chromatographic methods of analysis. Fixed (stationary) and mobile phases. Sorbates. "Dead Time" chromatographic column. Eluent chromatogram. Retention time of the substance. Evaluation of the effectiveness of the chromatographic column (the concepts of retention factor, selectivity coefficient and resolution). Number of theoretical plates  $N$ . Van Deemter equation. Chromatographic systems and detectors. Types of protein chromatography. Absolute calibration method. Method of internal normalization. Internal standard method.

**Topic 5.** Optical microscopy: histological and immunohistochemical studies in diagnostics. Probe and electron microscopy (2 hours).

Histological and histochemical studies in diagnostics. Biological material suitable for microscopic examination, preparation stages: preparation of smears, paraffin blocks and sections. Painting methods. Staining with antibodies. Theory of image formation. Microscope device. Classification of microscope objectives. Tuning the microscope according to Köhler. Light-field and dark-field microscopy. Methods of image contrast. Microscopy in transmitted and reflected light. Luminescence microscopy: principle of the method, illumination sources in luminescence microscopy, bioluminescence. Use of fluorescent labeling methods. Confocal and multiphoton microscopy. Probe, electron and X-ray microscopies and: types and principles, the design of microscopes, biological samples, parameters determined. Significance in biomedical research. Sample preparation.

**Topic 6.** Flow cytometry: opportunities, objects (2 hours).

Basics and principles of flow cytometry, light scattering and fluorescence, objects of research. Cytometer and cell sorter: device device (optical system, liquid system, electronic system), the principle of operation, calibration. Properties and parameters of biological, analyzed by methods of flow cytometry, signal parameters. Review of methods of flow cytometry. Experiment planning: sample preparation, controls, standardization methods, reagents used. Ensuring the reproducibility of the results obtained. Immunophenotyping, cell cycle analysis and cell proliferation. Analysis of cell viability, apoptosis, necrosis. Cytotoxic test, cell

degranulation. Visualization of results. Types of scales. Adjustment and calibration of the instrument for the selected characteristic.

#### **IV. STRUCTURE AND CONTENT OF THE PRACTICAL PART OF THE COURSE AND INDEPENDENT WORK**

Laboratory work (18 hours)

Laboratory work No. 1 "Genotyping of the cell line. Search for mutations by Sanger sequencing" (12 hours)

Equipment required to perform the work:

1. A set of mechanical single-channel dispensers of different volumes: 0.1-10  $\mu\text{l}$ , 10-100  $\mu\text{l}$ , 100-1000  $\mu\text{l}$ ;
2. A centrifuge with a cooling and acceleration of at least 10000 g, allowing centrifugation of test tubes with a volume of 0.2 ml, 0.5 ml, 1.5 ml, 2 ml;
3. Vortex;
4. Thermocycler with 0.2 ml thermoblock for test tubes, heatable lid and temperature gradient function;
5. Nanospectrophotometer, which allows measuring optical density in the range from 200 to 600 nm;
6. Chamber for horizontal electrophoresis;
7. Filling table and combs for pouring gels;
8. Power supply designed for electrophoresis of nucleic acids in agarose gel;
9. Gel-documenting system for obtaining images of electrophoresis gels;
10. Capillary genetic analyzer;

Reagents and consumables necessary to perform the work:

1. Kit for isolating and purifying total DNA from whole blood and cell cultures;
2. Agarose;
3. TAE electrode buffer;
4. DNA length marker;
5. Loading buffer for NDT electrophoresis;
6. Deionized, nuclease-free water;
7. DNA polymerase for detection of single-nucleotide polymorphisms and high-precision amplification of DNA fragments up to 1 kb long and the corresponding buffer;
8. dNTP mixture for PCR;



9. Ethanol 96%;
10. Sodium acetate 3mM;
11. Set for DNA sequencing by the Sanger method;
12. EDTA 0.125M;
13. Formamide;
14. Disposable tips with filters for dispensers with a volume of 0.1-10  $\mu$ l, 10-100  $\mu$ l, 100-1000  $\mu$ l;
15. Test tubes of 0.2 ml, 0.5 ml, 1.5 ml, 2 ml, free of DNase and RNase.

Algorithm of work performance:

1. The design of primers of specified parameters, allowing amplification of a fragment of genomic DNA containing the mutation of interest. Download the reference sequence of the interest gene from NCBI. Introduction to the SnapGene program needed to visualize the nucleotide sequence of the interest gene and primer design.
2. Preparation of the PCR protocol: selection of enzymes, selection of time and temperature parameters of the reaction.
3. Isolation of DNA from cell culture using a commercial genomic DNA isolation kit according to the manufacturer's protocol.
4. Determination of the purity and concentration of the resulting DNA preparation using a spectrophotometer. Acquaintance with the device and the rules of use.
5. Unearthing a reaction PCR mixture containing a template -- genomic DNA, a pair of primers, a DNA polymerase and a corresponding buffer, a mixture of dNTP and deionized water. Programming the amplifier. Load samples into the amplifier.
6. Preparation and pouring of agarose gel. Digging up a MARKer of DNA lengths and samples mixed with a loading buffer into the gel wells. Detection of PCR results using gel electrophoresis and gel documentation system.
7. Purification of the PCR mixture with sodium acetate and ethanol, DNA deposition.
8. Excavation of the reaction mixture for sequencing reaction using a commercial kit according to the manufacturer's protocol. Programming of the amplifier, loading of samples.
9. Purification of the reaction mixture with ethanol and EDTA.
10. Loading samples into the sequencer, programming the sequencer.
11. Analysis of chromatograms obtained after Sanger sequencing.

