7.6.2 *Expiry date*

The expiry date of platelets processed in a closed system shall be 72 h after the original whole blood was collected, unless they are stored in a plastic container approved by the national control authority for a longer storage period.

Platelets prepared in an open system should be used within 4 h of preparation if stored at 22 ± 2 °C, unless the procedure used has been shown to allow a longer storage period.

Single-donor platelet concentrates may be pooled for one recipient under aseptic conditions before issue. Such small pools should be used as soon as possible, and within 4 h of preparation if stored at room temperature.

7.7 *Leukocytes*

Leukocytes are obtained by the separation of whole blood or by apheresis, and may contain a large number of platelets and red blood cells, depending on the method of preparation. When leukocytes are obtained from units of whole blood, such units shall comply with the requirements of Part A, section 5, and Part B, section 7.2.

The methods used to process leukocytes shall comply with the requirements and recommendations given in section 7.4.1 for the separation of red cells.

The label on the final container shall bear, in addition to customary data, instructions to use the leukocytes as soon as possible and in any case not more than 4 h after the container has been opened for pooling. The temperature of storage and transport shall be 22 ± 2 °C.

Leukocytes can be separated from blood by centrifugation, sedimentation or leukapheresis. To obtain a sufficient number, the leukocytes from units obtained from several healthy donors may have to be pooled.

Leukapheresis by continuous-flow filtration or centrifugation is the most efficient way of obtaining leukocytes, since it gives large numbers of high-quality cells from a single donor.

If centrifugation of whole blood is used, 30–60% of the leukocytes present in the original whole blood may be recovered.

Approximately 90% of the leukocytes present in the original whole blood can be separated by sedimentation of the red cells, accelerated by the addition of suitable substances with high relative molecular mass.

Leukocytes should be negative for cytomegalovirus.

The product should be ABO typed and, in countries where D (Rh_o) is polymorphic, D (Rh_o) typed; it may also be desirable to determine the HLA type. If not HLA typed, leukocytes should be irradiated.

The large number of red cells present in products prepared by some methods makes compatibility testing before transfusion necessary.

7.7.1 *Testing of leukocytes*

The number of units to be tested and the leukocyte yield (number) required shall be specified by the national control authority.

7.7.2 *Expiry date*

The expiry date of leukocytes shall be 24 h after collection of the original whole blood.

7.8 *Cryoprecipitated factor VIII*

Cryoprecipitated factor VIII is a crude preparation of factor VIII. It shall be obtained from single units or small pools of plasma derived either from units of whole blood that comply with the requirements of Part A, section 5, and Part B, section 7.2, or by plasmapheresis.

> The product may be prepared as a pool from a small number of donations, usually four to six but not exceeding ten. It may be freeze-dried. However, preparations of cryoprecipitated factor VIII carry the risk of viral transmission unless they have undergone specific virucidal procedures during manufacture.

The method of thawing and harvesting the cryoprecipitate shall have been shown to yield a product containing an adequate activity of factor VIII (see section 7.8.1).

In procuring source material for coagulation factors, the following technical considerations should be borne in mind:

- In order to prevent coagulation, venepuncture should performed in such a way that tissue damage is minimal. The blood should flow freely without interruption, and be mixed thoroughly with anticoagulant during collection.
- Microbial contamination should be avoided during separation of the plasma by using multiple-plastic-bag closed systems or laminar-flow cabinets if an open procedure is used.
- The recovery of factor VIII depends on the interval between venepuncture and freezing of the plasma, the temperature at which the plasma is held and the freezing method. While a useful product may be obtained with plasma frozen as late as 18–24 h after phlebotomy, freezing the plasma as early and as rapidly as possible is strongly recommended.
- Ideally, fresh-frozen plasma should be prepared by rapid freezing using a combination of solid carbon dioxide and an organic solvent such as ethanol. Fresh-frozen plasma should be stored at or below -20 °C. Contamination of the plasma by the solvent or leaching of substances from the container into the plasma should be avoided.
- If the temperature of the thawed plasma exceeds 2 °C, a high proportion of the factor VIII is lost in the supernatant. During thawing or separation of the supernatant plasma, therefore, the temperature should not be allowed to exceed 2 °C. The plasma may be separated while there is still a small quantity of the ice present in the plasma

container. Increasing the speed of thawing by circulating air or water at a temperature of 0 °C is believed to increase the yield of factor VIII.

