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 (moroctocog 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.

MANUFACTURER'S REFERENCE PREPARATIONS

During development, reference preparations are established for subsequent verification of batch consistency during production, and for control of bulk and final preparation. They are derived from representative batches of purified bulk factor VIII (rDNA) that are extensively characterised by tests including those described below and whose procoagulant and other relevant functional properties have been ascertained and compared, wherever possible, with the International Standard for factor VIII concentrate. The reference preparations are suitably characterised for their intended purpose and are stored in suitably sized aliquots under conditions ensuring their stability.

PURIFIED BULK FACTOR VIII (rDNA)

The purified bulk complies with a suitable combination of the following tests for characterisation of integrity of the factor VIII (rDNA). Where any substance added during preparation of the purified bulk interferes with a test, the test is carried out before addition of that substance. Where applicable, the characterisation tests may alternatively be carried out on the finished product.

Specific biological activity or ratio of factor VIII activity

to factor VIII antigen. Carry out the assay of human coagulation factor VIII (2.7.4). The protein content, or where a protein stabiliser is present, the factor VIII antigen content, is determined by a suitable method and the specific biological activity or the ratio of factor VIII activity to factor VIII antigen is calculated.

Protein composition. The protein composition is determined by a selection of appropriate characterisation techniques which may include peptide mapping, Western blots, HPLC, gel electrophoresis, capillary electrophoresis, mass spectrometry or other techniques to monitor integrity and purity. The protein composition is comparable to that of the manufacturer's reference preparation.

Molecular size distribution. Using size-exclusion chromatography (*2.2.30*), the molecular size distribution is comparable to that of the manufacturer's reference preparation.

Peptide mapping (2.2.55). There is no significant difference between the test protein and the manufacturer's reference preparation.

Carbohydrates/sialic acid. To monitor batch-to-batch consistency, the monosaccharide content and the degree of sialylation or the oligosaccharide profile are monitored and correspond to those of the manufacturer's reference preparation.

FINAL LOT

It complies with the requirements under Identification, Tests and Assay.

Excipients: 80 per cent to 120 per cent of the stated content, determined by a suitable method, where applicable.

CHARACTERS

Appearance: white or slightly yellow powder or friable mass.

IDENTIFICATION

A. It complies with the limits of the assay.

B. The distribution of characteristic peptide bands corresponds with that of the manufacturer's reference preparation (SDS-PAGE or Western blot).

TESTS

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

Solubility. It dissolves within 5 min at 20-25 $^{\circ}$ C, giving a clear or slightly opalescent solution.

pH (2.2.3): 6.5 to 7.5.

Osmolality (2.2.35): minimum 240 mosmol/kg.

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.

Bacterial endotoxins (*2.6.14*): less than 3 IU in the volume that contains 100 IU of factor VIII activity.

ASSAY

Carry out the assay of human coagulation factor VIII (2.7.4). 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 120 per cent of the estimated potency.

STORAGE

Protected from light.

LABELLING

The label states:

- the factor VIII content in International Units,
- the name and amount of any excipient,
- the composition and volume of the liquid to be used for reconstitution.

01/2008:1223

HUMAN COAGULATION FACTOR IX

Factor IX coagulationis humanus

DEFINITION

Human coagulation factor IX is a plasma protein fraction containing coagulation factor IX, prepared by a method that effectively separates factor IX from other prothrombin complex factors (factors II, VII and X). It is obtained 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 IX per millilitre.

PRODUCTION

The method of preparation is designed to maintain functional integrity of factor IX, to minimise activation of any coagulation factor (to minimise potential thrombogenicity) and includes a step or steps that have been shown to remove or to inactivate known agents of infection; if substances are used for 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.

The specific activity is not less than 50 IU of factor IX per milligram of total protein, before the addition of any protein stabiliser.