Laboratory work No. 2 "Determination of cell viability by vital staining with fluorescent dyes" (6 hours)

Equipment required to perform the work:

1. Laminar box of 2 protection class;
2. Medical centrifuge;
3. CO<sub>2</sub> incubator;
4. Flow cytofluorimeter;
5. A set of mechanical single-channel dispensers of different volumes: 0.1-10 µl, 10-100 µl, 100-1000 µl.

Reagents and consumables necessary to perform the work:

1. A mixture of Igla nutrient media modified by Dulbecco and F12 with L-glutamine;
2. Embryonic bovine serum;
3. Solution of natarium pyruvate;
4. Solution of antibiotics penicillin and streptomycin;
5. Phosphate-salt buffer;
6. Trypsin solution;
7. Solution of fluorescent dye calcein;
8. Solution of fluorescent prpidium iodide;
9. Sterile disposable tips with filters for dispensers with a volume of 0.1-10 µl, 10-100 µl, 100-1000 µl;
10. Sterile plastic test tubes with a volume of 15 ml, 50 ml;
11. Culture bottles with growth area from 25 to 75 cm<sup>2</sup>

Algorithm of work performance:

1. Design of the experiment, preparation for work. Preparation of the necessary solutions. Sterilization of solutions, consumables and equipment.
2. Introduction to the cultivation of eukaryotic cells. Familiarization with the device and rules of work with laminar boxing. Preparation of needle nutrient medium modified by Dulbecco and F12 with L-glutamine with the addition of embryonic bovine serum, sodium pyruvate, penicillin antibiotic solution and streptomycin.
3. Passing of the cell line with the replacement of the nutrient medium. Cell dissociation with trypsin solution, centrifuguguage and resuspendiration. Cell counting. Heresieswith a density of  $3 \times 10^4$  / cm<sup>2</sup> in the volume of the culture medium at the rate of 0.2 ml / cm<sup>2</sup>.
4. Vital staining of cells with fluorescent dyes calcein and propidium iodide. Dissociation of cells with trypsin, washing from the components of the nutrient medium with a solution of phosphate-salt buffer. Onstaining the cell suspension with a solution of dyes on a phosphate buffer with a concentration of calcein 1 µg / ml and propidium iodide 2 µg / ml for 40 minutes, followed by replacingthe dye with a buffer.

5. Familiarization with the device, the functionality of the flow cytometer, the rules for working with the device. Instrument setup, gatekeeping, controls. Loading samples into the instrument. Cytometry of stained cells.

6. Analysis of cytometry results. Graphs, scales and analyzed parameters. Statistical calculations.

Independent work (45 hours)

Topics of abstracts:

1. Eukaryotic cell culture: types of cell cultures, culture conditions.
2. Basics of optical microscopy: theory of image formation, optical scheme of the microscope.
3. Light-field and dark-field microscopy, methods of image contrast.
4. Luminescence microscopy: principles of the method, light sources.
5. Confocal microscopy: the use of laser scanning microscopy to visualize fluorescent objects.
6. Phenotypic analysis of cells by methods of immunocytochemistry and epifluorescence microscopy.
7. The concept and principles of high-performance microscopy. Application of high-throughput microscopy in medical, biological, chemical and materials research.
8. Analysis of cell behavior in culture by methods of automated quantitative microscopy.
9. Electron microscopy methods in biomedical research and development of nanotechnological solutions.
10. Comparative characteristics of the types of probe microscopy, opportunities, advantages and disadvantages.
11. Evaluation of the physical properties of biological objects by methods of atomic force microscopy.
12. Basics and principles of flow cytometry, light scattering and fluorescence, cytometer device, sample preparation.
13. Analysis of cell phenotypes, cell cycle, proliferation and apoptosis by flow cytometry methods.
14. Enzyme immunoassay: principle of method, place in biology and medicine.
15. Mass spectrometry: the study of the structure of proteins.
16. Surface plasmonic resonance in immunochemistry.
17. Electrophoresis of macromolecules: physicochemical bases of protein electrophoresis.

18. Principles of protein immunoblot. Methods of detection of target proteins.
19. Physicochemical bases and parameters of chromatographic process.
20. Theoretical aspects of liquid chromatography. Influence of various factors on chromatographic separation of substances.
21. Atomic and molecular spectroscopy in biomedical research.
22. Methods for studying ligand-receptor interaction.
23. Classification of chromatographic methods of protein separation.
24. Nucleic acid manipulation: basic tools and methods.
25. Real-time PCR: features of the method, prospects for use in biomedical research.
26. Use of PCR to determine single nucleotide polymorphisms. Droplet Digital PCR.
27. Massive Parallel Sequencing Technologies: From Roche to Nanopore, What's Next?
28. Transcriptome Research: The Future for Medicine.
29. An overview of NGS data analysis software.
30. Hybridization and probes. Application of methods based on hybridization in biology and medicine.

## **V. EDUCATIONAL AND METHODOLOGICAL SUPPORT OF INDEPENDENT WORK OF STUDENTS**

### Recommendations for independent work of students

The purpose of the independent work of the student is to work meaningfully and independently first with educational material, then with scientific information, to lay the foundations of self-organization and self-education in order to instill the ability to further continuously improve their professional qualifications.

The process of organizing the independent work of the student includes the following stages:

- preparatory (setting goals, drawing up a program, preparing methodological support, preparing equipment);
- basic (implementation of the program, the use of methods of information retrieval, assimilation, processing, application, transfer of knowledge, fixation of results, self-organization of the work process);
- final (assessment of the significance and analysis of the results, their systematization, assessment of the effectiveness of the program and methods of work, conclusions on the directions of labor optimization).

