

**Methodical recommendations For students
to the practice session on the topic:
«FEDERAL STATE BUDGETARY EDUCATIONAL INSTITUTION
OF HIGHER EDUCATION
«BASHKIR STATE MEDICAL UNIVERSITY»
OF THE MINISTRY OF HEALTHCARE OF RUSSIAN FEDERATION**

DEPARTMENT REPRODUCTIVE HUMAN HEALTH
WITH COURSE OF IMMUNOLOGY

APPROVED by
Head of the department

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**Methodical recommendations For students
to the practice session on the topic:
«Immuno-diagnostic reactions»**

Discipline: Clinical Immunology
Specialty: 31.05.01. «General education»
Course 4
Semester 7
Hours: 4

Methodological instructions for students for practical lessons in the discipline "Clinical Immunology " were developed by the faculty of the department in accordance with the work program of the academic discipline (Ufa, 2021), the curriculum (2021) and taking into account the requirements of the Federal State Educational Standard of Higher Education 3 ++ according to specialty 31.05.01 General education (M., 2020).

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1. The theme and its relevance: "Immuno-diagnostic reactions.»»»

With the help of immune reactions , the following tasks can be solved:

- determination of unknown antibodies by known antigens .
- determination of unknown antigens by known antibodies.

There are many types of immune reactions that differ in the technique of staging and the registered effect.

These are reactions of agglutination (RA), precipitation (RP), reactions involving complement (RIC), reactions using labeled components.

2. Learning purpose: mastering knowledge about the concept of Immunity, types. Factors of non-specific resistance, knowledge of pathogenesis of each type of reaction and clinical manifestations.

To form professional competencies, the student must know:

- principles of using immunological reactions in clinical practice;
- the methodology of staging immunological reactions (IR);
- tasks that are solved by setting immunological reactions.
- the concept of "immunogram", when prescribed, the volume of the immunogram.

To form professional competencies, the student must be able to:

- navigate diagnostic reactions.
- interpret the results of serodiagnostics

master the following competencies: GC 1, GC 6, GPC 5, PC 1, PC 5_.

3. Materials for self-preparation to master this topic:

Self-training questions:

- 1) Characteristics of antigen-antibody reactions
- 2) Mechanism, stages, components.
- 3) Stages of immunodiagnostic reactions.
- 4) Groups of serological reactions.
- 5) Application for the diagnosis of infectious diseases. Diagnostic immune serums, diagnosticums.
- 6) agglutination reaction, components, mechanism, methods of formulation, application.
- 7) Passive agglutination reaction , or indirect hemagglutination reaction .
- 8) Coagglutination reaction
- 9) Latex agglutination reaction
- 10) Precipitation reaction. Components. Kinds.
- 11) Ring-precipitation reaction
- 12) Double immunodiffusion reaction according to Ouchterloni
- 13) Radial immunodiffusion reaction
- 14) Immunoelectrophoresis
- 15) Serological diagnostics of infectious diseases: agglutination reactions of Vidal, Wright, Weigl, Coombs. Serodiagnostics criteria: diagnostic titer, antibody titer increase. Determination of the period of illness.
- 16) opsonophagocytic reaction, purpose, methods of formulation, mechanism.
- 17) Immunogram, indications for determination, volume, interpretation of results.

4. Type of lesson: practical lesson

5. Duration: 4 hours

6. Equipment: computer, projector

7. The content of the lesson.

7.1. Control of the initial level of knowledge and skills. Self-control assignments: students' decision on individual sets of test assignments on the topic

