MINISTRY OF HEALTH OF THE RUSSIAN FEDERATION

PHARMACOPOEIA MONOGRAPH

Normal human immunoglobulin **PM.3.3.2.0007.15**

Replaces the Pharmacopoeia Monograph 42-3198-95

The present Pharmacopoeia Monograph applies to normal human immunoglobulin preparations for intramuscular or subcutaneous administration.

Normal human immunoglobulin is an immunologically active protein fraction that contains a broad spectrum of antibodies isolated from human plasma; its principal active component is immunoglobulin G (Ig G), which exhibits antibody activity of various specificity against various antigens.

Normal human immunoglobulin preparations contain no preservatives and no antibiotics.

MANUFACTURE

The raw material for the manufacture of human immunoglobulin is plasma obtained from at least 1000 healthy donors and tested for the markers of blood-borne infections, in individual donations and pools. Obtained plasma should meet the requirements established by the Pharmacopoeia Monograph “Human plasma for fractionation”.

Normal human immunoglobulin is purified and concentrated using the modified alcohol method for low-temperature fractionation of serum proteins.

The manufacture of normal human immunoglobulin should be performed in accordance with established manufacture organization and medicinal product quality control requirements to guarantee preservation of the structure and function

of immunoglobulin proteins responsible for the specific and viral safety of the medicinal product and precluding contamination with foreign agents. Antibacterial and antiviral efficacy of medicinal products should be guaranteed by appropriate antibody concentration achieved during the manufacturing process (at least six-fold).

TESTS

Description. A transparent or slightly opalescent, colourless or light yellow solution (unless different requirements are included in the Normative Document); a minor sediment may form during storage that should disappear upon slight shaking. The test is carried out by visual examination.

Identification. Identity is confirmed by the presence of only human serum proteins. The test is carried out by gel immunoelectrophoresis, using sera against human, bovine, horse, and porcine serum proteins, as described in the General Pharmacopoeia Monograph “Agarose gel immunoelectrophoresis”. This test may also be performed with the gel immunodiffusion method. The test method used should be specified in the Normative Document. The test should produce precipitation lines only with the serum against human serum proteins.

Transparency. A transparent or slightly opalescent solution with an optical density of not more than 0.05 (the test is carried out in accordance with the General Pharmacopoeia Monograph “Transparency and turbidity of liquids” and the General Pharmacopoeia Monograph “Spectrophotometry in the ultraviolet and visible regions”, using cuvettes with an optical path length of 3 mm at wavelength 540 nm). The test method used should be specified in the Normative Document.

Colour intensity. A colourless or light yellow solution (the test is carried out in accordance with the General Pharmacopoeia Monograph “Colour intensity of liquids”) or a solution with an optical density of not more than 0.15 (the test is carried out in accordance with the General Pharmacopoeia Monograph “Spectrophotometry in the ultraviolet and visible regions”, using cuvettes with an optical path length of 3 mm at wavelength 400 nm). The test method used should be specified in the manufacturer’s Normative Document.

Particulate matter. Visible particulate matter should be absent. The test is carried out in accordance with the General Pharmacopoeia Monograph “Visible particulate matter in medicinal products for parenteral use and ophthalmic dosage forms”.

Extractable volume. The extractable volume should be not less than the nominal value. The test is carried out in accordance with the General Pharmacopoeia Monograph “Extractable volume for parenteral pharmaceutical forms”.

pH value. From 5.0 to 7.4. The tested sample is diluted to a 1 % concentration with 0.9 % sodium chloride solution. The test is carried out by potentiometry, in accordance with the General Pharmacopoeia Monograph “Ionometry”.

Protein content. From 9.5 % to 16.0 %, depending on the pharmaceutical form. The test is carried out by colourimetry with the Biuret reagent, in accordance with the General Pharmacopoeia Monograph “Determination of protein”.

Electrophoretic homogeneity. The immunoglobulin fraction should constitute not less than 95 % of the total protein content. The test is carried out in accordance with the General Pharmacopoeia Monograph “Homogeneity testing for medicinal products containing human or animal serum by cellulose acetate electrophoresis”.

Molecular parameters. The content of monomers and dimers should be not less than 85 %, and the content of polymers and aggregates not more than 10 %. The test is carried out in accordance with the General Pharmacopoeia Monograph “HPLC determination of immunoglobulin molecular parameters”.

