

Thrombin. If the preparation to be examined contains heparin, determine the amount present as described in the test for heparin and neutralise the heparin by addition of *protamine sulphate R* (10 µg of protamine sulphate neutralises 1 IU of heparin). In each of 2 test-tubes, mix equal volumes of the reconstituted preparation and a 3 g/l solution of *fibrinogen R*. Keep one of the tubes at 37 °C for 6 h and the other at room temperature for 24 h. In a third tube, mix a volume of the fibrinogen solution with an equal volume of a solution of *human thrombin R* (1 IU/ml) and place the tube in a water-bath at 37 °C. No coagulation occurs in the tubes containing the preparation to be examined. Coagulation occurs within 30 s in the tube containing thrombin.

Factor II. Carry out the assay of human coagulation factor II (2.7.18).

The estimated content is not more than 125 per cent of the stated content. The confidence limits ($P = 0.95$) are not less than 90 per cent and not more than 111 per cent of the estimated potency.

Factor IX. Carry out the assay of human coagulation factor IX (2.7.11).

The estimated content is not more than 125 per cent of the stated content. The confidence limits ($P = 0.95$) are not less than 80 per cent and not more than 125 per cent of the estimated potency.

Factor X. Carry out the assay of human coagulation factor X (2.7.19).

The estimated content is not more than 125 per cent of the stated content. The confidence limits ($P = 0.95$) are not less than 90 per cent and not more than 111 per cent of the estimated potency.

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 spectrometry (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 not less than 30 IU of factor VII.

ASSAY

Assay of human coagulation factor VII (2.7.10).

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

STORAGE

In an airtight container, protected from light.

LABELLING

The label states:

- the number of International Units of factor VII per container,
- the maximum content of International Units of factor II, factor IX and factor X per container,
- the amount of protein per container,
- the name and quantity of any added substances, including where applicable, heparin,
- the name and volume of the liquid to be used for reconstitution,

- that the transmission of infectious agents cannot be totally excluded when medicinal products prepared from human blood or plasma are administered.

01/2008:0275

HUMAN COAGULATION FACTOR VIII

Factor VIII coagulationis humanus

DEFINITION

Human coagulation factor VIII is a preparation of a plasma protein fraction that contains the glycoprotein coagulation factor VIII together with varying amounts of von Willebrand factor, depending on the method of preparation. It is prepared from human plasma that complies with the monograph on *Human plasma for fractionation (0853)*.

The potency of the preparation, reconstituted as stated on the label, is not less than 20 IU of factor VIII:C per millilitre.

PRODUCTION

The method of preparation includes a step or steps that have been shown to remove or to inactivate known agents of infection; if substances are used for the inactivation of viruses, 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.

The specific activity is not less than 1 IU of factor VIII:C per milligram of total protein before the addition of any protein stabiliser.

The factor VIII fraction is dissolved in a suitable liquid. Excipients such as a stabiliser may be added. No antimicrobial preservative is added. The solution is passed through a bacteria-retentive filter, distributed aseptically into the final containers and immediately frozen. It is subsequently freeze-dried and the containers are closed under vacuum or under an inert gas.

VALIDATION STUDIES

Products stated to have von Willebrand factor activity. For products intended for treatment of von Willebrand's disease it shall be demonstrated that the manufacturing process yields a product with a consistent composition with respect to von Willebrand factor. This composition may be characterised in a number of ways. For example, the number and the relative amount of the different multimers may be determined by sodium dodecyl sulphate (SDS) agarose gel electrophoresis (about 1 per cent agarose) with or without Western blot analysis, using a normal human plasma pool as reference; visualisation of the multimeric pattern may be performed using an immunoenzymatic technique and quantitative evaluation may be carried out by densitometric analysis or by other suitable methods.

Products that show flakes or particles after reconstitution for use. If a few small flakes or particles remain when the preparation is reconstituted, it shall be demonstrated during validation studies that the potency is not significantly affected after passage of the preparation through the filter provided.

CHARACTERS

Appearance: white or pale yellow, hygroscopic powder or friable solid.

Reconstitute the preparation to be examined as stated on the label immediately before carrying out the identification, tests (except those for solubility and water) and assay.

IDENTIFICATION

It complies with the limits of the assay.

