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BLOOD COMPONENTS AND BLOOD DERIVATIVES

FIBRIN GLUE
Fibrini glutinum

DEFINITION

Fibrin glue is a sterile, pyrogen-free pharmaceutical preparation, consisting of a set containing two components: component 1 (fibrinogen concentrate), a protein fraction containing human fibrinogen and human Factor XIII, and component 2, a preparation containing human thrombin; the latter converts the first one into fibrin after its reconstitution and mixing in the presence of calcium ions.

It may contain other ingredients such as human fibronectin and a plasmin inhibitor such as aprotinin and stabilizers such as human albumin added before or during thrombin-induced fibrin formation. No antimicrobials are added to the preparation.

The constituent elements are obtained from human plasma, which meets the requirements of the monograph *Human plasma for fractionation*.

Thawed or reconstituted with the volume of diluent indicated on the label, component 1 contains not less than 40 g/L of coagulation proteins; component 2 thrombin activity varies over a wide range (approximately 4 - 1000 IU/mL).

PRODUCTION

It must be obtained in accordance with the Good Manufacturing Practices for Biological Medicines and Good Practices for the Blood Production Cycle, in accordance with current legislation to guarantee its quality, safety and efficacy.

The preparation method includes one or more viral inactivation steps that demonstrate the elimination or inactivation of known viral infectious agents. If substances are used to inactivate viruses during production, the subsequent purification process must demonstrate, through validation, that the concentration of inactivating substances has been eliminated or reduced to acceptable levels according to current regulations and that any residues may not pose risks to patients.

The constituents or their mixtures must be sterile and pyrogen-free, being then distributed aseptically in the final containers and immediately frozen. They must be lyophilized and the containers closed under vacuum or inert gas to avoid any microbial contamination.

If component 1 contains Factor XIII, the concentration of the latter should be not more than 10 IU/mL. If the label indicates that the activity of coagulation Factor XIII is greater than 10 IU/mL, the estimated activity must be not less than 80% and not more than 120% of the activity stated on the label.

CHARACTERISTICS

Aspect. Lyophilized constituents are white or pale yellow friable powders or solids. Frozen constituents are opaque, colorless or pale yellow solids. Liquid constituents are colorless or pale yellow.

COMPONENT 1 (FIBRINOGEN CONCENTRATE)

Note: *reconstitute lyophilized constituents and thaw frozen constituents as indicated on the label immediately before performing identification and testing, except for solubility and water.*

IDENTIFICATION

A. Perform precipitation assays with the finished product sample, using specific sera from not less than four different animal species and an anti-human serum. The assay is carried out with specific sera against the plasma proteins of animal species that are normally used in the country for the preparation of products of biological origin. The sample contains only proteins of human origin and does not precipitate with specific sera against plasmatic proteins from other animal species, but it precipitates with anti-human serum.

B. The determination of fibrinogen, as described in *Assay*, contributes to the identification of component 1.

C. The determination of Factor XIII, as described in *Assay*, contributes to the identification of component 1.

CHARACTERISTICS

Aspect of the preparation. Lyophilized concentrates dissolve in 20 minutes in the volume of the diluent for reconstitution, at the temperature indicated on the label, resulting in a limpid or slightly turbid, practically colorless preparation.

pH (5.2.19). 6.5 to 8.0.

Dissolution stability. For 120 minutes after reconstitution or thawing, no gel is formed at room temperature.

Water. Determine by one of the following methods: *Determination of water by the semi-micro method* **(5.2.20.3)**, *Loss on drying* **(5.2.9.1)** or *Near infrared absorption spectrophotometry* **(5.2.14)**. Not more than 2.0%.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

ASSAY

Fibrinogen (coagulation proteins) The estimated amount of coagulation proteins, expressed in milligrams, is not less than 70% and not more than 130% of the declared amount.

Use one of the methods described below.

A. *Coagulation proteins.* Dose the nitrogen in the residue by the method *Determination of Nitrogen* **(5.3.3.2).** Mix 0.2 mL of the reconstituted preparation and 2 mL of an appropriate buffer solution (pH 6.6 to 7.4) containing a sufficient amount of human thrombin (approximately 3 IU/mL) and calcium (0.05 *M*). Keep the mixture at 37 °C for 20 minutes, separate the precipitate by centrifugation (5000 × g, for 20 minutes) and wash thoroughly with a 0.9% (w/v) sodium chloride solution. Dose the

nitrogen in the residue by the method *Determination of Nitrogen* **(5.3.3.2).** Calculate the protein content by multiplying the result by 6.

B. *Coagulation test.* Dilute the reconstituted preparation with a 0.9% (w/v) sodium chloride solution until a fibrinogen concentration between 0.1 mg/mL and 1 mg/mL is obtained. Use 0.2 mL of the dilution, maintaining at 37 °C for 60 seconds and add 0.2 mL of an appropriate human thrombin solution (approximately 20 IU/mL containing not less than 1 m*M* calcium). Determine the coagulation time by an appropriate method. Repeat the process with not less than three different dilutions, in the range indicated above, using an appropriate fibrinogen solution (for example, normal human plasma calibrated, by means of a determination, before a freshly prepared plasma mixture (> 100 donors). Plot a calibration curve with the measured coagulation times for standard dilutions and fibrinogen content; from the curve, determine the fibrinogen content in the preparation to be examined.

Factor XIII. Where the label indicates that the activity of coagulation Factor XIII is greater than 10 IU/mL, the estimated activity must be not less than 80% and not more than 120% of the activity stated on the label.

Make three dilutions of the thawed or reconstituted component 1 and the reference preparation of normal human plasma, using as diluent plasma deficient in coagulation Factor XIII or other appropriate diluent. Add appropriate amounts of the following reagents to each dilution:

Activation reagent: containing human or bovine thrombin, appropriate buffer, calcium chloride and an appropriate inhibitor such as Gli-Pro-Arg-Ala-Ala-NH_2, which will inhibit the sample clotting and will not prevent Factor XIII activation by thrombin.

Detection reagent: containing activated Factor XIII specific peptide substrate such as LeuGli-Pro-Gli-Glu-Ser-Lis-Val-Ile-Gli-NH_2 and glycine ethyl ester as second substrate in appropriate buffer solution.

NADH Reagent: containing glutamate dehydrogenase, α-ketoglutarate and NADH in a suitable buffer solution.

After mixing, the changes in absorbance (ΔA/minutes) at a wavelength of 340 nm **(5.2.14)** are measured after reaching the reaction linear phase. The Factor XIII unit is equal to the activity of 1 mL of normal human plasma. Calculate test preparation activity using regular statistical methods **(8.2).** Confidence intervals (P = 0.95) must be not less than 80% and not more than 125% of the estimated activity.

COMPONENT 2 (THROMBIN PREPARATION)

IDENTIFICATION

A. Perform precipitation assays with the finished product sample, using specific sera from not less than four different animal species and an anti-human serum. The assay is carried out with specific sera against the plasma proteins of animal species that are normally used in the country for the preparation of products of biological origin. The sample contains only proteins of human origin and does not precipitate with specific sera against plasmatic proteins from other animal species, but it precipitates with anti-human serum.

B. The determination of thrombin, as described in *Assay*, contributes to the identification of component 2.

CHARACTERISTICS

Aspect of the preparation. Lyophilized concentrates dissolve in 5 minutes in the volume of the diluent for reconstitution, at the temperature indicated on the label, resulting in a colorless and limpid or slightly turbid preparation.

pH (5.2.19). 5.0 to 8.0.

Water. Determine by one of the following methods: *Determination of water by the semi-micro method* **(5.2.20.3)**, *Loss on drying* **(5.2.9.1)** or *Near infrared absorption spectrophotometry* **(5.2.14)**. Not more than 2.0%.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

ASSAY

Thrombin. If necessary, dilute the reconstituted preparation to be examined to approximately 2 - 20 IU per milliliter of thrombin using a pH 7.3 to 7.5 buffer as diluent, such as a pH 7.3 imidazole buffer solution containing 10 g/L of human or bovine albumin. To an appropriate volume of the dilution, add an appropriate volume of fibrinogen (1 g/L of clotted protein) heated to 37 °C and start measuring the coagulation time immediately. Repeat the procedure with each of the three dilutions of a thrombin reference preparation calibrated in international units. Calculate test preparation activity through regular statistical methods **(8.2).** Confidence intervals (P = 0.95) must be not less than 80% and not more than 125% of the estimated activity.

STORAGE

Protect from light.

LABELLING

Comply with current legislation. The label must indicate:

- the amount of fibrinogen (milligrams of coagulation proteins) and thrombin (International Units) per vial;
- Factor XIII activity (units) when greater than 10 IU/mL;
- where applicable, the volume of diluent required to reconstitute the preparation.

TOTAL LYOPHILIZED HUMAN PROTHROMBIN COMPLEX

Prothrombinum multiplex total humanum cryodesiccatus

The total human prothrombin complex is a fraction of plasmatic proteins that mandatorily contains Factors II, VII, IX and X of the human coagulation. The presence and amounts of Factors II, VII and X depend on the fractionation method used, obtained from human plasma that meets the requirements of the monograph *Human plasma for fractionation*.

The preparation activity, reconstituted according to the indications provided on the label, is not less than 20 IU of Factor IX per milliliter. If the content of any coagulation factor is declared as a unit value, the estimated potency must be between 80% and 125% of the declared potency; if the content of any factor is declared in the range, the estimated potency must be within the stated minimum and maximum limits.

The preparation method should avoid, as much as possible, the activation of coagulation factors, in order to reduce their thrombogenic potential, and comprises one or several steps that demonstrate the elimination or inactivation of known and unknown infectious agents. If viral inactivation substances are added in the production step, a purification procedure must be validated to demonstrate that the concentration of these substances has been reduced to an acceptable level, and that such residues do not compromise the safety of the preparation.

The specific activity is not less than 0.6 IU of Factor IX per milligram of total protein, before the potential addition of a protein stabilizer. The fraction containing the prothrombin complex is dissolved in an appropriate diluent. Heparin, antithrombin and other auxiliary substances can be added as a stabilizer. Antimicrobial preservatives should not be added. The solution undergoes a sterilizing filtration and is then aseptically filled into containers and immediately frozen. It is then lyophilized, and the containers are closed under vacuum or in the presence of an inert gas.

Note: *reconstitute the sample as indicated on the label immediately before performing the Identification, the tests (except those for solubility and water content) and the Assay.*

IDENTIFICATION

The sample meets the limits established in *Assay* for Human blood coagulation Factors II, VII, IX and X.

CHARACTERISTICS

Aspect. White or slightly colored, friable powder or solid, highly hygroscopic.

Osmolality (5.2.28). Not less than 240 mosmol/kg.

pH (5.2.19). 6.5 to 7.5.

Solubility. Add the volume of liquid diluent specified on the label, observing the recommended temperature. Gently shake for not more than 10 minutes. The preparation completely dissolves, and the formation of a solution which may be slightly colored is observed.

PHYSICOCHEMICAL TESTS

Water. Determine by one of the following methods: *Determination of water by the semi-micro method* **(5.2.20.3)**, *Determination of loss on drying* **(5.2.9.1)** or *Infrared absorption spectrophotometry* **(5.2.14)**. The water content must be less than 2.0%.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

Pyrogens (5.5.2.1). Complies with the test. Inject into each rabbit, per kilogram of body weight, a volume of the reconstituted sample corresponding to not less than 30 IU of Factor IX.

ASSAY

Factor IX

Proceed as described in *Determination of human blood coagulation Factor IX* **(5.5.1.4).** The determined activity is not less than 80% and not more than 125% of the declared activity. Confidence interval (P = 0.95) of the determined activity is not less than 80% and not more than 125%.

Factor II

Proceed as described in *Determination of human blood coagulation Factor II* **(5.5.1.3).** The determined activity is not less than 80% and not more than 125% of the declared activity. Confidence interval (P = 0.95) of the determined activity is between 90% and 111%.

Factor VII

Proceed as described in *Determination of human blood coagulation Factor VII* **(5.5.1.5).** The determined activity is not less than 80% and not more than 125% of the declared activity. Confidence interval (P = 0.95) of the determined activity is between 80% and 125%.

Factor X

Proceed as described in *Determination of human blood coagulation Factor X* **(5.5.1.6).** The determined activity is not less than 80% and not more than 125% of the declared activity. Confidence interval (P = 0.95) of the determined activity is between 90% and 111%.

Activated coagulation factors

Proceed as described in *Determination of activated coagulation factors* **(5.5.1.8).** If necessary, dilute the sample to obtain a solution containing 20 IU of Factor IX per milliliter. For each of the dilutions, the coagulation time is not less than 150 seconds.

Heparin

If heparin was added during preparation, determine its content according to the method *Determination of heparin in coagulation factors* **(5.5.1.1).** The sample does not contain more than the amount of heparin indicated on the label and never contains more than 0.5 IU of heparin per international unit of Factor IX.

Total proteins

If necessary, dilute the reconstituted preparation with 0.9% (w/v) sodium chloride solution to obtain a solution containing approximately 15 mg of protein in 2 mL. In a round-bottom centrifuge tube, add 2 mL of this solution. Add 2 mL of 75 g/L sodium molybdate solution and 2 mL of a mixture of nitrogen-free sulfuric acid and water (1:30). Shake and centrifuge for five minutes. The supernatant liquid must be decanted allowing the tube to be dried on a filter paper. Determine the nitrogen in the residue by the method *Determination of Nitrogen* **(5.3.3.2).** Calculate the protein content by multiplying the result by 6,25.

Thrombin

If the sample contains heparin, determine its content as indicated in the *Heparin Biological Test* and neutralize it by adding protamine sulfate (10 µg of protamine sulfate neutralizes 1 IU of heparin). Use two test tubes and, in each one, mix equal volumes of the reconstituted sample of a 3 g/L fibrinogen solution. Keep one tube at 37 °C for six hours and the other one at room temperature for 24 hours. In a third tube, mix equal volumes of the fibrinogen solution and the human thrombin solution at 1 IU/mL and place the tube in a 37 °C water bath. No coagulation is produced in tubes containing the sample. Coagulation is produced within 30 seconds in the tube containing the thrombin.

PACKAGING AND STORAGE

Protect from light.

LABELLING

Comply with current legislation. The label must indicate:

- the name: total prothrombin complex;
- the number of International Units of Factors IX, VII and X and the number or range of International Units of Factor II per vial;
- the amount of protein in each container;
- the name and amount of any substance added, including heparin and thrombin, if applicable;
- the name and volume of the diluent needed to reconstitute the preparation.