In the process of independent work, the student acquires the skills of self-organization, self-control, self-government, self-reflection and becomes an active independent subject of educational activity. Independent work of students should have an important impact on the formation of the personality of the future specialist, it is planned by the student independently. Each student independently determines the mode of his work and the measure of work spent on mastering the educational content in each discipline. He performs extracurricular work according to a personal individual plan, depending on his preparation, time and other conditions.

#### Methodical recommendations for independent work of students

As the material on the subject of the discipline is mastered, it is planned to perform independent work of students on the collection and processing of literary material to expand the field of knowledge in the discipline under study, which allows you to deepen and consolidate specific practical knowledge gained in classroom classes. To study and fully master the program material on the discipline, educational, reference and other literature recommended by this program, as well as specialized periodicals, are used.

When independently preparing for classes, students take notes on the material, independently study the issues on the topics covered, using the educational literature from the proposed list, periodicals, scientific and methodological information, databases of information networks.

Independent work consists of such types of work as the study of material on textbooks, reference books, videos and presentations, as well as other reliable sources of information; preparation for the zechet. To consolidate the material, it is enough, flipping through the notes or reading it, mentally restore the material. If necessary, refer to the recommended educational and reference literature, write down incomprehensible moments in the questions to understand them in the upcoming lesson.

Preparation for practical exercises. This type of independent work consists of several stages:

1) Repetition of the studied material. For this purpose, lecture notes, recommended basic and additional literature are used;

2) Deepening knowledge on the proposed topics. It is necessary to differentiate the available material in lectures, textbooks in accordance with the points of the plan of the practical lesson. Separately write out unclear questions, terms. It is better to do this in the margins of the lecture notes or textbook. Clarification should be carried out with the help of reference literature (dictionaries, encyclopedic publications, etc.);

3) Drawing up a detailed plan for the speech, or conducting calculations, solving problems, exercises, etc. In preparation for practical exercises, students take notes on the material, prepare answers to the above questions on the topics of practical exercises. In addition to the practical material, students independently study questions on the proposed topics, using educational literature from the proposed list, periodicals, scientific and methodological information, databases of information networks (Internet, etc.).

Requirements for the presentation and design of the results of independent work

There are no special requirements for the provision and design of the results of this independent work.

Control over the implementation of the plan of independent work of students is carried out by the teacher in practical classes by interviewing and by including in the final tasks specified in the lesson from the plan of independent work.

## VI. MONITORING THE ACHIEVEMENT OF COURSE OBJECTIVES

No p/n	Supervised sections / topics of the discipline	Achievement indicator code and name	Learning outcomes	Assessment tools	
				current control	Intermediate-accurate certification
1.	Section 1. Purpose, objectives, methods and theoretical foundations of molecular and cellular diagnostics. Topic 1. Introduction. The purpose and objectives of methods of molecular and cellular diagnostics.	PC-2.1	Knows: Fundamental concepts of cell and molecular biology; methodology of setting up basic laboratory studies; approaches to the analysis of the information obtained. Can: Determine the goals and objectives of the experiment, choose the object and methods of research; develop and optimize the conditions for setting up an experiment, perform laboratory biological research; draw logical conclusions based on the results of the experiment. Owns: Skills, rules and algorithms for designing, performing laboratory research.	Oral questioning	Exam Questions

2.	Section 1. Purpose, objectives, methods and theoretical foundations of molecular and cellular diagnostics. Topic 2. Theoretical foundations of molecular diagnostics.	PC-2.3	<p>Knows: Basic principles and stages of modern methods of analysis of the structure and properties of biological objects; with the possession of the main regulatory documents that ensure the conduct of research and production-technological biological work.</p> <p>He can: Translate in practice the knowledge of the basics of the organization and planning of research and production work using regulatory documents; analyze the data and operate with the information obtained; collect the necessary theoretical and practical material for the implementation of research work.</p> <p>Owens: For the organization and conduct of research and production-technological biological works; methods of self-analysis of available biological information; Work with library catalogues.</p>	Oral questioning	Exam Questions
3.	Section 1. Purpose, objectives, methods and theoretical foundations of molecular and cellular diagnostics. Topic 3. Theoretical foundations of cell diagnostics	PC-2.3	<p>Knows: Basic principles and stages of modern methods of analysis of the structure and properties of biological objects; with the possession of the main regulatory documents that ensure the conduct of research and production-technological biological work.</p> <p>He can: Translate in practice the knowledge of the basics of the organization and planning of research and production work using regulatory documents; analyze the data and operate with the information obtained; collect the necessary theoretical and practical material for the implementation of research work.</p> <p>Owens: For the organization and conduct of research and</p>	Oral questioning	Exam Questions

			production-technological biological works; methods of self-analysis of available biological information; Workwith library catalogues.		
4.	Section 2. Methods of molecular and cellular diagnostics and their application. Topic 1. Cytogenetic research methods: karyotyping, hybridization in situ.	PC-2.2	Knows: Fundamentals of molecular and cellular biology, biochemistry and biotechnology, fundamental principles of organization and functioning of living systems, methods and approaches to the study of the structure and properties of biological objects, organic and inorganic compounds. Can: Identify the problem in the analysis of scientific literature and / or the results of their own experimental work, set original goals and objectives of the scientific experiment, choose the appropriate object and method of research; perform laboratory biological research using modern equipment and computing facilities; analyze the biological data obtained during the experiment, draw logical conclusions and conclusions. Owns: Skills in performing laboratory research using the scientific methodological foundations of fundamental research.	Oral questioning	Exam Questions
5.	Section 2. Methods of molecular and cellular diagnostics and their application. Topic 2: Methods based on the use of amplification Polymerase chain reaction. Ligase chain reaction.	PC-2.2	Knows: Fundamentals of molecular and cellular biology, biochemistry and biotechnology, fundamental principles of organization and functioning of living systems, methods and approaches to the study of the structure and properties of biological objects, organic and inorganic compounds. Can: Identify the problem in the analysis of scientific literature and / or the results of their own experimental work, set	Oral questioning	Exam Questions



