**Course Program of "Modern morphological research methods in neurobiology: histology, immunohistochemistry, electron microscopy"**

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1. **The name of the educational program in which the discipline is read.**

The discipline "Modern morphological research methods in neurobiology: histology, immunohistochemistry, electron microscopy" is delivered in the framework of the Master's program "Human ecology with fundamentals of biomedicine" in the direction of training 06.04.01 "Biology", master’s degree level.

1. **The overall complexity.**

The total complexity of the discipline is 5 credit units, 180 hours. The program of the discipline provides:

The fall semester of the 1st course: lectures-18 hrs., laborotary work-54 hrs., student individual work-81 hrs, 27 hours preparation for the exam.

1. **The place of discipline in the structure of the educational program.**

The discipline "Modern morphological research methods in neurobiology: histology, immunohistochemistry, electron microscopy " concerns to the variable part of the block of disciplines.

In order to learn the discipline the following knowledge and skills are necessary: mathematics, physics, structure of substances, computational methods in chemistry, physical chemistry

1. **The purpose of studying the discipline.**

The aim of the discipline "Modern morphological research methods in neurobiology: histology, immunohistochemistry, electron microscopy" is to study the methods of studying the nervous tissue or their combination including:

1) histology (in different colors) - qualitative and quantitative;

2) electron microscopy - qualitative and quantitative, analysis of serial sections with subsequent three-dimensional reconstruction;

3) immunohistochemical study (immunomorphology and electronic immunohistochemistry).

1. **Requirements for the results of mastering the discipline**

In accordance with the federal state educational standard of higher education in the direction of training 06.04.01 Biology (master’s degree level) discipline is aimed at the formation of the following competencies:

readiness to use fundamental biological ideas in the field of professional activity for setting and solving new problems

the ability to independently analyze available information, identify fundamental problems, set a task and carry out field and laboratory biological research in solving specific problems using modern equipment and computing tools, be responsible for the quality of work and scientific accuracy of the results

the ability to apply the methodological foundations of design, field and laboratory biological, environmental research, use of modern equipment and computing systems (in accordance with the direction (profile) of the Master’s program)

the ability to generate new ideas and methodical solutions

**As a result of mastering the discipline, the student must:**

**Know:**

Modern neuromorphological methods (immunohistochemistry), the basics of morphological analysis at the level of antigens specific for nerve tissue - the receptor apparatus of cells

**be able to:**

Reveal qualitative and quantitative alterations (changes) in individual neurons and glial cells, their populations, in cell processes, intercellular contacts (synapses) that have arisen as a result of the conducted experiments, that is, to establish the morphological basis of the observed biochemical, electrophysiological and other shifts.

**Have skills:**

To study the ultrastructure of cells, to evaluate the intracellular changes in the organelles of nerve and glial cells, the synaptic apparatus, on the serial sections, the structure of the cell organelles and bonds between cells in the volume is studied by constructing three-dimensional models;

**The content of the discipline "The physical chemical fundamentals of natural process’s unity" is built on a modular principle, with two main modules.**

1. **Immunohistochemistry** is a method of detecting and determining the exact location of a particular cellular or tissue component (antigen) in tissues in situ by means of immunological and histochemical reactions, which is based on the antigen-antibody reaction. The molecules of cellular structures (surface glycoproteins, structural proteins of cells, oncoproteins, viral proteins, chimeric proteins resulting from cytogenetic breakages, etc.) or the intercellular substance of the tissue act as an antigen. Antibodies are obtained from the blood serum of animals immunized with the antigen of interest, or from hybridoma tissue culture. The uniqueness of "hybridoid" technology is that all cells in tissue culture are descendants of a single cell and therefore synthesize absolutely identical antibody molecules. These antibodies are called monoclonal. As a result of immunization of animals from the serum, polyclonal antibodies are obtained, the polypeptide chains of which differ from each other. The task of histochemical reactions is to make the product of antigen-antibody binding visible to the eye, for which labels of different types are used. Fluorescent dyes, which were widely used in the first stages of the development of immunohistochemistry, are now used primarily in the analysis of cytological material, especially with flow cytotoxicometry, as well as in the study of autoimmune and immunocomplex diseases. Currently, when working with histological sections of tissues, which have been fixed in formalin and paraffin fill, enzyme labels are used. The chemical conversion of the chromogen caused by the enzyme at the final stage of the immunohistochemical analysis leads to the deposition at the sites of formation of the immune complex of the colored product. The most common label is horseradish peroxidase and 3,3'-diaminobenzidine tetrachloride (DAB) as the substrate chromogen. The product of the polymer nature of brown color formed during the reaction is insoluble in organic solvents, which makes it possible to enclose the stained sections in optically transparent media (Canadian balsam, synthetic polymers) and to obtain permanent preparations suitable for long-term use.
2. **The electronic microscopy.** The electron microscope is a device that allows you to obtain an image of objects with magnification from x 10 000 to 100 000 thanks to the use, unlike an optical microscope, instead of a light beam, an electron beam. There are scanning and transmission electron microscopes. Their difference lies in the direction of the beam of electrons. For example, in a scanning microscope, which is designed to examine the surface of an object, the electrons bounce off the object, and get on a special electron detector, then it is possible to obtain a relief map of the analyzed zone.The transmission microscope consists of a column in which a vacuum is created. The column consist the cathode - electron radiation source. The resulting electron beam is accelerated, then is focused by a system of magnetic lenses (sometimes electrostatic lenses), passes through the sample so that some of the electrons are scattered on the sample, and some are not. Thus, the electron beam transmitted through the sample bears information about the structure of the sample. Further, the beam passes through a system of magnifying lenses and forms an image on a luminescent screen, a photographic plate or a CCD camera. In addition to the usual electron microscopic study, electronic immunohistochemistry can also be used, this is the detection of antigens in tissues at the ultrastructural level. It can be carried out according to two protocols: pre-embedding (before the enbedding into resin) and post-embedding (after embedding into resin, and in this case we will talk about immunogold labeling).

**Basic educational technology**

Discipline teaching provides the following forms of organization of the educational process: score-rating system of knowledge assessment during the current control, mid-term control and intermediate certification, interactive lectures, independent student work, testing, project method, presentation method.

**Forms of control**

The discipline program provides for the following types of control: monitoring progress in the form of a test, a report with a presentation and a project assignment, mid-term monitoring of progress in the form of testing, intermediate control in the form of an exam.

**GRADING SCHEME**

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| A | EXCELLENT - outstanding performance with only minor errors |
| B | VERY GOOD - above the average standard but with some errors |
| C | GOOD - generally sound work with a number of notable errors |
| D | SATISFACTORY- fair but with significant shortcomings |
| E | SUFFICIENT - performance meets the minimum criteria |
| FX | FAIL - some more work required before the credit can be awarded |
| F | FAIL - considerable further work is required |