1. Phagocytic number:

- A. % of non-phagocytic cells
- B. % of phagocytic cells

- C. average number of absorbed particles per 1 cell
 D. average number of cells mixed with phagocytosis objects
1. Complement titration by 50% hemolysis allows us to judge the state of individual components of the complement system:
 A. No
 B. Yes
 C. Partially
 D. Approximately
2. In which reaction peroxidase is used as a label:
 A. radioimmunological method
 B. neutralization reaction
 C. complement binding reactions
 D. indirect immunofluorescence reactions
 E. enzyme immunoassay
3. The number of T-lymphocytes of healthy people's helpers:
 A. 55-65%
 B. 45-55%
 C. 35-45%
 D. 25-35%
4. Immunodiffusion in gel of AT and AT solutions poured into different wells, towards each other, is:
 A. Lysis reaction
 B. Precipitation reaction
 C. Agglutination reaction
 D. Neutralization reaction
5. Phagocytic index:
 A. % of non-phagocytic cells
 B. % of phagocytic cells
 C. average number of absorbed particles per 1 cell
 D. average number of cells, mixed with phagocytosis objects
6. Determination of the serum complement level is based on:
 A. The phenomenon of complement-dependent lysis of rabbit erythrocytes loaded with antibodies
 B. The phenomenon of complement-dependent lysis of sheep erythrocytes loaded with antibodies
 C. The phenomenon of interaction of a mixture of rabbit erythrocytes with ram hemolytic serum
 D. The phenomenon of interaction of a mixture of sheep erythrocytes with rabbit hemolytic serum
7. The phenomenon of flakes, or sediment in the agglutination reaction is caused by:
 A. cells "glued" with antibodies having two or more antigen-binding centers
 B. cells "glued" with antibodies having one antigen-binding center
 C. cells "glued" with antibodies having two or more complement molecules
 D. cells "glued" with antibodies, having one complement molecule each
8. The number of T-lymphocytes in healthy people:
 A. 40-70%
 B. 10-30%
 C. 20-50%
 D. 60-90%
9. Spontaneous spontaneous agglutination is caused by:

- A. an increase in the external temperature above 37 ° C for 24 hours
- B. a change in the pH of the reaction to the alkaline side
- C. the addition of microbes to the agar culture suspension in a saline solution of 0.2% formalin
- D. R-form of bacteria

11. The precipitation reaction involves:

- A. bacterial cell;
- B. bacterial cell, toxin;
- C. bacterial cell, toxin, antitoxic serum;
- D. toxin, antimicrobial serum, electrolyte;
- E. toxin, antitoxic serum, electrolyte.

12. Specific drugs used to detect antibodies in the patient's blood serum:

- A. immune diagnostic serums;
- B. antitoxins;
- C. allergens;
- D. anatoxins;
- E. diagnosticums.

13. To determine the blood type and Rh factor, use:

- A. enzyme immunoassay,
- B. Agglutination reaction,
- C. complement binding reaction,
- D. immunoblotting,
- E. immunofluorescence analysis.

Typical tasks.

Task 1. A person who had typhoid fever was discharged from the infectious diseases department of the hospital after a three-time negative bacteriological examination of feces. A month later, the same disease was registered in his family.

- 1) Could the patient have been the source of infection?
- 2) What kind of research should be conducted to verify this assumption?

The answer to task 1:

- 1) The patient could be a source of infection.
- 2) To confirm this assumption, it is necessary to use a serological diagnostic method (ELISA or RPGA) in order to detect Vi-antibodies. Additionally, to determine the phagotype of the typhoid culture in a newly ill person and compare it with the phagotype according to the patient's medical history. If the phagotypes match and Vi-antibodies are detected, then the patient is the source of infection. You can also conduct a bacteriological study of bile to isolate biculture.

Task number 2.

With the serum of a 10-year-old child who was admitted to the infectious department with a preliminary diagnosis of whooping cough, RA was diagnosed with whooping cough. The reaction titer is 1:400, controls are normal.

1. What is the purpose of the agglutination reaction?
2. Evaluate the result. It is known from the anamnesis that the child is vaccinated by age.

The answer to problem #2.

For the purpose of serodiagnostics - diagnostics of infectious diseases and resistance to them by detecting them and establishing antibody titers, detecting antigen pathogens in the body, determining cellular reactions to these antigens. Accounting: incubation is carried out at 37 ° C for 2 hours, then at room temperature 18-20 hours. The reaction is taken into account with the naked eye before shaking the test tubes, and then when they are lightly shaken with the finger of the right hand. In doubtful cases, an agglutinoscope or a magnifying glass is used. Test tubes are compared with controls:

KA - uniform turbidity, spontaneous agglutination of the antigen is excluded. CS - the contents are transparent,

there is no spontaneous agglutination of antibodies. With such controls, the results of the agglutination reaction, positive or negative, will be reliable.