LYOPHILIZED HUMAN BLOOD COAGULATION FACTOR IX

Factor IX coagulationis sanguinis humanus cryodesiccatus

coagulation factor IX; 03806

Lyophilized human blood coagulation factor IX is the protein fraction of plasma that contains human blood coagulation factor IX, obtained by a method that allows the separation of factor IX from other factors in the human prothrombin complex (Factors II, VII and X). It is prepared from human plasma in accordance with the monograph *Human plasma for fractionation*. The preparation activity, reconstituted according to the indications provided on the label, is not less than 20 IU of factor IX per milliliter.

The preparation method must be developed so as to maintain the functional integrity of factor IX, minimize the activation of any coagulation factor (to limit thrombogenic potential) and must include one or several steps that demonstrate the elimination or inactivation of known and unknown infectious agents. If viral inactivation substances are added in the production step, a purification procedure must be validated to demonstrate that the concentrations of these substances have been reduced to an acceptable level, and that such residues do not compromise the safety of the preparation. The specific activity is not less than 50 IU of factor IX per milligram of total protein, before the eventual addition of a protein stabilizer.

The fraction containing factor IX is diluted in an appropriate diluent. Heparin, antithrombin and other auxiliary substances can be added as a stabilizer. Antimicrobial preservatives should not be added. The solution is filtered through a sterilizing filter and distributed aseptically into the final containers and immediately frozen. It is then lyophilized, and the containers are closed under vacuum or inert gas.

The regularity of the production method is assessed by appropriate analytical procedures during development studies, which usually include:

- determination of factor IX;
- determination of activated coagulation factors;
- determination of the activity of coagulation factors II, VII and X, which is not more than 5% of the activity of factor IX.

IDENTIFICATION

Reconstitute the sample as indicated on the label immediately before performing the Identification, the tests (except those for solubility and water content) and the assay.

A. Perform sample precipitation assays using an appropriate range of animal species-specific sera. The assay is carried out with specific sera containing the plasma proteins of animal species that are normally used in the country for the preparation of products of biological origin. The sample contains proteins of human origin and does not precipitate with specific sera containing plasmatic proteins from other animal species.

B. Determination of factor IX coagulant activity helps to identify the preparation.

CHARACTERISTICS

Aspect. Friable, white or pale yellow powder or solid.

pH (5.2.19). 6.5 to 7.5.

Osmolality (5.2.28). Not less than 240 mosmol/kg.

Solubility. Add the volume of diluent indicated on the label to the contents of a sample container and gently shake for 10 minutes at room temperature. Total dissolution occurs, thus forming a clear or slightly opalescent and colorless solution.

PHYSICOCHEMICAL TESTS

Water. Determine by an appropriate method such as *Determination of water by the semi-micro method* **(5.2.20.3)**, *Loss on drying* **(5.2.9.1)** or *Infrared absorption spectrophotometry* **(5.2.14)**. Not more than 2.0%.

BIOLOGICAL SAFETY TESTS

Pyrogens (5.5.2.1). Complies with the test. Inject into each rabbit, per kilogram of body weight, a volume of the reconstituted sample corresponding to not less than 30 IU of Factor IX and not more than of 50 IU of Factor IX.

Sterility (5.5.3.2.1). Complies with the test.

ASSAY

Blood coagulation factor IX

Proceed as described in *Determination of blood coagulation Factor IX* **(5.5.1.4).** The determined activity is not less than 80% and not more than 125% of the declared activity. Confidence interval (P = 0.95) of the determined activity is not greater than 80% to 125%.

Activated coagulation factors

Proceed as described in *Determination of activated coagulation factors* **(5.5.1.8).** If necessary, dilute the sample to obtain a solution containing 20 IU of factor IX per milliliter. For each of the dilutions, the coagulation time is not less than 150 seconds.

Heparin

If heparin was added during production, determine its amount according to the test *Determination of heparin in coagulation factors* **(5.5.1.1).** The sample contains not more than the amount of heparin indicated on the label and not more than 0.5 IU of heparin per international unit of factor IX.

Total proteins

If necessary, dilute the reconstituted preparation with 0.9% (w/v) sodium chloride solution to obtain a solution containing approximately 15 mg of protein in 2 mL. In a round-bottom centrifuge tube, add 2,0 mL of this solution. Add 2 mL of 7.5% (w/v) sodium molybdate solution and 2 mL of a mixture of nitrogen-free sulfuric acid and water (1:30). Shake and centrifuge for five minutes. The supernatant liquid must be decanted allowing the tube to be dried on a filter paper. Determine the

nitrogen in the residue by the method *Determination of Nitrogen by the Kjeldahl method* **(5.3.3.2).** Calculate the protein content by multiplying the result by 6.25. This method may not be applicable to certain products, especially those that do not contain a protein stabilizer, such as albumin, with another validated method being used for the assay.

STORAGE

Protect from light.

LABELLING

The label indicates at least:

- the number of international units of factor IX in each vial; the amount of protein in each vial;
- the name and amount of any substance added, including heparin, when applicable;
- the name and volume of the diluent needed to reconstitute the preparation; the storage conditions;
- the expiration date; that the transmission of infectious agents cannot be totally excluded when administering human blood or plasma medicinal products (the latter may alternatively be indicated in the package insert text).

LYOPHILIZED HUMAN BLOOD COAGULATION FACTOR VII

Factor VII coagulationis humanus cryodesiccatus

coagulation factor VII; 03808

Lyophilized human blood coagulation factor VII is a protein fraction of plasma that contains factor VII (a glycoprotein single-chain derivative) and may also contain small amounts of its activated form (two chains derivative or Factor VIIa), as well as Factors II, IX, and X, Protein C and Protein S. It is prepared from human plasma according to the monograph *Human plasma for fractionation*. The preparation activity, reconstituted according to the indications provided for on the label, is not less than 15 IU of factor VII per milliliter.

The preparation method must be developed so as to maintain the functional integrity of factor VII, minimize the activation of any coagulation factor (to limit thrombogenic potential) and must include one or several steps that demonstrate the elimination or inactivation of known and unknown infectious agents. If viral inactivation substances are added in the production step, a purification procedure must be validated to demonstrate that the concentrations of these substances have been reduced to an acceptable level, and that such residues do not compromise the safety of the preparation. The specific activity is not less than 2 IU of factor VII per milligram of total protein, before the eventual addition of a protein stabilizer.

The fraction containing factor VII is dissolved in an appropriate diluent. Heparin, antithrombin and other auxiliary substances can be added as a stabilizer.

No antimicrobial preservative is added. The solution is filtered through a sterilizing filter and then distributed aseptically into the final containers and immediately frozen. It is then lyophilized, and the containers are closed under vacuum or inert gas.

The regularity of the production method is demonstrated, with regard to the activities of the preparation factors II, IX and X, expressed in international units and in relation to the activity of factor VII.

The regularity of the production method with regard to the activity of factor VIIa in the preparation is demonstrated. Factor VIIa activity can be determined with a recombinant soluble tissue factor, which does not activate factor VII to factor VIIa, but which has a specific factor VIIa cofactor function: after incubation of the mixture of soluble recombinant tissue factor and phospholipids with a dilution of the sample and plasma deficient in factor VII, calcium chloride is added and the coagulation time is determined; the coagulation time is inversely proportional to the factor VIIa activity of the sample.

IDENTIFICATION

Reconstitute the sample as indicated on the label immediately before performing the Identification, the tests (except those for solubility and water content) and the assay.

A. Carry out precipitation assays with the sample, with an appropriate series of specific sera for the various species. It is recommended that the assay be performed with specific serum protein from each of the domestic species normally used in the preparation of biological products. The preparation is shown to contain proteins of human origin and show negative results with specific sera for plasmatic proteins from other species.

B. The determination of factor VII coagulation activity helps to identify the preparation.

CHARACTERISTICS

Aspect. Friable powder or solid, which can be white, pale yellow, green or blue.

pH (5.2.19). 6.5 to 7.5.

Osmolality (5.2.28). Not less than 240 mosmol/kg.

Solubility. Add the volume of diluent indicated on the label to the contents of a sample vial, at the recommended temperature, and gently shake for not more than 10 minutes. The sample completely dissolves forming a clear or slightly opalescent preparation, which can be stained.

PHYSICOCHEMICAL TESTS

Water. Determine by an appropriate method such as *Determination of water by the semi-micro method* **(5.2.20.3)**, *Loss on drying* **(5.2.9.1)** or *Infrared absorption spectrophotometry* **(5.2.14)**. Not more than 2.0%.

BIOLOGICAL SAFETY TESTS

Pyrogens (5.5.2.1). Complies with the test. Inject into each rabbit, per kilogram of body weight, a volume of the reconstituted sample corresponding to not less than 30 IU of factor VII.

Sterility (5.5.3.2.1). Complies with the test.

ASSAY

Blood coagulation factor VII

Proceed as described in *Determination of blood coagulation Factor VII* **(5.5.1.5).** The determined activity is not less than 80% and not more than 125% of the declared activity. Confidence interval (P = 0.95) of the determined activity is not greater than 80% to 125%.

Activated coagulation factors

Proceed as described in *Determination of activated coagulation factors* **(5.5.1.8).** For each of the dilutions, the coagulation time is not less than 150 seconds.

Heparin

If heparin was added during production, determine its quality according to test *Determination of heparin in coagulation factors* **(5.5.1.1).** The sample contains not more than the amount of heparin indicated on the label and not more than 0.5 IU of heparin per international unit of factor VII.

Total proteins

If necessary, dilute the reconstituted preparation with 0.9% (w/v) sodium chloride solution to obtain a solution containing approximately 15 mg of protein in 2 mL. In a round-bottom centrifuge tube,

add 2 mL of this solution . Add 2 mL of 7.5% (w/v) sodium molybdate solution and 2 mL of a mixture of nitrogen-free sulfuric acid and water (1:30). Shake and centrifuge for five minutes. The supernatant liquid must be decanted, allowing the tube to be dried on a filter paper. Determine the nitrogen in the residue by the method *Determination of Nitrogen by the Kjeldahl method* **(5.3.3.2).** Calculate the protein content by multiplying the result by 6.25.

Thrombin

If the sample contains heparin, determine the amount present, as indicated in the *Determination of heparin in coagulation factors* **(5.5.1.1)**, and neutralize it by adding protamine sulfate (10 µg of protamine sulfate neutralize 1 IU of heparin). Use two test tubes and, in each one, mix equal volumes of the reconstituted sample of a 0.3% fibrinogen solution (w/v). Keep one tube at 37 °C for six hours and the other one at room temperature for 24 hours. In a third tube, mix one volume of the fibrinogen solution with one volume of human thrombin solution containing 1 IU per milliliter and place the tube in a 37 °C water bath. No coagulation is produced in the sample tubes. Coagulation is produced within 30 seconds in the tube containing thrombin.

STORAGE

Protect from light.

LABELLING

The label indicates at least:

- the number of international units of factor VII in each vial;
- the amount of protein in each vial;
- the name and amount of any substance added, including heparin, when applicable;
- the name and volume of the diluent needed to reconstitute the preparation; the storage conditions;
- the expiration date; that the transmission of infectious agents cannot be totally excluded when administering human blood or plasma medicinal products (the latter may alternatively be indicated in the package insert text).

LYOPHILIZED HUMAN BLOOD COAGULATION FACTOR VIII

Factor VIII coagulationis sanguinis humanus cryodesiccatus

coagulation factor VIII; 03809

Lyophilized human blood coagulation factor VIII is a protein fraction of plasma that contains a glycoprotein called coagulation factor VIII and, depending on the purification method, variable amounts of von Willebrand factor. It is prepared from a mixture of plasma obtained from healthy donors. The activity of the preparation, reconstituted according to the indications provided on the label, is not less than 20 IU of factor VIII:C per milliliter.

The method of preparation includes two or more viral inactivation steps that demonstrate the elimination or inactivation of known viral infectious agents. If viral inactivation substances are added in the production step, a purification procedure must be validated to demonstrate that the concentrations of these substances have been reduced to an acceptable level, and that such residues do not compromise the safety of the preparation. The specific activity is not less than 1 IU of factor VIII:C per milligram of total protein, before the eventual addition of a protein stabilizer.

Lyophilized factor VIII is dissolved in a diluent specified by the manufacturer. Auxiliary substances, such as a stabilizer, can be added. No antimicrobial preservatives are added. The solution is filtered to provide retention of bacteria, then being aseptically distributed to final containers and immediately frozen. It is then lyophilized, and the containers are closed under vacuum or inert gas.

Validation applied to products with an indication of having an activity of von Willebrand factor. In products intended for the treatment of Von Willebrand disease, it has been shown that the manufacturing process gives rise to a product with a constant composition with regard to the Von Willebrand factor. This composition can be demonstrated in several manners. For example, the number and the various multimers of von Willebrand factor may be determined by agarose gel electrophoresis (approximately 1% agarose) in the presence of sodium dodecyl sulfate (SDS), with or without *Western Blot* analysis, by using a mixture of common human plasma as reference. Visualization of the multimeric profile can be performed by an immunoenzymatic technique and quantitative densitometry evaluation or other appropriate methods.

Products with flakes or particles after reconstitution for use. If tiny particles or flakes remain after the reconstituted preparation, it must be demonstrated during the validation study that the potency is not significantly influenced after filtration of the preparation.

IDENTIFICATION

It complies with the test *Lyophilized human blood coagulation Factor VIII* described in *Assay*.

CHARACTERISTICS

Aspect. Hygroscopic white or slightly yellow friable powder or solid.

pH (5.2.19). 6.5 to 7.5.

Osmolality (5.2.28). Not less than 240 mosmol/kg.

Solubility. Add the volume of the diluent indicated on the label to the contents of a vial containing the sample, at the recommended temperature and gently shake for not more than 10 minutes. The sample dissolves completely forming a clear or slightly opalescent, colorless or slightly yellow solution. When the product vial presents tiny particles or flakes after reconstitution, the preparation must be reconstituted and filtered, as described on the label. The filtered solution is clear or slightly opalescent.

PHYSICOCHEMICAL TESTS

Water. Determine by an appropriate method such as *Determination of water by the semi-micro method* **(5.2.20.3)**, *Loss on drying* **(5.2.9.1)** or *Infrared absorption spectrophotometry* **(5.2.14)**. Not more than 2.0%.

