**IMMUNOLOGY**

*Lesson № 11*

**IMMUNOLOGY AS A SCIENCE. INNATE IMMUNITY.**

**Evaluation of the functional state of phagocytes.**

**Practical work that student has to learn at the lesson and to be able to perform at concluding session**

1. Calculation of lysozyme titre in saliva.
2. Evaluation of the activity of phagocytes using preparations stained by Romanovsky-Giemsa: counting phagocytes and indexing of phagocytes.

**CALCULATION OF LYSOZYME TITRE IN SALIVA**

Two-fold dilutions of human saliva (**1:2 1:4 1:8 1:16 1:32 1:64 1:128**) are normally prepared in the reading plates or test tubes from fresh saliva using broth. The same volume of standard bacterial suspension has to be added to each well or tube, mix and after the incubation the change in turbidity should be detected by eye or using special equipment.

If the lysozyme in the tested saliva is active it will prevent growth and kill bacteria, so the turbidity in the samples will be decreased. When you have prepared the dilutions of the saliva it is necessary to leave the two last wells of the reading plate or the two last tubes as control ones: control of the bacterial growth in broth (we don’t add saliva to this well or tube) and control of the saliva (we don’t add bacteria to this sample).

We can read the results of the test only if our control samples showed that the test was set up properly: the sample of bacteria grown in broth without saliva has to show high turbidity that means active growth, and the sample of saliva has to be absolutely clear that means there was no bacterial contamination in the preparation of saliva.

To calculate the titre of the lysozyme in the preparation we use the highest dilution of the saliva that produces pronounced clearance of the bacterial growth in the broth.

**Evaluation of the activity of phagocytes using the preparations stained by Romanovsky-Giemsa: countING of phagocytes and indexING of phagocytes**

To evaluate the phagocytic activity a sample of blood is mixed with bacteria (for example with *Staphylococcus aureus)* and incubated for 20 min at room temperature. A drop of this solution is placed on a clean glass slide and a smear is prepared, air dried, fixed and stained with the Romanovsky-Giemsa technique.

Usually a 100x oil-immersion objective of the microscope is used to calculate the count of phagocytes by detecting a minimum 100 phagocytic cells including the phagocytizing cells containing ingested bacteria. The number of phagocytizing cells and the number of bacteria engulfed by the phagocyte were counted.

Activity of phagocytes is calculated as follows:

**Count of phagocytes** – the number of phagocytizing cells divided by the total number of phagocytes counted (100 cells).

**Index of phagocytes** – the total number of bacteria (staphylococci) engulfed by the phagocytes, divided by the total number of phagocytes containing engulfed bacteria.

*Lesson № 12*

**THE HUMAN IMMUNE SYSTEM.**

**Determination of the activity of complement in complement**

**fixation reaction.**

**Practical work that student has to learn at the lesson and to be able to perform at concluding session**

## 1. Determination of the activity of the complement – the scheme of titration of the complement (determination of working concentration) and reading of the results.

## **Determination of the activity of**

## **the complement – the scheme of titration of complement (determination of working concentration) and reading of the results**

## To obtain the preparations of the complement blood of guinea pigs is normally used. The concentration of the complement in different animals is different so it is necessary to determine these concentrations and to prepare the complement in **working titre** which could be used in immunological tests.

1. Firstly, serial ten-fold dilutions of the blood serum in tubes containing buffer have to be prepared.
2. To every tube a standard amount of sheep red blood cells (erythrocytes) pre-coated with anti-erythrocyte antibodies (the red blood cells pre-bound to anti-red blood cells antibodies called **haemolytic system**) are added.
3. Tubes are placed in a thermostated incubator for 30 minutes at 37 0C to permit the complement to be activated and to produce haemolysis of erythrocytes.
4. Reading of the results of the test: haemolysis is detected by appearance of clear scarlet blood in the tubes.
5. The tube with the highest dilution of the complement that contains clear scarlet blood used for the calculation of the complement titre.

*Theme№ 13*

**CELL – MEDIATED IMMUNITY. ANTIGENS.**

**Practical work that student has to learn in the lesson and to be able to perform at the concluding session**

1. Agglutination reaction performed on the glass slide.

**Agglutination reaction performed**

**on the glass slide**

This agglutination test is performed on a glass slide by mixing a loopful of microorganisms with a specific diagnostic antiserum on a slide and inspecting the result by naked eye or through the low-power objective (8x). As a control of the serological reaction it is necessary to mix a loopful of microorganisms with saline (physiological solution) on the same glass slide and compare the results.

If the agglutination reaction is positive visible clumps will appear in the drop containing microorganisms and antiserum. specific clumps of agglutinated microorganisms In the control drop there will be no such clumps and the microorganisms mixed in saline are usually giving to the suspension even turbid look.