			<p>original goals and objectives of the scientific experiment, choose the appropriate object and method of research; perform laboratory biological research using modern equipment and computing facilities; analyze the biological data obtained during the experiment, draw logical conclusions and conclusions.</p> <p>Owns: Skills in performing laboratory research using the scientific methodological foundations of fundamental research.</p>		
6.	<p>Section 2. Methods of molecular and cellular diagnostics and their application. Topic 3: Determination of the primary structure of nucleic acids, search for mutations.</p>	PC-2.2	<p>Knows: Fundamentals of molecular and cellular biology, biochemistry and biotechnology, fundamental principles of organization and functioning of living systems, methods and approaches to the study of the structure and properties of biological objects, organic and inorganic compounds.</p> <p>Can: Identify the problem in the analysis of scientific literature and / or the results of their own experimental work, set original goals and objectives of the scientific experiment, choose the appropriate object and method of research; perform laboratory biological research using modern equipment and computing facilities; analyze the biological data obtained during the experiment, draw logical conclusions and conclusions.</p> <p>Owns: Skills in performing laboratory research using the scientific methodological foundations of fundamental research.</p>	Oral questioning	Exam Questions
7.	<p>Section 2. Methods of molecular and cellular diagnostics and their application. Topic 4. Electrophoretic</p>	PC-2.2	<p>Knows: Fundamentals of molecular and cellular biology, biochemistry and biotechnology, fundamental principles of organization and</p>	Oral questioning	Exam Questions

	<p>separation of proteins. Western blotting. Chromatographic methods of protein purification.</p>		<p>functioning of living systems, methods and approaches to the study of the structure and properties of biological objects, organic and inorganic compounds.</p> <p>Can: Identify the problem in the analysis of scientific literature and / or the results of their own experimental work, set original goals and objectives of the scientific experiment, choose the appropriate object and method of research; perform laboratory biological research using modern equipment and computing facilities; analyze the biological data obtained during the experiment, draw logical conclusions and conclusions.</p> <p>Owns: Skills in performing laboratory research using the scientific methodological foundations of fundamental research.</p>		
8.	<p>Section 2. Methods of molecular and cellular diagnostics and their application. Topic 5. Optical microscopy: histological and immunohistochemical studies in diagnostics. Probe and electron microscopy.</p>	PC-2.2	<p>Knows: Fundamentals of molecular and cellular biology, biochemistry and biotechnology, fundamental principles of organization and functioning of living systems, methods and approaches to the study of the structure and properties of biological objects, organic and inorganic compounds.</p> <p>Can: Identify the problem in the analysis of scientific literature and / or the results of their own experimental work, set original goals and objectives of the scientific experiment, choose the appropriate object and method of research; perform laboratory biological research using modern equipment and computing facilities; analyze the biological data obtained during the experiment, draw logical conclusions and conclusions.</p>	Oral questioning	Exam Questions

			Owns: Skills in performing laboratory research using the scientific methodological foundations of fundamental research.		
9.	Section 2. Methods of molecular and cellular diagnostics and their application. Topic 6. Flow cytometry: possibilities, objects.	PC-2.2	Knows: Fundamentals of molecular and cellular biology, biochemistry and biotechnology, fundamental principles of organization and functioning of living systems, methods and approaches to the study of the structure and properties of biological objects, organic and inorganic compounds. Can: Identify the problem in the analysis of scientific literature and / or the results of their own experimental work, set original goals and objectives of the scientific experiment, choose the appropriate object and method of research; perform laboratory biological research using modern equipment and computing facilities; analyze the biological data obtained during the experiment, draw logical conclusions and conclusions. Owns: Skills in performing laboratory research using the scientific methodological foundations of fundamental research.	Oral questioning	Exam Questions

## VII. LIST OF REFERENCES AND INFORMATION AND METHODOLOGICAL SUPPORT OF THE DISCIPLINE

### Main literature

1. Histology, cytology and embryology : textbook / T.M. Studenikina, T.A. Vylegzhanina, T.I. Ostrovskaya, I.A. Stelmakh ; ed. by T.M. Studenikina. — Moscow : INFRA-M, 2023. — 574 p. — (Higher education: Bachelor's degree). - ISBN 978-5-16-006767-4. - Text : electronic. - URL: <https://znanium.com/catalog/product/1916106>
2. Lenchenko, E. M. Cytology, histology and embryology : a textbook for secondary vocational education / E. M. Lenchenko. — 2nd ed., ispr. and add.

— Moscow : Izdatelstvo Yurayt, 2023. — 347 p. — (Vocational education). — ISBN 978-5-534-08617-1. — Text : electronic // Educational platform Yurait [site]. — URL: <https://urait.ru/bcode/514046>

3. Polyakova, T. I. *Biologiya celli : uchebnoe posobie* / T. I. Polyakova, I. B. Sukhov. — St. Petersburg : Sankt-Peterburgskii medico-sotsial'nyi institut, 2015. — 56 p. — Text : electronic // Digital educational resource IPR SMART : [site]. — URL: <https://www.iprbookshop.ru/74246.html>

4. Ivanishchev, V. V. *Molecular biology : textbook* / V.V. Ivanishchev. — Moscow : RIOR : INFRA-M, 2019. — (Higher education). — 225 p. — DOI: <https://doi.org/10.12737/1731-9>. - ISBN 978-5-369-01731-9. - Text : electronic. - URL: <https://znanium.com/catalog/product/1019421>

