HUMAN NORMAL IMMUNOGLOBULIN (IM)

DEFINITION

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

Human Normal Immunoglobulin 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 a protein concentration of 160 g 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 10 times that in the initial pooled material.

Human Normal Immunoglobulin is prepared as a stabilized 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. Multi-dose preparation contains an antimicrobial preservative.

Single dose preparation do not contain an antimicrobial preservative. Any antimicrobial preservative or stabilizing 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 test carried during development studies.

If the human normal immunoglobulin is intended for subcutaneous administration, the production method shall have been shown to consistently yield products that comply with the test for Fc function (as detailed in the test for Fc function in the monograph for Human normal immunoglobulin for intravenous use) of immunoglobulin.

DESCRIPTION

The liquid preparation is clear and colourless or 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

A. Examine by a suitable immuno-electrophoresis 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 the protein of human origin and negative results with antisera specific to plasma protein for other species.

TESTS

Solubility:

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

pH (2.4.24): 5.0 – 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.

Assay for total protein:

Human normal immunoglobulin contains not less than 90% and not more than 110% of the quantity of protein stated on the label and in any case not less than 10% w/v and not more than 18% w/v of protein.

Dilute a suitable volume with water to produce a solution containing 1% w/v of protein. Take 1.5 ml of the dilution in a round bottom centrifuge tube. Add 5 ml of water, mix add 0.2 ml of 7.5% w/v solution of sodium molybdate and 2 ml of mixture consisting of 1 volume nitrogen-free sulphuric acid and 30 volumes of water. Shake, centrifuge for 5 minutes, decant the supernatant liquid and allow the inverted tube to drain on filter paper. To the residue in the tube add 3 drops of 30% w/v solution of copper sulphate and 1 ml of nitrogen-free sulphuric acid and boil gently for 10 minutes; cool, add 1 g of anhydrous sodium sulphate and 10 mg of selenium, boil gently for 1 hour and cool. Transfer to an ammonia distillation apparatus, add 6 ml of a saturated solution of sodium hydroxide and pass steam through the flask; distil for seven minutes, collecting the distillate in a mixture of 5 ml of a saturated solution of boric acid, 5 ml of water, and 1 drop saturated solution of methyl red in alcohol containing 0.1% of methylene blue and titrate with 0.02M hydrochloric acid.

Protein composition

Determine by zone electrophoresis ((2.4.12)

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

Test solution:

Dilute the preparation under examination 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 a10mm band or apply 0.25 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 R for 5 minutes. Decolorize 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 electropherogram obtained with the tests solution on cellulose acetate or on agarose gels, not more than 10% of protein has mobility different from that of the principal band.

Molecular size

Determine by liquid chromatography (2.4.16).

Test solution:

Dilute the preparation under examination 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.

Chromatographic system

- Size: 60cm X 7.5mm or 30cm X 7.8mm.
- Stationary phase: Hydrophilic silica gel for chromatography R, of a grade suitable for fractionation of globular proteins with relative molecular masses in the range 10,000-500,000.
- 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 1000 ml of water R.
- 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 85.0% of the total area of the chromatogram and the sum of the peak area of polymers and aggregates represents not more than 10% of the total area of the chromatogram.

Loss on drying (2.4.19): Not more than 2%, determined on 0.5 g, by drying over
phosphorous pentoxide at a pressure not exceeding 3 pa for 24 hours

**Sterility** (2.2.11)

It complies with the test for sterility.

**Pyrogen** (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.

**Pyrogen**

It complies with the test of pyrogens, using 1 ml of the preparation under examination per kilogram of the rabbit’s weight.

**Bacterial Endotoxin-BET:**

Where the bacterial endotoxin test is used, the product contains less than 5 IU of endotoxin per milliliter.

**Abnormal Toxicity** (2.2.1) –

Complies the test for abnormal toxicity, Method A, injecting 0.5 ml into each mouse and 5 ml into each guinea-pig.

**Antibody to hepatitis B surface antigen**

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

**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. 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 WHO. *Human hepatitis A immunoglobulin BRP* is calibrated in International Units by comparison with the International Standards. 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% and not more than 125%.

**Tests only for human normal immunoglobulin for subcutaneous administration:**

**Anti-A and Anti-B haemagglutinin:**

If Human normal Immunoglobulin is intended for subcutaneous administration, it complies with the test (as stated in the monograph for Dried human hemophilic fraction) for anti-A and anti-B haemagglutinin.

**Anti-D antibodies:**

If Human normal Immunoglobulin is intended for subcutaneous administration, it complies with the test for anti-D antibodies (as stated in the monograph for human normal immunoglobulin intravenous) in human immunoglobulin

**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.

**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.