

14.5 *Control tests on the final product*

The tests specified below shall be performed on representative samples from every filling lot. If the product is processed further after filling, e.g. by freeze-drying, the tests shall be performed on samples from each drying chamber.

14.5.1 *Identity test*

An identity test shall be performed on at least one labelled container from each filling lot to verify that the preparation is of human origin. The test shall be one approved by the national control authority. Additional tests shall be made to determine that the protein is predominantly albumin or plasma protein fraction as appropriate. The tests mentioned in section 14.3.2 shall be used.

14.5.2 *Protein concentration and purity*

The protein concentration and purity of each filling lot shall be within the limits prescribed in section 14.3.2.

Tests to determine the concentration of additives (such as polyethylene glycol, porcine enzymes and reducing and alkylating agents) used during production shall be carried out if required by the national control authority.

14.5.3 *Sterility test*

Each filling lot shall be tested for sterility. Part A, section 5, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (9, p.48) shall apply. Samples for sterility testing shall be taken from final containers selected at random after heating at 60 °C for 10-11 h.

In one country, the sterility test is carried out at least 10 days after heating at 60 °C for 10 h. In some countries, the sterility test is carried out both before and after heating at 60 °C for 10 h.

14.5.4 *General safety test*

In some countries a general safety test may be required, whereby each filling lot is tested for extraneous toxic contaminants by appropriate tests involving injection into mice and guinea-pigs. The injection shall cause neither significant untoward reactions nor death within an observation period of seven days. The tests shall be approved by the national control authority.

The tests generally used are the intraperitoneal injection of 0.5 ml into each of at least two mice weighing approximately 20 g and the injection of 5.0 ml into each of at least two guinea-pigs weighing approximately 350 g. In some countries, if one of the animals dies or shows signs of ill-health, such as weight loss, during a specified period, the test is repeated. The substance passes the test if none of the animals of the second group dies or shows signs of ill-health, such as weight loss, during that period.

14.5.5 Freedom from pyrogenicity

Each filling lot shall be tested for pyrogenicity by the intravenous injection of the test dose into three or more rabbits that have not previously received blood products. In general, the dose shall be at least equivalent proportionally, on a rabbit body-weight basis, to the maximum single human dose recommended, but not more than 10 ml/kg of body weight. For albumin at concentrations of 200 g/l and 250 g/l, the test dose for each rabbit shall be at least 3 ml/kg of body weight, and for albumin at concentrations of 35 g/l and 50 g/l and plasma protein fraction, 10 ml/kg of body weight.

A filling lot shall pass the test if it satisfies the requirements specified by the national control authority.

14.5.6 Moisture content

The residual moisture content shall, where appropriate, be determined by a method approved by the national control authority.

The methods in use are: (a) drying over phosphorus pentoxide for at least 24 h at a pressure not exceeding 2.7 Pa (0.02 mmHg); and (b) the Karl Fischer method.

The acceptable moisture content shall be determined by the national control authority.

14.5.7 Prekallikrein activator

An assay shall be performed for prekallikrein activator. The product shall contain not more than 35 IU of prekallikrein activator per ml.

14.5.8 Hydrogen ion concentration

The final product, reconstituted if necessary and diluted with 0.15 mol/l sodium chloride to give a protein concentration of 10 g/l, shall, when measured at a temperature of 20-27 °C, have a pH of 6.9 ± 0.5 (albumin) or 7.0 ± 0.3 (plasma protein fraction).

In some countries, different ranges of pH values are permitted.

14.5.9 Absorbance

A sample taken from the final solutions of albumin and plasma protein fraction, when diluted with water to a concentration of 10 g/l of protein and placed in a cell with a 1-cm light path, shall have an absorbance not exceeding 0.25 when measured in a spectrophotometer set at 403 nm.

14.5.10 Inspection of filled containers

All final containers shall be inspected for abnormalities, such as non-uniform colour, turbidity, microbial contamination and the presence of atypical particles, after storage at 20-35 °C for at least 14 days following heat treatment at 60 °C for 10 h. Containers showing abnormalities shall not be distributed.