5. Temnov, M. S. *Introduction to Molecular Biology*. In 2 parts. Ch.1 : *uchebnoe posobie* / M. S. Temnov, D. S. Dvoretzky. — Tambov : Tambov State Technical University, EBS ASV, 2021. — 81 c. — ISBN 978-5-8265-2390-2. — Text : electronic // Digital educational resource IPR SMART : [site]. — URL: <https://www.iprbookshop.ru/123024.html>

6. Konichev, A. S. *Molecular biology : textbook for universities* / A. S. Konichev, G. A. Sevastyanova, I. L. Tsvetkov. — 5th ed. — Moscow : Izdatelstvo Yurait, 2023. — 422 p. — (Higher education). — ISBN 978-5-534-13468-1. — Text : electronic // Educational platform Yurait [site]. — URL: <https://urait.ru/bcode/517095>

7. Ershov, Y. A. *Biochemistry : textbook and practicum for secondary vocational education* / Y. A. Ershov, N. I. Zaitseva ; edited by S. I. Shchukin. — 2nd ed., ispr. and add. — Moscow : Izdatelstvo Yurayt, 2023. — 323 p. — (Vocational education). — ISBN 978-5-534-10400-4. — Text : electronic // Educational platform Yurait [site]. — URL: <https://urait.ru/bcode/517755>

#### Further reading

1. Barysheva E.S. *Biochemistry [Elektronnyi resurs]: uchebnoe posobie* / Barysheva E.S.— Electron. text data.— Orenburg: Orenburg State University, EBS ASV, 2017.— 142 p. <http://lib.dvfu.ru:8080/lib/item?id=IPRbooks:IPRbooks-78767&theme=FEFU>

2. *Biology of stem cells and cell technologies: for medical universities in 2 vol.: v. 1* / M. A. Paltsev, R. S. Akchurin, M. A. Alexandrova [et al.] ; ed. by M. A. Paltsev. — Moscow: Medicine, Shiko, 2009. — 272 p. <http://lib.dvfu.ru:8080/lib/item?id=chamo:779352&theme=FEFU>

3. *Biology of stem cells and cell technologies: for medical universities in 2 vols.: v. 2* / M. A. Paltsev, R. S. Akchurin, M. A. Alexandrova [et al.] ; ed. by M.

A. Paltsev. – Moscow: Medicine, Shiko, 2009. – 455 p.  
<http://lib.dvfu.ru:8080/lib/item?id=chamo:779355&theme=FEFU>

4. Antitumor potential of hematopoietic stem cells on the model of experimental glioblastoma: abstract of the dissertation for the scientific degree of candidate of biological sciences: 03.03.04 / P. V. Mishchenko. – Vladivostok, 2015. – 23 p. <http://lib.dvfu.ru:8080/lib/item?id=chamo:799674&theme=FEFU>

5. Revischin, A.V. Cell therapy for neurodegenerative diseases [Electronic resource]: monograph / A.V. Revischin — Electron. text data. — M.: Moskovskii pedagogicheskii gosudarstvennyi universiteta, 2017. — 160 s. — Mode of access: <http://www.iprbookshop.ru/75971.html>. — EBS «IPRbooks»

6. Tarantul, V.Z. Genno-cell biotechnology of the XXI century and man / V.Z. Tarantul // Russia and the modern world. – № 1 – 2009. – S. 188-203. <http://lib.dvfu.ru:8080/lib/item?id=chamo:641555&theme=FEFU>

7. Romanovsky, G.B. Biomedical law in Russia and abroad / G.B. Romanovsky, N.N. Tarusina, A.A. Mokhov [et al.]. – Moscow: Prospekt, 2016. – 364 p. <http://lib.dvfu.ru:8080/lib/item?id=chamo:813279&theme=FEFU>.

List of resources of the information and telecommunication network  
"Internet"

1. Ministry of Health of the Russian Federation – official website: <https://www.rosminzdrav.ru/>

2. Central Research Institute of Organization and Informatization of Healthcare – official website: <http://mednet.ru/>

3. Research Institute of Biomedical Chemistry named after V.N. Orekhovich – official site: <http://www.ibmc.msk.ru/>

## VIII. METHODOLOGICAL INSTRUCTIONS FOR MASTERING THE DISCIPLINE

**The lecture** is the main active form of classroom classes, explanations of the fundamental theoretical sections, which involves intensive mental activity of the student. The lecture is cognitive, developmental, educational and organizing in nature. The lecture notes help to assimilate the theoretical material of the discipline. When listening to the lecture, it is necessary to note its rubrication, terminology, keywords, definitions, formulas, graphic schemes.

When working at home with lecture notes, it is necessary to use the main textbook and additional literature that are recommended for this discipline.

When presenting a lecture course, the following are used as forms of interactive learning: lecture-conversation, lecture-visualization, which are built on

the basis of previous knowledge, including related disciplines. Presentations, an interactive whiteboard, tables, and diagrams are used to illustrate. In the course of the presentation of the lecture material, problematic and provoking questions are raised, elements of discussion are included.

**Lecture-visualization.** The lecture is accompanied by a computer presentation with basic texts (headings, formulations, keywords and terms), illustrations of microscopic and ultramicroscopic images of cells, drawing diagrams and writing formulas on an interactive whiteboard, visual tables and slides are demonstrated, which contributes to a better perception of the material presented.

**Lecture-conversation** - "dialogue with the audience" - is a common form of interactive learning and allows you to involve students in the educational process, as it creates direct contact of the teacher with the audience. Students are asked questions of a problematic, provoking or informational nature. Students themselves can also ask questions. Any of the students can offer his answer, another can supplement it. This form of lecture allows you to involve all students in the work, activate their attention, thinking, gain collective experience, learn to formulate questions.

