HUMAN NORMAL IMMUNOGLOBULIN FOR INTRAVENOUS USE

Human Normal Immunoglobulin for Intravenous Administration

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 plasma for fractionation. 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 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 1,000 donors by a method that has been shown to yield a product that (a) does not transmit infection; (b) at an immunoglobulin concentration of 50g per litre, 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;(c) has a defined distribution of immunoglobulin G subclasses; (d) complies with the test for Fc function of immunoglobulin.

Test for Fc function of immunoglobulin

The Fc function for the preparation under the examination, its value is not less than that stated in the leaflet accompanying the reference preparation.

Human Normal Immunoglobulin for Intravenous Administration is prepared as a stabilized solution or as a freeze-dried preparation. A stabilizer 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.

[Detail already given in the monograph to be retained]

Description

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

A. Examine by a suitable immunoelectrophoresis technique. Using anti serum to normal human serum compare normal human serum and the preparation under examination both diluted to contain 1% w/v of protein. The main component of the preparation under examination
corresponds to the IgG component of normal serum. The preparation under examination may show the presence of small quantities of other plasma proteins. If human albumin has been added as a stabilizer it may be seen as a major component.

OR

B. Double Immuno Diffusion:

Precipitation test with a suitable range of species specific antisera which gives positive results for the presence of protein of human origin and negative results with antisera specific to plasma protein for other species

Tests

pH (2.4.24): 4.0 to 7.4

Dilute the preparation under examination with a 0.9% w/v solution of sodium chloride to obtain a solution containing 1.0% of protein.

Osmolality (2.4.23): minimum 240 mosmol per kg.

Total protein:

Minimum 3% w/v and between 90 -110% of the quantity of protein stated on the label.

Dilute the preparation under the examination with a 0.9% solution of sodium chloride to obtain a solution containing about 15 mg of protein in 2 mL. Take 2 mL of the solution in a round bottom centrifuge tube. Add 2 mL of 7.5% w/v solution of sodium molybdate and 2 mL of mixture of 30 volumes of water and 1 volume of nitrogen free sulphuric acid.

Shake, centrifuge for 5 mins. Decant the supernatant liquid and let the inverted tube stand on a filter paper to drain the fluid. Determine the nitrogen in the centrifugation residue by the method of sulphuric acid digestion (2.3.30) and calculate the content of the protein by multiplying the result by 6.25.

Protein composition

Determine by zone electrophoresis (2.4.12)

Use strips of suitable cellulose acetate gel as the supporting medium and barbital buffer pH8.6 as the electrolyte solution.

Test solution:

Dilute the preparation under examination with a 0.9% w/v solution of sodium chloride to an immunoglobulin concentration of 3.0% w/v.

Reference solution:

Reconstitute human immunoglobulin for electrophoresis reference preparation and dilute with a 0.9% w/v solution of sodium chloride to a protein concentration of 3.0% w/v.

To a strip apply 4 µL of the test solution as a10mm band or apply 0.4 µL per mm 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 30mm. stain the strips with amido black 10B solution for 5 minutes. Decolorize with a mixture of 10 volumes of glacial acetic acid and 90 volumes of methanol 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 and 81 volumes of methanol. 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 preparation, the proportion of protein in the principal band is within the limits stated in the leaflet accompanying the reference preparation.

Results: In electropherogram obtained with the tests solution, not more than 5.0% of protein has mobility different from that of the principal band. This limit is not applicable if albumin has been added to the preparation as a stabilizer; for such preparations, a test for proteins compositions is
carried out during manufacture before addition of the stabilizer.

**Molecular size**

Determine by liquid chromatography (2.4.14).

**Test solution:** Dilute the preparation under examination with a 0.9% w/v solution of sodium chloride to obtain a concentration in the range of 0.4 to 1.2% w/v and injection of 50-600 µg of protein are usually suitable.

**Reference solution:** Dilute human immunoglobulin RS with a 0.9% w/v solution of sodium chloride to the same protein concentration as the test solution.