 

Figure. Agglutination on the slide test: “**+**” – positive agglutination reaction, “**–**“– control (no agglutination).

*Theme№ 14*

**HUMORAL IMMUNITY. IMMUNOGLOBULINS.**

**Practical work that student has to learn at the lesson and to be able to perform at the concluding session**

1. Agglutination reaction performed in the test tubes containing serial dilutions of patient’s sera for the estimation of the "titre" of agglutinating antibodies – the scheme of setting up the reaction of agglutination performed in the tubes and reading of the results.

**agglutination Reaction performed in the test tubes containing serial dilutions of patient’s sera for the estimation of the "titre" of agglutinating Antibodies – The scheme of PERFORMING the reaction of agglutination performed in the tubes and reading of the results.**

For the estimation of the "titre" of agglutinating antibodies (Abs) in the serum of a patient, a tube dilution test is usually applied.

1. First of all it is necessary to prepare series of ten-fold serum dilutions in tubes containing saline:
2. Then a standard amount of Ag (called diagnosticum) has to be added to each tube with dilutions.
3. To mix Ag and Abs it is necessary to shake thoroughly, and then the tubes have to be incubated at 37° C for 1-2 hours.
4. Two control tubes should be included:
	1. the tube for control of antigen (C Ag) containing a standard amount of diagnosticum dissolved in saline
	2. the tube for control of the patient’s serum (C Abs) containing only patients serum
5. The result is determined by the appearance of clumps leaving supernatant fluid clear.
6. The “titre" of specific antibodies in the tested serum is evaluated as the highest dilution of the serum with clearly visible agglutination.

*Theme№ 15*

**ALLERGY.**

**Reaction of precipitation.**

**Practical work that student has to learn at the lesson and to be able to perform at the concluding session**

1. Ascoli's ring precipitation test – the scheme of setting up of the reaction of precipitation in tubes and reading of the results.
2. Immunoprecipitation Mancini test – reading of the results of precipitation in gel (gel diffusion test).

**Ascoli's ring precipitation test –**

**the scheme FOR setting up of the reaction of precipitation in tubes and reading of the results**

This precipitation test is performed by layering the antigen solution over a column of specific antiserum in a narrow test tube. The result of positive reaction is the formation of precipitate at the junction of the two liquids clearly visible as precipitation ring (figure 29).



Figure. Ascoli's ring precipitation test.

**Immunoprecipitation Mancini test –**

**reading of the results of THE precipitation in gel (gel diffusion test).**

Immunoprecipitation (called also immunodiffusion) Mancini test is based on diffusion of antigen solutions in in semi-solid matrices such as agar gel. The technique includes incorporation of antibodies by pouring specific serum into the liquefied gel. When the gel is solidified the holes are punched in it and different dilutions of the antigen are placed in holes.

As the antigen solution diffuses into the gel it reacts with the antibody and a ring-shaped bound of precipitation is formed around every hole .

The test is a quantitative one since the diameter of the ring around the hole is proportional to the concentration of antigen. If using different concentrations of an antigen and plotting the amount of precipitate against increasing antigen concentrations it is easy to generate a precipitin curve which can help to quantitate the concentration of an antigen in an unknown sample.

This test is commonly used in the clinical laboratory for the determination of immunoglobulin levels in patient sera samples. In this case the preparation of serum will be the antigen in this precipitation reaction and it is necessary to incorporate specific anti-immunoglobulin antiserum into the agarose gel.

*Theme№ 16*

**IMMUNOPATHOLOGY AND CLINICAL IMMUNOLOGY.**

**IMMUNITY IN TRANSPLANTATION, CANCER. AND IMMUNITY IN MYCOSES.**

**Complement fixation (activation) reaction.**

**Practical work that student has to learn at the lesson and to be able to perform at the concluding session**

1. The scheme for setting up of the complement fixation reaction and reading of the results.
2. Evaluation of the immunogram.

**The scheme For setting up of the complement fixation reaction and reading of the results**

The principle of the complement fixation reaction is illustrated in the table 1.

Table 1 - Thescheme of setting up of the complement fixation reaction and reading of the results

|  |  |
| --- | --- |
| Reagents of the CF reaction (0.2 ml per tube) | Reagents of CF reaction in every tube, ml |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Main test | Positivecontrol | Negativecontrol | Control of the serum | Control of Ag | Control of complement | Control of haemolytic system |
| Serum of a patient | + | - | - | + | - | - | - |
| Positive serum | - | + | - | - | - | - | - |
| Negative serum | - | - | + | - | - | - | - |
| Antigen | + | + | + | - | + | - | - |
| Complement | + | + | + | + | + | + | - |
| Incubation at 370 C for 30 minutes |
| Haemolytic system (0.6 ml per tube) | + | + | + | + | + | + | + |
| Haemolysis | + or - | + | + | + | + | + | - |
| Result of CF reaction | + or - | + | - | - | - | - | - |

The variant when the complement fixation reaction is performed in tubes is recommended to use seven test tubes and the whole volume of the mixture in every tube is 1 ml.

