**POOLED PLASMA**
During the manufacture of plasma products, the first homogeneous pool of plasma (for example, after removal of cryoprecipitate) is tested for 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/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. The plasma pool complies with the test if it is found non-reactive for hepatitis C virus RNA.

**Hepatitis C virus RNA for NAT testing BRP** is suitable for use as a positive control.

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

**STORAGE**
Store and transport frozen plasma at or below −20 °C; the plasma may still be used for fractionation if the temperature is between −20 °C and −15 °C for not more than a total of 72 h without exceeding −15 °C on more than one occasion as long as the temperature is at all times −5 °C or lower.

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

**Human plasma (pooled and treated for virus inactivation)**

**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** is suitable for use as a positive control.

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).

A positive control with 10^4 IU of B19 virus DNA 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 plasma pool contains not more than 10^4 IU of B19 virus DNA per millilitre.

**B19 virus DNA for NAT testing BRP** is suitable for use as a positive control.

The method of preparation is designed to minimise activation of any coagulation factor (to minimise potential thrombogenicity) and includes a step, or steps that have been shown to inactivate known agents of infection; if substances are used for the inactivation of viruses during production, the subsequent purification procedure must be validated to demonstrate that the concentration of these substances is reduced to a suitable level and that any residues are such as not to compromise the safety of the preparation for patients.

A typical method to inactivate enveloped viruses is the solvent-detergent process which uses treatment with a combination of tributyl phosphate and octoxinol 10; these reagents are subsequently removed by oil extraction or by solid phase extraction so that the amount in the final product is less than 2 µg/ml for tributyl phosphate and less than 5 µg/ml for octoxinol 10.

No antimicrobial preservative is added.

The solution is passed through a bacteria-retentive filter, distributed aseptically into the final containers and immediately frozen; it may subsequently be freeze-dried.

Plastic containers comply with the requirements for sterile plastic containers for human blood and blood components (3.2.3).

Glass containers comply with the requirements for glass containers for pharmaceutical use (3.2.1).

**CHARACTERS**
The frozen preparation, after thawing, is a clear or slightly opalescent liquid free from solid and gelatinous particles. The freeze-dried preparation is an almost white or slightly yellow powder or friable solid.

**Thaw or reconstitute the preparation to be examined as stated on the label immediately before carrying out the identification, tests and assay.**

**IDENTIFICATION**
A. Examine by electrophoresis (2.2.23) comparing with normal human plasma. The electropherograms show the same bands.

B. It complies with the test for anti-A and anti-B haemagglutinins (see Tests).

**TESTS**

**pH** (2.2.3): 6.5 to 7.6.
**Human prothrombin complex**

**DEFINITION**

Human prothrombin complex is a plasma protein fraction containing blood coagulation factor IX together with variable amounts of coagulation factors II, VII and X; the presence and proportion of these additional factors depends on the process used for virus inactivation.

**ASSAY**

**Factor VIII.** Carry out the assay of coagulation factor VIII (2.7.4) using a reference plasma calibrated against the International Standard for blood coagulation factor VIII in plasma.

The estimated potency is not less than 0.5 IU/ml. The confidence limits (P = 0.95) are not less than 80 per cent and not more than 120 per cent of the estimated potency.

**Factor V.** Using imidazole buffer solution pH 7.3 R, prepare 3 twofold dilutions of the preparation to be examined, preferably in duplicate, from 1 in 10 to 1 in 40. Test each dilution as follows: mix 0.1 ml of plasma substrate deficient in factor V R, 0.1 ml of the dilution to be examined, 0.1 ml of thromboplastin R and 0.1 ml of a 3.5 g/l solution of calcium chloride R; measure the coagulation times, i.e., the interval between the moment at which the calcium chloride solution is added and the first indication of the formation of fibrin, which may be observed visually or by means of a suitable apparatus.

In the same manner, determine the coagulation time of 4 twofold dilutions (1 in 10 to 1 in 80) of human normal plasma in imidazole buffer solution pH 7.3 R. 1 unit of factor V is equal to the activity of 1 ml of human normal plasma. Human normal plasma is prepared by pooling plasma units from not fewer than 30 donors and stored at −30 °C or lower.

Check the validity of the assay and calculate the potency of the test preparation by the usual statistical methods for a parallel-line assay (for example, 5.3).

The estimated potency is not less than 0.5 IU/ml. The confidence limits (P = 0.95) are not less than 80 per cent and not more than 120 per cent of the estimated potency.

**Factor XI.** Carry out the assay of human coagulation factor XI (2.7.22) using as reference human normal plasma (see above under Factor V).

The estimated potency is not less than 0.5 IU/ml. The confidence limits (P = 0.95) are not less than 80 per cent and not more than 125 per cent of the estimated potency.

**LABELLING**

The label states:

– the ABO blood group,
– the method used for virus inactivation.