7.8.1 *Testing of cryoprecipitated factor VIII*

Randomly selected units shall be tested for potency and sterility on a regular basis. The number of units to be tested shall be specified by the national control authority. The freeze-dried preparation shall dissolve without any signs of precipitation in the solvent recommended by the manufacturer within 30 min when held at a temperature not exceeding 37 °C.

The potency of cryoprecipitated factor VIII shall be compared with that of an appropriate plasma or intermediate-purity standard, by measuring its ability to correct the prolonged activated partial thromboplastin time of haemophilia A plasma or by another suitable method.

When cryoprecipitated factor VIII is produced from fresh-frozen plasma (frozen within 8 h of donation), the yield should be greater than 400 IU/l of starting plasma. Plasma frozen after this time will yield less cryoprecipitated factor VIII.

> In many laboratories, the average yield of factor VIII is 400 IU/l of starting plasma. The average yield of factor VIII as freeze-dried cryoprecipitate is then at least 300 IU/l of starting plasma. Whether this yield can be obtained elsewhere will depend on local technical possibilities. In some countries, the yields will be much lower, and the national control authority should decide as to the yield that is acceptable.

7.8.2 *Expiry date*

The frozen product shall be stored at or below -20 °C (if possible below -30 °C) and shall have an expiry date one year from the date of collection. The freeze-dried product shall be stored at 5 ± 3 °C and shall also have an expiry date one year from the date of collection. After thawing or reconstitution, cryoprecipitated factor VIII should be kept at 20–24 °C. It shall be used as soon as possible and in any case not more than 4 h after its container has been opened for pooling or reconstitution.

7.9 *Labelling*

After having been tested and before being issued for transfusion, units of single-donor and small-pool products shall be identified by means of container labels that clearly state at least the following information:

- the proper name of the product;
- the unique number or symbol identifying the donor(s);
- the expiry date, and when appropriate, the expiry time after reconstitution;
- any special storage conditions or handling precautions that are necessary;
- a reference to a package insert containing instructions for use, warnings and precautions;

- the name and address of the blood donor centre and, where applicable, the manufacturer and distributor;
- the average content in International Units of activity, where appropriate.

The results of red cell grouping shall be stated on the label of whole blood, red cells, fresh-frozen plasma (for clinical use), platelets and leukocytes but not necessarily on that of cryoprecipitated factor VIII.

Part C. Requirements for large-pool products

8. Introduction

A number of requirements common to albumin, plasma protein fraction, immunoglobulin preparations and coagulation-factor concentrates are given in Parts A and B, sections 3-7. However, for clarity, it has proved convenient to bring together in Part C certain specific requirements applicable to these products when manufactured on a large scale.

The source material for the large-scale preparation of blood products should comply with the relevant provisions of Parts A and B.

9. Buildings

The buildings used for the fractionation of plasma shall be of suitable size, construction and location to facilitate their proper operation, cleaning and maintenance in accordance with the requirements of Good Manufacturing Practices for Pharmaceutical (*7*) and Biological (*8*) Products. They shall comply with the Guidelines for National Authorities on Quality Assurance for Biological Products (*6*) and in addition provide adequate space, lighting and ventilation for the activities listed below.

> Each of listed activities is an important integral part of the production procedure, and countries wishing to start manufacturing large-pool blood products and related substances should not do so unless adequate provision can be made for all of them.

9.1 *Storage of whole blood and plasma*

Whole human blood and plasma shall be stored frozen or refrigerated in separate facilities that are used only for this purpose. The source materials shall remain in quarantine until the results of testing show that they are suitable for introduction into the fractionation premises.

9.2 *Separation of cells and fractionation of plasma*

Cells shall be separated and plasma fractionated in a building isolated from those where non-human proteins or microbiological materials, such as vaccines, are manufactured or processed and separate from the animal house.

> In some countries, cell constituents are separated in an area separate from that where plasma is fractionated.

9.3 *Supply and recovery of ancillary materials*

Adequate facilities shall be provided for the supply of ancillary materials, such as ethanol, water, salts and polyethylene glycol.