0.2 ml of corresponding reagent for CF reaction is taken and mixed in the tubes as shown in the table 1. The first tube contains test serum of the patient, specific antigen and complement which are mixed to assay patient’s serum for presence of specific antibodies. The other six tubes are controls of the reagents of the reaction. We don’t add any of the reagents into these tubes (see table 1).

 When all reagents are added and mixed in the tubes they are left at 37° C to permit interaction of antigen with antibody and "fixation" of comple­ment. After allowing for complement fixation by the Ag/Ab complex, a standard suspension of sheep red blood cells (erythrocytes), which have been pre-coated with anti-erythrocyte antibodies (anti-sheep rabbit serum) – the mixture called **haemolytic system** is added to every tube. The tubes are then incubated at 37° C for 30 minutes and read for haemolysis.

 The red blood cells lysis is normally detected by the release of hemoglobin into the medium resulting in the appearance of specific scarlet colour of the mixture in the tubes. All control tubes excluding the positive control and control of haemolytic system should produce some haemolysis. The result of the CF reaction in the first tube containing test serum of the patient could be positive if no haemolysis occurred or negative when haemolysis is present in this tube.

**Evaluation of the immunogram**

Table 2 – The principle scheme of evaluation of the immunogram

|  |  |
| --- | --- |
| **Indexes** | **Characteristics of the immune response** |
|
| **CD3** | All populations of Т cells, cell-mediated immunity |
| **CD4** | Т cells – helpers, cell-mediated immunity |
| **CD8** | Т cells – killers/suppressors, cell-mediated immunity |
| **CD16** | NK cells, naturally acquired immunity  |
| **CD19 (CD20, CD22)** | В cells, humoral immunity |
| **CD25** | Activated Т and В cells, cell-mediated immunity and humoral immunity |
| **CD95** | Receptors for apoptosis on differentiated Т and В cells, cell-mediated immunity and humoral immunity  |
| **Immune regulatory index CD4/CD8** | Correlation Т helpers/Т supressors, cell-mediated immunity |
| **Count of phagocytes**  | Evaluation of functional activity of phagocytes, naturally acquired immunity  |
| **Index of phagocytes**  | Evaluation of functional activity of phagocytes, naturally acquired immunity  |
| **CH50** | Complement activity – concentration producing 50% haemolysis, naturally acquired immunity  |
| **Circulatory immune** **complexes** | Evaluation of humoral immunity and functional activity of phagocytes (naturally acquired immunity ) |
| **NBT-test** | Nitroblue tetrazolium test – evaluation of functional activity of phagocytes (naturally acquired immunity ) |
| **IgM** | Immunoglobulin М, humoral immunity |
| **IgG** | Immunoglobulin G, humoral immunity |
| **IgA** | Immunoglobulin А, humoral immunity |
| **BTR** | PHA | Phytohaemagglutinin – mitogen triggering BTR of Т cells  |
| LPS | Lipopolysaccharide – mitogen triggering BTR of В cells  |
| Con A | Concanavalin А – mitogen triggering BTR of Т cells |

Abbreviations:

 **CD** – differentiation clusters, molecules – markers anchored at the membranes of immune cells.

 **BTR** – blast – transformation reaction of lymphocytes.

*Lesson № 17*

**IMMUNE PROPHYLAXIS AND IMMUNE THERAPY OF INFECTIOUS DISEASES. IMMUNITY AND AGE.
Serological tests using labelled antibodies or antigens.**

**Practical work that student has to learn at the lesson and to be able to perform at the concluding session**

1. Reading of the results of immune phenotyping of lymphocytes.

**Reading of the results of immune phenotyping**

**of lymphocytes**

 The “rosette” assay for immune phenotyping of lymphocytes is applied to calculate functionally active Т- and В lymphocytes isolated from peripheral blood of patients. According to the difference in the composition of their cell membranes Т lymphocytes and В lymphocytes are capable to bind erythrocytes in a different way.

 To set up the test it is necessary to mix human peripheral blood lymphocytes with sheep (or mouse) erythrocyte diagnosticum carrying antilymphosyte monoclonal antibodies and to calculate the number of rosettes formed by lymphocytes surrounded by bound erythrocytes using the microscope. Functionally active lymphocytes (B or T) have to be able to bind at least three erythrocytes.