BIOLOGICAL SAFETY TESTS

Pyrogens (5.5.2.1). Complies with the test. Inject into each rabbit, per kilogram of body weight, a volume of the reconstituted sample corresponding to not less than 30 IU of factor VIII:C.

Sterility (5.5.3.2.1). Complies with the test.

ASSAY

Hepatitis B surface antigens

Proceed as described in *Immunochemical methods* **(5.6).** Examine the reconstituted sample. Hepatitis B surface antigen is not detected.

Human von Willebrand factor

Use one of the methods described below.

A. Proceed as described in *Determination of human von Willebrand factor* **(5.5.1.2).**

B. Determine the activity of ristocetin cofactor. Prepare appropriate dilutions of the reconstituted sample and reference preparation by using a 0.9% sodium chloride (w/v) and 5% human albumin (w/v) solution as diluent. Add to each preparation an appropriate amount of a mixture containing stabilized human platelets and ristocetin A. Mix on a glass slide with gentle circular movements for one minute. Allow to stand for one minute and read the result in a dark background and side lighting. The last dilution showing a clearly visible agglutination will indicate the sample titer. Diluent should be used as negative evidence. The determined activity is not less than 60% and not more than 140% of the activity approved for the product.

Lyophilized human blood coagulation factor VIII

Proceed as described in *Determination of lyophilized human blood coagulation factor VIII* **(5.5.1.7).** The determined activity is not less than 80% and not more than120% of the indicated activity.

Anti-A and anti-B hemagglutinins

Proceed as described in *Determination of anti-A and anti-B hemagglutinin titers* **(5.5.1.9).** Dilute the reconstituted sample with 0.9% sodium chloride solution (w/v) up to a 3 IU/mL concentration. The dilutions to 1/64 show no signs of agglutination. Complies with the test.

Total proteins

Proceed as described in *Determination of nitrogen by the Kjeldahl method* **(5.3.3.2).** If necessary, dilute the reconstituted preparation with a 0.9% sodium chloride solution (w/v) to obtain a solution containing approximately 15 mg of protein in 2 mL. Add 2 mL of this solution, 2 mL of 7.5% sodium molybdate solution (w/v), and 2 mL of a mixture of nitrogen-free sulfuric acid and water (1:30) to a round-bottom centrifuge tube. Shake and centrifuge for five minutes. The supernatant liquid must be decanted allowing the tube to be dried on a filter paper. Calculate the protein content multiplying the result by 6.25. This method may not be applicable to certain products, especially those that do not contain a protein stabilizer, such as albumin, with another validated method being used for the assay.

STORAGE

Protect from light.

LABELLING

Comply with current legislation. The label indicates:

- the number of international units of factor VIII:C and, if appropriate, of von Willebrand factor;
- the amount of protein in each container;
- the name and amount of any substance added;
- the name and volume of the liquid needed to reconstitute the preparation; the storage conditions;
- the expiration date; that the transmission of infectious agents cannot be totally excluded when administering human blood or plasma-derived drugs (the latter may alternatively be indicated in the package insert text).

LYOPHILIZED HUMAN FIBRINOGEN

Fibrinogenum humanum cryodesiccatus

fibrinogen; 04045

Lyophilized human fibrinogen contains the soluble fraction of human plasma that, by adding thrombin, is transformed into fibrin. Fibrinogen must be obtained from *Human plasma for fractionation.* The preparation may contain additives such as salts, buffers or stabilizers. The preparation reconstituted with the volume of diluent indicated on the label must contain not less than 10 g/L of fibrinogen.

The preparation method comprises one or more steps that have been shown to eliminate known infectious agents; it has been shown that the residues in the final product of substances eventually used in processes intended for viral inactivation or in further validated purification processes do not have any undesirable effect on patients.

No antibiotics should be added to the plasma, and the preparation should not contain any antimicrobial preservatives.

The preparation method must be such that the specific activity (fibrinogen content in relation to the total protein content) is not less than 80%. If a protein stabilizer (e.g. human albumin) is added to the preparation, it must meet the requirements for the specific activity of fibrinogen prior to the stabilizer addition.

During the fractionation of human plasma, fibrinogen and albumin can be obtained at the same time. The determination of the albumin specific activity must then be determined by an appropriate immunochemical method, and the determined amount must be subtracted from the amount of total protein for the calculation of specific activity.

IDENTIFICATION

Reconstitute the sample, according to the indications on the label, carry out precipitation tests with several specific sera from different species. The test is recommended to be carried out with specific serum of plasmatic proteins from each species of domestic animal currently used for the preparation of products of biological origin. The sample must contain proteins of human origin and provides negative results with serum specific for plasmatic proteins from other species. Sample assay contributes to identifying the preparation.

CHARACTERISTICS

Aspect. Friable, white or pale yellow powder or solid.

pH (5.2.19). 6.5 to 7.5.

Osmolality (5.2.28). Not less than 240 mosmol/kg.

Solubility. Add the volume of diluent indicated on the label to the vial content. At a temperature of 20 °C to 25 °C, fibrinogen dissolves within 30 minutes, resulting in an almost colorless and slightly turbid preparation.

Preparation stability. After reconstituting the preparation at a temperature of 20 °C to 25 °C, allow to stand. No sign of gelation should appear within 60 minutes upon reconstitution.

PHYSICOCHEMICAL TESTS

Water. Determine by one of the following methods: *Determination of water by the semi-micro method* **(5.2.20.3)**, *Loss on drying* **(5.2.9.1)** or *Infrared absorption spectrophotometry* **(5.2.14)**. Not more than 2.0%.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

Pyrogens (5.5.2.1). Complies with the test. Inject into each rabbit, per kilogram of body weight, a volume corresponding to not less than 30 mg of fibrinogen, calculated in relation to the amount indicated on the label.

ASSAY

Hepatitis B surface antigens

Examine the reconstituted specimen as described in *Immunochemical methods* **(5.6).** Hepatitis B surface antigen should not be detected.

Fibrinogen

Mix 0.2 mL of the reconstituted sample with 2 mL of appropriate buffer (pH 6.6 to 6.8) containing a sufficient amount of thrombin (about 3 IU/mL) and calcium (0.05 mol/L). Keep the mixture at 37 °C for 20 minutes, separate the precipitate by centrifugation (5000 × *g* for 20 minutes) and wash carefully with a 0.9% (w/v) sodium chloride solution. Determine the nitrogen content by the method *Determination of nitrogen by the Kjeldahl method* **(5.3.3.2)** and calculate the amount of fibrinogen (coagulation proteins) by multiplying the result by 6. The content is not less than 70.0% and not more than 130.0% of the amount indicated on the label. Also, kits are available on the market for manual or automated quantitative determinations of fibrinogen in citrated plasma, by the clot formation method. These kits are based on an optimum amount of bovine thrombin that is added to a 1:10 diluted plasma. The measured coagulation time must be inversely related to the concentration of fibrinogen in the tested sample. These kits must be registered with the competent body and duly validated in accordance with the standards of the *National Committee for Clinical Standards*: *Collection Transport and Processing of Blood Specimens for Coagulation Testing and Performance of Coagulation Assays.* 1991. NCCLS *Document* H21 - A2 can be used in hemotherapy units that produce cryoprecipitate from fresh frozen plasma, and need to quantify plasma fibrinogen.

STORAGE

Protect from light.

LABELLING

The label must state the amount of fibrinogen contained in the flask, the name and volume of the diluent to be used to reconstitute the preparation, where appropriate; the name and amount of protein stabilizer used in the preparation.

ANTI-D HUMAN IMMUNOGLOBULIN
Immunoglobulinum humanum anti-D

anti-D human immunoglobulin; 11440

Anti-D human immunoglobulin is a sterile, liquid or lyophilized preparation containing immunoglobulins, especially immunoglobulin G. The preparation is for intramuscular administration. It is obtained from plasma from D-negative donors containing high titer of specific anti-D immunoglobulins and small amounts of antibodies against other blood groups. Normal human immunoglobulin can be added to it. Anti-D human immunoglobulin meets the requirements set in the monograph *Normal Human Immunoglobulin*, except with regard to the minimum number of donors and the minimum content in total proteins.

Stability. For the liquid preparation, conduct an accelerated degradation test, performed by heating at 37 °C for four weeks in the final product; the loss of anti-D activity is not more than 20% of the initial value.

To limit B19 viral load in plasma pools used by manufacturers of anti-D immunoglobulin, the plasma pool is tested for the presence of the B19 virus using techniques for nucleic acid and amplification properly validated. Not more than 10 IU/µL.

A positive control with 10 IU of B19 virus DNA per microliter and an internal control prepared by adding an appropriate marker in a sample from the plasma pool are used in the test. The test is invalid if the positive control is not reagent or if the result obtained with the internal control indicates the presence of inhibitors.

If human immunoglobulin and/or human albumin solution is added to the preparation, the plasma pool or origin pools must meet the requirements stated above for B19 virus DNA.

ASSAY

Proceed as described in *Determination of anti-D human immunoglobulin* **(5.5.1.15)**. The estimated potency is not less than 90% of the stated potency. Confidence intervals (P = 0.95) are not less than 80% and not more than 120% of the estimated potency.

PACKAGING AND STORAGE

See monograph *Normal human immunoglobulin*.

LABELLING

Comply with current legislation. See monograph *Normal human immunoglobulin*. On the label it is indicated the number of International Units per container.

ANTI-HEPATITIS A HUMAN IMMUNOGLOBULIN

Immunoglobulinum humanum hepatitidis A

Anti-hepatitis A human immunoglobulin; 11441

Anti-hepatitis A human immunoglobulin is a sterile, liquid or lyophilized preparation containing immunoglobulins, especially immunoglobulin G. The preparation is for intramuscular administration. It is obtained from plasma from selected donors containing antibodies against hepatitis A. Normal human immunoglobulin can be added to it. Anti-hepatitis A human immunoglobulin meets the requirements set in the monograph *Normal Human Immunoglobulin*, except with regard to the minimum number of donors and the minimum content in total proteins.

ASSAY

The activity of anti-Hepatitis A human immunoglobulin is evaluated by comparing to the activity of a reference preparation measured in International Units, by means of a test with appropriate sensitivity and specificity (Proceed as described in *Immunochemical methods* **(5.6)**. The International Unit corresponds to the activity of a certain quantity of international reference preparation of anti-hepatitis A human immunoglobulin. The correspondence between the International Unit and the preparation of international reference is indicated by the World Health Organization.

The activity indicated is not less than 600 IU/mL. The activity estimated is not less than the activity indicated. Confidence intervals (P = 0.95) are not less than 80% and not more than 125% of the estimated activity.

PACKAGING AND STORAGE

See monograph *Normal human immunoglobulin*.

LABELLING

Comply with current legislation. See monograph *Normal human immunoglobulin*. On the label it is indicated the number of International Units per milliliter.

ANTI-HEPATITIS B HUMAN IMMUNOGLOBULIN

Immunoglobulinum humanum hepatitidis B

Anti-hepatitis B human immunoglobulin; 10809

Anti-hepatitis B human immunoglobulin is a sterile, liquid or lyophilized preparation containing immunoglobulins, especially immunoglobulin G. The preparation is for intramuscular administration. It is obtained from plasma from selected donors containing antibodies against hepatitis B. Normal human immunoglobulin can be added to it. Anti-hepatitis B human immunoglobulin meets the requirements set in the monograph *Normal Human Immunoglobulin*, except with regard to the minimum number of donors and the minimum content in total proteins.

ASSAY

The activity of anti-hepatitis B human immunoglobulin is evaluated by comparison with the activity of a reference preparation measured in International Units, by means of an appropriate sensitivity and specificity assay (Proceed as described in *Immunochemical methods* **(5.6)**). The International Unit corresponds to the activity of a certain quantity of international reference preparation of anti-hepatitis B human immunoglobulin. The correspondence between the International Unit and the preparation of reference is indicated by the World Health Organization.

The activity indicated is not less than 100 IU/mL. The activity estimated is not less than the activity indicated. Confidence intervals (P = 0.95) are not less than 80% and not more than 125% of the estimated activity.

PACKAGING AND STORAGE

Comply with the monograph *Vaccines for human use*.

LABELLING

Comply with current legislation.

ANTI-HEPATITIS B HUMAN IMMUNOGLOBULIN FOR INTRAVENOUS ADMINISTRATION

Immunoglobulinum humanum hepatitidis B ad usum intravenosum

Anti-hepatitis B human immunoglobulin for intravenous administration is a sterile liquid or lyophilized preparation containing immunoglobulins, especially immunoglobulin G. It is obtained from plasma from plasma from donors carrying specific antibodies against the surface antigen of hepatitis B. It can be added to normal human immunoglobulin for intravenous administration. Anti-hepatitis B human immunoglobulin for intravenous administration meets the requirements set in the monograph *Normal Human Immunoglobulin for intravenous administration*, except with regard to the minimum number of donors and the minimum content in total proteins and the limit of osmolality.

ASSAY

The activity of anti-hepatitis B human immunoglobulin for intravenous administration is evaluated by comparison of the antibody titer of the sample and the activity of a reference preparation measured in International Units, by means of an immunoassay with appropriate sensitivity and specificity (Proceed as described in *Immunochemical methods* **(5.6)**). The International Unit corresponds to the activity of a certain quantity of international reference preparation of anti-hepatitis B human immunoglobulin. The correspondence between the International Unit and the preparation of international reference is indicated by the World Health Organization.

The activity indicated is not less than 50 IU/mL. The activity estimated is not less than the activity indicated. Confidence intervals (P = 0.95) are not less than 80% and not more than 125% of the estimated activity.

PACKAGING AND STORAGE

See monograph *Normal human immunoglobulin for intravenous administration*.

LABELLING

Comply with current legislation. See monograph *Normal human immunoglobulin for intravenous administration*. On the label it is indicated the minimum number of International Units per container.

ANTI-RABIES HUMAN IMMUNOGLOBULIN
Immunoglobulinum humanum rabicum

Anti-rabies human immunoglobulin; 11442

Anti-rabies human immunoglobulin is a sterile, liquid or lyophilized preparation containing immunoglobulins, especially immunoglobulin G. The preparation is for intramuscular administration. It is obtained from plasma from donors carrying specific antibodies against rabies. Normal human immunoglobulin can be added. Anti-rabies human immunoglobulin meets the requirements set in the monograph *Normal Human Immunoglobulin*, except with regard to the minimum number of donors and the minimum content in total proteins.