Facilities for the recovery of organic solvents used in fractionation may also be provided.

9.4 *Viral inactivation*

A separate area shall be provided for all processing subsequent to the completion of viral inactivation procedures when these are carried out at a stage in production before aseptic dispensing and filling (see section 9.5).

9.5 *Freeze-drying, filling, packaging, labelling and storage*

Separate facilities shall be used for the freeze-drying, filling, labelling and packaging of containers. A separate area shall be provided for the storage of labels, package inserts and packages. Another separate area shall be used for the storage of final containers before dispatch.

9.6 *Keeping of records*

Adequate provision shall be made for keeping records of all donors, materials, fractionation steps, quality-control procedures and results, of the distribution of the final products and of the disposal of potentially infectious materials. Records should be retained for at least two years beyond the expiry date of the products to which they relate.

Some manufacturers might wish to extend this period to cover any future legal disputes.

9.7 *Quality control*

Separate facilities shall be provided for quality control, including haematological, biochemical, physicochemical, microbiological, pyrogen and safety testing.

9.8 *Disposal of infective material*

Provision shall be made for the suitable disposal of potentially infectious materials by autoclaving or incineration according to good manufacturing practices.

The disposal of these materials should comply with local legislation.

10. Equipment

Equipment used for the collection, processing, storage and distribution of source materials and large-pool blood products shall comply with the requirements of Good Manufacturing Practices for Pharmaceutical (*7*) and Biological (*8*) Products.

Particular attention shall be paid to:

- The maintenance, monitoring and recording of the operation of continuously operating equipment, the validation of its reliability and the provision of stand-by equipment.
- The suitability and compatibility of the surfaces of all materials (e.g. filter medium, glass, stainless steel, plastic and rubber) that come into contact with the products.

  Metal surfaces that come into contact with proteins should be resistant to scratching. The surfaces of some materials can denature certain proteins or activate certain coagulation factors.

- The ease and efficiency with which equipment can be cleaned and, where necessary, sterilized. Any bactericidal agent used shall be capable of being completely eliminated before the equipment is used.

  Caution should be exercised in the use of detergents because of their possible effects on the final product; tests should be made to ensure that they do not have any adverse effect on it.

- The provision of suitable facilities for decontamination and for the disposal of potentially infective materials and equipment.

11. Provision of support services

A number of support services are essential for the fractionation of source materials.

11.1 *Water supply*

An adequate supply of suitable pyrogen-free water shall be provided for use during the fractionation process and for the reconstitution and/or dilution of the plasma fractions before filling and freeze-drying.

The two most commonly used types of water are pyrogen-free distilled water and pyrogen-free deionized water, each of which should be maintained at 80°C. Water preparation and delivery systems should be tested at regular intervals for endotoxin content and conductance. The water system should be a continuously circulating one and should have no dead ends.

Water for injections is generally used for the preparation of final products (*14*).

11.2 *Steam supply*

An adequate supply of steam shall be provided for the operation of sterilizing and cleaning equipment. The steam shall be clean and have the quality of water for injections.

11.3 *Other support facilities*

Other support facilities required are:

- A supply of electrical and thermal energy.

- A means of refrigeration for:
  - storing various source materials and fractions;
  - keeping the various fractionation areas at the correct temperature;
  - keeping the process equipment at the correct temperature;
  - storing final products under test;
  - storing final products awaiting dispatch.
- A system of ventilation providing the following two grades of filtered air:
  - air filtered to remove particles of 5 μm or greater in diameter, which shall be supplied to the entire work area; and
  - air passed through a filter with a retention capacity of more than 99.95% for particles greater than 0.5 μm in diameter, which shall be supplied at a positive pressure to areas where aseptic dispensing is to take place.

Other support facilities may include solvent recovery and a sewage disposal service. Sewage disposal must be carried out in accordance with the sanitary standards of the competent health authority.

Proteinaceous sewage from a plasma processing plant is highly nitrogenous and has a high biological oxygen demand; it should therefore not be discharged untreated.