The normal colour of albumin solutions may range from colourless to yellow or green to brown.

When turbidity or non-uniform colour raises the possibility of microbial contamination, testing should be done to isolate and identify the microorganisms.

14.6 **Records**

The requirements of Good Manufacturing Practices for Biological Products (8, pages 27-28) shall apply.

14.7 **Samples**

The requirements of Good Manufacturing Practices for Biological Products (8, page 29, paragraph 9.5) shall apply.

14.8 **Labelling**

The requirements of Good Manufacturing Practices for Biological Products (8, pages 26-27) and the national control authority's requirements for parenteral solutions shall apply.

In addition, the label on the container should state:

- the type of source material,
- the protein concentration,
- the oncotic equivalent in terms of plasma,
- that preservatives are absent
- the warning "Do not use if turbid",
- the sodium and potassium concentrations.

14.9 **Distribution and shipping**

The requirements of Good Manufacturing Practices for Biological Products (8) shall apply.

14.10 **Storage and shelf-life**

The requirements of Good Manufacturing Practices for Biological Products (8, pages 26-27) shall apply.

Final containers of albumin solution shall have a maximum shelf-life of three years if they are stored at or below 30 °C, and of five years if they are stored at 5 ± 3 °C.

Other storage conditions and shelf-lives may be approved by the national control authority.

Final containers of plasma protein fraction solution shall have a maximum shelf-life of three years if they are stored at or below 30 °C, and of five years if they are stored at 5 ± 3 °C.

Other storage conditions and shelf-lives may be approved by the national control authority.

15. **Control of immunoglobulins**

The final bulk solution of normal immunoglobulin shall be made from material from at least 1000 donors. If normal immunoglobulin is to be used for preventing or treating a particular infection, the titre of specific antibody should be measured.

For normal immunoglobulins, a large number of donors are needed if the final product is to contain adequate amounts of the various desired antibodies.

For specific immunoglobulins, whether intended for intravenous or intramuscular injection, the number of donors represented is less important because the requirement for specific antibody in the final product will be defined.

The immunoglobulin concentration in the final bulk of normal and specific immunoglobulin preparations for intramuscular use shall be 100-180 g/l. Concentrations lower than 100 g/l shall require the approval of the national control authority.

The immunoglobulin concentration in the final bulk of intravenous immunoglobulin shall be at least 30 g/l. If, in a specific immunoglobulin preparation, the concentration is lower than 30 g/l, it shall require the approval of the national control authority.

The immunoglobulin preparation shall be composed of not less than 90% of immunoglobulin, as determined by a method approved by the national control authority.

Tests shall be conducted on each filling lot of immunoglobulin solution to determine the proportion of aggregated and fragmented immunoglobulin. The recommended distribution shall be that at least 90% of the protein, other than proteins added as stabilizers to intravenous immunoglobulins, shall have the molecular size of immunoglobulin monomer and dimer. Not more than 10% shall consist of split products together with aggregates (oligomers of relative molecular mass equal to or greater than that of immunoglobulin trimer). This requirement shall not apply to products deliberately fragmented. The tests and limits shall be approved by the national control authority. Of the material having the molecular size of immunoglobulin monomer and dimer, most will consist of monomer. If a minimum level of monomer *per se* is to be established, the time and temperature at which samples must be incubated before analysis shall be specified.

Gel-permeation chromatography and high-performance exclusion chromatography are useful techniques for determining molecular size distribution and can be standardized for making these measurements.

For intravenous immunoglobulin, the following tests shall be performed on a sample from each filling lot:

- A test for hypotensive activity.

An appropriate test is that for prekallikrein activator content. In some countries the kallikrein test is also used.

- **A test for anticomplement activity.**

Several methods are available. The test method used and the maximum level of anticomplement activity permitted should be approved by the national control authority.

