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HUMAN NORMAL IMMUNOGLOBULIN FOR INTRAVENOUS ADMINISTRATION

Immunoglobulinum humanum normale ad usum intravenosum

DEFINITION

Human normal immunoglobulin for intravenous administration is a sterile liquid or freeze-dried preparation containing immunoglobulins, mainly immunoglobulin G (IgG). Other proteins may be present. Human normal immunoglobulin for intravenous administration contains the IgG antibodies of normal subjects. This monograph does not apply to products intentionally prepared to contain fragments or chemically modified IgG.

Human normal immunoglobulin for intravenous administration is obtained from plasma that complies with the requirements of the monograph *Human plasma for fractionation (0853)*. The preparation may contain excipients such as stabilisers.

PRODUCTION

The method of preparation includes a step or steps that have been shown to remove or to inactivate known agents of infection; if substances are used for inactivation of viruses, it shall have been shown that any residues present in the final product have no adverse effects on the patients treated with the immunoglobulin. The method of preparation also includes a step or steps that have been shown to remove thrombosis-generating agents. Emphasis is given to the identification of activated coagulation factors and their zymogens and process steps that may cause their activation. Consideration is also to be given to other procoagulant agents that could be introduced by the manufacturing process.

The product shall have been shown, by suitable tests in animals and evaluation during clinical trials, to be well tolerated when administered intravenously.

Human normal immunoglobulin for intravenous administration is prepared from pooled material from not fewer than 1000 donors by a method that has been shown to yield a product that:

- does not transmit infection;
- at an immunoglobulin concentration of 50 g/L, contains antibodies for at least 2 of which (1 viral and 1 bacterial) an International Standard or Reference Preparation is available, the concentration of such antibodies being at least 3 times that in the initial pooled material;
- has a defined distribution of immunoglobulin G subclasses;
- complies with the test for Fc function of immunoglobulin (*2.7.9*);
- does not exhibit thrombogenic (procoagulant) activity.

Human normal immunoglobulin for intravenous administration is prepared as a stabilised solution or as a freeze-dried preparation. In both cases the preparation is passed through a bacteria-retentive filter. The preparation may subsequently be freeze-dried and the

containers closed under vacuum or under an inert gas. No antibiotic is added to the plasma used. No antimicrobial preservative is added either during fractionation or at the stage of the final bulk solution.

The stability of the preparation is demonstrated by suitable tests carried out during development studies.

CHARACTERS

Appearance:

– *liquid preparation*: clear or slightly opalescent and colourless or pale yellow liquid;
– *freeze-dried preparation*: hygroscopic, white or slightly yellow powder or solid friable mass.

For the freeze-dried preparation, reconstitute as stated on the label immediately before carrying out the identification and the tests, except those for solubility and water.

IDENTIFICATION

Examine by a suitable immunoelectrophoresis technique. Using antiserum to normal human serum, compare normal human serum and the preparation to be examined, both diluted to contain 10 g/L of protein. The main component of the preparation to be examined corresponds to the IgG component of normal human serum. The preparation to be examined may show the presence of small quantities of other plasma proteins; if human albumin has been added as a stabiliser, it may be seen as a major component.

TESTS

Solubility. For the freeze-dried preparation, add to the container the volume of the liquid stated on the label at the recommended temperature. The preparation dissolves completely within 30 min at 20-25 °C.

pH (*2.2.3*): 4.0 to 7.4.

Dilute the preparation to be examined with a 9 g/L solution of *sodium chloride R* to obtain a solution containing 10 g/L of protein.

Osmolality (*2.2.35*): minimum 240 mosmol/kg.

Total protein. The preparation contains not less than 30 g/L and between 90 per cent and 110 per cent of the quantity of protein stated on the label.

Dilute the preparation to be examined with a 9 g/L solution of *sodium chloride R* to obtain a solution containing about 15 mg of protein in 2 mL. To 2.0 mL of this solution in a round-bottomed centrifuge tube add 2 mL of a 75 g/L solution of *sodium molybdate R* and 2 mL of a mixture of 1 volume of *nitrogen-free sulfuric acid R* and 30 volumes of *water R*. Shake, centrifuge for 5 min, decant the supernatant liquid and allow the inverted tube to drain on filter paper. Determine the nitrogen in the centrifugation residue by the method of sulfuric acid digestion (*2.5.9*) and calculate the content of protein by multiplying the result by 6.25.

Protein composition. Zone electrophoresis (*2.2.31*).

Use strips of suitable cellulose acetate gel or suitable agarose gel as the supporting medium and *barbital buffer solution pH 8.6 R1* as the electrolyte solution.

If cellulose acetate is the supporting material, the method described below can be used. If agarose gels are used, and because they are normally part of an automated system, the manufacturer's instructions are followed instead.

Test solution. Dilute the preparation to be examined with a 9 g/L solution of *sodium chloride R* to an immunoglobulin concentration of 30 g/L.

Reference solution. Reconstitute *human immunoglobulin for electrophoresis BRP* and dilute with a 9 g/L solution of *sodium chloride R* to a protein concentration of 30 g/L.

To a strip apply 4.0 µL of the test solution as a 10 mm band or apply 0.4 µL per millimetre if a narrower strip is used. To another strip apply in the same manner the same volume of the reference solution. Apply a suitable electric field such that the albumin band of normal human serum applied on a control strip migrates at least 30 mm. Stain the strips with *amido black 10B solution R* for 5 min. Decolourise with a mixture of 10 volumes of *glacial acetic acid R* and 90 volumes of *methanol R* so that the background is just free of colour. Develop the transparency of the strips with a mixture of 19 volumes of *glacial acetic acid R* and 81 volumes of *methanol R*. Measure the absorbance of the bands at 600 nm in an instrument having a linear response over the range of measurement. Calculate the result as the mean of 3 measurements of each strip.