ASSAY

The activity of anti-rabies human immunoglobulin is evaluated by comparing the dose required to neutralize the infectious power of rabies virus and the dose of a reference preparation measured in International Units required to ensure the same degree of neutralization (*Immunochemical methods* **(5.6)**). Perform the measurement in sensitive cell cultures and reveal the presence of the virus not neutralized by immunofluorescence. The International Unit corresponds to the specific neutralizing activity for the rabies virus of a given amount of an international reference preparation of anti-rabies human immunoglobulin.

The correspondence between the International Unit and the international reference preparation is indicated by the World Health Organization.

Perform the measurement in appropriate sensitive cells. Use the cell line BHK 21, multiplied in the culture medium described below, and submit from 18 to 30 passages from ATCC seed lot. Collect the cells after incubation for 2 to 4 days. Treat the cells with trypsin. Prepare a suspension of 500 000 cells per milliliter (cell suspension). To increase the sensitivity of cells add, if necessary, 10 minutes before the use of this suspension, 10 µg of diethylaminoethyl dextran per milliliter.

Use a fixed strain of virus multiplied in sensitive cells, for example, the CVS strain adapted to culture in cell line BHK 21 (virus stock suspension). Titrate the virus stock suspension as follows:

Prepare a series of virus suspension dilutions. On plates with cell culture chambers (eight chambers per plate), distribute 0.1 mL of each dilution. Add 0.1 mL of the culture medium and 0.2 mL of the cell suspension. Incubate at 37°C in an atmosphere of carbon dioxide for 24 hours. Fix, stain with immunofluorescence and calculate as described below. Determine the titer of the virus stock suspension and prepare a virus working dilution corresponding to 100 $TCID_{50}$ per 0.1 mL.

In each test, check the amount of virus by performing a control titration: from the dilution corresponding to 100 $TCID_{50}$ per 0.1 mL, perform three successive dilutions of ratio 10. Distribute respectively 0.1 mL of each dilution in four chambers containing 0. 1 mL of the culture medium and add 0.2 mL of the cell suspension. The test is valid only if the titration is between 30 and 300 $TCID_{50}$.

Dilute the reference preparation with non- supplemented culture medium to a concentration of 2 IU/mL (reference stock dilution and store below -80 °C). Prepare two appropriate pre-dilutions (1/8 and 1/10) of the reference stock dilution so that the dilution of the reference preparation, which reduces by 50% the number of fluorescent fields in the cell culture plate, is among the four dilutions. Add 0.1 mL of medium to each chamber, except in the 1st of each of the two rows to which it is added,

respectively, 0.2 mL of the two pre-dilutions of the reference stock dilution, and then transfer 0.1 mL successively to the other chambers.

Dilute the sample at 1/100 with non-supplemented culture medium (immunoglobulin stock dilution) to minimize errors due to the viscosity of the undiluted preparation. Prepare three appropriate pre-dilutions of the immunoglobulin stock dilution so that the sample dilution, which reduces by 50% the number of fluorescent fields in the cell culture plate, is among the four dilutions.

Add 0.1 mL of medium to each chamber, except in the 1st of each of the three rows to which it is added, respectively, 0.2 mL of the three pre-dilutions of the immunoglobulin stock dilution. Prepare a series of dilution of ratio 2 successively transferring 0.1 mL to the other chambers.

To all chambers containing the dilutions of the reference preparation and the sample dilutions, add 0.1 mL of virus suspension corresponding to 100 $TCID_{50}$ per 0.1 mL (working dilution), shake manually and allow to stand at 37° C in an atmosphere of carbon dioxide for 90 minutes, add 0.2 mL of the cell suspension, shake manually and allow to stand at 37 °C in an atmosphere of carbon dioxide for 24 hours. After 24 hours, remove the medium and withdraw the plastic walls.

Wash the monocellular chambers with pH 7.4 phosphate saline buffer and then with a mixture of 20 volumes of water and 80 volumes of acetone and fix for 3 minutes with a mixture of 20 volumes of water and 80 volumes of acetone at -20 °C. Spread the ready-to-use fluorescent anti-rabies serum conjugate onto the slides.

Allow to stand for 30 minutes at 37 °C in an atmosphere with very high humidity. Wash with pH 7.4 phosphate saline buffer and dry. Examine 20 fields of each chamber at a magnification of 250 times with a microscope equipped for fluorescence reading. Record the number of fields containing not less than one fluorescent cell. Check the dose of the test virus used on the plate for virus titration and determine the preparation dilution of the reference and sample, which reduces by 50% the number of fluorescent fields, performing the calculations for the set of two or three dilutions, through iterative probability analysis. The test is only valid when the statistical analysis demonstrates a significant slope of the dose/effect curve and does not reveal deviation of linearity or parallelism.

The activity indicated is not less than 150 IU/mL. The estimated activity is not inferior to the declared activity or superior to twice the activity indicated. Confidence intervals (P = 0.95) are not less than 80% and not more than 125% of the estimated activity.

CULTURE MEDIUM FOR GROWTH OF BHK 21 CELLS

Media sold with a slightly different composition from that indicated can also be used.

sodium chloride	6.4 g
potassium chloride	0.40 g
calcium chloride, anhydrous	0.20 g
magnesium sulfate, heptahydrate	0.20 g
sodium phosphate monohydrate	0.124
glucose monohydrate	4.5 g
ferric nitrate nonahydrate	0.10 mg
L-arginine hydrochloride	42.0 mg
L-cystine	24.0 mg
L-histidine	16.0 mg
L-isoleucine	52.0 mg
L-leucine	52.0 mg

L-lysine hydrochloride	74.0 mg
L-phenylalanine	33.0 mg
L-threonine	48.0 mg
L-tryptophan	8.0 mg
L-tyrosine	36.0 mg
L-valine	47.0 mg
L-methionine	15.0 mg
L-glutamine	0.292 g
I-inositol	3.60 mg
choline chloride	2.0 mg
folic acid	2.0 mg
nicotinamide	2.0 mg
calcium pantothenate	2.0 mg
pyridoxal hydrochloride	2.0 mg
thiamine hydrochloride	2.0 mg
riboflavin	2.0 mg
phenol red	15.0 mg
sodium bicarbonate	2.75 g
water q.s.p.	1000 mL

Add to the medium the following supplement:

fetal calf serum (heated at 56°C for 30 minutes)	10%
tryptose phosphate broth	10%
benzylpenicillin sodium	60 mg/L
streptomycin	0.1 g/L

PACKAGING AND STORAGE

See monograph *Normal human immunoglobulin*.

LABELLING

See monograph *Normal human immunoglobulin*. On the label it is indicated the number of International Units per container.

ANTI-RUBELLA HUMAN IMMUNOGLOBULIN
Immunoglobulinum humanum rubellae

Anti-rubella human immunoglobulin; 11443

Anti-rubella human immunoglobulin is a sterile, liquid or lyophilized preparation containing immunoglobulins, especially immunoglobulin G. The preparation is for intramuscular administration. It is obtained from plasma containing specific antibodies against the rubella virus. Normal human immunoglobulin can be added. Anti-rubella human immunoglobulin meets the requirements set in the monograph *Normal Human Immunoglobulin*, except with regard to the minimum number of donors and the minimum content in total proteins.

ASSAY

The activity of anti-rubella human immunoglobulin is evaluated by comparison with the activity of a reference preparation measured in International Units, through appropriate test of hemagglutination inhibition. The International Unit corresponds to the activity of a certain amount of international reference preparation of anti-rubella human serum. The correspondence between the International Unit and the international reference preparation is indicated by the World Health Organization.

The estimated activity is not less than 4500 IU/mL. Confidence intervals (P = 0.95) of the estimated activity are not less than 50% and not more than 200% of the declared activity.

PACKAGING AND STORAGE

See monograph *Normal human immunoglobulin*.

LABELLING

Comply with current legislation. See monograph *Normal human immunoglobulin*. On the label it is indicated the number of International Units per milliliter.

ANTI-MEASLES HUMAN IMMUNOGLOBULIN
Immunoglobulinum humanum morbillicum

Anti-measles human immunoglobulin; 11444

Anti-measles human immunoglobulin is a sterile, liquid or lyophilized preparation containing immunoglobulins, especially immunoglobulin G. The preparation is for intramuscular administration. It is obtained from plasma containing specific antibodies against the measles virus. Normal human immunoglobulin can be added. Anti-measles human immunoglobulin meets the requirements set in the monograph *Normal Human Immunoglobulin*, except with regard to the minimum number of donors and the minimum content in total proteins.

ASSAY

The activity of the liquid preparation, or lyophilized preparation reconstituted according to the indications on the label is not less than 50 IU of neutralizing antibodies of the measles virus per milliliter.

The activity is evaluated by comparing the titration of antibodies in the sample and a reference preparation measured in International Units, using a test dose of measles virus in appropriate cell culture.

The International Unit corresponds to the specific neutralizing activity for the measles virus of a given amount of the international reference preparation of anti-measles human serum.

The correspondence between the International Unit and the international reference preparation is indicated by the World Health Organization.

Prepare serial dilutions of the sample and the reference preparation. Mix equal volumes of each dilution and a measles virus suspension containing approximately 100 $TCID_{50}$ in 0.1 mL. Incubate these mixtures protected from light at 37 °C for 2 hours. Use not less than six cell cultures for each mixture and inoculate 0.2 mL of the mixture per culture. Incubate for not less than 10 days. Examine cultures as to the virus development.

Determine the activity comparing the dilution that contains the lowest amount of the sample that has neutralized the virus with the reference preparation that manifests similar activity. Calculate sample activity in international units of neutralizing antibodies of the measles virus per milliliter.

PACKAGING AND STORAGE

See monograph *Normal human immunoglobulin*.

LABELLING

See monograph *Normal human immunoglobulin*. On the label it is indicated the number of International Units per container.

ANTI-TETANUS HUMAN IMMUNOGLOBULIN
Immunoglobulinum humanum tetanicum

anti-tetanus human immunoglobulin; 11445

The anti-tetanus human immunoglobulin is a sterile, liquid or lyophilized preparation containing immunoglobulins, especially immunoglobulin G. It is obtained from plasma containing specific antibodies against the *Clostridium tetani* toxin. Normal human immunoglobulin can be added. Anti-tetanus human immunoglobulin meets the requirements set in the monograph *Normal Human Immunoglobulin*, except with regard to the minimum number of donors and the minimum content in total proteins.

During production, it is necessary to establish a satisfactory relation between the activity determined by immunoassay with the method *Determination of human activity against tetanus* and the activity determined by the method *Antitoxic activity in mice* in *Assay*.

ASSAY

Antitoxic activity in mice

Evaluate the activity by determining the dose that ensures the protection of mice against the paralyzing effects of a given dose of tetanus toxin. This dose is compared with a reference preparation of a tetanus human immunoglobulin measured in international units required to provide the same protection. The International Unit of antitoxin corresponds to specific neutralizing activity regard to tetanus toxin contained in a certain amount of international standard consisting of lyophilized human immunoglobulin. The correspondence between the International Unit and the International Standard is indicated by the World Health Organization. The tetanus human immunoglobulin is measured in International Units in comparison with the International Standard.

Choice of animals. Use mice weighing between 16 and 20 g.

Preparation of test toxin. Prepare the toxin test by a suitable method from the sterile filtrate of a culture of *C. tetani* in liquid medium. The two methods mentioned below are given as an example, but any other appropriate method may be used.

(1) To a culture filtrate of about nine days, add 1 to 2 volumes of glycerin and maintain the mixture in a liquid state at a temperature slightly lower than 0 °C.

(2) Precipitate the toxin by adding ammonium sulfate to the culture, dry the precipitate under vacuum in the presence of phosphorus pentoxide, pulverize and keep it dry in sealed ampoules under vacuum in the presence of phosphorus pentoxide.

Determination of test toxin dose (dose Lp/10).
Prepare a solution of the reference preparation in a suitable liquid so that it contains 0.5 IU of antitoxin per milliliter. If the toxin is stored in a dry state, reconstitute using a suitable liquid. Prepare a series of mixtures of the solution preparation of the sample and reference so that each one contains 2 mL of the solution of the reference preparation and a variable amount of the sample. Complete each mixture with the same final volume of 5 mL using a suitable liquid. Allow to stand for 60 minutes protected from light. Use a group of six mice for each mixture. Inject each of them subcutaneously with 0.5 mL of the mixture assigned to the group. Keep the mice under observation for 96 hours. The ones suffering from paralysis can be sacrificed. The toxin test dose corresponds to the amount present in 0.5 mL of the mixture containing the lowest amount of toxin that causes, during the period of

observation, paralysis in 6 mice to which it was administered, despite partial neutralization due to reference preparation.

Determination of the immunoglobulin activity

Prepare a solution of the reference preparation in a suitable liquid so that it contains 0.5 IU of antitoxin per milliliter. Prepare a solution of the test toxin in a suitable liquid so that it contains 5 doses/mL. Prepare a series of mixtures of the solution of the test toxin and of the sample so that each one contains 2 mL of the solution of the text toxin and a variable amount of the sample. Complete each mixture with the same final volume of 5 mL with a suitable liquid. Prepare a second series of mixtures of the toxin test solution and reference preparation solution so that each one contains 2 mL of solution of the test toxin and a variable amount of the reference preparation. In this second series, the average dilution of the reference preparation corresponds to the mixture containing 1 IU of anti-toxin (2 mL of solution of the reference preparation). Complete each mixture with the same final volume of 5 mL using a suitable liquid. Allow the mixtures of the two series to stand for 60 minutes protected from light. Use a group of six mice for each mixture. Inject each one of them subcutaneously with 0.5 mL of the mixture assigned to the group. Keep the mice under observation for 96 hours. The ones suffering from paralysis can be sacrificed. The mixture containing the maximum amount of immunoglobulin that does not protect any mice from paralysis corresponds to 1 IU. This amount is used to calculate the immunoglobulin activity in international Units per milliliter.

The test is valid only if all the mice inoculated with the mixture containing up to 2 mL of the solution of the reference preparation are affected by paralysis and if all mice inoculated with mixtures containing larger amounts of this solution do not show paralysis symptoms.

Determination of the activity of the anti-tetanus human immunoglobulin

The activity of anti-tetanus human immunoglobulin is evaluated by comparing the antibody titer of the sample and that of a reference preparation, measured in International Units with the aid of a immunoassay test with appropriate sensitivity and specificity (*Immunochemical methods)*. The tetanus human immunoglobulin is measured in International Units in comparison with the International Standard. The indicated activity is not less than 100 IU of tetanus antitoxin per milliliter. The determined activity is not inferior to the indicated activity. The confidence intervals (P = 0.95) of the determined activity are not less than 80% and not greater than 125%.