These support facilities shall be located separately from the main process areas and in a place where the conditions (light, physical access, etc.) are conducive to the establishment of effective and routine preventive maintenance programmes. The equipment shall incorporate devices capable of monitoring and recording its operation so as to ensure the safety both of the material being processed and of the process operators. In this way a proper record of the operations of support facilities can be kept and, where necessary, entered into the process record of the product batches.

The equipment should be such as to ensure that both the fractionation process and the proteins are protected if the support services are interrupted. To this end, adequate spare equipment and emergency reserve systems should be available, serviced by engineering staff skilled in the maintenance and repair of such equipment.

12. Personnel

The plasma fractionation plant shall be under the direction of a designated qualified person who shall be responsible for ensuring that all operations are carried out properly and competently. The director shall have a good working knowledge of the scientific principles involved and shall be responsible for ensuring that employees are adequately trained, have adequate practical experience and are aware that accepted good practices should be applied in their work.

The personnel involved in quality-control functions shall be separate from those involved in production. The head of the quality-control department shall be responsible only to the director.

Where appropriate, personnel shall wear gowns, masks, boots, gloves and eye protectors.

Personnel should be medically examined at regular intervals. Those known to be carriers of specific pathogenic organisms that may adversely affect the product shall be excluded from the production area.

Vaccination against hepatitis B is strongly recommended for employees routinely exposed to blood or blood products.

13. Production control

13.1 *Fractionation of source materials*

The general conditions for the large-scale fractionation of source materials to prepare prophylactic or therapeutic blood products shall comply with Good Manufacturing Practices for Pharmaceutical (*7*) and Biological (*8*) Products and shall be approved by the national control authority.

Most physical and chemical techniques of protein separation may be used for the preparation of plasma fractions, provided that they yield protein preparations that have previously been shown to be safe and effective.

The fractionation procedures used shall give a good yield of products meeting the quality requirements of international or national authorities. Fractionation shall be carried out in such a manner that the risk of microbiological contamination and protein denaturation is minimized.

> The safety of fractionation steps may be increased by using protected or closed systems. Reproducibility may be increased by the use of automation.
>
> The biological characteristics of the products (such as antibody activity, biological half-life and *in vivo* recovery of the proteins) should not be affected by the fractionation procedures to the extent that they are unacceptable for clinical use.

Methods shall be used that exclude or inactivate pathogenic organisms, in particular hepatitis viruses and human retroviruses, from the final products intended for clinical use. Manufacturers shall validate the ability of their manufacturing processes to inactivate and/or remove potential contaminating viruses by the use of relevant model viruses.

There is increasing evidence that certain manufacturing procedures, coupled with strict control to ensure that the final product complies with precise specifications, result in a product free from HIV, hepatitis B and hepatitis C infectivity.

For coagulation products, viral inactivation and removal methods such as chromatography or treatment with dry heat, wet heat, steam under pressure, heated organic solvents or solvents/detergents shall be used, in combination with other methods that have been shown to be successful in reducing or eliminating the risk of HIV and hepatitis virus transmission.

Donor screening and viral inactivation procedures used in manufacturing plasma coagulation concentrates have significantly improved the safety of these products.

Fibrinogen prepared from plasma pools continues to carry a risk of infection unless it is treated to remove or inactivate viruses. Where large-pool, virally inactivated fibrinogen concentrates are not available, cryoprecipitated factor VIII prepared from individual units or small pools of plasma is preferred as a source of fibrinogen. Approximately 150 mg of fibrinogen is contained in the cryoprecipitate from one unit of plasma (200 ml) frozen within 8 h of collection from the donor.

The operating manual for the fractionation procedure shall specify the times of sampling of the products and the volumes to be taken at each stage of the process as well as the tests to be made on the samples.

Where appropriate, all materials used for fractionation shall be tested for microbiological contamination, identity, purity, endotoxin content and toxicity in accordance with *The international pharmacopoeia* (*14, 15*) or national pharmacopoeia.

Certain procedures, equipment and materials may introduce contaminants into the final product that can induce allergenic or immunogenic responses in recipients. The quantities of such contaminants in the final product shall be minimized. For example, where monoclonal antibodies are used for product purification, the residual concentration in the final product must be below clinically reactive levels.

> It is advisable to use air filtration under positive pressure during fractionation, to exclude airborne allergenic dust.