- **A test for haemagglutinins by the antiglobulin (Coombs) technique.**

In such tests, group OD(Rh₀)-positive cells should be used to test for anti-D (anti-Rh₀); group A and group B D(Rh₀)-negative cells should be used for anti-A and anti-B, respectively.

The purpose of the test is to ensure that the use of the product will not give rise to haemolytic reactions. The upper limit of activity should be specified by the national control authority.

15.1 **Potency of normal immunoglobulins**

A 160 g/l solution of normal immunoglobulin shall be prepared from final bulk solution by a method that has been shown to be capable of concentrating, by a factor of 10 from source material, at least two different antibodies, one viral and one bacterial, for which an international standard or reference preparation is available (16) (e.g. antibodies against poliomyelitis virus, measles virus, streptolysin O, diphtheria toxin, tetanus toxin, staphylococcal α -toxin).

For immunoglobulins formulated at an immunoglobulin concentration lower than 16%, the concentrating factor for antibodies from source material may be proportionally lower.

The immunoglobulin solution shall be tested for potency at the concentration at which it will be present in the final container.

Since preparations of normal immunoglobulins produced in different countries can be expected to differ in their content of various antibodies, depending upon the antigenic stimulation to which the general population has been subjected (either by natural infection or by deliberate immunization), at least two antibodies should be chosen for the potency test by the national control authority. The final product passes the test if it contains at least the minimum antibody levels required by the national control authority.

15.2 **Potency of specific immunoglobulins**

The potency of each final lot of specific immunoglobulin shall be tested with respect to the particular antibody that the preparation has been specified to contain. For intramuscular immunoglobulins, the following levels shall apply:

- For tetanus immunoglobulin, at least 100 IU/ml of tetanus antitoxin, as determined by a neutralization protection test in animals or by a method shown to be equivalent.
- For rabies immunoglobulin, at least 100 IU/ml of anti-rabies antibody,

as determined by an appropriate neutralization test in animals or by a method shown to be equivalent.

- For hepatitis B immunoglobulin, at least 100 IU/ml of anti-hepatitis antibody.
- For varicella zoster immunoglobulin, at least 100 IU/ml of anti-varicella zoster antibody, as measured by a comparative enzyme-linked immunosorbent assay or by a method shown to be equivalent.
- For anti-D (anti-Rh₀) immunoglobulin, the estimated potency shall be expressed in International Units and shall be not less than 90% and not more than 120% of the stated potency, and the fiducial limits of error shall be within 80% and 125% of the estimated potency.

The national control authority shall specify the antibody limits for other immunoglobulins.

After the potency tests, a test for immunoglobulin subclass may be performed. Different manufacturing steps have been shown to reduce the concentration of specific immunoglobulin subclasses (e.g. IgG1, IgG2, IgG3 and IgG4) in immunoglobulin preparations. The distribution of the four subclasses of IgG may be a factor in the efficacy of intravenous immunoglobulin preparations, since specific antibodies belonging to particular subclasses have been identified as being important in several infectious diseases.

In some countries the distribution of IgG subclasses has been measured by radial immunodiffusion. Enzyme-linked immunosorbent assays have also been described, and may be used if properly validated. Assays should be calibrated against the appropriate international reference materials.

15.3 ***Sterility and safety***

Each filling lot shall be tested for sterility. Part A, section 5, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (9, p. 48) shall apply.

In some countries a general safety test may be required, whereby each filling lot is tested for extraneous toxic contaminants by appropriate tests involving injection into mice and guinea-pigs. The injection shall cause neither significant toxic reactions nor death within an observation period of seven days. The tests shall be approved by the national control authority.

The tests generally used are the intraperitoneal injection of 0.5 ml into each of at least two mice weighing approximately 20 g and the injection of 5.0 ml into each of at least two guinea-pigs weighing approximately 350 g. In some countries, if one of the animals dies or shows signs of ill-health, such as weight loss, during a specified period, the test is repeated. The substance passes the test if none of the animals of the second group dies or shows signs of ill-health, such as weight loss, during that period.

