DRIED HUMAN ANTIHAEMOPHILIC FRACTION (Human Coagulation Factor VIII)

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 plasma for fractionation.

The potency of the preparation, reconstituted as stated on the label, is not less than 20 IU of factor VIII: C per milliliter. The specific activity is not less than 1 IU of Factor VIII: C per mg of total protein before the addition of any protein stabilizer.

PRODUCTION:

Dried human antihaemophilic fraction may be prepared from human plasma so obtained by precipitation under controlled conditions of pH, ionic strength and temperature with organic solvents, or by freezing and thawing. The precipitate may be washed by extraction with suitable solvents, dissolved in a solution of sodium citrate adjusted to a pH of 6.8-7.2, which may also contain sodium chloride. The solution is sterilized by filtration through a membrane filter, distributed in sterile containers and dried from the frozen state. The air is removed or replaced by oxygen free nitrogen and the containers are sealed so as to exclude microorganisms. No antibacterial preservative is added but an antiviral agent may be added provided that it can be demonstrated to have no deleterious effect on the final product in the amount present and to cause no adverse reaction in man. Heparin may be used.

For the following tests, where it is directed that a solution is to be used, dissolve the contents of sealed container in a volume of appropriate solvent equal to the volume of water for injection stated on the label.

DESCRIPTION:

A white or pale yellow powder or friable soluble.

IDENTIFICATION:

It complies with the limits of the assay.

Solubility: To a container of the preparation to be examined at the volume of the solvent stated on the label at the recommended temperature. The preparation dissolves completely with gentle swirling within 10 minute, giving a clear or slightly opalescent, colorless 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 (2.4. 24) : 6.5-7.5, determined on the reconstituted solution.

Osmolality: Minimum 240 mosmol/kg.

Loss on drying (2.4.19); Not more than 2.0%, determine by drying 0.5g over phosphate pentaoxide at a pressure not exceeding 3 kPa for 24 hours.

Haemagglutinins, anti-A and anti-B:

Dissolve in water. Dilute the solution with saline solution to produce a solution containing 3 units/ml. carry out the test for
Haemagglutinins, anti-A and anti-B using a suitable indirect method such as that described below.

Prepare in duplicate serial dilution of the preparation under examination in saline solution. To each dilution of one series add an equal volume of a 5% v/v suspension of group A red blood cells previously washed 3 times with saline solution. To each dilution of the other series add an equal volume of a 5% v/v suspension of group B red blood cells previously washed 3 times with saline solution. Incubate the suspension at 37º for 30 minutes and then wash the cells 3 times with saline solution. Leave the cells in contact with a polyvalent anti human globulin reagent for 30 minutes. With centrifugation, examine each suspension for agglutination under a microscope. The 1 to 64 dilution do not show agglutination.

**Hepatitis B surface antigen:**

Dissolve in water. Examine the solution by a suitably sensitive method such as radioimmunoassay. Hepatitis B surface antigen is not detected.

**Abnormal toxicity (2.2.1):**

When dissolved in water for injection, complies with a test for abnormal toxicity, method B, injecting into each mouse a volume containing 1.5 units and into each guinea pig a volume containing 15 units.

**Pyrogen (2.2.8) / or Bacterial Endotoxin (BET) (2.2.3):**

It complies with the test for pyrogen or, preferably and where justified and authorized with a validated in-vitro test such as the Bacterial Endotoxin test.

**Pyrogen:** for the pyrogen test inject per kilogram of rabbit’s mass a volume equivalent to not less than 50 IU of Factor VIII: C

**BET:** where the bacterial endotoxin test is used the preparation to be examined contains less than 0.03 IU of endotoxin per international unit of Factor VIII: C

**Sterility (2.2.11):**

Complies with a test for sterility.

**Assay for Factor VIII activity:**

The estimated potency is not less than 80% and not more than 120% of the stated potency. The confidence limits (P = 0.95) are not less than 80 % and not more than 120% of the estimated potency.

Method A : Clotting assay (to be retained as such, as in IP 2010)

Method B : Chromogenic assay (2.8.7)

Human coagulation factor VIII is assayed by its biological activity as a cofactor in the activation of factor X by activated factor IX (factor IXa) in the presence of calcium ions and phospholipid. The potency of a factor VIII preparation is estimated by comparing the quantity necessary to achieve a certain rate of factor Xa formation in a test mixture containing the substance that take part in the activation of factor X, and the quantity of the International Standard, or of a reference preparation calibrated in the International Units, required to produce the same rate of factor Xa formation. The International Units is the factor VIII activity of a stated amount of the International Standard, which consists of a freeze-dried human coagulation factor VIII concentrate. The equivalence in International Units of the International Standard is stated by the World Health Organization.

**Human coagulation factor VIII BRP** is calibrated in International Unites by comparison with the International Standard. The chromogenic assay method consists of 2 consecutive steps: the factor VIII-dependent activation of factor X in a coagulation-factor
reagent composed of purified components, and the enzymatic cleavage of a chromogenic factor Xa substrate to yield a chromophore that can be quantified spectrophotometrically. Under appropriate assay conditions, there is a linear relation between the rate of factor Xa formation and the factor VIII concentration. The assay is summarized by the scheme.

