**Instructions for the check of reagents for antigen from AB0 blood group system testing**

1. The test is performed manually. The nacryl whiteplates, reagent blood cells and monoclonal reagents are used.
2. Put 1 drop of monoclonal anti-A reagent in 3 places vertically in a line on the nacryl whiteplate. Put the 1 drop of monoclonal anti-B reagent in 3 places vertically in a parallel line on the nacryl whiteplate.
3. Next add one drop of reagent blood cells per well horizontally in a line, appropriately: 0, A1, B groups.
4. Put the whiteplate in circular movement and leave it for 3-5 minutes in room temperature.
5. A set of reagents is correct if strong agglutination has been found according to the scheme:

Monoclonal reagents anti-A anti-B

0 ○ ○

Reagent blood cells A ● ○

B ○ ●

**Instructions for the check of reagents for antigen from Rh blood group system testing**

1. Prepare 4 plastic test tubes.
2. Add one drop of anti-D BLEND reagent to two of the test tubes and one drop of anti-D RUM1 reagent to the remaining two test tubes.
3. Next add one drop of Rh positive reagent blood cells and one drop of Rh negative reagent blood cells appropriately to the anti – D BLEND test tubes and to anti – D RUM1 test tubes. Centrifuge for 1 minute.
4. A set of reagents is correct if strong agglutination has been found between Rh positive reagent blood cells and anti-D reagent and agglutination has not been found between Rh negative reagent blood cells and anti-D reagent.

**Instructions for AB0 and Rh typing in a tested blood sample**

1. The test is performed manually. The nacryl whiteplate, reagent blood cells and monoclonal reagents are used.
2. Prepare two additional test tubes with the same number as the tested blood.
3. Put the serum in the first of the test tubes. Put a few drops of red blood cells in the other test tube with a pipette. The red blood cells should be suspended in PBS, centrifuged, the supernatant should be removed. The red blood cells should be again suspended in PBS in order to obtain 10 % solution (1 drop of red blood cells and 9 drops of PBS).
4. Put one drop of monoclonal anti-A and anti-B reagents and 3 drops of tested serum, 1 per well, on the nacryl whiteplate. Next add one drop of suspension of red blood cells to anti-A and anti-B reagents and add one drop of A, B and 0 reagent blood cells to the tested serum.

Monoclonal reagents: tested serum:

anti-A anti-B

10% suspension of red blood cells 0 A B

in PBS reagent blood cells

1. Put the whiteplate in circular movement and leave it for 3-5 minutes in room temperature.
2. Examine the results of agglutination and identify the group according to the table:

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Monoclonal reagents | | Reagents blood cells | | | GROUP |
| anti-A | anti-B | 0 | A | B |
|  |  |  |  |  | 0 |
|  |  |  |  |  | A |
|  |  |  |  |  | B |
|  |  |  |  |  | AB |

1. To identify the Rh group add one drop of anti-D BLEND reagent to the first of the test tubes and add one drop of anti-D RUM1 reagent to the other test tube.
2. Next add one drop of red blood cells to anti-D reagents. Centrifuge for 1 minute.
3. Agglutination between anti-D reagent and tested cells shows that the patient is Rh positive and has the Rh antigen on red blood cells. Lack of agglutination between anti-D reagent and tested cells shows that the patient is Rh negative and does not have the Rh antigen on red blood cells.

**Instructions for testing immunological antibodies in indirect antiglobulin test: IAT**

1. Prepare a microcard LISS/COOMBS with 6 reaction columns – there are three columns needed, numbered as: I, II, III and with the number of tested blood.
2. Add 50 µl of reagent blood cells I, II, III to each column (using an automatic pipette).
3. Next add 25 µl of tested serum (using an automatic pipette).
4. Incubate for 15 minutes at 37 ° C, next centrifuge for 10 minutes.
5. Examine the results:

If the cells get across the antiglobulin serum – there is lack of Ag-Ab reaction (there are not immunological antibodies in serum).

If the cells stop above/in the antiglobulin serum – there is Ag-Ab reaction (there are immunological antibodies in serum).

**Instructions for crossmatch**

1. The test is performed by two students. The sample of blood from one student will be recipient sample and the drains of packed RBCs will be donor samples.
2. Prepare 5% suspension of donor blood cells in LISS ID-2.
3. Place the recipient number, the donor number and the number of panel blood cells I, II, III on LISS/COOMBS microcard.
4. Add 50 µl panel blood cells I, II, III and 50µl donor blood cells (use a pipette).
5. Add 25µl recipient serum to each microcolumn (use a pipette).
6. Incubate the microcard for 15 minutes at 37 ° C, next centrifuge it.
7. Examine the results:

If cells get across the antiglobulin serum – there is lack of Ag-Ab reaction (there are not immunological antibodies in serum). The crossmatch is compatible.

If cells stop above/in the antiglobulin serum – there is Ag-Ab reaction (there are immunological antibodies in serum). The crossmatch is incompatible.