**Seminar-colloquium.** Colloquium is a collective form of consideration and consolidation of educational material. Colloquiums are one of the types of practical classes designed for in-depth study of the discipline, are held in an interactive mode. In classes on the topic of the colloquium, issues are analyzed, together with the teacher, their discussion is held, which is aimed at consolidating the material, forming the skills to conduct polemics, developing independence and critical thinking, the ability of students to discuss them. navigate in large information flows, develop and defend their own position on problematic issues of the academic discipline.

As methods of interactive learning at colloquia, the following are used: a detailed conversation, discussion, press conference.

**A detailed conversation** involves the preparation of students for each issue of the lesson plan with a single list of recommended mandatory and additional literature for all. Reports are prepared by students on a pre-proposed topic.

**Discussion** in a group has a number of advantages. Discussion can be caused by the teacher during the lesson or planned in advance by him.

**Control tests.** Blank or computer testing is used in the mode of selecting the correct answers, establishing the correspondence of concepts, marking details on diagrams, etc.

## Methodical instructions for working with literature

An initial list of sources should be compiled. The basis may be the list of references recommended in the work program of the course. For the convenience of work, you can make your own file cabinet of selected sources (surname of the authors, title, characteristics of the publication) in the form of a working file in the computer. Such a file cabinet has an advantage, because it allows you to add sources, replace one with another if necessary, the Initial list of references can be supplemented using the electronic catalog of the FEFU library.

Working with literature on a particular topic, it is necessary not only to read, but also to learn the method of its study: make a brief summary, an algorithm, a scheme of the material read, which allows you to quickly understand it, remember it. It is not recommended to rewrite the text verbatim.

## IX. MATERIAL AND TECHNICAL SUPPORT OF DISCIPLINE

Training sessions on the discipline are held in rooms equipped with appropriate equipment and software.

The list of material and technical and software of the discipline is given in the table.

### Logistics and Software Discipline

Name of special premises and premises for independent work	Equipment special premises and rooms for independent work	List of licensed software. Details of the supporting document
Laboratory auditorium equipped with a multimedia complex Vladivostok, Russky Island, Ajax village, 10, aud. M420, area 74,6 m <sup>2</sup>	Screen with electric drive 236 * 147 cm Trim Screen Line; Projector DLP, 3000 ANSI Lm, WXGA 1280x800, 2000:1 EW330U Mitsubishi; Subsystem of specialized fasteners of equipment CORSA-2007 Tuarex; Video switching subsystem: DVI DXP 44 DVI Pro Extron matrix switch; DVI twisted pair extender DVI 201 Tx/Rx Extron; Subsystem of audio switching and sound amplification; acoustic system for ceiling mounting SI 3CT LP Extron; digital audio processor DMP 44 LC Extron; extension for IPL T CR48 control controller Aqua distiller PE-2205 (5l/h); Analytical scales Acculab ATL-2200d2-I; Laboratory scale Vibra SJ-6200CE (LSE=6200 g/0,1 g); Moisture meter AGS100; Dual-beam spectrophotometer UV-1800 manufactured by Shimadzu; Rotary evaporator Hei-VAP Advantage ML/G3B; Magnetic stirrer PE-6100 (10 pcs); Magnetic	-

	stirrer PE-6110 M with heating (5pcs); Electric heating tiles; Infrared spectrophotometer IRAffinity-1S with Fourier; Form for the formation of suppositories for 100 cells; Pharmaceutical refrigerator; Liquid chromatograph LC-20 Prominence with spectrophotometric and refractometric detector; Laboratory centrifuge PE-6926 with a rotor of 10×5 ml, a set of automatic dosers Ecochem, a set of porcelain mortars, manual machines for packing capsules in size "0", "00", "1".	
Reading rooms of the FEFU Scientific Library with open access to the fund (building A – level 10)	HP All-in-One 400 All-in-One 19,5 (1600x900), Core i3-4150T, 4GB DDR3-1600 (1x4GB), 1TB HDD 7200 SATA, DVD+/-RW,GigEth,Wi-Fi,WT,usb kbd/mse,Win7Pro (64-bit)+Win8.1Pro(64-bit),1-1-1 Wty Internet access speed 500 Mbps. Workplaces for people with disabilities are equipped with Braille displays and printers; equipped with: portable devices for reading flat-printed texts, scanning and reading machines video magnifier with the ability to regulate color spectra; magnifying electronic magnifiers and ultrasonic markers	-
Laboratory auditorium Vladivostok, Russky Island, Ajax village, 10, aud. L406, area 30 m <sup>2</sup>	Aqua distiller PE-2205 (5l/h); mixer; Laboratory scale AGN100; Magnetic stirrer PE-6100 (5 pcs); Magnetic stirrer PE-6110 M with heating (2 pcs); Electric heating tiles; a set of laboratory utensils, a set of porcelain mortars with pistils.	-

## **X. VALUATION FUNDS**

For discipline, oral information is used as an evaluative means.

Oral questioning allows you to assess the knowledge and logic of the student, the ability to use terminology, speech skills and other communication skills.

The training function is to identify details that for some reason were not sufficiently understood during the training sessions and in preparation for the test.

A survey is a means of control, organized as a special conversation of the teacher with the student on topics related to the discipline being studied, and designed to clarify the amount of knowledge of the student on a certain section, topic, problem, etc.

### **Examples of topics for oral inquiry**

1. Basics of cultivation of microorganisms: principles and growth needs. Nutrient media, their types, preparation, additives. Principles of manipulation with



microorganizes, reseeded, obtaining a clean culture.