PACKAGING AND STORAGE

See monograph *Normal human immunoglobulin*.

LABELLING

Comply with current legislation. See monograph *Normal human immunoglobulin*. The label must indicate the number of International Units contained in the vial.

ANTI-VARICELLA HUMAN IMMUNOGLOBULIN
Immunoglobulinum humanum varicellae

anti-varicella human immunoglobulin; 11446

Anti-varicella human immunoglobulin is a sterile, liquid or lyophilized preparation containing immunoglobulins, especially immunoglobulin G. The preparation is for intramuscular administration. It is obtained from plasma containing specific antibodies against the *Herpesvirus varicellae*. Normal human immunoglobulin can be added. Anti-varicella human immunoglobulin meets the requirements set in the monograph *Normal Human Immunoglobulin*, except with regard to the minimum number of donors, to the minimum content in total proteins and, in authorized cases, to the test of antibodies against the hepatitis B surface antigen.

ASSAY

The activity of anti-varicella human immunoglobulin is evaluated by comparison with the activity of a reference preparation measured in International Units, through an appropriate sensitivity and specificity assay (Proceed as described in *Immunochemical methods* **(5.6)**). The International Unit corresponds to the activity of a certain amount of international reference preparation of anti-varicella human immunoglobulin. The correspondence between the International Unit and the international reference preparation is indicated by the World Health Organization.

The activity indicated is not less than 100 IU/mL. The activity estimated is not inferior to the activity indicated. Confidence intervals (P = 0.95) are not less than 80% and not more than 125% of the estimated activity.

PACKAGING AND STORAGE

See monograph *Normal human immunoglobulin*.

LABELLING

Comply with current legislation. See monograph *Normal human immunoglobulin*. On the label it is indicated the number of International Units per milliliter.

ANTI-VARICELLA HUMAN IMMUNOGLOBULIN FOR INTRAVENOUS ADMINISTRATION

Immunoglobulinum humanum varicellae ad usum intravenosum

The anti-varicella human immunoglobulin for intravenous administration is a sterile, liquid or lyophilized preparation containing immunoglobulins, especially immunoglobulin G. It is obtained from plasma containing specific antibodies against the human herpes virus 3 (varicella-zoster virus 1). Normal human immunoglobulin for intravenous administration can be added. Anti-varicella human immunoglobulin for intravenous administration meets the requirements set in the monograph *Normal Human Immunoglobulin for intravenous administration*, except with regard to the minimum number of donors and the minimum content in total proteins and the osmolality limit.

ASSAY

The activity of anti-varicella human immunoglobulin for intravenous administration is evaluated by comparison of the antibody titer of the sample and the activity of a reference preparation measured in International Units, through an immunoassay with appropriate sensitivity and specificity (Proceed as described in *Immunochemical methods* **(5.6)**). The International Unit corresponds to the activity of a certain amount of international reference preparation of anti-varicella human immunoglobulin. The correspondence between the International Unit and the international reference preparation is indicated by the World Health Organization.

The activity indicated is not less than 25 IU/mL. The activity estimated is not less than the activity indicated. Confidence intervals (P = 0.95) are not less than 80% and not more than 125% of the estimated activity.

PACKAGING AND STORAGE

See monograph *Normal human immunoglobulin for intravenous administration*.

LABELLING

Comply with current legislation. See monograph *Normal human immunoglobulin for intravenous administration*. On the label it is indicated the number of International Units per milliliter.

NORMAL HUMAN IMMUNOGLOBULIN
Immunoglobulinum humanum normale

human immunoglobulin; 04865

Normal human immunoglobulin is a sterile, lyophilized or liquid preparation, containing mainly IgG. Other proteins may also be present. Normal human immunoglobulin containing IgG antibodies can be administered intramuscularly or subcutaneously. Normal human immunoglobulin is obtained from human plasma, which meets the requirements of the monograph *Human Plasma for Fractionation*. Antibiotics should not be added in the preparation.

The preparation method should include one or more steps demonstrating the removal or inactivation of known infectious agents. If substances are used for viral inactivation, it must be demonstrated that there is no residue in the final preparation showing adverse effects in patients treated with immunoglobulin. It is necessary to demonstrate, through appropriate testing in animals and evaluation during clinical trials, that the product is well tolerated when administered intramuscularly of subcutaneously. Normal human immunoglobulin is prepared from a pool of plasma from not less than 1000 donors, by a known method providing a sterile final product and with a protein concentration of 160 g/L, containing antibodies for not less than two agents (of which one is viral and one is bacterial) available in Reference Preparation, or International Standard. The concentration of each antibody should be not less than ten times higher than in the original plasma pool.

If normal human immunoglobulin is prepared for subcutaneous administration, the production method should be suitable for product consistent efficiency that complies with the test for immunoglobulin F_c function. Normal human immunoglobulin is prepared as a stabilized solution, for example, in a solution of sodium chloride at 0.9% (w/v); in glycin solution 2.25% (w/v), or if the preparation is lyophilized, in glycin solution of 6% (w/v). Multi-dose preparations should contain an antimicrobial agent. Single-dose preparations should not contain antimicrobial agents. In the final product, the amount of antimicrobial agents or stabilizers used must not present adverse events harmful to health. The substance should be filtered through a bacterial-retaining filter (sterilizing filtration). The preparation may subsequently be lyophilized, and the vials sealed under vacuum or inert gas.

The preparation stability must be established through appropriate testing during the development of the stability study.

IDENTIFICATION

A. Run sample precipitation tests by using a suitable range of specific sera from different species of domestic animals. The test must be carried out with specific sera of plasmatic proteins from each species of domestic animal currently used for the preparation of products with biological origin. The normal human immunoglobulin contains human proteins and provides negative results with specific sera with plasma proteins from other species.

B. Perform in the sample a test of immunoelectrophoresis following proper technique. Using a normal human antiserum, compare the normal human serum with the sample diluted in order to contain 10 g/L of proteins. The main component of the sample corresponds to the IgG component of normal human serum, and there may be other plasma proteins in small amounts. If the human albumin has been added as a stabilizer it may be regarded as an important compound.

CHARACTERISTICS

Aspect. The liquid preparation is clear or pale yellow or slightly brown during storage, and may be slightly turbid or have a small amount of particle formation. The lyophilized preparation is a powder or solid friable mass, white or slightly yellowish. For the lyophilized preparation, reconstitution should take place according to labelling immediately before the *identification* and other tests, except for *Solubility* and *Water*.

pH (5.2.19). 5.0 to 7.2. Dilute the preparation to be examined in a solution of sodium chloride at 0.9% (w/v) at a protein concentration of 1% (w/v).

Osmolality (5.2.28). Not less than 240 mosmol/kg.

Solubility. For the lyophilized preparation, add the volume of the diluent according to the label. The preparation dissolves completely within 20 minutes at a temperature from 20 °C to 25 °C.

Protein composition. Proceed as described in *Electrophoresis* **(5.2.22)**, use the *Zone electrophoresis* technique. Use suitable strips of cellulose acetate gel or agarose as the support medium, and pH 8.6 barbital buffer as electrolyte solution. If the cellulose acetate is the support material, use the method described below. If the support material is the agarose gel, and as it is usually part of the automatic system, follow manufacturer's instructions.

Sample solution: dilute the sample with 0.9% sodium chloride solution (w/v) to a concentration of 50 IU/mL proteins.

Reference solution: reconstitute a reference standard for human immunoglobulin electrophoresis and dilute with sodium chloride at 0.9% (w/v) up to a concentration of 5% (w/v) in proteins.

System suitability: in the electrophoretogram obtained with the *Reference solution*, cellulose acetate or agarose gel, the protein proportion in the main band is in accordance with the limits set in the instructions accompanying the reference preparation.

Procedure: apply to the strip 2.5 µL of *Sample solution* or 0.25 µL per milliliter if a narrower strip is used. For other strips, apply likewise the same amount of the *Reference solution*. Apply a suitable electric field so that the band of human serum albumin, applied to the control strip, migrates not less than 30 mm. Stain the strip with amido black 10B SR for 5 minutes. Discolor with a mixture of glacial acetic acid and methyl alcohol (10:90) so that the bottom is free from staining. Develop transparency of the strips with a mixture of glacial acetic acid and methyl alcohol (19:81). Measure the band absorbance in linear response instruments and wavelength of 600 nm. Calculate the result as the average of three measurements for each band.

The protein mobility is not greater than 10% of the main protein band.

Distribution of molecular size. Proceed as described in *High efficiency liquid chromatography* **(5.2.17.4)**. Use chromatograph equipped with an ultraviolet detector at 280 nm, column 600 mm length and 7.5 mm inner diameter or 300 mm length and 7.8 mm inner diameter, packed with hydrophilic silica gel (for an adequate degree for fractionation of globular proteins with relative molecular mass between 10,000 and 500,000), *Mobile phase* flow of 0.5 mL/minuto.

Mobile phase: dissolve 4.873 g of dibasic dihydrate sodium phosphate, 1.741 g monobasic monohydrate sodium phosphate, 11.688 g of sodium chloride and 50 mg of sodium azide in 1000 mL of water.

Sample solution: dilute the sample with sodium chloride solution at 0.9% (w/v) up to concentration suitable to the chromatographic system used. The concentration range of 4 g/L to 12 g/L, and the injection from 50 µg to 600 µg of protein is usually adequate.

Standard solution: dilute the human immunoglobulin standard with sodium chloride solution at 0.9% (w/v) to obtain a concentration in proteins equal to the *Sample solution*.

In the chromatogram obtained with the *Standard solution*, the main peak corresponds to IgG monomers and there is a peak corresponding to the dimer with relative retention of the main peak of 0.85. Identify peaks in the chromatogram obtained with the *Sample solution* compared to the chromatogram of the *Standard solution*. No peak with retention time lower than that of the dimer corresponds to polymers and aggregates. The preparation to be examined complies with the test if the chromatogram obtained with the *Sample solution* meets the following items:

- the relative retention time, compared to the corresponding peak of the chromatogram obtained with the *Standard solution* is 1 ± 0.02 for the monomer and dimer;
- *area under the peak:* the sum of the area under the peaks of the monomer and dimer is not less than 85% of the total area of the chromatogram and the total area under the peaks of polymers and aggregates is not more than 10% of the total area of the chromatogram. This requirement does not apply to preparations to which albumin is added as a stabilizer. In the case of preparations stabilized with albumin, a molecular size distribution test is performed during manufacturing before adding the stabilizer.

PHYSICOCHEMICAL TESTS

Water. Determined by an appropriate method such as the *Semi-micro method* **(5.2.20.3)**, *Loss on drying* **(5.2.9.1)** or *Infrared absorption spectrophotometry* **(5.2.14)**. Not more than 2%.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

Pyrogens (5.5.2.1). Complies with the test. Inject each rabbit, per kilogram of body weight, with volume corresponding to not less than 1 mL of immunoglobulin.

ASSAY

Anti-D antibody

If normal immunoglobulin is for subcutaneous use, it must comply with *Determination of anti-D human immunoglobulin* **(5.5.1.15)** in normal immunoglobulin for intravenous administration.

Antibody for Hepatitis B surface antigen

Determine through a suitable *Immunochemical method* **(5.6).** Not less than 0.5 UI/g of immunoglobulin.

Antibody for Hepatitis A virus

If intended for Hepatitis A prophylaxis, it must meet the following additional requirements. Determine the content of antibodies in comparison with the preparation of a reference standard measured in IU by using a suitable, specific and sensitive *Immunochemical method* **(5.6).**

The International Unit is the amount of activity of the international Standard of anti-hepatitis A immunoglobulin. The equivalent in international Standard unit is declared by the World Health Organization.

The reference standard of human immunoglobulin against Hepatitis A is measured in international Units compared to the international Standard. The stated potency is not less than 100 IU/mL. The estimated potency is not less than the stated potency. Confidence interval (P = 0.95) of the estimated potency is not less than 80% and not more than 125%.

Anti-A and anti-B hemagglutinins

Run the test if the normal human immunoglobulin is for subcutaneous preparation. Proceed as described in *Determination of anti-A and anti-B hemagglutinin titers* **(5.5.1.9).** Dilute the preparation to be examined at a concentration of 30 g/L of immunoglobulin prior to the preparation of serial dilutions to be used in the test. The agglutination is less than the 1:64 dilution.

Total proteins

Proceed as described in *Determination of nitrogen by the Kjeldahl method* **(5.3.3.2).** Dilute the sample with a 0.9% sodium chloride solution (w/v) to obtain a concentration of about 15 IU/mL of proteins in 2 mL. Add 2 mL of this solution, 2 mL of 7.5% sodium molybdate (w/v), and 2 mL of a mixture of nitrogen-free sulfuric acid and water (1:30) to a round-bottom centrifuge tube. Shake and centrifuge for five minutes. The supernatant liquid must be decanted allowing the tube to be dried on a filter paper. Calculate the protein content by multiplying the nitrogen content by 6.25. The protein content is not less than 100 g/L and not more than 180 g/L. It contains not less than 90% and not more than 100% of the amount indicated on the label.

PACKAGING AND STORAGE

Keep the liquid preparation in a colorless glass container, protected from light and at the temperature indicated on the label. Keep the lyophilized preparation in a colorless glass container under reduced pressure or under inert gas, protected from light and at a temperature not exceeding 25 °C.

LABELLING

Comply with current legislation. The label must indicate:

- for the liquid preparation, the volume of the preparation and protein content should be expressed in g/L;
- for the lyophilized preparation, the amount of protein in the vial;
- the route of administration;
- for the lyophilized preparation, the name of composition and diluent volume for reconstitution to be added;
- where applicable, that the preparation is suitable for use in the prophylaxis of hepatitis A infection;
- where applicable, the activity of anti-hepatitis A immunoglobulin in IU/mL;
- in multi-dose preparations, the name and concentration of the antimicrobial agent.

NORMAL HUMAN IMMUNOGLOBULIN FOR INTRAVENOUS ADMINISTRATION

Immunoglobulinum humanum normale ad usum intravenosum

Normal human immunoglobulin for intravenous administration is a sterile, liquid or lyophilized preparation containing immunoglobulins, especially immunoglobulin G (IgG). Other proteins may also be present. It contains IgG antibodies from normal individuals. This monograph does not apply to preparations produced by a process intended to obtain a preparation containing fragments or chemically modified. Normal human immunoglobulin for intravenous administration is obtained from plasma that meets the requirements of the monograph *Human Plasma for Fractionation*. No antimicrobial is added to the plasma used.