The intensity of the reaction is expressed by the pluses:

++++ - the reaction is sharply positive; all the agglutinate is at the bottom of the tube in the form of an umbrella, the liquid above it is transparent;

+++ - the reaction is positive; the sediment is pronounced, the supravaginal fluid is slightly cloudy;

++ - partial agglutination with a small sediment, the supravaginal fluid is cloudy;

+ - doubtful agglutination, the liquid is cloudy.

In case of a negative reaction, there is no agglutination, the suspension remains uniformly cloudy and indistinguishable in appearance from the contents of the test tube with antigen control. The titer of the agglutination reaction is considered to be the maximum dilution of serum with a reaction intensity of at least ++.

Evaluation of the results: to confirm or refute the diagnosis of the disease, the obtained titer of the agglutination reaction is compared with the diagnostic titer. For example: the diagnostic titer of the agglutination reaction in typhoid fever is 1:100 in unvaccinated children, 1:200 in unvaccinated adults.

Task number 3.

A patient was admitted to the medical center who was clinically diagnosed with acute glomerulonephritis. It is known that this disease in most cases manifests itself as a secondary immunopathological condition against the background of chronic pharyngeal disease of streptococcal etiology.

1. What is the mechanism of development of this immunopathological condition?

2. What laboratory tests would help you confirm the possibility of developing an immunopathological condition in an infectious process caused by group A streptococci?

The answer to task No. 3.

1. The development of glomerulonephritis is associated with acute and chronic diseases of various organs, mainly of streptococcal nature. Most often these are sore throats, scarlet fever, purulent skin lesions (streptoderma), pneumonia. The cause of the development of glomerulonephritis can also be ARVI, measles, chickenpox. There are reports of the causal role of microorganisms such as Staphylococcus aureus, Streptococcus pneumoniae, Neisseria meningitidis, Plasmodium of malaria, Toxoplasma gondii and some Viruses. Develops 2-3 weeks after an infectious disease. In the development of glomerulonephritis, previous diseases, reactivity of the body, living conditions and nutrition have a certain importance.

2. Group A streptococci can be easily detected in throat smears using commercial kits: group A antigens are extracted with chemical reagents or enzymes and identified in latex agglutination, coagglutination or ELISA reactions. For the express diagnosis of glomerulonephritis, antibodies to streptolysin O or streptodorzane can be determined; serological studies also allow to identify carriers. It should be noted that antibodies to streptolysin O are not formed in skin infections caused by group A streptococci.

7.2. Analysis with the teacher of the key questions necessary for the development of the topic of the lesson.

7.3. Presentation by the teacher of the methodology for assessing the state of factors of non-specific protection of the body in the laboratory.

7.4. Independent work of students under the supervision of a teacher (draw in a notebook the stages of phagocytosis, the main schemes of complement activation).

7.5. Control of the final level of assimilation of the topic:

The teacher checks the students' oral answers to the questions of self-preparation.

Checking the presence of drawings of phagocytosis stages and the main schemes of complement activation in the notebooks.

Materials for monitoring the level of development of the topic:

- a set of test tasks,

- situational tasks.

Place of self-training: study room for independent work of students.

Educational and research work of students on this topic (conducted during school hours): working with the main and additional literature.

The main literature

Serial№	Title	Author(s)	Year, place of	Number of copies	
				In library	At the

			publication		department
1	2	3	4	7	8
	Basic Immunology: Functions and Disorders of the Immune System [Текст] : [учебноиздание]	A. K. Abbas, A. H. Lichtman, S. Pillai.	Elsevier, 2016 – 335 p.	80	0

Additional literature

Serial №	Title	Author(s)	Year, place of publication	Number of copies	
				In library	At the department
1	2	3	4	7	8
•	Lectures in immunology: курс лекций	Maianskii, A. N.	N. Novgorod: Publishing house NSMA, 2004 – 256 p.	40	0
•	IMMUNOLOGY	Khaitov R.M.	2008 – 256 c.on-line.	access mode: ЭБС «Консультант студента» http://www.studmedlib.ru/book/ISBN9785970407042.html	unlimited access
•	Fundamental Immunology.	Lippincott Williams & Wilkins	2008 –on-line	access mode: Database«LWW Medical Book Collection 2011» http://ovidsp.ovid.com	unlimited access