2. Ensuring biological safety when working with micro-organizations, regulatory and legal documentation. Selective and marker media. Sensitivity and resistance to antibiotics. Phenotypic characteristics of cells and clones.

3. Cell cultures of eukaryotes. Types of cell cultures. Assessment of the state of cell cells in culture. Phases of cell growth. Confluence.

4. Eukaryotic cell culture conditions: laboratory organization principles, requirements and instruments for compliance with aseptic conditions, growth needs of cells, nutrient media, serums, growth additives, serum culture, serum-free cultivation and growth factors.

5. Methods of cytogenetic diagnosis. Karyotyping: sample preparation, metaphase plate staining, chromosomal banding, microscopy, kariogram counting and analysis of results.

6. In situ hybridization technique. Overview of methods and approaches, varieties of FISH. Probes for in situ hybridization: preparation, direct and indirect tagging of probes, types of fluorochromes commercial solutions. Features of the enzyme label. Types of substrates for enzyme labels. Theoretical basis of using different types of probes for FISH. Hybridization in situ of the labeled DNA probe.

7. Principles of PCR diagnostics. Basics of PCR: mechanism, stages. Devices, reagents and consumables for PCR. Development of the PCR protocol, primer design and necessary software. Biological material. Types and technologies of PCR: classical, allele-specific PCR, real-time PCR. Detection of amplification results.

8. Identification of mutations. DNA sequencing. Sanger DNA Sequencing: Principle of Method, Steps, Components of Reaction Mixtures. DNA sample preparation and PCR purification of the sequencing mixture. Accounting for PCR results using Sanger sequencing. Analysis of chromatograms.

9. Mass parallel sequencing: platforms, equipment, application in diagnostics. Platforms for NGS, advantages and disadvantages of various techniques. NGS Data Analysis: NgS Processing and Data Interpretation Steps. Search for mutations and analysis of gene expression. Genomics. Transcriptomics.

10. The principle of the method of electrophoresis of proteins. Electrophoretic mobility. Classification of electrophoretic methods.

11. Features of electrophoresis in polyacrylamide gel. Native and SDS-PAGE electrophoresis. Disc electrophoresis. Basics of protein immunoblotting. Methods of immunodetection. Qualitative and quantitative processing of electrophoregrams.

12. Chromatographic methods of analysis. Fixed (stationary) and mobile phases. Sorbates. "Dead Time" chromatographic column. Eleuent chromatogram.

Retention time of the substance. Evaluation of the effectiveness of the chromatographic column (the concepts of retention factor, selectivity coefficient and resolution). Number of theoretical plates  $N$ . Van Deemter equation. Chromatographic systems and detectors. Types of protein chromatography. Absolute calibration method. Method of internal normalization. Internal standard method.

13. Fundamentals of optical microscopy: history, principles, possibilities, resolution of the method and its limitations, specialized variants of light microscopy (phase-contrast, interference, polarization, fluorescent / luminescent). Principles of the design of optical microscopes, the main components of optical microscopes: illuminators and radiation sources (lamps, LEDs, lasers), condensers and their purpose, objectives, their magnification and numerical aperture.

14. Fluorescent microscopy. Laser scanning microscopy: confocal principle and multiphoton (two-photon) principle. Microscopy of the second harmonic. Microscopy of total internal reflection.

15. Preparation of samples for research using optical microscopy. Types of micropreparations: total drugs, slice preparations, smear preparations. Types of microtome devices: rotary and sled microtomes, cryotomes, vibratomes. Principles of preparation of histological preparations-sections. Staining of drugs with routine dyes, low molecular weight fluorochromes, direct and indirect immunocoloration (immunohistochemistry, immunocytochemistry). Application of fluorescent proteins and genetic constructs with fluorescent reporters in biological research.

16. Flow cytometry and cell sorting: principles, features of the device design, possibilities, application in research practice and medical laboratory diagnostics.

17. Electron microscopy. Scanning (scanning) microscopy. Transmission (transmission) microscopy.

18. Probe microscopy, atomic force microscopy in biomedical research: the principle of the method, its capabilities and prospects.

**Methodological recommendations that determine the procedures for assessing the results of mastering the discipline**

### **Assessment tools for intermediate attestation**

Intermediate certification of students in the discipline is carried out in accordance with local university regulations and is mandatory. The form of reporting on the discipline is an exam.

### **Methodical instructions for passing the exam**

The exam is taken by the leading teacher (associate professor, professor), for whom this type of educational load is assigned in an individual plan. The form of the exam is oral.

The time allowed to the student to prepare for the answer to the exam should be no more than 40 minutes. After this time, the student should be ready to respond.

The presence at the examination of unauthorized persons (except for persons carrying out the inspection) without the permission of the relevant persons (rector or vice-rector for academic affairs, director of the School, head of the OBOR or director of the department) is not allowed. Disabled persons and persons with disabilities who do not have the opportunity to move independently are allowed to take the exam with accompanying persons.

With an intermediate assessment, students are given a grade of "excellent", "good", "satisfactory" or "unsatisfactory". If the student does not appear for the exam, an entry "did not appear" is made in the statement.

### **Exam Questions**

1. Cultivation of microorganisms: principles and growth needs. Liquid and solid (gel) nutrient media. Principles of manipulation with microorganisms, ensuring biological safety.

2. Cultivation of microorganisms: selective and marker media. Sensitivity and resistance to antibiotics. Phenotypic characteristics of cells and clones.

3. Cell cultures of humans and animals. Primary, secondary cultures and cell lines. Genotyping and morphotyping of cells in culture.