**Step 1**

![Factor x (activated) factor VIII](factorx activates factor VIII to IXa, phospholipid, Ca\(^{2+}\))

**Step 2**

Chromogenic substrate factor Xa peptide + Chromophore

Both steps employ reagents that may be obtained commercially from a variety of sources. Although the composition of individual reagents may be subject to some variation, their essential features are described in the following specification. Deviations from this description may be permissible provided that it has been shown, using the International Standard for human blood coagulation factor VIII concentrate as the standard, that the results obtained do not differ significantly.

It is important to demonstrate by validation the suitability of the kit used, notably by checking the time course of factor Xa generation in order to determine the time taken to reach 50 per cent the maximal factor Xa generation.

**Reagents**

The coagulation factor reagents comprises purified proteins derived from human or bovine sources. These include factor X, factor IXa, and a factor VIII activator, usually thrombin. These proteins are partially purified, preferably to at least 50 per cent, and do not contain impurities that interfere with the activation of factor VIII or factor X. Thrombin may be present in its precursor form prothrombin, provided that its activation in the reagent is sufficiently rapid to give almost instantaneous activation of factor VIII in the assay.

Phospholipid may be obtained from natural sources or be synthetically prepared, and must, to a substantial extent, consist of the species phosphatidylserine. The components of the complete reagent are usually divided into at least 2 separate reagents, each lacking the ability to generate a factor Xa on its own. One of the reagent contains calcium ions. After reconstitution the reagents may be combined provided that no substantial amounts of Xa are generated in the absence of factor VIII. In the final incubation mixture, factor VIII must be the only rate limiting component.

2nd step comprises a quantification of a formed factor Xa, employing a chromogenic substrate that is specific factor Xa. Generally this consist of a derivative short peptide of between 3 and 5 amino acids joined chromophore groupe. On cleavage of this group from the peptide substrate, its chromophoric properties shit to a wavelength allowing its spectrophotometric quantification. The substrate must also contain appropriate inhibitors to stop further factor Xa generation, e.g. chelating agents, and to suppress thrombin activity.

**Assay procedures:**

Reconstitute the entire contains of one ampule of the reference preparation and of the preparations to be examined; use immediately. Add sufficient prediluent to the reconstituted preparations to produce solutions containing 0.5 – 2.0 IU per mL. The prediluent consists of haemophilia A plasma, or of an artificially prepared reagent that contains sufficient vonWille Brand factor and that gives results that do not defer significantly from those obtained employing haemophilia plasma. The pre diluted materials must be stable beyond the time required for the assay. Prepare further dilutions of the reference and test preparations using a non-chelating, appropriately buffered solution, for example, Tris (Hydroxymethyl) aminomethane or imidazole, containing 1% of human or bovine albumin. Prepare at least 2 dilution series of at least 3 further dilution for each material. Prepare dilution such that the final factor VIII...
concentration in the reaction mixture is preferably below 0.1 IU mL, during the step of Xa generation.

Prepare a control solution that includes all components except factor VIII.

Prepare all dilution in plastic tubes and use immediately.

**Step 1:** Mix pre-warmed dilution of the factor VIII reference preparation and the of the preparations to be examined with an appropriate volume of the pre-warmed coagulation factor reagent or combination of its separate constituents and incubate the mixture in plastic tubes or microplate wells at 37º C. Allow the activation of factor X to proceed for a suitable time, terminating the reaction (Step 2) when the factor Xa concentration has reached approximately 50% of the maximal (plateau) level. Appropriate activation times or usually between 2 min. and 5 min.

**Step 2:** Terminate the activation by addition of a pre-warmed reagent containing a chromogenic substrate. Quantify the rate of substrate cleavage, which must be linear with a concentration of factor Xa formed, by measuring the absorbance change at an appropriate wavelength using a spectrophotometer, either monitoring the absorbance continuously, thus allowing the initial rate of substrate cleavage to be calculated or terminating the hydrolysis reaction after a suitable interval by lowering the pH by addition of a suitable reagent, such as a 50% v/v solution of acitic acid, or a 1 Mol pH 3 citrate buffer solution. Adjust the hydrolysis time to achieve a linear development of a chromophore overtine. Appropriate hydrolysis times or usually between 3 min. and 15 min, but deviations are permissible if better linearity of the dose – response relationship is thus obtained.

Calculate the potency of the test preparation by the statistical methods

**For total protein:** To be retained as per IP 2010

**For sodium ions:** To 10 ml of the solution add sufficient water to produce 100 ml, dilute 10 ml to 500 ml with water and determine the content of sodium ions by method B for flame photometry, measuring at about 589nm and using sodium solution FP suitably diluted with water as the standard solution.

The preparation contains not more than 200 mmoles of sodium ions per liter

**Assay for von Willebrand Factor**


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 risocetitn cofactor assay may be used*

**STORAGE:**

Store protected from light, in an atmosphere of nitrogen at a temperature below 8º. The containers are sterile and sealed so as to exclude micro-organisms.

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