with a mixture of 19 volumes of glacial acetic acid R and 81 volumes of methanol R. Measure the absorbance of the bands at 600 nm in an instrument having a linear response over the range of measurement. Calculate the result as the mean of 3 measurements of each strip.

System suitability: in the electropherogram obtained with the reference solution on cellulose acetate or on agarose gels, the proportion of protein in the principal band is within the limits stated in the leaflet accompanying the reference preparation.

Results: in the electropherogram obtained with the test solution on cellulose acetate or on agarose gels, not more than 5 per cent of protein has a mobility different from that of the principal band. This limit is not applicable if albumin has been added to the preparation as a stabiliser; for such preparations, a test for protein composition is carried out during manufacture before addition of the stabiliser.

**Distribution of molecular size.** Liquid chromatography (2.2.29).

**Test solution.** Dilute the preparation to be examined with a 9 g/l solution of sodium chloride R to a concentration suitable for the chromatographic system used. A concentration in the range of 4-12 g/l and injection of 50-600 µg of protein are usually suitable.

**Reference solution.** Dilute human immunoglobulin (molecular size) BRP with a 9 g/l solution of sodium chloride R to the same protein concentration as the test solution.

**Column:**
- size: l = 0.6 m, Ø = 7.5 mm, or l = 0.3 m, Ø = 7.8 mm;
- stationary phase: hydrophilic silica gel for chromatography R of a grade suitable for fractionation of globular proteins with relative molecular masses in the range 10 000 to 500 000.

**Mobile phase:** dissolve 4.873 g of disodium hydrogen phosphate dihydrate R, 1.741 g of sodium dihydrogen phosphate monohydrate R, 11.688 g of sodium chloride R and 50 mg of sodium azide R in 1 litre of water R.

**Flow rate:** 0.5 ml/min.

**Detection:** spectrophotometer at 280 nm.

In the chromatogram obtained with the reference solution, the principal peak corresponds to the IgG monomer and there is a peak corresponding to the dimer with a relative retention to the principal peak of about 0.85. Identify the peaks in the chromatogram obtained with the test solution by comparison with the chromatogram obtained with the reference solution; any peak with a retention time shorter than that of the dimer corresponds to polymers and aggregates.

**Results:** in the chromatogram obtained with the test solution:
- relative retention: for the monomer and for the dimer, the relative retention to the corresponding peak in the chromatogram obtained with the reference solution is 1 ± 0.02;
- peak area: the sum of the peak areas of the monomer and the dimer represent not less than 90 per cent of the total area of the chromatogram and the sum of the peak areas of polymers and aggregates represents not more than 3 per cent of the total area of the chromatogram. This requirement does not apply to products where albumin has been added as a stabiliser; for products stabilised with albumin, a test for distribution of molecular size is carried out during manufacture before addition of the stabiliser.

**Anticomplementary activity** (2.6.17). The consumption of complement is not greater than 50 per cent (1 CH50 per milligram of immunoglobulin).

**Prekallikrein activator** (2.6.15): maximum 35 IU/ml, calculated with reference to a dilution of the preparation to be examined containing 30 g/l of immunoglobulin.

**Anti-A and anti-B haemagglutinins** (2.6.20). Carry out the tests for anti-A and anti-B haemagglutinins. If the preparation to be examined contains more than 30 g/l of immunoglobulin, dilute to this concentration before preparing the dilutions to be used in the test. The 1 to 64 dilutions do not show agglutination.

**Anti-D antibodies** (2.6.26). It complies with the test for anti-D antibodies in human immunoglobulin for intravenous administration.

**Antibody to hepatitis B surface antigen:** minimum 0.5 IU/g of immunoglobulin, determined by a suitable immunochemical method (2.7.1).

**Water.** Determined by a suitable method, such as the semi-micro determination of water (2.5.12), loss on drying (2.2.32) or near infrared spectrophotometry (2.2.40), the water content is within the limits approved by the competent authority.

**Sterility** (2.6.1). It complies with the test for sterility.

**Pyrogens** (2.6.8). It complies with the test for pyrogens. Inject per kilogram of the rabbit’s mass a volume equivalent to 0.5 g of immunoglobulin but not more than 10 ml per kilogram of body mass.

