

# MINISTRY OF HEALTH OF THE RUSSIAN FEDERATION

## PHARMACOPOEIA MONOGRAPH

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**Normal human immunoglobulin  
for intravenous administration**  
Monograph 42-3159-95

**PM.3.3.2.0008.15**  
Replaces the Pharmacopoeia

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The present Pharmacopoeia Monograph applies to normal human immunoglobulin preparations for intravenous administration.

Normal human immunoglobulin for intravenous administration is an immunologically active protein fraction isolated from human plasma and containing a broad spectrum of antibodies; its principal active component is immunoglobulin G (Ig G), which exhibits activity against various antigens.

Normal human immunoglobulin preparations for intravenous administration 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 for intravenous administration is purified and concentrated using the Cohn ethanol fractionation method (cold water – alcohol precipitation). This method is based on physicochemical differences of various proteins and their different solubility in the presence of ethanol at low temperatures, different ionic strength, dielectric constants, and medium pH values,

using additional methods for chromatographic purification and advanced virus inactivation technology that guarantee viral safety of the medicinal product.

The manufacture of normal human immunoglobulin for intravenous administration should be performed in accordance with established requirements to guarantee preservation of the structure and function of immunoglobulin proteins responsible for the specific and viral safety of the medicinal product. Antibacterial and antiviral efficacy of normal human immunoglobulin preparations for intravenous administration should be guaranteed by appropriate antibody concentration achieved during the manufacturing process (at least three-fold for medicinal products with protein concentrations in the range of 4.5 % to 5.5 % and at least six-fold for medicinal products with protein concentrations in the range of 9.0 % to 11.0 %).

## TESTS

**Description.** A transparent or slightly opalescent, colourless or light yellow solution. Lyophilized products are an amorphous hygroscopic cake appearing as a tablet or white powder; a light yellow colour is acceptable (unless different requirements are included in the Normative Document). 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, in accordance with the General Pharmacopoeia Monograph “Gel immunodiffusion”. The test should produce precipitation lines only with the serum against human serum proteins.

**Dissolution time** (for lyophilized medicinal products). Not more than 20 minutes (unless otherwise specified in the Normative Document). A description of the method should be included, along with the solvent used, its volume, and the dissolution conditions (solvent temperature, need for mixing, etc.).

**Transparency.** A transparent or slightly opalescent solution (the test is carried out in accordance with the General Pharmacopoeia Monograph “Transparency and turbidity of liquids”) or a 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 “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.** The medicinal product should be a colourless or light yellow solution, with a colour intensity not exceeding that of the Reference Solution Y5 (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 0.05 (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.

**Weight loss on drying** (for lyophilized medicinal products). Not more than 3 %. Two simultaneous tests are performed on a 0.15 – 0.20 g weight of the tested sample. The test is carried out by gravimetry, in accordance with the General Pharmacopoeia Monograph “Weight loss on drying”.

If the obtained results are unsatisfactory, this control is repeated for twice as many specimens. If the repeat control test produces unsatisfactory results, the medicinal product should be discarded.

**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** (for liquid medicinal products). 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 4.0 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”. For dry pharmaceutical forms, the Normative Document should specify the name of the solvent and provide a description of the method used to reconstitute the medicinal product.

**Protein content.** From 4.5 % to 5.5 % or from 9.5 % to 11.0 %. 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”.

**Electrophoretic homogeneity.** The main IgG immunoglobulin fraction should constitute not less than 95 % of the total protein content (unless otherwise specified in the Normative Document). 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 immunoglobulin G monomers and dimers should be not less than 90 %, and the content of polymers and aggregates not more than 3 %. 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** (for liquid medicinal products). The medicinal product should remain liquid and form no gel after being exposed to a temperature of  $(56 \pm 1) ^\circ\text{C}$  for 4 hours in a water bath or water thermostat.

**Stabilizers.** The stabilizers added to the medicinal product are quantified using the methods 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 manufacturer’s Normative Document.

**Virus-inactivating agents.** The residual content of virus-inactivating agents in the medicinal product is quantified using the methods 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 virus-inactivating agents should be specified in the manufacturer’s Normative Document.

**Osmolality.** The osmolality value should be not less than 240 mOsmol/kg. The test is carried out in accordance with the General Pharmacopoeia Monograph “Osmolarity”.

**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 0.5 EU/mL (for medicinal products with a protein concentration not exceeding 50 g/L) or less than 1.0 EU/mL (for medicinal products with a protein concentration higher than 50 g/L).

The test is carried out in accordance with the General Pharmacopoeia Monograph “Pyrogenicity” (not less than 0.5 g of immunoglobulin protein per kilogramme of rabbit body weight; the administered volume of the medicinal product should not exceed 10 mL per kilogramme of rabbit body weight) or in accordance with the General Pharmacopoeia Monograph “Bacterial endotoxins”, using the method described in the Normative Document of the manufacturer.

**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 (intravenously) and 5.0 mL for guinea-pigs (subcutaneously, into both sides of the body, 2.5 mL for each). 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 methods 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.

#### **Specific safety**

**Anticomplementary activity.** The upper complement binding limit is 1 CH<sub>50</sub>/mg of protein, i. e. 1 mg of immunoglobulin protein should not bind more than 1 CH<sub>50</sub> complement. The test is carried out in accordance with the General Pharmacopoeia Monograph “Determination of the anticomplementary activity of human immunoglobulin-containing medicinal products for intravenous administration”.

**Anti-A and anti-B haemagglutinins.** 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.** 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”.

### **Viral safety**

**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.