15.4 ***Identity test***

An identity test shall be performed on at least one labelled container from each filling lot to verify that the preparation is of human origin. The test shall be one approved by the national control authority.

Additional tests shall be made to determine that the protein is predominantly immunoglobulin.

The methods in most common use are radial immunodiffusion and electrophoresis.

15.5 **Freedom from pyrogenicity**

Each filling lot shall be tested for pyrogenicity by the intravenous injection of the test dose into three or more rabbits that have not previously received blood products. In general, the dose shall be at least equivalent proportionally, on a rabbit body-weight basis, to the maximum single human dose recommended, but not more than 10 ml/kg of body weight. The recommended test doses are 1 ml/kg and 10 ml/kg of body weight for intramuscular and intravenous preparations, respectively.

A filling lot shall pass the test if it satisfies the requirements specified by the national control authority.

15.6 **Moisture content**

The residual moisture content of a sample from each filling lot shall, where appropriate, be determined by a method approved by the national control authority.

The methods in use are: (a) drying over phosphorus pentoxide for at least 24 h at a pressure not exceeding 2.7 Pa (0.02 mmHg); and (b) the Karl Fischer method.

The acceptable moisture content shall be determined by the national control authority.

15.7 **Hydrogen ion concentration**

The final product, reconstituted if necessary and diluted with 0.15 mol/l sodium chloride to give a protein concentration of 10 g/l, should, when measured at a temperature of 20-27 °C, have a pH of 6.9 ± 0.5 .

In some countries, a different range of pH values is permitted for intravenous immunoglobulins.

15.8 **Stability**

For immunoglobulin solutions, a stability test shall be performed on each filling lot by heating an adequate sample at 37 °C for four weeks. No gelation or flocculation shall occur.

Alternatively (or in addition), the molecular size distribution of the immunoglobulin or assays of enzymes such as plasmin (fibrinolysin) may be used, when shown to predict stability reliably and when approved by the national control authority.

15.9 Records

The requirements of Good Manufacturing Practices for Biological Products (8, pages 27-28) shall apply.

15.10 Samples

The requirements of Good Manufacturing Practices for Biological Products (8, page 29, paragraph 9.5) shall apply.

15.11 Labelling

The requirements of Good Manufacturing Practices for Biological Products (8, pages 26-27) shall apply.

In addition, the label on the container shall state:

- the type of source material;
- the protein concentration;
- the concentration of preservative, if any;
- "For intramuscular use only" (if the immunoglobulins are not specially prepared for intravenous use);
- "For intravenous use", when appropriate;
- for specific immunoglobulin, the content of specific antibody expressed in International Units or equivalent national units;
- for freeze-dried preparations, the name and volume of reconstituting liquid to be added.

The label on the package or the package insert shall show:

- the approximate concentration of electrolytes and excipients and, for intravenous preparations, the approximate osmolality;
- the buffering capacity when the pH of the diluted product is lower than that specified in section 15.7;
- the concentration of preservative, if any;
- the recommended dose for each particular disease or condition;
- the warning "Do not use if turbid";
- the sodium and potassium concentrations (if the immunoglobulin is intended for intravenous use).

15.12 Distribution and shipping

The requirements of Good Manufacturing Practices for Biological Products (8) shall apply.

15.13 Storage and shelf-life

The requirements of Good Manufacturing Practices for Biological Products (8, pages 26-27) shall apply.

Liquid immunoglobulin shall be stored at $5 \pm 3^\circ\text{C}$ and shall have a shelf-life of not more than three years. Freeze-dried preparations shall be stored below 25°C and shall have a shelf-life of not more than five years.

Other storage conditions and shelf-lives may be approved by the national control authority.

16. Control of preparations of coagulation-factor concentrates (factor VIII, factor IX and fibrinogen)

Factor VIII preparations are available as both frozen products and freeze-dried concentrates. The frozen products are usually derived from a single donation and consist of the cryoprecipitated factor VIII from the donor concerned prepared in a closed separation system. The control of this product and the freeze-dried product from fewer than 10 plasma donations is covered in Part B, section 7.8.1.