13.1.1 *Preservatives and stabilizers*

No preservatives shall be added to albumin, plasma protein fraction, intravenous immunoglobulin or coagulation-factor concentrates either during fractionation or at the stage of the final bulk solution. Antibiotics shall not be used as preservatives or for any other purpose in the fractionation of plasma.

To prevent protein denaturation, stabilizers may be added. Such substances shall have been shown to the satisfaction of the national control authority not to have any deleterious effect on the final product in the amounts present and to cause no untoward reactions in humans.

> Stable solutions of immunoglobulins may be prepared in approximately 0.3 mol/l glycine or 0.15 mol/l sodium chloride. In some countries, thiomersal and sodium timerfonate are not permitted as preservatives in intramuscular immunoglobulins.

13.2 *Storage and control of source materials*

At all stages of the manufacturing process, the source materials and resulting fractions shall be stored at temperatures and under conditions

shown to prevent further contamination and the growth of micro-organisms, to protect the identity and the integrity of the proteins and to preserve the biological activity and safety of the products.

If similar materials are stored together, the places allocated to them shall be clearly demarcated.

All source materials and resulting fractions shall be fully identified at all times; such identification shall include the batch number of all in-process fractions and final containers awaiting labelling.

13.2.1 *In-process control*

Source materials are subject to biological variability and the products resulting from protein separation will contain various amounts of other protein components of plasma. It is essential, therefore, to establish a monitoring system such that the safe operating limits of each process are maintained.

The main information collected is on variations in physical conditions (temperature, pH, ionic strength, timing, etc.) and in the number and species of contaminating organisms.

Owing to the numerous and interdependent factors involved, there are no universally accepted specifications for such in-process quality-assurance systems. For this reason, the information collected should be combined with data from previous experience with the same manufacturing process to ensure production control appropriate to the quality requirements of the final product.

13.2.2 *Record-keeping*

Records shall be kept of the performance of all steps in the manufacture, quality control and distribution of large-pool blood products and related substances (*7, 8*).

These records shall:

- be original (not a transcription), indelible, legible and dated;
- be made at the time that the specific operations and tests are performed;
- identify the person recording the data as well as the person checking them or authorizing the continuation of processing;
- be detailed enough to allow all the relevant procedures performed to be clearly reconstructed and understood;
- permit the tracing of all successive steps and identify the relationships between dependent procedures, products and waste materials;
- be maintained in an orderly fashion that will permit the retrieval of data for a period consistent with shelf-lives and the legal requirements of the national control authority and, if necessary, allow a prompt and complete recall of any particular lot;
- show the lot numbers of the materials used for specified lots of products;
- indicate that processing and testing were carried out in accordance with procedures established and approved by the designated responsible authority.

14. Control of albumin and plasma protein fraction

Source materials should be processed in such a manner that the albumin in the solutions manufactured will be changed as little as possible and will not cause undesirable reactions in the recipients. Source materials may contain either vasoactive substances or substances capable of generating or releasing endogenous vasoactive substances. Such substances may also be formed in the course of fractionation, and consequently contaminate the albumin and plasma protein fraction. To guard against this possibility, adequate in-process controls and the testing before release for prekallikrein activator activity are mandatory for albumin solutions of purity less than 95% (such as plasma protein fraction) containing 35–50 g of protein per litre. Such testing is also recommended for highly purified albumin products (purity greater than 95%).

Within 24 h of the start of filling, albumin and plasma protein fraction in solution shall be heated in the final container to 60 ± 0.5 °C and maintained at that temperature for not less than 10 h but not more than 11 h by a method that ensures uniform heat distribution throughout the batch. Although pasteurization at the final bulk stage may be possible, this approach requires careful validation before use.

Special attention should be given to microbial contamination of source material and intermediates, since soluble microbial substances, especially endotoxins, may accumulate in the finished albumin solution. In addition, it is possible that small amounts of endotoxin, present even in products for which satisfactory results have been obtained in tests for pyrogens, may have a cumulative effect in recipients receiving large product volumes in relatively short periods of time, as, for example, in therapeutic plasma exchange.

> In some countries, information is being collected about the usefulness of quantitative *Limulus* assays for the presence of endotoxin.

