

equivalence in International Units of the International Reference Preparation is stated by the World Health Organisation.

Prepare serial two-fold dilutions of the immunoglobulin to be examined and of the reference preparation. Mix each dilution with an equal volume of a suspension of measles virus containing about 100 CCID₅₀ in 0.1 ml and incubate protected from light at 37 °C for 2 h. Using not fewer than six cell cultures per mixture, inoculate 0.2 ml of each mixture into each of the cell cultures allocated to that mixture and incubate for not less than 10 days. Examine the cultures for viral activity and compare the dilution containing the smallest quantity of the immunoglobulin which neutralises the virus with that of the corresponding dilution of the reference preparation.

Calculate the potency of the immunoglobulin to be examined in International Units per millilitre of neutralising antibody against measles virus.

STORAGE

See *Human normal immunoglobulin (0338)*.

LABELLING

See *Human normal immunoglobulin (0338)*.

The label states the number of International Units per container.

01/2008:0338

HUMAN NORMAL IMMUNOGLOBULIN

Immunoglobulinum humanum normale

[232-706-1]

DEFINITION

Human normal immunoglobulin is a liquid or freeze-dried preparation containing immunoglobulins, mainly immunoglobulin G (IgG). Other proteins may be present. Human normal immunoglobulin contains the IgG antibodies of normal subjects. It is intended for intramuscular injection.

Human normal immunoglobulin is obtained from plasma that complies with the requirements of the monograph on *Human plasma for fractionation (0853)*. No antibiotic is added to the plasma used.

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

Human normal immunoglobulin is prepared from pooled material from at least 1000 donors by a method that has been shown to yield a product that:

- does not transmit infection;
- at a protein concentration of 160 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 10 times that in the initial pooled material.

Human normal immunoglobulin is prepared as a stabilised solution, for example in a 9 g/l solution of sodium chloride, a 22.5 g/l solution of glycine or, if the preparation is to be freeze-dried, a 60 g/l solution of glycine. Multidose preparations contain an antimicrobial preservative.

Single-dose preparations do not contain an antimicrobial preservative. Any antimicrobial preservative or stabilising agent used shall have been shown to have no deleterious effect on the final product in the amount present. The solution 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.

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

CHARACTERS

The liquid preparation is clear and pale-yellow or light-brown; during storage it may show formation of slight turbidity or a small amount of particulate matter. The freeze-dried preparation is a 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 solution may show the presence of small quantities of other plasma proteins.

TESTS

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

pH (2.2.3): 5.0 to 7.2.

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.

Total protein. 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 sulphuric 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 residue by the method of sulphuric acid digestion (2.5.9) and calculate the content of protein by multiplying the result by 6.25. The preparation contains not less than 100 g/l and not more than 180 g/l of protein and not less than 90 per cent and not more than 110 per cent of the quantity of protein stated on the label.

Protein composition. Examine by 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 a protein concentration of 50 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 50 g/l.

To a strip apply 2.5 µl of the test solution as a 10 mm band or apply 0.25 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 strip 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 on cellulose acetate or on agarose gels, 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 on cellulose acetate or on agarose gels, not more than 10 per cent of protein has a mobility different from that of the principal band.

Distribution of molecular size. Liquid chromatography (2.2.29).

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, $\varnothing = 7.5$ mm; or $l = 0.3$ m, $\varnothing = 7.8$ mm;
- stationary phase: *hydrophilic silica gel for chromatography R*, 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 litre of *water R*.

Flow rate: 0.5 ml/min.

Detection: spectrophotometer at 280 nm.

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. The preparation to be examined complies with the test if, in the chromatogram obtained with the test solution:

- **relative retention:** for the monomer and for the dimer, the relative retention 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 85 per cent of the total area of the chromatogram and the sum of the peak areas of polymers and aggregates represents not more than 10 per cent of the total area of the chromatogram.

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 for sterility.

Pyrogens (2.6.8). It complies with the test for pyrogens. Inject 1 ml per kilogram of the rabbit's mass.

Antibody to hepatitis B surface antigen. Not less than 0.5 IU/g of immunoglobulin, determined by a suitable immunochemical method (2.7.1).

Antibody to hepatitis A virus. If intended for use in the prophylaxis of hepatitis A, it complies with the following additional requirement. Determine the antibody content by comparison with a reference preparation calibrated in International Units, using an immunoassay of suitable sensitivity and specificity (2.7.1).

The International Unit is the activity contained in a stated amount of the International Standard for anti-hepatitis A immunoglobulin. The equivalence in International Units of the International Standard is stated by the World Health Organisation.

Human hepatitis A immunoglobulin BRP is calibrated in International Units by comparison with the International Standard.

The stated potency is not less than 100 IU/ml. The estimated potency is not less than the stated potency. The confidence limits ($P = 0.95$) of the estimated potency are not less than 80 per cent and not more than 125 per cent.

STORAGE

For the liquid preparation, in a colourless glass container, protected from light. For the freeze-dried preparation, in an airtight colourless glass container, protected from light.

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 route of administration;
- for freeze-dried preparations, the name or composition and the volume of the reconstituting liquid to be added;

- where applicable, that the preparation is suitable for use in the prophylaxis of hepatitis A infection;
- where applicable, the anti-hepatitis A virus activity in International Units per millilitre;
- where applicable, the name and amount of antimicrobial preservative in the preparation.

01/2008:0918

HUMAN NORMAL IMMUNOGLOBULIN FOR INTRAVENOUS ADMINISTRATION

Immunoglobulinum humanum normale ad usum intravenosum

DEFINITION

Human normal immunoglobulin for intravenous administration is a 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 on *Human plasma for fractionation (0853)*. No antibiotic is added to the plasma used.

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 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).

Human normal immunoglobulin for intravenous administration is prepared as a stabilised solution or as a freeze-dried preparation. A stabiliser may be added. 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 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

The liquid preparation is clear or slightly opalescent and colourless or pale yellow. The freeze-dried preparation is a 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 the volume of the liquid stated on the label. 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: minimum 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 sulphuric 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 sulphuric 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