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

**LABELLING**
The label states:
- for liquid preparations, the volume of the preparation in the container and the protein content expressed in grams per litre;
- for freeze-dried preparations, the quantity of protein in the container;
- the amount of immunoglobulin in the container;
- the route of administration;
- for freeze-dried preparations, the name or composition and the volume of the reconstituting liquid to be added;
- the distribution of subclasses of immunoglobulin G present in the preparation;
- where applicable, the amount of albumin added as a stabiliser;
- the maximum content of immunoglobulin A.

**01/2008:0853**

**HUMAN PLASMA FOR FRACTIONATION**

**Plasma humanum ad separationem**

**DEFINITION**
Human plasma for fractionation is the liquid part of human blood remaining after separation of the cellular elements from blood collected in a receptacle containing an anticoagulant,
or separated by continuous filtration or centrifugation of anticoagulated blood in an apheresis procedure; it is intended for the manufacture of plasma-derived products.

**PRODUCTION**

**DONORS**

Only a carefully selected, healthy donor who, as far as can be ascertained after medical examination, laboratory blood tests and a study of the donor's medical history, is free from detectable agents of infection transmissible by plasma-derived products may be used. Recommendations in this field are made by the Council of Europe [Recommendation No. R (95) 15 on the preparation, use and quality assurance of blood components, or subsequent revision]; a directive of the European Union also deals with the matter: Commission Directive 2004/33/EC of 22 March 2004 implementing Directive 2002/98/EC of the European Parliament and of the Council as regards certain technical requirements for blood and blood components.

**Immunisation of donors.** Immunisation of donors to obtain immunoglobulins with specific activities may be carried out when sufficient supplies of material of suitable quality can be obtained from naturally immunised donors. Recommendations for such immunisations are formulated by the World Health Organisation (Requirements for the collection, processing and quality control of blood, blood components and plasma derivatives, WHO Technical Report Series, No. 840, 1994 or subsequent revision).

**Records.** Records of donors and donations made are kept in such a way that, while maintaining the required degree of confidentiality concerning the donor’s identity, the origin of each donation in a plasma pool and the results of the corresponding acceptance procedures and laboratory tests can be traced.

**Laboratory tests.** Laboratory tests are carried out for each donation to detect the following viral markers:

1. antibodies against human immunodeficiency virus 1 (anti-HIV-1);
2. antibodies against human immunodeficiency virus 2 (anti-HIV-2);
3. hepatitis B surface antigen (HBsAg);
4. antibodies against hepatitis C virus (anti-HCV).

Pending complete harmonisation of the laboratory tests to be carried out, the competent authority may require that a test for alanine aminotransferase (ALT) also be carried out. When complete reactive result is found in any of these tests, the donation is not accepted.

**INDIVIDUAL PLASMA UNITS**

The plasma is prepared by a method that removes cells and cell debris as completely as possible. Whether prepared from whole blood or by plasmapheresis, the plasma is separated from the cells by a method designed to prevent the introduction of micro-organisms. No antibacterial or antifungal agent is added to the plasma. The containers comply with the requirements for glass containers (3.2.1) or for plastic containers for blood and blood components (3.2.3). The containers are closed so as to prevent any possibility of contamination.

If 2 or more units are pooled prior to freezing, the operations are carried out using sterile connecting devices or under aseptic conditions and using containers that have not previously been used. When obtained by plasmapheresis or from whole blood (after separation from cellular elements), plasma intended for the recovery of proteins that are labile in plasma is frozen within 24 h of collection by cooling rapidly in conditions validated to ensure that a temperature of −25 °C or below is attained at the core of each plasma unit within 12 h of placing in the freezing apparatus.

When obtained by plasmapheresis, plasma intended solely for the recovery of proteins that are not labile in plasma is frozen by cooling rapidly in a chamber at −20 °C or below as soon as possible and at the latest within 24 h of collection. When obtained from whole blood, plasma intended solely for the recovery of proteins that are not labile in plasma is separated from cellular elements and frozen in a chamber at −20 °C or below as soon as possible and at the latest within 72 h of collection.

It is not intended that the determination of total protein and factor VIII shown below be carried out on each unit of plasma. They are rather given as guidelines for good manufacturing practice, the test for factor VIII being relevant for plasma intended for use in the preparation of concentrates of labile proteins.

**Total protein.** Carry out the test using a pool of not fewer than 10 units. Dilute the pool with a 9 g/l solution of sodium chloride R to obtain a solution containing about 15 mg of protein in 2 ml. To 2.0 ml of this solution in a round-bottomed centrifuge tube add 2 ml of a 75 g/l solution of sodium molybdate R and 2 ml of a mixture of 1 volume of nitrogen-free sulphuric acid R and 30 volumes of water R. Shake, centrifuge for 5 min, decant the supernatant liquid and allow the inverted tube to drain on filter paper. Determine the nitrogen in the residue by the method of sulphuric acid digestion (2.5.9) and calculate the protein content by multiplying the quantity of nitrogen by 6.25. The total protein content is not less than 50 g/l.