The preparation method comprises one or several stages that inactivate or eliminate known infectious agents. It must be shown that residues in the final product of the substances used in processes to inactivate viruses do not have any undesirable effect on patients treated with the immunoglobulin. The safety of the preparation ready for intravenous administration has been shown by suitable tests on animals and by a study during clinical trials. Normal human immunoglobulin for intravenous administration is prepared from plasma collected from not less than 1000 donors, using a method that enables to obtain a preparation which:

- will not transmit infection;
- in the immunoglobulin concentration of 5% (w/v) contains not less than two antibodies (one bacterial and another viral) for which there is an international standard or a reference preparation, the concentration of such antibodies is not less than three times greater than the initial raw material;
- has a distribution defined in subclasses of immunoglobulin G;
- meets the test for *Determination of immunoglobulin Fc function* **(5.5.1.16)**.

Normal human immunoglobulin for intravenous administration is prepared either in the form of a stabilized or of a lyophilized solution. A stabilizer can be added. In both cases, the preparation is subject to a sterilizing filtration. Antimicrobial preservative is not added during plasma fractionation and in the final plasma pool. The stability of the final product is demonstrated by tests performed during the development studies.

IDENTIFICATION

A. Carry out sample precipitation tests by using a suitable range of specific sera from different species of domestic animals. The test must be carried out with specific sera of plasmatic proteins from each species of domestic animal currently used for the preparation of products with biological origin. The normal human immunoglobulin contains human proteins and provides negative results with specific sera with plasma proteins from other species.

B. Perform in the sample a test of immunoelectrophoresis following proper technique described in *Immunochemical methods* **(5.6)**. Use a normal human antiserum, compare the normal human serum with the sample diluted in order to contain 10 g/L of proteins. The main component of the sample corresponds to the IgG component of normal human serum, and there may be other plasma proteins in small amounts. If the human albumin has been added as a stabilizer it may be detected as an important compound.

CHARACTERISTICS

Aspect. The liquid preparation is clear or slightly opalescent, colorless or pale yellow. The lyophilized preparation is a white or slightly yellowish powder or a solid friable mass. In a lyophilized preparation, its reconstitution is carried out according to the indications on the label immediately before performing the identification and testing, except for solubility and water content.

pH (5.2.19). Between 4.0 and 7.4. Dilute the sample with 0.9% sodium chloride solution (w/v) to a concentration of 1% (w/v) in proteins.

Osmolality (5.2.28). Not less than 240 mosmol/kg.

Solubility. In a lyophilized sample, add the volume of the diluent indicated on the label. The sample dissolves completely in 30 minutes at a temperature of 20 °C to 25 °C.

Composition in Proteins. Proceed as described in *Electrophoresis* **(5.2.22)**, use the *Zone electrophoresis* technique. Use suitable strips of cellulose acetate gel as support, and pH 8.6 barbital buffer as electrolyte solution.

Sample solution: dilute the sample with 0.9% sodium chloride solution (w/v) to a concentration of 3% (w/v) in immunoglobulin.

Standard solution: reconstitute a reference standard for human immunoglobulin electrophoresis and dilute with sodium chloride at 0.9% (w/v) up to a concentration of 3% (w/v) in proteins.

Apply on a strip 4µL of the *Sample solution* in a 10 mm spot or apply 0.4 µL per millimeter when using a narrower strip. In another strip apply, under the same conditions, the same volume of the *Standard solution*. Apply a suitable electric field so that the normal human serum albumin band in a standard electrophoretogram migrates not less than 30 mm. Treat strips with amido black 10B SR for 5 minutes with a mixture of 10 volumes of glacial acetic acid with 90 volumes of methyl alcohol for the time strictly necessary to achieve the discoloration of the frame. Develop the frame transparency with a mixture of 19 volumes of glacial acetic acid with 81 volumes of methyl alcohol. Determine the absorbance of the bands at 600 nm with the aid of an apparatus that in this wavelength provides linear response in the measurement range. Perform three determinations on each strip and calculate the readings average on each strip. In the electrophoretogram of the sample, not more than 5% of the protein may have different mobility from the main band. This limit does not apply if albumin was added to the preparation as a stabilizer, in preparations stabilized with albumin, there will be a composition in proteins test during production, before adding the stabilizer. The test is only valid if, in the electrophoretogram obtained with the *Standard solution*, the proportion of proteins contained in the main band is within the limits indicated in the literature accompanying the preparation of the reference standard.

Distribution of molecular size. Proceed as described in *High efficiency liquid chromatography* **(5.2.17.4)**. Use a chromatograph with ultraviolet detector at 280 nm; column with 300 mm of length and 7.8 mm of inner diameter, packed with hydrophilic silica gel for chromatograph. Flow of *Mobile phase* of 0.5 mL/min.

Mobile phase: dissolve 4.873 g of dibasic dihydrate sodium phosphate, 1.741 g monobasic monohydrate sodium phosphate, 11.688 g of sodium chloride and 50 mg of sodium azide in 1000 mL of water.

Sample solution: dilute the amount of *Sample solution* with sodium chloride solution at 0.9% (w/v) up to concentration suitable to the chromatographic system used. Generally, it is convenient a concentration between 4 g/L and 12 g/L and injection of 500 µg to 600 µg of protein.

Standard solution: dilute the human immunoglobulin standard with sodium chloride solution at 0.9% (w/v) to obtain a concentration in proteins equal to the *Sample solution*.

Inject the *Sample solution* and the *Standard solution*. In the chromatogram obtained with the *Standard solution*, the main peak corresponds to IgG monomers and there is a peak corresponding to the dimer with retention time in relation to the monometer of about 0.85. Identify peaks in the chromatogram obtained with the *Sample solution* compared to the chromatogram obtained with the *Standard solution*, the peaks with the retention time lower than the dimer correspond to polymers and aggregates. The sample meets the test if, in the chromatogram obtained with the *Sample solution,* the retention time, compared to the corresponding peak in the chromatogram obtained with the *Standard solutio*n, is 1 ± 0.02 for the monomer and dimer, and the sum of the monomer and the dimmer represents not less than 90.0% of the total area of the chromatogram and aggregates and polymers represent not more than 3.0% of the total area. This requirement does not apply to preparations with added albumin as a stabilizer, in albumim stabilized preparations, perform a test of molecular size distribution during manufacturing, before adding the stabilizer.

PHYSICOCHEMICAL TESTS

Water. Determine by one of the following methods: *Determination of water by the semi-micro method* **(5.2.20.3)**, *Loss on drying* **(5.2.9.1)** or *Infrared absorption spectrophotometry* **(5.2.14)**. Not more than 2.0%.

Pre-kallikrein activator. Proceed as described in *Determination of pre-kallikrein activator* **(5.5.1.11)**. Not more than 35 IU/mL, calculated in relation to a sample dilution containing 30 g/L of immunoglobulin.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

Pyrogens (5.5.2.1). Complies with the test. Inject each rabbit, per kilogram of body weight, with a volume corresponding to not less than 1 mL of immunoglobulin.

ASSAY

Antibody against Hepatitis B surface antigen

The content of the sample in antibodies against the hepatitis-B surface antigen determined by a suitable *Immunochemical method* **(5.6)** is not less than 0.5 IU/g of immunoglobulin.

Anticomplementary activity

Proceed as described in *Determination of anti-complementary activity of immunoglobulin* **(5.5.1.13)**. The proportion of the consumed complement is not more than 50.0% (1 CH_{50} per milligram of immunoglobulin).

Anti-A and anti-B hemagglutinins

Proceed as described in *Determination of anti-A and anti-B hemagglutinin titers* **(5.5.1.9).** Run tests of anti-A and anti-B Hemagglutinins. If the sample has a content of immunoglobulins greater than 30 g/L dilute to this concentration before preparing dilutions for the test. The dilutions to 1/64 show no signs of agglutination.

Immunoglobulin A

Use a suitable *Immunochemical method* **(5.6).** The immunoglobulin A content is not greater than that indicated on the label.

Total proteins

Dilute the sample with a 0.9% sodium chloride solution (w/v) to obtain a concentration of about 15 IU/mL of proteins in 2 mL. In a round-bottom centrifuge tube, add 2 mL of this solution. Add 2 mL of 7.5% (w/v) sodium molybdate solution and 2 mL of a mixture of nitrogen-free sulfuric acid and 30 volumes of water. Shake and centrifuge for five minutes. The supernatant liquid must be decanted allowing the tube to be dried on a filter paper. Dose the nitrogen in the residue by the method *Determination of Nitrogen by the Kjeldahl method* **(5.3.3.2).** Calculate the protein content by multiplying the nitrogen content by 6.25. The preparation contains not less than 30 g/L and between 90.0% and 110.0% of the amount indicated on the label.

PACKAGING AND STORAGE

Keep the liquid preparation in a colorless glass container, protected from light and at the temperature indicated on the label. Keep the lyophilized preparation in a colorless glass container under reduced pressure or under inert gas, protected from light and at a temperature not exceeding 25 °C.

LABELLING

The label indicates:

- in a liquid product, the preparation volume in the container and the protein content in grams per liter;
- in a lyophilized product, the amount of protein;
- the amount of immunoglobulin in the vial;
- the route of administration;
- storage conditions;
- the expiry date;
- in the lyophilized product, the diluent name or the composition and volume;
- the distribution of immunoglobulin G subclasses in the preparation,
- where appropriate, the amount of albumin added as a stabilizer;
- the maximum content of immunoglobulin A.

MIXTURES OF SURPLUS HUMAN PLASMA TREATED BY VIRAL INACTIVATION

Plasma humanum collectum excederem deinde conditum ad viros exstinguendos

Frozen or lyophilized, sterile, pyrogen-free preparation obtained from surplus human plasma from donors having the same ABO and Rh(Du) blood group. The preparation is thawed or reconstituted prior to use, in order to obtain an injectable solution. The human plasma used should meet the requirements of the monograph *Human Plasma for Fractionation*.

The plasma units for production are frozen at a temperature equal to or less than -30 °C within 6 hours following the separation of blood cell fractions and within not more than 24 hours after collection. The mixture is prepared from plasma units having the same ABO and Rh (Du) blood group.

The plasma mixture is examined by employing appropriate sensitivity and specificity methods for the presence of hepatitis B virus surface antigen virus (HBsAg), hepatitis C antibodies, and HIV antibodies. In these tests, the mixture of plasma must provide negative results.

The mixture of plasma must also be submitted to RNA research of hepatitis C virus, as described in *Amplification Techniques for Nucleic Acids* **(5.5.1.10)** duly validated. The test includes a positive standard containing 100 IU of RNA from hepatitis C virus per mililiter and, to identify the presence of inhibitors, an internal standard prepared by adding an appropriate marker in the plasma mixture sample. The test is only valid if the positive standard is reactive or if the result obtained with the internal standard does not indicate the presence of inhibitors. The mixture meets the test if it is not reactive to RNA of hepatitis C virus.

The plasma mixture must also be submitted to DNA research of the B19 virus, as described in *Amplification Techniques for Nucleic Acids* **(5.5.1.10)** duly validated. The pool should contain not more than 10 IU/µL. The test includes a positive control of 10 IU per microliter of DNA for B19 virus and, to identify the presence of inhibitors, an internal standard prepared by adding an appropriate marker in the plasma mixture sample. The test is only valid if the positive standard is reactive or if the result obtained with the internal standard does not indicate the presence of inhibitors.

The method of preparation is carried out to avoid activating any coagulation factor and thus limit their potential thrombogenic action. It comprises one or more stages related to the elimination or inactivation of known infectious agents. Should substances be used for viral inactivation during the production, the subsequent purification process must be validated to demonstrate that the concentration of these substances is at an appropriate level and potential residues do not compromise the safety of the preparation.

The typical method used for the inactivation of enveloped viruses is the solvent-detergent process, which comprises a treatment with a mixture of tributyl phosphate and octoxynol 10; subsequently, these reagents are removed by solid or oily phase extraction, so that the residual content in the final product is less than 2 µg/mL for tributyl phosphate, and 5 µg/mL for octoxynol 10. Antimicrobial preservatives should not be added.

The solution is filtered through a sterilizing membrane and distributed aseptically into the final containers and immediately frozen. The final containers are composed of plastic material and comply with the requirements for *Plastic containers* **(6.2)**, or glass, complying with the requirements for *Glass containers* **(6.1)**. It may then be lyophilized.

IDENTIFICATION

Reconstitute or thaw the sample as stated on the label immediately before carrying out identification, tests and assays.

A. Perform electrophoresis examination by comparing the sample with normal human plasma. The electrophoretograms have the same bands

B. Perform precipitation tests from a suitable range of specific sera from different species of domestic animals. The assay must be performed with specific serum protein from each of the domestic species normally used in the preparation of biological products. The sample must contain proteins of human origin and provides negative results to specific sera with plasma proteins from other species.

C. The mixture complies with the *Determination of anti-A and anti-B Hemagglutinins* titer (see *Assay*).

CHARACTERISTICS

Aspect. After thawing, the preparation is clear or slightly opalescent, free from solid particles, and gelatinous. The lyophilized preparation is a white or light yellow powder or friable solid.

pH (5.2.19). 6.5 to 7.6.

Osmolality (5.2.28). Not less than 240 mosmol/kg.

PHYSICOCHEMICAL TESTS

Water. Determine by one of the following methods: *Determination of water by the semi-micro method* **(5.2.20.3)**, *Determination of loss on drying* **(5.2.9.1)** or *by Infrared absorption spectrophotometry* **(5.2.14)**. The content is within the limits approved by the competent authorities.

Citrate. Not more than 25 mmol/L. Proceed as described in *High efficiency liquid chromatography* **(5.2.17.4)**. Use a chromatograph equipped with an ultraviolet detector at 215 nm; 300 mm column and 7.8 mm of inner diameter, packed with cation-exchange resin (9 µm), flow of *Mobile phase* of 0.5 mL/min.

Mobile phase: 0.051% sulfuric acid solution (w/v).

Sample solution: dilute the sample with an equal volume of a 0.9% sodium chloride solution (w/v). Filter through a 0.45 µm filter.

Standard solution: dissolve 0.3 g of sodium citrate in water and dilute to 100 mL with the same solvent.

Procedure: separately inject 10 µL of *Standard solution* and *Sample solution.* The citrate retention time is about 10 minutes. Column equilibrium time: 15 minutes.

