MINISTRY OF HEALTH OF THE RUSSIAN FEDERATION

GENERAL PHARMACOPOEIA MONOGRAPH

Determination of anti-A and anti-B haemagglutinins in medicinal products containing human immunoglobulins	**GPM**.1.8.2.0005.15 First Edition

The present General Pharmacopoeia Monograph applies to the method used to determine anti-A and anti-B haemagglutinins in medicinal products containing human immunoglobulins.

The planar indirect haemagglutination method (Method A) and the gel method (Method B) are based on the fact that anti-A and anti-B haemagglutinins contained in the tested medicinal product, being incomplete antibodies of the Ig G Class, promote red blood cell sensitization. Addition of an antiglobulin serum in a saline medium at a temperature of (37 ± 0.5) °C causes agglutination of sensitized red blood cells and permits visualization of the reaction's results.

Procedure description

The content of haemagglutinins is determined using human Blood Group A_1 (II), Rhesus negative, and Blood Group B (III), Rhesus negative, red blood cells. Both recently prepared suspension (in the indirect planar method (Method A)) and standard red blood cells included in blood group test kits (for Methods A and B) may be utilized.

In the planar haemagglutinin assay (Method A), 5 % red blood cell suspension should be used. When determining the content of haemagglutinins in the gel method (Method B), 0.8 % red blood cell suspension should be employed.

The planar indirect haemagglutination method (Method A)

Equal amounts of the appropriate tested sample dilution and 5 % human Blood Group A_1 (II) red blood cell suspension (first row) or 5 % human Blood Group B (III) red blood cell suspension (second row) are transferred into glass test tubes or wells of a microtitre plate. The samples are carefully stirred and incubated for 30 minutes at (37 ± 0.5) °C. After the end of incubation, the samples are centrifuged at 1500 – 2000 rpm for 10 minutes. After that, the supernatant is removed, the obtained sediment is resuspended in ten volumes of 0.9 % sodium chloride solution and centrifuged again at 1500 – 2000 rpm for 10 minutes. This procedure should be repeated not less than 3 times. The supernatant is removed, the polyvalent antiglobulin serum (Coombs' serum) in a volume equal to that of the red blood cell sediment is added, and then the mixture is carefully mixed. The samples are incubated for 30 minutes at (37 ± 0.5) °C. After the end of incubation, the samples are examined under a microscope or with the naked eye; any agglutination of red blood cells should be noticed.

The haemagglutinin titre is determined as the maximum dilution of the tested sample at which red blood cell agglutination of any intensity is observed. The dilution to a protein content of 30 g/L is disregarded when determining the titre.

Each sample with no agglutination in it is mixed with an equal amount (volume) of control Coombs' cells, then the mixture is carefully mixed and an agglutination assessment is performed in 2 to 4 minutes.

Results acceptance criteria:

– agglutination should be observed in samples with a negative result (no agglutination) after the addition of control Coombs' cells.

The gel method (Method B)

The gel method is based on the use of a gel card, which is a plastic plate with microtubes containing gel columns. Each microtube consists of a dosing / incubation chamber and a column containing polymerized dextran microspheres in

a buffer low ionic strength solution (LISS) mixed with the polyvalent antiglobulin serum (Coombs' serum).

One drop of 0.8 % human Blood Group A_1 (II) red blood cell suspension (first row) or 0.8 % human Blood Group B (III) red blood cell suspension (second row) and 25.0 μL of the respective dilution of the tested sample are transferred into the dosing / incubation chamber of the microtube. The samples are incubated at (37 ± 0.5) °C for 15 minutes. After the end of incubation, the samples are centrifuged on a specially designed centrifuge (for gel cards) under standard conditions (programmed mode) and the agglutination is assessed.

The haemagglutinin titre is determined as the maximum dilution of the tested sample, at which agglutinated red blood cells are distributed throughout the gel column or in its upper part. Non-agglutinated red blood cells precipitate to the bottom of the microtube. The dilution to a protein content of 30 g/L is disregarded when determining the titre.

Notes

1. Preparation of the tested sample. The tested medicinal product is diluted with the saline solution (0.9 % sodium chloride solution or a buffer low ionic strength solution (LISS)) to a 30 g/L protein content.

Two rows of twofold dilutions of the tested sample should be prepared using 1 % bovine serum albumin solution (1:2; 1:4; 1:8; 1:16; 1:32; 1:64; 1:128). When using the gel method (Method B), dilutions of the medicinal product should be prepared using 0.9 % sodium chloride solution or a buffer low ionic strength solution (LISS).

2. Preparation of the standard red blood cell suspension. Human Blood Group A_1 (II), Rhesus negative, red blood cells (recently prepared, provided that they have been stored for not more than 3 days), are centrifuged for 10 minutes at 1500 – 2000 rpm at room temperature, and then the supernatant is poured out. The obtained sediment is resuspended in ten volumes of 0.9 % sodium chloride solution and centrifuged again for 10 minutes at 1500 – 2000 rpm at room temperature. This procedure should be repeated not less than 3 times, until a transparent supernatant is obtained. To obtain the 5 % suspension, one volume of the red blood cell suspension should be resuspended in 19 volumes of 0.9 % sodium chloride solution.

The same manner should be used to prepare the 5 % human Blood Group B (III) red blood cell suspension, Rhesus negative.