Fractional composition. The tested sample is diluted to a 1 % concentration with 0.9 % sodium chloride solution. An intensive IgG precipitation line should be observed, and there should be not more than four additional lines. The test is carried out by gel immunoelectrophoresis, using a serum against human serum

proteins in accordance with the General Pharmacopoeia Monograph “Agarose gel immunoelectrophoresis”.

Heat stability. The medicinal product should remain liquid and form no gel after being exposed to a temperature of (56 ± 1) °C for 4 hours in a water bath or water thermostat.

Stabilizers. The stabilizer(s) added to the medicinal product is / are quantified using the method(s) described in the General Pharmacopoeia Monograph “Gas chromatography” and / or in accordance with the General Pharmacopoeia Monograph “High-performance liquid chromatography (HPLC)” (unless a different method is specified in the Normative Document). Acceptable limits for the content of stabilizers should be specified in the Normative Document.

Sterility. The medicinal product is required to be sterile. The test is carried out in accordance with the General Pharmacopoeia Monograph “Sterility”.

Pyrogenicity or **Bacterial endotoxins.** The medicinal product is required to be non-pyrogenic or its content of bacterial endotoxins should be less than 5 EU/ml.

The test is carried out in accordance with the General Pharmacopoeia Monograph “Pyrogenicity” (the required test dose is 1.0 mL of the medicinal product per kilogramme of rabbit body weight) or in accordance with the General Pharmacopoeia Monograph “Bacterial endotoxins”, using the method described in the Normative Document.

Abnormal toxicity. The medicinal product is required to be non-toxic. The test is carried out in accordance with the General Pharmacopoeia Monograph “Abnormal toxicity”. The test is performed on five healthy white mice with a body weight in the range of 18 to 20 g and on two guinea-pigs with a body weight of 250 to 300 g. The test dose is 0.5 mL for white mice (intraperitoneally) and 5.0 mL for guinea-pigs (subcutaneously, into both sides of the body, 2.5 mL for each) (unless otherwise specified in the Normative Document). Experimental animals should be followed up for 7 days.

Antibody content (specific activity). The document should specify the quantity of antibacterial antibodies (against at least one pathogen) and antiviral antibodies (against at least one pathogen). The test is carried out in accordance with the method specified in the Normative Document (for instance, anti-measles antibody content is determined by the passive haemagglutination assay, and anti-alpha-staphylolysin content is found in the reaction neutralizing the haemolytic properties of staphylococcal alpha-toxin), using appropriate standard samples.

Anti-A and anti-B haemagglutinins (for the subcutaneous pharmaceutical form)**.** No agglutination should be observed at the 1:64 dilution of the medicinal product. The test is carried out in accordance with the General Pharmacopoeia Monograph "Determination of anti-A and anti-B haemagglutinins in medicinal products containing human immunoglobulins".

Anti-D antibodies (for the subcutaneous pharmaceutical form). The content of anti-D antibodies in the medicinal product should not exceed that of the positive standard sample. The test is carried out in accordance with the General Pharmacopoeia Monograph "Testing for anti-D antibodies in medicinal products containing human immunoglobulins".

Hepatitis B virus surface antigen (HBsAg). The medicinal product should contain no hepatitis B virus surface antigen. The test is carried out by enzyme immunoassay, using test systems approved for use in Russian health care practice and having a sensitivity not less than 0.1 IU/mL according to the Instructions for Use.

Anti-hepatitis C virus antibodies. The medicinal product should contain no anti-hepatitis C virus antibodies. The test is carried out by enzyme immunoassay, using test systems approved for use in Russian health care practice and having 100 % sensitivity and specificity according to the Instructions for Use.

Anti-human immunodeficiency virus (HIV-1 and HIV-2) antibodies and HIV-1 p24 antigen. The medicinal product should contain no anti-human immunodeficiency virus (HIV-1 and HIV-2) antibodies and no HIV-1 p24 antigen. The test is carried out by enzyme immunoassay, using test systems approved for

use in Russian health care practice and having 100 % sensitivity and specificity according to the Instructions for Use.

Packaging and Labeling. In accordance with the General Pharmacopoeia Monograph “Medicinal products containing human plasma”.

The secondary (consumer) package of the medicinal product should contain the following inscription: «Contains no antibodies to HIV-1, HIV-2, hepatitis C virus and no hepatitis B virus surface antigen».

Storage. In accordance with the General Pharmacopoeia Monograph “Immunobiological medicinal products”. The medicinal product should be stored away from light, in the temperature range of 2 to 8 °C, unless otherwise specified in the Normative Document. The medicinal product should not be frozen.