Generally, the small-pool product undergoes little or no purification and is handled and subdivided in such a way that many control tests are inappropriate. However, freeze-dried factor VIII concentrates prepared from more than 10 donations may be purified.

Source material for factor VIII preparations shall meet the general criteria for donor selection and testing for disease markers as specified in Parts A and B. It shall preferably be plasma frozen within 8 h of collection or frozen cryoprecipitate. Such material shall be kept frozen at such a temperature that the activity of the factor VIII is maintained.

16.1 Tests on final containers

16.1.1 Sterility and safety

Each filling lot shall be tested for sterility. Part A, section 5, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (9, p. 48) shall apply.

In some countries a general safety test may be required, whereby each filling lot is tested for extraneous toxic contaminants by appropriate tests involving injection into mice and guinea-pigs. The injection shall cause neither significant toxic reactions nor death within an observation period of seven days. The tests shall be approved by the national control authority.

The tests generally used are the intraperitoneal injection of 0.5 ml into each of at least two mice weighing approximately 20 g and the injection of 5.0 ml into each of at least two guinea-pigs weighing approximately 350 g. In some countries, if one of the animals dies or shows signs of ill-health, such as weight loss, during a specified period, the test is repeated. The substance passes the test if none of the animals of the second group dies or shows signs of ill-health, such as weight loss, during that period. For factor VIII and factor IX concentrates, the test dose should not exceed 500 IU of the coagulation factor per kg of body weight of the test animal.

16.1.2 Freedom from pyrogenicity

Each filling lot shall be tested for pyrogenicity by the intravenous injection of the test dose into three or more rabbits that have not previously received blood products. In general, the dose shall be at least equivalent

proportionally, on a rabbit body-weight basis, to the maximum single human dose recommended, but not more than 10 ml/kg of body weight.

The following test doses are suggested: factor VIII, 10 IU/kg of body weight; factor IX, 50 IU/kg of body weight; and fibrinogen, 30 mg/kg of body weight.

16.1.3 Solubility and clarity

Factor VIII preparations shall dissolve in the solvent recommended by the manufacturer within 30 min when held at a temperature not exceeding 37°C. Factor IX preparations shall dissolve in the solvent recommended by the manufacturer within 15 min when held at 20-25°C. The solutions, when kept at room temperature, shall not show any sign of precipitation or gel formation within 3 h of dissolution of the coagulation factors.

16.1.4 Protein content

The amount of protein in a final container shall be determined by a method approved by the national control authority.

16.1.5 Additives

Tests to determine the concentration of additives (such as heparin, polyethylene glycol, sodium citrate and glycine) used during production shall be carried out if required by the national control authority.

16.1.6 Moisture content

The residual moisture content shall be determined by a method approved by the national control authority. The acceptable moisture content shall be determined by the national control authority.

The methods available are: (a) drying over phosphorus pentoxide for 24 h at a pressure not exceeding 2.7 Pa (0.02 mmHg); and (b) the Karl Fischer method.

16.1.7 Hydrogen ion concentration

When the product is dissolved in a volume of water equal to the volume stated on the label, the pH of the resulting solution shall be 7.2 ± 0.4 .

In some countries, different pH values are approved.

16.2 Test applicable to factor VIII concentrates

Each filling lot shall be assayed for factor VIII activity by a test approved by the national control authority, using a standard calibrated against the International Standard for Blood Coagulation Factor VIII: Concentrate.

The national standard and the manufacturer's house standard should be a concentrate rather than a plasma because the former has better long-term stability and provides more homogeneous assay results.

The specific activity shall be at least 500 IU/g of protein. The estimated potency shall be not less than 80% and not more than 125% of the stated potency. The confidence limits of error shall be not less than 64% and not more than 156% of the estimated potency.