**Factor VIII.** Carry out the test using a pool of not fewer than 10 units. Thaw the samples to be examined, if necessary, at 37 °C. Carry out the assay of factor VIII (2.7.4), using a reference plasma calibrated against the International Standard for human coagulation factor VIII in plasma. The activity is not less than 0.7 IU/ml.

**STORAGE AND TRANSPORT**

Frozen plasma is stored and transported in conditions designed to maintain the temperature at or below −20 °C; for accidental reasons, the storage temperature may rise above −20 °C on one or more occasions during storage and transport but the plasma is nevertheless considered suitable for fractionation if all the following conditions are fulfilled:
the total period of time during which the temperature exceeds – 20 °C does not exceed 72 h;
– the temperature does not exceed – 15 °C on more than one occasion;
– the temperature at no time exceeds – 5 °C.

**POOLED PLASMA**

During the manufacture of plasma products, the first homogeneous pool of plasma (for example, after removal of cryoprecipitate) is tested for HBsAg and for HIV antibodies using test methods of suitable sensitivity and specificity; the pool must give negative results in these tests.

The plasma pool is also tested for hepatitis C virus RNA using a validated nucleic acid amplification technique (2.6.21). A positive control with 100 IU/ml of hepatitis C virus RNA and, to test for inhibitors, an internal control prepared by addition of a suitable marker to a sample of the plasma pool are included in the test. The test is invalid if the positive control is non-reactive or if the result obtained with the internal control indicates the presence of inhibitors.

**CHARACTERS**

Before freezing, a clear to slightly turbid liquid without visible signs of haemolysis; it may vary in colour from light yellow to green.

**LABELLING**

The label enables each individual unit to be traced to a specific donor.

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**HUMAN PLASMA (POOLED AND TREATED FOR VIRUS INACTIVATION)**

Plasma humanum coagmentatum conditumque ad exstinguendum virum

**DEFINITION**

Human plasma (pooled and treated for virus inactivation) is a frozen or freeze-dried, sterile, non-pyrogenic preparation obtained from human plasma derived from donors belonging to the same ABO blood group. The preparation is thawed or reconstituted before use to give a solution for infusion.

The human plasma used complies with the monograph on Human plasma for fractionation (0853).

**PRODUCTION**

The units of plasma to be used are cooled to – 30 °C or lower within 6 h of separation of cells and in any case within 24 h of collection.

The pool is prepared by mixing units of plasma belonging to the same ABO blood group.

The pool of plasma is tested for hepatitis B surface antigen (HBsAg), for hepatitis C virus antibodies and for HIV antibodies using test methods of suitable sensitivity and specificity; the pool must give negative results in these tests.

The plasma pool is also tested for hepatitis C virus RNA using a validated nucleic acid amplification technique (2.6.21). A positive control with 100 IU of hepatitis C virus RNA per millilitre and, to test for inhibitors, an internal control prepared by addition of a suitable marker to a sample of the plasma pool are included in the test. The test is invalid if the positive control is non-reactive or if the result obtained with the internal control indicates the presence of inhibitors. The pool complies with the test if it is found non-reactive for hepatitis C virus RNA.

**Hepatitis C virus RNA for NAT testing BRP**

**BIOLOGICALS**

**B19 virus DNA**

The plasma pool contains not more than 10.0 IU/µl.

A positive control with 10.0 IU of B19 virus DNA per microlitre and, to test for inhibitors, an internal control prepared by addition of a suitable marker to a sample of the plasma pool are included in the test. The test is invalid if the positive control is non-reactive or if the result obtained with the internal control indicates the presence of inhibitors.

**B19 virus DNA for NAT testing BRP**

To limit the potential burden of B19 virus in plasma pools, the plasma pool is also tested for B19 virus using a validated nucleic acid amplification technique (2.6.21).

**TESTS**

- **pH** (2.2.3): 6.5 to 7.6.
- **Osmolality** (2.2.35): minimum 240 mosmol/kg.
- **Total protein**: minimum 45 g/l.

Dilute with a 9 g/l solution of sodium chloride R to obtain a solution containing about 15 mg of protein in 2 ml. Place