Human normal immunoglobulin for intravenous administration

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.

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 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 (one viral and one 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. 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. The preparation contains not less than 30 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), using strips of suitable cellulose acetate gel as the supporting medium and barbital buffer solution pH 8.6 R1 as the electrolyte solution.

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. 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. The test is not valid unless, in the electropherogram obtained with the reference preparation, the proportion of protein in the principal band is within the limits stated in the leaflet accompanying the reference preparation.

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 4 g/l to 12 g/l and injection of 50 µg to 600 µg of protein are usually suitable.

Reference solution. Dilute human immunoglobulin 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,
- 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 µg 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 IgG monomer and there is a peak corresponding to 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 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 monomer and 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 monomer and dimer represent not less than 90 per cent of the total area of the chromatogram and the sum of the peak area 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 CH50 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). Carry out the tests for anti-A and anti-B haemagglutinins. If the preparation to be examined contains more than 30 g/l of immunoglobulin, dilute to this concentration before preparing the dilutions to be used in the test. The 1:64 dilutions do not show agglutination.

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 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 body mass.

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

STORAGE
For the liquid preparation, store in a colourless glass container, protected from light, at the temperature stated on the label. For the freeze-dried preparation, store 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.