MINISTRY OF HEALTH OF THE RUSSIAN FEDERATION

GENERAL PHARMACOPOEIA MONOGRAPH

Test for anti-D antibodies in medicinal products containing human immunoglobulins

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First Edition

The present General Pharmacopoeia Monograph applies to the method used to determine anti-D antibodies in medicinal products containing human immunoglobulins. Anti-D antibodies are determined either by planar haemagglutination (Method A) or by haemagglutination in gel (Method B).

The principle of this method is based on the fact that anti-D antibodies contained in the tested medicinal product are Class G immunoglobulins that can cause agglutination of D-positive red blood cells.

Procedure description

The content of anti-D antibodies is determined using papainized D-positive human red blood cells belonging to the I (0) blood group. Erythrocytes of the $0R_2R_2$ should be preferred, but the $0R_1R_1$ or $0R_1R_2$ erythrocyte phenotypes may be used as well. Mixing red blood cells of different phenotypes is unacceptable.

D-negative red blood cells of the 0rr phenotype should be used for analysis specificity control.

The analysis may be performed on either recently prepared red blood cell suspension (when testing with the planar haemagglutination method, Method A) or standard erythrocytes provided in test kits (Methods A and B). When using Method A for the determination of anti-D antibodies, 3 % red blood cell

suspension should be used; the gel method (Method B) necessitates the use of 0.8 % red blood cell suspension to determine the content of haemagglutinins.

The planar haemagglutination method (Method A)

Equal volumes of the appropriate medicinal product dilution and 3 % D-positive human Blood Group I (0) red blood cell suspension (first row) or 3 % D-negative human Blood Group I (0) red blood cell suspension (second row) are transferred into glass test tubes or wells of a microtitre plate. The prepared dilution of the standard sample is also mixed with an equal volume of 3 % D-positive human Blood Group I (0) red blood cell suspension (first row) or 3 % D-negative human Blood Group I (0) red blood cell suspension (second row). The samples are carefully stirred (on a shaker) for 10 seconds, and then centrifuged for 1 minute at 80 rpm. The test tubes (microtitre plates) are placed at an angle of 70° to the horizontal plane, and the agglutination of red blood cells is evaluated by visual examination in 4 to 5 minutes (not later than in 10 minutes).

The content of anti-D antibodies determined by the maximum medicinal product dilution producing agglutination of any intensity is compared with the content of anti-D antibodies in the positive standard sample.

The haemagglutination in 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).

One drop of 0.8 % standard D-positive human Blood Group I (0) red blood cell suspension (first row) or one drop of 0.8 % D-negative human Blood Group I (0) 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. At the same time, samples of positive and negative anti-D antibody standard samples: one drop of 0.8 % standard D-positive human Blood Group I (0) red blood cell suspension (first row) or one drop of 0.8 % D-negative human Blood Group I (0) 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 30 minutes. After the end of incubation, the samples are centrifuged (on a centrifuge specially designed for gel cards) under standard conditions (programmed mode) and the agglutination is assessed. 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 content of anti-D antibodies determined by the maximum medicinal product dilution producing agglutination of any intensity is compared with the content of anti-D antibodies in the positive standard sample.

Results acceptance criteria:

– No agglutination should be observed in the test tubes (microtitre plates, microtubes) containing the D-negative human red blood cell suspension. If an agglutination is observed in these test tubes (microtitre plates, microtubes), this indicates a non-specific reaction and the test should be repeated in this case;

– the content of anti-D antibodies in the positive standard sample should correspond to the certified values.

Notes

1. Preparation of the tested sample. The tested sample is diluted with a phosphate buffer solution (pH 7.4 ± 0.1) containing 2 g/L bovine serum albumin until the sample has a 25 g/L protein content. This tested sample dilution is regarded as a twofold dilution (1:2), even if the true dilution is different.

Prepare two rows of twofold dilutions of the tested sample using a phosphate buffer solution (pH 7.4 ± 0.1) containing 2 g/L bovine serum albumin (1:4; 1:8; 1:16; 1:32; 1:64; 1:128; 1:256). If the gel method is used (Method B), the tested sample dilutions are prepared with 0.9 % sodium chloride solution or a buffer low ionic strength solution (LISS).

2. Preparation of the anti-D antibody standard samples. Use 5 % human immunoglobulin solution containing 0.0475 IU/mL anti-D antibodies with a nominal haemagglutination assay titre of 1:8 (positive standard sample) and 5 % human immunoglobulin solution containing anti-D antibodies at a dilution not exceeding 1:2 (negative standard sample) as standard samples. The content of anti-D antibodies in the positive standard sample is the maximum acceptable level for human immunoglobulin preparations for intravenous administration. The standard samples should be prepared as required by the Instructions for Use. The analysis

should be performed on samples diluted with the phosphate buffer solution (pH 7.4 ± 0.1) containing 2 g/L bovine serum albumin, until the sample has a 25 g/L protein content.

3. Preparation of the papain solution. Transfer freeze-dried papain in accordance with the manufacturer's Instructions for Use. Incubate at (37 ± 0.5) ^{0}C for 10 to 15 minutes. This solution should be used while it is fresh. The solution may be stored at (6 ± 2) ^{0}C for 5 days or at minus (21 ± 1) °C for 6 months. After thawing, the solution must not be re-frozen.

4. Preparation of the standard red blood cell suspension. Take recently prepared D-positive red blood cells (that have been stored for not more than 3 days) obtained from at least 3 donors, and centrifuge at room temperature for 10 minutes at 1500 – 2000 rpm. Pour the supernatant out, resuspend the sediment in ten volumes of the phosphate buffer solution, and centrifuge for 10 minutes under the same conditions. Repeat this procedure not less than 3 times, until a transparent supernatant is obtained.

Transfer equal volumes of the washed red blood cells and papain solution into a glass test tube, incubate for 15 minutes at (37 ± 0.5) ^{0}C, and then centrifuge for 10 minutes at 1500 – 2000 rpm. After removing the supernatant, the sediment is resuspended in ten volumes of the phosphate buffer solution and centrifuged under the same conditions.

To prepare the 3 % suspension, one volume of the papainized red blood cells sediment is resuspended in 32 volumes of the phosphate buffer solution containing 2 g/L bovine serum albumin.

The 3 % suspension of D-negative red blood cells obtained from 1 to 3 donors is prepared in the same fashion.

5. Preparation of the phosphate buffer solution. Transfer 8.0 g of sodium chloride, 0.76 g of sodium hydrogen phosphate anhydrous, 0.2 g of potassium chloride, and 0.2 of potassium dihydrogen phosphate into a 1000 mL volumetric flask. Add 900 mL of purified water. Bring the pH value of this solution to 7.4 ± 0.1 with 1 M sodium hydroxide solution or 1 M hydrochloric acid solution, stir, bring the volume of the solution to the mark with purified water, and stir again.