4. Ensuring the conditions for the cultivation of eukaryotic cells: the principles of laboratory organization, requirements and devices for compliance with aseptic conditions, the growth needs of cells, nutrient media, serums, growth additives, serum-free cultivation and growth factors.

5. Optical microscopy: history, principles, possibilities, resolution of the method and its limitations, specialized variants of light microscopy (phase-contrast, interference, polarization, fluorescent / luminescent).

6. Principles of the design of optical microscopes, the main components of optical microscopes: illuminators and radiation sources (lamps, LEDs, lasers), condensers and their purpose, objectives, their magnification and numerical aperture.

7. Skills of working on optical microscopes: the use of a micro- and macro-screw, a stage table and a preparer, setting up the light according to Köller, working with diaphragms, features of working at various magnifications, immersion media, refractive index.

8. Flow cytometry and cell sorting: principles, features of the device design, possibilities, application in research practice and medical laboratory diagnostics.

9. Fluorescent microscopy. Laser scanning microscopy: confocal principle and multiphoton (two-photon) principle. Microscopy of the second harmonic. Microscopy of total internal reflection.

10. Ultra-high resolution fluorescence microscopy. Three-dimensional reconstruction of microobjects. Automated quantitative microscopy (high-content imaging).

11. Electron microscopy. Scanning (scanning) microscopy. Transmission (transmission) microscopy.

12. Probe microscopy, atomic force microscopy in biomedical research: the principle of the method, its capabilities and prospects.

13. Preparation of biological samples for research using various variants of optical microscopy. Types of micropreparations: total drugs, slice preparations, smear preparations. Types of microtome devices: rotary and sled microtomes, cryotoms, vibratomes.

14. Principles of preparation of histological preparations-sections. Staining of drugs with routine dyes, low molecular weight fluorochromes, direct and indirect immunocoloration (immunohistochemistry, immunocytochemistry). Application of fluorescent proteins and genetic constructs with fluorescent reporters in biological research.

15. Cell fractionation by centrifugation methods, centrifugation in medium density gradients. Principles of centrifugation and arrangement of centrifuges, low-speed, high-speed centrifuges, ultracentrifuges (preparative and analytical).

16. The principle of the method of electrophoresis of proteins. Electrophoretic mobility. Classification of electrophoretic methods.

17. Features of electrophoresis in polyacrylamide gel. Native and SDS-PAGE electrophoresis. Disc electrophoresis. Basics of protein immunoblotting. Methods of immunodetection. Qualitative and quantitative processing of electrophoregrams.

18. Chromatographic methods of analysis. Fixed (stationary) and mobile phases. Sorbates. "Dead Time" chromatographic column. Eluent chromatogram. Retention time of the substance. Evaluation of the effectiveness of the

chromatographic column (the concepts of retention factor, selectivity coefficient and resolution). Number of theoretical plates  $N$ . Van Deemmer equation. Chromatographic systems and detectors.

19. Types of protein chromatography. Absolute calibration method. Method of internal normalization. Internal standard method.

20. Methods of cytogenetic diagnosis. Karyotyping: sample preparation, metaphase plate staining, chromosomal banding, microscopy, kariogram counting and analysis of results.

21. Methods of cytogenetic diagnosis. In situ hybridization technique. Overview of methods and approaches, varieties of FISH. Probes for in situ hybridization: preparation, direct and indirect tagging of probes, types of fluorochromes commercial solutions. Features of the enzyme label. Types of substrates for enzyme labels. Theoretical basis of using different types of probes for FISH. Hybridization in situ of the labeled DNA probe.

22. Principles of PCR diagnostics. Basics of PCR: mechanism, stages. Devices, reagents and consumables for PCR. Development of the PCR protocol, primer design and necessary software. Biological material. Types and technologies of PCR: classical, allele-specific PCR, real-time PCR. Detection of amplification results.

23. Identification of mutations. DNA sequencing. Sanger DNA Sequencing: Principle of Method, Steps, Components of Reaction Mixtures. DNA sample preparation and PCR purification of the sequencing mixture. Accounting for PCR results using Sanger sequencing. Analysis of chromatograms.

24. Mass parallel sequencing: platforms, equipment, application in diagnostics. Platforms for NGS, advantages and disadvantages of various techniques. NGS Data Analysis: NgS Processing and Data Interpretation Steps. Search for mutations and analysis of gene expression. Genomics. Transcriptomics.

### Criteria for grading a student on the exam

Evaluation of the test	Requirements for the formed competencies
"Excellent"	The "excellent" grade is given to the student if he has deeply and firmly mastered the program material, exhaustively, consistently, clearly and logically coherently presents it, is able to closely link the theory with practice, freely copes with tasks, questions and other types of application of knowledge, and does not find it difficult to answer when modifying tasks, uses the material of monographic literature in the answer, correctly justifies the decision made, has versatile skills and techniques implementation of practical tasks on the methodology of scientific research.
"Good"	The "good" grade is given to the student if he firmly knows the material, correctly and substantively presents it, avoiding significant inaccuracies in the answer to the question, correctly applies theoretical provisions when solving practical questions and problems, possesses the necessary skills and techniques for their implementation.

"satisfactory"	The grade "satisfactory" is given to the student if he has knowledge only of the basic material, but has not mastered its details, admits inaccuracies, insufficiently correct wording, violations of the logical sequence in the presentation of the program material, has difficulties in performing practical work.
"unsatisfactory"	The grade "unsatisfactory" is given to a student who does not know a significant part of the program material, makes significant mistakes, uncertainly, with great difficulties performs practical work. As a rule, the grade "unsatisfactory" is given to students who cannot continue their studies without additional classes in the relevant discipline.