Chromatographic system
- A stainless steel column 60cm X 7.5mm or 30cm X 7.8mm packed with hydrophilic silica.
- **Mobile phase:** Dissolve 4.873 g of disodium hydrogen phosphate dehydrate, 1.741 g of sodium dihydrogen phosphate monohydrate, 11.688 g of sodium chloride and 50 mg of sodium azide in 1000 ml of water.
- **Flow rate:** 0.5 ml per minute,
- Spectrophotometer set 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 a chromatogram obtained with the reference solution; any peak with a retention time shorter than that a dimer corresponds to polymers and aggregates.

**Results:**
In the chromatogram obtained with the test solution:
(a) **relative retention:** for monomer and dimer, the relative retention to the corresponding peak to the chromatogram obtained with the reference solution is 1±0.02;
(b) **peak area:** the sum of the peak areas of monomer and dimer represent not less than 90.0% of the total area of the chromatogram and the sum of the peak area of polymers and aggregates represents not more than 3.0% of the total area of the chromatogram. This requirement does not apply to products where albumin has been added as a stabilizer; for products stabilized with albumin, a test for distribution of molecular size is carried out during manufacture before addition of the stabilizer.

**Anticomplementary activity**

The consumption of complement is not greater than 50% (1 CH₅₀ per milligram of immunoglobulin).

[Detail already given in the monograph to be retained]

**Prekallikrein activator:**
Maximum 35 IU/ml calculated with the reference to a dilution of the preparation to be examined containing 30 g/l of immunoglobulin.

[Detail already given in the monograph to be retained]

**Anti-A and anti-B haemagglutinins:**

Carry out the tests for anti-A and anti-B haemagglutinins as stated in the monograph for Dried human haemophilic fraction. If the preparation to be examined contains more than 30 g/l of immunoglobulin, dilute to this concentration before preparing the dilution to be used in the test. The 1 to 64 dilutions do not show agglutination.

**Anti-D antibodies**
It complies with the test for anti-D antibodies in human immunoglobulin for intravenous administration.

[Detail already given in the monograph to be retained]
Antibody to hepatitis B surface antigen

Minimum 0.5 IU/g of immunoglobulin, determine by a suitable immunochemical method.

Immunoglobulin A

As determined by a suitable immunochemical method, the content of the 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 (2.3.43), loss on drying (2.4.19) or near infrared spectrophotometry (2.4.6), the water content is within the limits approved by the competent authority.

Sterility (2.2.11)

It complies with the test for sterility.

Pyrogens (2.2.8) / or Bacterial Endotoxin (BET) (2.2.3)

It complies with the test for Pyrogens or, preferably and where justified and authorized with a validated in-vitro test such as the Bacterial Endotoxin test.

Pyrogens:

Complies with the test for pyrogens. Inject per kg of the rabbit’s mass a volume equivalent to 0.5 g of immunoglobulin but not more than 10 ml/kg of body mass.

Bacterial Endotoxin- BET:

Where the bacterial endotoxin test is used, the product contains less than 0.5 IU of endotoxin per milliliter for solution with protein content not greater than 50 g/L, and less than 1 IU of endotoxin per milliliter for solutions with the protein content greater than 50 g/L but not greater than 100 g/L.

STORAGE

For the liquid preparation, store in a colorless glass container, protected from light, at the temperature stated on the label. For the freeze-dried preparation, store in an airtight colorless glass container, protected from light, at a temperature not exceeding 25º C.

LABELLING

The label states:

- for liquid preparation, the volume of the preparation in the container and the protein content expressed in g/l.
- for freeze-dried preparation, the quantity of protein in the container.
- The amount of immunoglobulin in the container.
- The route of administration.
- For freeze-dried preparation, the name or composition and the volume of the reconstituting liquid to be added.
- The distribution of subclasses of IgG present in the preparation.
- Where applicable, the amount of albumin added as a stabilizer.
- The maximum content of IgA.
-Recommended storage temperature