The in-process controls should be capable of detecting contamination with bacteria and moulds. In addition, care should be taken to ensure, by a method that shall be validated, that all equipment and reagents used in the manufacturing process are scrupulously clean and free from toxic materials.

14.1 *Stability of albumin solutions*

The stability of solutions of albumin and plasma protein fraction (that have been heated for 10–11 h at 60 °C) shall be tested by heating adequate samples at 57 °C for 50 h. The test solutions shall remain visually unchanged when compared to control samples that have been heated for only 10–11 h at 60 °C.

The thermal stability of albumin solutions shall be taken into consideration by the national control authority in determining the expiry dates.

The physicochemical quality of stored albumin solutions, as measured by the formation of dimers and particularly polymers, is influenced by:

— the quality of the starting plasma;
— the quality of the fractionation, particularly with respect to the degree of purity achieved and the number of reprecipitation and reheating procedures involved; and
— the storage conditions with respect not only to temperature and time but also to the physical state and concentration of the solutions.

With regard to the thermal stability of albumin solutions, the following general statements may be made:

- The addition of stabilizing chemicals is necessary. Commonly used products are sodium octanoate and sodium acetyltryptophanate.
- Albumin prepared from aged liquid or dried plasma is less stable than albumin made from fresh-frozen plasma.
- Reprocessing steps, such as reprecipitation and reheating, may reduce the stability of albumin solutions.
- On long-term storage, albumin solutions are more stable at 5 ± 3 °C than at 32–35 °C. Long-term storage above 30 °C should be avoided.

14.2 *Control of bulk material*

14.2.1 *Tests on bulk material*

Tests on the bulk powder or solution shall be made if the manufacturer sends the material to another institution for further processing. Samples for these tests shall be taken under conditions that do not impair the quality of the bulk material. Tests shall be carried out on a specially dissolved sample processed to a stage equivalent to the final product, after sterilization by filtration. The tests shall be those listed in sections 14.3.2 to 14.3.7 inclusive.

14.2.2 *Storage*

The bulk material shall be stored as liquid or powder in sealed containers under conditions that minimize denaturation and the multiplication of microbial agents.

14.3 *Control of the final bulk solution*

14.3.1 *Preparation*

The final bulk solution shall be prepared from bulk powder or by the dilution of concentrates by a method approved by the national control authority. It shall meet all of the requirements of sections 14.3.2 to 14.3.7 inclusive.

14.3.2 *Concentration and purity*

The albumin concentration in final bulk albumin solutions shall be between 35 and 265 g/l. Not less than 95% of the proteins present shall be albumin, as determined by a suitable electrophoretic method after the sample has been heated for 10–11 h at 60 °C.

The protein concentration in final bulk solutions of plasma protein fraction shall be at least 35 g/l. Plasma protein fraction shall contain at least 83% albumin and not more than 17% globulins. Not more than 1% of the protein in plasma protein fraction shall be γ-globulin.

14.3.3 *Hydrogen ion concentration*

The final bulk solution, 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 and temperatures are permitted.

14.3.4 *Sterility and safety*

The final bulk shall be sterile. If required by the national control authority, it shall be tested for sterility; samples shall be taken for such testing in a manner that does not compromise the sterility of the bulk material. 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.

14.3.5 *Sodium content*

The final bulk solutions of albumin and plasma protein fraction shall have a maximum sodium concentration of 160 mmol/l.

14.3.6 *Potassium content*

The final bulk solutions of albumin and plasma protein fraction shall have a maximum potassium concentration of 2.0 mmol/l.

14.3.7 *Aluminium content*

The final bulk solutions of albumin and plasma protein fraction shall have a maximum aluminium concentration of 7.5 μmol/l (200 μg/l).

14.4 *Filling and containers*

The requiremens concerning filling and containers given in Good Manufacturing Practices for Biological Products (*8*) shall apply.

Special attention shall be paid to the requirement that solutions of albumin and plasma protein fraction in the closed final containers shall be heated to inactivate any infectious agents that may be present (see section 14, paragraph 2). In order to prevent protein denaturation, a stabilizer shall be added to albumin solution before heating (see section 13.1.1).

In some countries, the national control authority may authorize an interval longer than 24 h between filling and heating to 60 °C.

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.