Calcium. Proceed as described in *Atomic absorption spectrometry* **(5.2.13.1)**. Determine in wavelength at 622 nm. Not more than 5 mmol/L.

Potassium. Proceed as described in *Atomic emission spectrometry* **(5.2.13.2)**. Determine in wavelength at 766.5 nm. Not more than 5 mmol/L.

Sodium. Proceed as described in *Atomic emission spectrometry* **(5.2.13.2)**. Determine in the wavelength at 589 nm. Not more than 200 mmol/L.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

Pyrogens (5.5.2.1). Complies with the test. Inject each rabbit, per kilogram of body weight, with volume corresponding to 3 mL of sample.

ASSAY

Irregular erythrocyte antibodies.

When examined by indirect antiglobulin test, the undiluted sample shows no signs of irregular erythrocyte antibodies.

Hepatitis A virus antibodies.

Not less than 2 IU/mL, determined by suitable *Immunochemical methods* **(5.6)**. The standard of human immunoglobulin for hepatitis A is suitable for use as a reference preparation.

Anti-A and anti-B hemagglutinins.

Proceed as described in *Determination of anti-A and anti-B hemagglutinin titers* **(5.5.1.9).** The presence of the Hemagglutinins (anti-A or anti-B) corresponds to blood group on the label.

Activated coagulation factors.

Proceed as described in *Determination of activated coagulation factors* **(5.5.1.8)**. Perform the test with 0.1 mL of sample instead of dilutions at 1/10 and 1/100. The coagulation time related to the tube containing the sample is not less than 150 seconds. Complies with the test.

Factor V.

Prepare 3 dilutions at 1/10 and 1/40 of the sample by using pH 7.4 imidazole buffer, preferably prepared in duplicate. Proceed as follows for each dilution: mix 0.1 mL of substrate for Factor V deficient plasma, 0.1 mL of dilution from sample, 0.1 mL of thromboplastin reagent, and 0.1 mL of 0.35% calcium chloride solution (w/v). Record the coagulation time, that is, the time interval between the addition of calcium chloride solution and the first signs of fibrin formation. Observe by using appropriate equipment. Determine, in duplicate and under the same conditions, the coagulation times of 4 dilutions between 1/10 and 1/80 of normal human plasma in the pH 7.4 imidazole buffer. A unit of Factor V corresponds to activity of 1 mL of normal human plasma. The normal human plasma is prepared from a mixture of plasma units from not less than 30 donors and kept at a temperature of -30 °C or below. Check the test validity and calculate the sample activity through *Statistical procedures applicable to biological tests* **(8.2).** The activity determined is not less than 0.5 units/mL. Confidence interval (P = 0.95) of the determined activity is not less than 80% and not more than 120%.

Factor VIII.

Proceed as described in *Determination of Blood Coagulation Factor VIII, lyophilized* **(5.5.1.7)** by using a standard plasma calibrated against the international standard Factor VIII for human blood coagulation. The determined activity is not inferior to 0.5 UI/mL. Confidence interval (P = 0.95) of the determined activity is not less than 80% and not more than 120%.

Total proteins.

Dilute the sample with a 0.9% sodium chloride solution (w/v) to obtain a solution containing about 15 mg of protein in 2 mL. In a round-bottom centrifuge tube, add 2 mL of this solution. Add 2 mL of 7.5% sodium molybdate solution (w/v) and 2 mL of a mixture of 1 volume nitrogen-free sulfuric acid and 30 volumes of water. Shake, centrifuge for 5 minutes, decant the supernatant, and invert the tube on a filter paper to drain. Carry out the nitrogen assay in the residue by the digestion method with sulfuric acid, as described in *Determination of nitrogen by the Kjeldahl method* **(5.3.3.2)** and calculate the protein content multiplying the result by 6.25. The total protein content is not less than 45 g/L.

LABELLING

The label should indicate the ABO and Rh (Du) blood group and the method used for viral inactivation. Comply with current legislation.

MIXTURES OF HUMAN PLASMA TREATED BY VIRAL INACTIVATION

Plasma humanum collectum deinde conditum ad viros exstinguendos

Frozen or lyophilized, sterile, pyrogen-free preparation obtained from human plasma from donors having the same ABO and Rh(Du) blood group. The preparation is thawed or reconstituted prior to use, in order to obtain an injectable solution. The human plasma used should meet the requirements of the monograph *Human Plasma for Fractionation*.

The plasma units for production are frozen at a temperature equal to or less than 30 °C within 6 hours following the separation of blood cell fractions and, within not more than 24 hours after collection. The mixture is prepared from plasma units having the same ABO and Rh (Du) blood group.

The plasma mixture is examined by employing appropriate sensitivity and specificity methods for the presence of hepatitis B virus surface antigen (HBsAg), hepatitis C antibodies, and HIV antibodies. In these tests, the mixture of plasma must provide negative results.

The plasma mixture must also be submitted to RNA research of hepatitis C, according to monograph *Amplification Techniques for Nucleic Acids* **(5.5.1.10)** duly validated. The test includes a positive standard containing 100 IU of RNA from hepatitis C virus per mililiter and, to identify the presence of inhibitors, an internal standard prepared by adding an appropriate marker in the plasma mixture sample. The test is only valid if the positive standard is reactive or if the result obtained with the internal standard does not indicate the presence of inhibitors. The mixture meets the test if it is not reactive to RNA of hepatitis C virus.

The plasma mixture must also be submitted to DNA research of the B19 virus, according to monograph *Amplification Techniques for Nucleic Acids* **(5.5.1.10)** duly validated. The pool should contain not more than 10 IU/µL. The test includes a positive control of 10 IU per microliter of DNA for B19 virus and, to identify the presence of inhibitors, an internal standard prepared by adding an appropriate marker in the plasma mixture sample. The test is only valid if the positive standard is reactive or if the result obtained with the internal standard does not indicate the presence of inhibitors.

The method of preparation is carried out to avoid activating any coagulation factor and thus limit their potential thrombogenic action. It comprises one or more stages to which it is demonstrated the elimination or inactivation of known infectious agents. Should substances be used for viral inactivation during the production, the subsequent purification process must be validated to demonstrate that the concentration of these substances is at an appropriate level and potential residues do not compromise the safety of the preparation.

The typical method used for the inactivation of enveloped viruses is the solvent-detergent process, which comprises a treatment with a mixture of tributyl phosphate and octoxynol 10; subsequently, these reagents are removed by solid or oily phase extraction, so that the residual content in the final product is less than 2 µg/mL for tributyl phosphate, and less than 5 µg/mL for octoxynol 10. Antimicrobial preservatives should not be added.

The solution is filtered through a sterilizing membrane and distributed aseptically into the final containers and immediately frozen. The final containers are composed of plastic material and comply with the requirements for *Plastic containers* **(6.2)**, or glass, complying with the requirements for *Glass containers* **(6.1)**. It may then be lyophilized.

IDENTIFICATION

Reconstitute or thaw the sample as stated on the label immediately before carrying out identification, tests and assays.

A. Perform electrophoresis examination by comparing the sample with normal human plasma. The electrophoretograms have the same bands

B. Perform precipitation tests from a suitable range of specific sera from different species of domestic animals. The assay must be performed with specific serum protein from each of the domestic species normally used in the preparation of biological products. The sample must contain proteins of human origin and provides negative results to specific sera with plasma proteins from other species.

C. The mixture complies with the *Determination of anti-A and anti-B Hemagglutinins* titer (see *Assay*).

CHARACTERISTICS

Aspect. After thawing, the solution is a clear or slightly opalescent liquid, free from solid particles, and gelatinous. The lyophilized preparation is a white or light yellow powder or friable solid.

pH (5.2.19). 6.5 to 7.6.

Osmolality (5.2.28). Not less than 240 mosmol/kg.

PHYSICOCHEMICAL TESTS

Water. Determine by one of the following methods: *Determination of water by the semi-micro method* **(5.2.20.3)**, *Determination of loss on drying* **(5.2.9.1)** or *by Infrared absorption spectrophotometry* **(5.2.14)**. The content is within the limits approved by the competent authorities.

Citrate. Not more than 25 mmol/L. Proceed as described in *High efficiency liquid chromatography* **(5.2.17.4)**. Use a chromatograph equipped with an ultraviolet detector at 215 nm; 300 mm column and 7.8 mm of inner diameter, packed with cation-exchange resin (9 µm), flow of *Mobile phase* of 0.5 mL/min.

Mobile phase: 0.051% sulfuric acid solution (w/v).

Sample solution: dilute the sample with an equal volume of a 0.9% sodium chloride solution (w/v). Filter through a 0.45 µm filter.

Standard solution: dissolve 0.3 g of sodium citrate in water and dilute to 100 mL with the same solvent.

Procedure: separately inject 10 µL of Standard solution and Sample solution. The citrate retention time is about 10 minutes. The column equilibrium time is about 15 minutes.

Calcium. Proceed as described in *Atomic absorption spectrometry* **(5.2.13.1)**. Determine in wavelength at 622 nm. Not more than 5 mmol/L.

Potassium. Proceed as described in *Atomic emission spectrometry* **(5.2.13.2)**. Determine in wavelength at 766.5 nm. Not more than 5 mmol/L.

Sodium. Proceed as described in *Atomic emission spectrometry* **(5.2.13.2)**. Determine in the wavelength at 589 nm. Not more than 200 mmol/L.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

Pyrogens (5.5.2.1). Inject each rabbit, per kilogram of body weight, with volume corresponding to 3 mL of sample. Complies with the test.

ASSAY

Irregular erythrocyte antibodies.

When examined by indirect antiglobulin test, the undiluted sample shows no signs of irregular erythrocyte antibodies.

Hepatitis A virus antibodies.

Not less than 2 IU/mL, determined by the suitable *Immunochemical method* **(5.6)**. The standard of human immunoglobulin for hepatitis A is suitable for use as a reference preparation.

Anti-A and anti-B hemagglutinins.

Proceed as described in *Determination of anti-A and anti-B hemagglutinin titers* **(5.5.1.9).** The presence of the Hemagglutinins (anti-A or anti-B) corresponds to blood group on the label.

Activated coagulation factors.

Proceed as described in *Determination of activated coagulation factors* **(5.5.1.8)**. Perform the test with 0.1 mL of sample instead of dilutions at 1/10 and 1/100. The coagulation time related to the tube containing the sample is not less than 150 seconds. Complies with the test.

Factor V.

Prepare 3 dilutions at 1/10 and 1/40 of the sample by using pH 7.4 imidazole buffer, preferably prepared in duplicate. Proceed as follows for each dilution: mix 0.1 mL of substrate for Factor V deficient plasma, 0.1 mL of dilution from sample, 0.1 mL of thromboplastin reagent, and 0.1 mL of 0.35% calcium chloride solution (w/v). Record the coagulation time, that is, the time interval between the addition of calcium chloride solution and the first signs of fibrin formation. Observe by using appropriate equipment. Determine in duplicate and under the same conditions, the coagulation times of 4 dilutions between 1/10 and 1/80 of normal human plasma in the pH 7.4 imidazole buffer. A unit of Factor V corresponds to activity of 1 mL of normal human plasma. The normal human plasma is prepared from a mixture of plasma units from not less than 30 donors and kept at a temperature of -30 °C or below. Check the test validity and calculate the sample activity through *Statistical procedures applicable to biological tests* **(8.2).** The activity determined is not less than 0.5 units/mL. Confidence interval (P = 0.95) of the determined activity is not less than 80% and not more than 120%.

Factor VIII.

Proceed as described in *Determination of Blood Coagulation Factor VIII, lyophilized* **(5.5.1.7)** by using a standard plasma calibrated against the international standard Factor VIII for human blood coagulation. The activity determined is not less than 0.5 IU/mL. Confidence interval (P = 0.95) of the determined activity is not less than 80% and not more than 120%.

Total proteins.

Dilute the sample with a 0.9% sodium chloride solution (w/v) to obtain a solution containing about 15 mg of protein in 2 mL. In a round-bottom centrifuge tube, add 2 mL of this solution. Add 2 mL of 7.5% sodium molybdate solution (w/v) and 2 mL of a mixture of 1 volume nitrogen-free sulfuric acid and 30 volumes of water. Shake, centrifuge for 5 minutes, decant the supernatant, and invert the tube on a filter paper to drain. Carry out the nitrogen assay in the residue by the digestion method with sulfuric acid, as described in *Determination of nitrogen by the Kjeldahl method* **(5.3.3.2),** and calculate the protein content multiplying the result by 6.25. The total protein content is not less than 45 g/L.

LABELLING

The label should indicate the ABO and Rh (Du) blood group and the method used for viral inactivation. Comply with current legislation.

HUMAN PLASMA FOR FRACTIONATION
Plasma humanum ad separationem

Human plasma for fractionation is the liquid portion of whole blood remaining after separation of blood cell fractions, using appropriate closed system of blood collection that meets the requirements for plastic containers used to collect human blood, containing anticoagulant, conservative, preservative solution or separated by continuous filtration or centrifugation of anticoagulated blood in an apheresis procedure for obtaining products derived from human plasma.

DONORS

Only plasma from a healthy donor and carefully selected that, after medical examination, laboratory blood tests, study of medical history and free from infectious agents transmitted by the plasma, may be accepted for plasma collection for fractionation. Refer to the legislation in force for blood products.

Immunization of donors. Plasma from deliberate immunization of donors to obtain hyperimmune globulins may be used for fractionation when sufficient amounts of this material may not be naturally obtained from immunized donors. It is recommended that immunization of donors is carried out in accordance with the procedures adopted by the World Health Organization (WHO).

Records. Data and information about donors and donations should be maintained to enable the confidentiality of the donor identity, the origin of each donation in plasma pool and traceability corresponding to laboratory tests.

Laboratory tests. Properly validated laboratory tests are performed at each donation to detect viral markers and other infectious agents, such as those described below.

A. Antibodies against the type 1 and type 2 of human immunodeficiency virus (HIV-1 and HIV-2).

B. Surface antigen of Hepatitis B virus (HBsAg).

C. Antibodies against Hepatitis C virus (anti-HCV).

The analytical methods used must have adequate sensitivity and specificity. If a positive repeated result is found in any of the tests, the donation should be rejected.

INDIVIDUAL UNITS OF PLASMA

The plasma should be prepared by a method which completely removes, as much as possible, other cell fractions by centrifugation of whole blood. It should be obtained from whole blood or by apheresis. The plasma should be separated from its cell by a method designed to prevent the introduction of microorganisms. No antibacterial or antifungal agent may be added to the plasma. The filling systems for collecting and processing human blood should meet the requirements for closed systems for collection of human blood and should prevent any possibility of contamination.