TESTS

Solubility. To a container of the preparation to be examined add the volume of the solvent stated on the label at the recommended temperature. The preparation dissolves completely with gentle swirling within 10 min, giving a clear or slightly opalescent, colourless or slightly yellow solution.

Where the label states that the product may show a few small flakes or particles after reconstitution, reconstitute the preparation as described on the label and pass it through the filter provided: the filtered solution is clear or slightly opalescent.

pH (2.2.3): 6.5 to 7.5.

Osmolality (2.2.35): minimum 240 mosmol/kg.

Total protein. If necessary, dilute an accurately measured volume of the preparation to be examined with a 9 g/l solution of *sodium chloride R* to obtain a solution containing about 15 mg of protein in 2 ml. Place 2.0 ml of this solution in a round-bottomed centrifuge tube and 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 amount of protein by multiplying the result by 6.25. *For some products, especially those without a protein stabiliser such as albumin, this method may not be applicable and another validated method for protein determination must therefore be performed.*

Anti-A and anti-B haemagglutinins (2.6.20). Dilute the preparation to be examined with a 9 g/l solution of *sodium chloride R* to contain 3 IU of factor VIII:C per millilitre. The 1 to 64 dilutions do not show agglutination.

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 of the preparation to be examined equivalent to not less than 50 IU of factor VIII:C.

ASSAY

Factor VIII (2.7.4). The estimated potency is not less than 80 per cent and not more than 120 per cent of the stated potency. The confidence limits ($P = 0.95$) are not less than 80 per cent and not more than 120 per cent of the estimated potency.

von Willebrand factor (2.7.21). Carry out the assay of human von Willebrand factor for preparations intended for the treatment of von Willebrand's disease.

The estimated potency is not less than 60 per cent and not more than 140 per cent of the stated potency.

Pending the availability of an International Standard for von Willebrand factor concentrate calibrated for use in the collagen-binding assay, only the ristocetin cofactor assay may be used.

STORAGE

In an airtight container, protected from light.

LABELLING

The label states:

- the number of International Units of factor VIII:C and, where applicable, of von Willebrand factor in the container;
- the amount of protein in the container;
- the name and quantity of any added substance;
- the name and volume of the liquid to be used for reconstitution;
- where applicable, that the preparation may show the presence of a few small flakes or particles after reconstitution;
- that the transmission of infectious agents cannot be totally excluded when medicinal products prepared from human blood or plasma are administered.

01/2008:1643

HUMAN COAGULATION FACTOR VIII (rDNA)

Factor VIII coagulationis humanus (ADNr)

DEFINITION

Human coagulation factor VIII (rDNA) is a freeze-dried preparation of glycoproteins having the same activity as coagulation factor VIII in human plasma. It acts as a cofactor of the activation of factor X in the presence of factor IXa, phospholipids and calcium ions.

Human coagulation factor VIII circulates in plasma mainly as a two-chain glycosylated protein with 1 heavy (relative molecular mass of about 200 000) and 1 light (relative molecular mass 80 000) chain held together by divalent metal ions. Human coagulation factor VIII (rDNA) is prepared as full-length factor VIII (octocog alfa), or as a shortened two-chain structure (relative molecular mass 90 000 and 80 000), in which the B-domain has been deleted from the heavy chain (morococog alfa).

Full-length human rDNA coagulation factor VIII contains 25 potential *N*-glycosylation sites, 19 in the B domain of the heavy chain, 3 in the remaining part of the heavy chain (relative molecular mass 90 000) and 3 in the light chain (relative molecular mass 80 000). The different products are characterised by their molecular size and post-translational modification and/or other modifications.

PRODUCTION

Human coagulation factor VIII (rDNA) is produced by recombinant DNA technology in mammalian cell culture. It is produced under conditions designed to minimise microbial contamination.

Purified bulk factor VIII (rDNA) may contain added human albumin and/or other stabilising agents, as well as other auxiliary substances to provide, for example, correct pH and osmolality.

The specific activity is not less than 2000 IU of factor VIII:C per milligram of total protein before the addition of any protein stabiliser, and varies depending on purity and the type of modification of molecular structure of factor VIII.

The quality of the bulk preparation is controlled using one or more manufacturer's reference preparations as reference.