System suitability: in the electropherogram obtained with the reference solution, the proportion of protein in the principal band is within the limits stated in the leaflet accompanying the reference preparation.

Results: in the electropherogram obtained with the test solution, not more than 5 per cent of protein has a mobility different from that of the principal band. This limit is not applicable if albumin has been added to the preparation as a stabiliser; for such preparations, a test for protein composition is carried out during manufacture before addition of the stabiliser.

Molecular size distribution. Size exclusion chromatography (*2.2.30*).

Test solution. Dilute the preparation to be examined with a 9 g/L solution of *sodium chloride R* to a concentration suitable for the chromatographic system used. A concentration in the range of 4-12 g/L and injection of 50-600 µg of protein are usually suitable.

Reference solution. Dilute *human immunoglobulin (molecular size) BRP* with a 9 g/L solution of *sodium chloride R* to the same protein concentration as the test solution.

Column:

- *size*: *l* = 0.6 m, Ø = 7.5 mm, or *l* = 0.3 m, Ø = 7.8 mm;
- *stationary phase*: *hydrophilic silica gel for chromatography R*(1) of a grade suitable for fractionation of globular proteins with relative molecular masses in the range 10 000 to 500 000.

Mobile phase: dissolve 4.873 g of *disodium hydrogen phosphate dihydrate R*, 1.741 g of *sodium dihydrogen phosphate monohydrate R*, 11.688 g of *sodium chloride R* and 50 mg of *sodium azide R* in 1 L of *water R*.

Flow rate: 0.5 mL/min.

Detection: spectrophotometer at 280 nm.

(1) TSK G3000 SW (*l* = 0.6 m, Ø = 7.5 mm) and TSK G3000 SW_{XL} (*l* = 0.3 m, Ø = 7.8 mm) are suitable.

Identification of peaks: in the chromatogram obtained with the reference solution, the principal peak corresponds to the IgG monomer and there is a peak corresponding to the dimer with a relative retention to the principal peak of about 0.85; identify the peaks in the chromatogram obtained with the test solution by comparison with the chromatogram obtained with the reference solution; any peak with a retention time shorter than that of the dimer corresponds to polymers and aggregates.

Results: in the chromatogram obtained with the test solution:

– *retention time*: for the monomer and for the dimer, the retention time relative to the corresponding peak in the chromatogram obtained with the reference solution is 1 ± 0.02;

– *peak area*: the sum of the peak areas of the monomer and the dimer represent not less than 90 per cent of the total area of the chromatogram and the sum of the peak areas of polymers and aggregates represents not more than 3 per cent of the total area of the chromatogram. This requirement does not apply to products where albumin has been added as a stabiliser; for products stabilised with albumin, a test for distribution of molecular size is carried out during manufacture before addition of the stabiliser.

Anticomplementary activity (*2.6.17*). The consumption of complement is not greater than 50 per cent (1 CH_{50} per milligram of immunoglobulin).

Prekallikrein activator (*2.6.15*): maximum 35 IU/mL, calculated with reference to a dilution of the preparation to be examined containing 30 g/L of immunoglobulin.

Anti-A and anti-B haemagglutinins (*2.6.20, method B*). It complies with the test for anti-A and anti-B haemagglutinins (direct method).

Anti-D antibodies (*2.6.26*). It complies with the test for anti-D antibodies in human immunoglobulin.

Antibody to hepatitis B surface antigen: minimum 0.5 IU per gram of immunoglobulin, determined by a suitable immunochemical method (*2.7.1*).

Immunoglobulin A. As determined by a suitable immunochemical method (*2.7.1*), the content of immunoglobulin A is not greater than the maximum content stated on the label.

Water. Determined by a suitable method, such as the semi-micro determination of water (*2.5.12*), loss on drying (*2.2.32*) or near-infrared spectrophotometry (*2.2.40*), the water content is within the limits approved by the competent authority.

Sterility (*2.6.1*). It complies with the test.

Pyrogens (*2.6.8*) **or Bacterial endotoxins** (*2.6.14*). It complies with the test for pyrogens or, preferably and where justified and authorised, with a validated *in vitro* test such as the bacterial endotoxin test.

For the pyrogen test, inject per kilogram of the rabbit's mass a volume equivalent to 0.5 g of immunoglobulin, but not more than 10 mL per kilogram of the rabbit's mass.

Where the bacterial endotoxin test is used, the preparation to be examined contains less than 0.5 IU of endotoxin per millilitre for solutions with a protein content not greater than 50 g/L, and less than 1.0 IU of endotoxin per millilitre for solutions with a protein content greater than 50 g/L but not greater than 100 g/L.

STORAGE

Liquid preparation: in a colourless glass container, protected from light, at the temperature stated on the label.

Freeze-dried preparation: in an airtight colourless glass container, protected from light, at a temperature not exceeding 25 °C.

LABELLING

The label states:

- for liquid preparations, the volume of the preparation in the container and the protein content expressed in grams per litre;
- for freeze-dried preparations, the quantity of protein in the container;
- the amount of immunoglobulin in the container;
- the route of administration;
- for freeze-dried preparations, the name or composition and the volume of the reconstituting liquid to be added;
- the distribution of subclasses of immunoglobulin G present in the preparation;
- where applicable, the amount of albumin added as a stabiliser;
- the maximum content of immunoglobulin A.