If two or more units are mixed before freezing, the operation should be performed using sterile connectors or under aseptic conditions, with containers that have not been previously used.

When it is obtained by plasmapheresis or whole blood (after separation of the cellular elements), the plasma may be used for recovering labile proteins when frozen within 24 hours after collection, with

rapid cooling under conditions validated to ensure that the temperature at -25°C or lower is achieved within each plasma unit within 12 hours of the start of insertion in the freezer.

When it is obtained by plasmapheresis, plasma intended only for the recovery of non-labile proteins must be frozen by rapid cooling in cold chamber at -20°C or below, as soon as possible and not later than 24 hours after collection.

When it is obtained by plasmapheresis, plasma intended only for the recovery of non-labile proteins must be frozen by rapid cooling in cold chamber at -20°C or below, as soon as possible and not later than 72 hours after collection. It is not necessary to determine the total protein and factor VIII, described in *Assay* in each plasma unit. These determinations are parameters of the good manufacturing practices, being the *Factor VIII* test relevant for use in the preparations of labile protein concentrates.

The total protein content in each plasma unit depends on the content of proteins in the donor's serum and on the degree of dilution inherent to the donation procedure.

When plasma is obtained from a selected donor and using an appropriate proportion of anticoagulant conservative and preservative solution, the total protein content obtained is within the limit of 50 g/L. If the volume of blood or plasma collected together with the conservative and preservative anticoagulant solution is less than the established, the resulting plasma is not necessarily suitable for fractionation. The intended goal with the good manufacturing practices should be reaching the limit prescribed for all normal donations.

The preservation of factor VIII of human coagulation depends on the procedure of collection and subsequent handling of the plasma unit. With good practices, 0.7 UI/mL may be usually achieved in plasma units, but plasma units with lower factor VIII activity may be still suitable for the production of coagulation factor concentrates. The goal intended with good manufacturing practices is to preserve, as much as possible, the labile proteins.

MIXTURES OF PLASMA (PLASMA POOL)

During the manufacture of plasma derivatives, the first mixture of plasma pool (for example, after removal of cryoprecipitate) should be tested for surface antigen of Hepatitis B virus (HBsAg) and antibodies against HIV by using appropriate sensitivity and specificity methods. The results should be negative in all tests.

Also, a test should be performed for RNA of Hepatitis C virus by using a validated technique of nucleic acid amplification. In the test it is included a positive control with 100 UI/mL of RNA from Hepatitis C virus, and to test for inhibitors, an internal control prepared by adding the appropriate marker to a sample of plasma pool. The test is not valid if the positive control is not reactive or if the result obtained indicates the presence of inhibitors.

The plasma mixture satisfies the test if it is not reactive to RNA of Hepatitis C virus. The test should be performed compared to an international standard recognized by WHO.

CHARACTERISTICS

Aspect. Before freezing, the plasma for fractionation, a clear or slightly turbid liquid without visible signs of hemolysis, may vary in color from slightly yellow to greenish.

ASSAY

Factor VIII:C

Proceed as described in *Determination of lyophilized human blood coagulation factor VIII* **(5.5.1.7).** Perform the test by using plasma pool with not less than 10 units of plasma sample. If necessary, thaw the samples to be examined at a temperature not exceeding 37°C. Use a calibrated reference plasma against an *International Standard* of Factor VIII. The activity is not less than 0.7 UI/mL.

Total proteins

Proceed as described in *Determination of nitrogen by the Kjeldahl method* **(5.3.3.2).** Perform the test by using the mixture with not less than 10 units of plasma. Dilute the plasma mixture with a 0.9% sodium chloride solution (w/v) to obtain a solution containing approximately 15 mg of protein in 2 mL. Add 2 mL of this solution, 2 mL of 7.5% sodium molybdate solution (w/v), and 2 mL of a mixture of nitrogen-free sulfuric acid and water (1:30) to a rounded-bottom centrifuge tube. Shake, centrifuge for 5 minutes, decant the supernatant liquid, and invert the tube allowing its contents to drain on filter paper. Determine the nitrogen content in the residue after mineralization and calculate the protein content by multiplying the amount of nitrogen by 6.25. The total protein content is not less than 50 g/L.

STORAGE AND TRANSPORTATION

The frozen plasma should be stored and transported under conditions developed to maintain the temperature at -20°C or lower; for accidental reasons, the storage temperature may rise above -20°C on one or more occasions during the storage and transportation, however, plasma is acceptable for fractionation if all the following conditions are met:

- the total time during which the temperature exceeds -20°C may not be greater than 72 hours;
- the temperature should not exceed -15°C in more than one occasion;
- in no occasion may the temperature exceed -5°C.

LABELLING

Comply with current legislation. The label should enable each individual unit to be traceable to its specific donor.

HUMAN ALBUMIN SOLUTION
Albumini humani solutio

Human albumin solution is a protein, sterile and pyrogen-free solution obtained from human plasma that complies with the requirements of the monograph *Human Plasma for Fractionation*.

Albumin is obtained under controlled conditions, particularly with regard to pH, ionic strength and temperature, so that the albumin concentration in the final product is not less than 96% of the total protein content.

The human albumin solution is prepared as a concentrated solution containing 150 g/L to 250 g/L of total protein or as an isotonic solution containing 35 g/L to 50 g/L of total protein. A stabilizer such as sodium caprylate (sodium octanoate) or *N*-acetyltryptophan or a combination of these two at a suitable concentration can be added against the effects of heat. If viral inactivation substances are added in the production step, a purification procedure must be validated to demonstrate that the concentrations of these substances have been reduced to an acceptable level, and that such residues do not compromise the safety of the preparation.

No antimicrobial preservatives are added at any stage of preparation. The final solution is subject to sterilizing filtration and is aseptically distributed into sterile containers which are then closed to prevent contamination. The solution in its final container is heated to (60.0 ± 1.0) °C and held at that temperature for a time of not less than 10 hours. The containers are then incubated at a temperature between 30°C and 32°C for not less than 14 days or between 20°C and 25°C for not less than four weeks and visually analyzed for possible microbial contamination.

IDENTIFICATION

A. Perform precipitation assays using anti-albumin sera from different species. The test must be carried out with specific sera for human albumin from each species of domestic animal currently used in the country for the preparation of products of biological origin. The solution contains human proteins and provides negative results with anti-albumin sera from other species.

B. Perform an assay using one of the *Immunochemical Methods* **(5.6)**, following proper technique. With the aid of a normal human serum, compare a normal serum with the sample, after previously diluting both, so as to contain 10 g/L of protein. The main component of the sample corresponds to the main component of normal human serum and there may be other plasma proteins in small amounts.

CHARACTERISTICS

Aspect. Clear, slightly viscous liquid, usually colorless, brownish-yellow or greenish.

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 6.7 to 7.3. Dilute the preparation to be examined with a 0.9% sodium chloride solution (w/v) to obtain a solution containing 10 g/L of protein.

Protein composition. Proceed as described in *Electrophoresis* **(5.2.22)**, using strips of cellulose acetate gel or agarose as the support medium, and pH 8.6 barbital buffer pH as the electrolyte solution.

Note: *If the cellulose acetate strip is chosen for the run, the method described below may be used. If agarose gels are used, it is because they are part of an electrophoresis automatic system and the manufacturer's instructions should be followed instead.*

Sample solution: dilute the sample with 0.9% sodium chloride solution (w/v) to obtain a concentration of 20 g/L in proteins.

Standard solution: dilute the human albumin standard for electrophoresis with sodium chloride solution at 0.9% (w/v) to obtain a concentration of 20 g/L in proteins.

Apply to the strip 2.5 µL of *Sample solution* in 10 mm traces, or deposit 0.25 µl per milliliter if a narrower strip is used. To another strip apply, under the same conditions, the same volume of the *Standard solution*. Apply a suitable electric field so that the fastest moving compound migrates not less than 30 mm. Treat strips with amido black 10B SR for 5 minutes and then with a mixture of 10 volumes of glacial acetic acid and 90 volumes of methyl alcohol for the time strictly necessary to achieve the support discoloration. Provoke the transparency frame with a mixture of 19 volumes of glacial acetic acid and 81 volumes of methyl alcohol. Determine the absorbance of the bands at 600 nm with the aid of an apparatus that in this wavelength provides a linear response in the measurement range. Perform three determinations on each strip and calculate the readings average for each strip. In the electrophoretogram of the sample solution, not more than 5% of the proteins may have different mobility from the main band. The test is only valid if, in the electrophoretogram obtained with the reference solution, the proportion of proteins contained in the main band is within the limits established by the manufacturer accompanying the reference preparation.

Distribution of molecular size. Proceed as described in *High efficiency liquid chromatography* **(5.2.17.4)**. Use chromatograph equipped with an ultraviolet detector at 280 nm, column 600 mm length and 7.5 mm inner diameter or 300 mm length and 7.8 mm inner diameter packed with hydrophilic silica gel (adequate to the fractionation of globular proteins with a molecular weight ratio in the range of 10,000 to 500,000); *Mobile phase* flow of 0.5 mL/minuto.

Mobile phase: solution containing 4.873 g of dibasic sodium phosphate dihydrate, 1.741 g of monobasic sodium phosphate monohydrate, 11.688 g of sodium chloride and 50 mg of sodium azide, per liter of ultra-purified water.

Sample solution: dilute the sample in a sodium chloride solution at 0.9% (w/v) up to a concentration suitable to the chromatographic system used. A concentration of between 4 g/L and 12 g/L and injection of 50 µg to 600 µg of protein are generally suitable.

The retention time will be defined by the equipment and the size of the column used, and the stabilization peak must be disregarded. The peak produced by albumin must be symmetrical and equal to or greater than 95% of the total area of the chromatogram. After a brief empty space, the peaks produced by the presence of polymers and aggregates should appear. The area of this peak divided by two should be not more than 5% of the total area of the chromatogram.

PHYSICOCHEMICAL TESTS

Aluminum. Proceed as described in *Atomic absorption spectrometry* **(5.2.13.1)**. Meet the following conditions: use a graphite furnace as an atom generator, flame between readings, 309.3 nm wavelength or any other suitable wavelength, 0.5 nm slit width, pyrolytically coated tube, with integrated platform and priority of correction turned off. Prepare solutions as described below.

Note: *use plastic containers for the preparation of plastic solutions and equipment, whenever possible. Wash the appliance with 200 g/L nitric acid before use.*

Sample solution: use the sample to be analyzed, diluted if necessary.

Validation solution: use an albumim international standard for aluminum assay validation.

Reference solutions: prepare not less than three reference solutions on a scale that measures the expected aluminum concentration in the preparation to be examined, e.g. by diluting the *Aluminum standard solution* (10 ppm aluminum Al) with a 10 to 1.0 g/L octoxynol solution.

Monitoring solution: add *Aluminum standard solution (10 ppm aluminum Al)* or a certified reference material to the proper solution in an amount sufficient to increase the aluminum concentration to 20 µg/L.

Blank solution: 10 to 1.0 g/L octoxynol solution.

The operating conditions found in **Table 1** are cited as an example of suitable conditions found for a particular equipment and can be modified for better results.

Table 1 – **Appropriate operating conditions found, cited as an example.**

Step	***Final temperature (°C)***	***Time of displacement (s)***	***Time elapsed (s)***	***Gas***
1	120	10	80	Argon
2	200	5	20	Argon
3	650	5	10	Argon
4	1300	5	10	Argon
5	1300	1	10	No gas
6	2500	0.7	4	No gas
7	2600	0.5	3	Argon
8	20	12.9	3	No gas

Procedure: Inject the *Blank Solution, Reference Solutions, Sample Solution and Monitoring Solution* three times. The aluminum recovery added in the preparation of the *Monitoring solution* is within the range of 80% to 120%. Determine absorbances. Build an analytical curve from the average of the readings obtained with the *Reference Solutions* and determine the aluminum content in the preparation to be analyzed using the analytical curve. Not more than 200 µg/L.

Pre-kallikrein activator. Proceed as described in *Determination of pre-kallikrein activator* **(5.5.1.11)**. The sample contains not more than 35 IU of pre-kallikrein activator per milliliter.

Hemoglobin. Dilute the sample with 0.9% sodium chloride solution (w/v) up to a concentration of 10 g/L in proteins. Determine the absorbance **(5.2.14)** at 403 nm using water as blank. The absorbance is not more than 0.15.

Potassium. Proceed as described in *Atomic emission spectrometry* **(5.2.13.2)**. Determine the emitted intensity at 766.5 nm. The sample contains not more than 0.05 millimol of K^{+1} per gram of protein.

Sodium. Proceed as described in *Atomic emission spectrometry* **(5.2.13.2)**. Determine the emitted intensity at 589 nm. The sample contains not less than 95% and not more than105% of the sodium content indicated on the label and not more than 160 millimol of Na^{+1} per liter.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

Pyrogens (5.5.2.1). Complies with the test.

Note: *in a solution containing 35 g/L to 50 g/L of protein, inject each rabbit, per kilogram of body weight, with 10 mL of the preparation to be examined.* ***I****n a solution containing 150 g/L to 250 g/L of protein, inject each rabbit, per kilogram of body weight, with 5 mL of the preparation to be examined.*

ASSAY

Total proteins

Dilute the preparation with a 0.9% sodium chloride solution (w/v) to obtain a solution expected to contain approximately 15 mg of protein in 2 mL. In a round-bottomed centrifuge tube, add 2 mL of this solution. Add 2 mL of 75 g/L sodium molybdate solution and 2 mL of a mixture of nitrogen-free sulfuric acid and water (1:30). Shake, centrifuge for 5 minutes, decant the supernatant liquid and invert the tube, allowing its content to drain on filter paper. Determine the nitrogen content present in the residue after mineralization according to the *Determination of nitrogen by the Kjeldahl method* **(5.3.3.2)** and calculate the protein content by multiplying the amount of nitrogen by 6.25. The preparation contains not less than 96% and not more than105% of the amount of protein informed on the label.

PACKAGING AND STORAGE

Protect from light.

LABELLING

Comply with current legislation. The label must contain the name of the preparation, volume of the preparation, protein content expressed in grams per liter, sodium content expressed in millimol per liter, name and concentration of any substance added to the preparation (example: stabilizer) and that the product should not be used if there is turbidity or deposit.