Human prothrombin complex

DEFINITION

Prothrombinum multiplex humanum

HUMAN PROTHROMBIN COMPLEX

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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
method of fractionation. 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 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 0.6 IU of factor IX per milligram of total protein, before the addition of any protein stabiliser.

The prothrombin complex fraction is dissolved in a suitable liquid. Heparin, antithrombin and other auxiliary substances 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.

CHARACTERS

A white or slightly coloured powder or friable solid, very hygroscopic.

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 for coagulation factor IX activity and, where applicable, those for factors II, VII and X.

TESTS

Solubility. To a container of the preparation to be examined add the volume of the liquid stated on the label at the recommended temperature. The preparation dissolves completely with gentle swirling within 10 min, giving a clear solution that may be coloured.

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 reconstituted preparation with a 9 g/1 solution of sodium chloride R to obtain a solution expected to contain about 15 mg of protein in 2 ml. To 2.0 ml of the solution in a round-bottomed centrifuge tube add 2 ml of a 75 g/1 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.

Activated coagulation factors (2.6.22). If necessary, dilute the preparation to be examined to contain 20 IU of factor IX per millilitre. For each of the dilutions, the coagulation time is not less than 150 s.

Heparin. If heparin has been added during preparation, determine the amount present by the assay of heparin in coagulation factor concentrates (2.7.12). The preparation to be examined contains not more than the amount of heparin stated on the label and in any case not more than 0.5 IU of heparin per International Unit of factor IX.

Thrombin. If the preparation to be examined contains heparin, determine the amount present as described in the test for heparin and neutralise it 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.

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 of the reconstituted preparation equivalent to not less than 30 IU of factor IX.

ASSAY

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

The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The confidence interval (P = 0.95) of the estimated potency is not greater than 80 per cent to 125 per cent.

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

The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The confidence interval (P = 0.95) of the estimated potency is not greater than 90 per cent to 111 per cent.

Factor VII. If the label states that the preparation contains factor VII, carry out the 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 interval (P = 0.95) of the estimated potency is not greater than 80 per cent to 125 per cent.

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

The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The confidence interval (P = 0.95) of the estimated potency is not greater than 90 per cent to 111 per cent.

STORAGE

In an airtight container, protected from light.

LABELLING

The label states:

– the number of International Units of factor IX, factor II and factor X per container,

– where applicable, the number of International Units of factor VII per container,
where applicable, that the preparation contains protein C and/or protein S,

— the amount of protein per container,
— the name and quantity of any added substances, including where applicable, heparin,
— the name and quantity 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.

**HUMAN RABIES IMMUNOGLOBULIN**

**DEFINITION**

Human rabies immunoglobulin is a liquid or freeze-dried preparation containing immunoglobulins, mainly immunoglobulin G. The preparation is intended for intramuscular administration. It is obtained from plasma from donors immunised against rabies. It contains specific antibodies neutralising the rabies virus. *Human normal immunoglobulin* (0338) may be added. It complies with the monograph on *Human normal immunoglobulin* (0338), except for the minimum number of donors and the minimum total protein content.

**POTENCY**

The potency is determined by comparing the dose of immunoglobulin required to neutralise the infectivity of a rabies virus suspension with the dose of a reference preparation, calibrated in International Units, required to produce the same degree of neutralisation (2.7.1). The test is performed in sensitive cell cultures and the presence of unneutralised virus is revealed by immunofluorescence. The International Unit is the specific neutralising activity for rabies virus in a stated amount of the International Standard for anti-rabies immunoglobulin. The equivalence in International Units of the International Standard is stated by the World Health Organisation.

*Human rabies immunoglobulin* BRP is calibrated in International Units by comparison with the International Standard.

Carry out the test in suitable sensitive cells. It is usual to use the BHK 21 cell line, grown in the medium described below, between the 18th and 30th passage levels counted from the ATCC seed lot. Harvest the cells after 2 to 4 days of growth, treat with trypsin and prepare a suspension containing 500 000 cells per millilitre (cell suspension). 10 min before using this suspension add 10 µg of diethylamino-ethyl dextran R per millilitre, if necessary, to increase the sensitivity of the cells.

Use a fixed virus strain grown in sensitive cells, such as the CVS strain of rabies virus adapted to growth in the BHK 21 cell line (seed virus suspension). Estimate the titre of the seed virus suspension as follows.

Prepare a series of dilutions of the viral suspension. In the chambers of cell-culture slides (8 chambers per slide), place 0.1 ml of each dilution and 0.1 ml of medium and add 0.2 ml of the cell suspension. Incubate in an atmosphere of carbon dioxide at 37 °C for 24 h. Carry out fixation, immunofluorescence staining and evaluation as described below. Determine the end-point titre of the seed virus suspension and prepare the working virus dilution corresponding to 100 CCID<sub>50</sub> per 0.1 ml.

For each assay, check the amount of virus used by performing a control titration: from the dilution corresponding to 100 CCID<sub>50</sub> per 0.1 ml, make three tenfold dilutions. Add 0.1 ml of each dilution to four chambers containing 0.1 ml of medium and add 0.2 ml of the cell suspension. The test is not valid unless the titre lies between 30 CCID<sub>50</sub> and 300 CCID<sub>50</sub>.

Dilute the reference preparation to a concentration of 2 IU/ml using non-supplemented culture medium (stock reference dilution, stored below −80 °C). Prepare two suitable predilutions (1:8 and 1:10) of the stock reference dilution so that the dilution of the reference preparation that reduces the number of fluorescent fields by 50 per cent lies within the four dilutions of the cell-culture slide. Add 0.1 ml of the medium to each chamber, except the first in each of two rows, to which add respectively 0.2 ml of the two predilutions of the stock reference dilution transferring successively 0.1 ml to the other chambers.

Dilute the preparation to be examined 1 in 100 using non-supplemented medium (stock immunoglobulin dilution) to reduce to a minimum errors due to viscosity of the undiluted preparation and make three suitable predilutions so that the dilution of the preparation to be examined that reduces the number of fluorescent fields by 50 per cent lies within the four dilutions of the cell-culture slide. Add 0.1 ml of the medium to all the chambers except the first in each of three rows, to which add respectively 0.2 ml of the three predilutions of the stock immunoglobulin dilution. Prepare a series of twofold dilutions transferring successively 0.1 ml to the other chambers.

To all the chambers containing the dilutions of the reference preparation and the dilutions of the preparation to be examined, add 0.1 ml of the virus suspension corresponding to 100 CCID<sub>50</sub> per 0.1 ml (working virus dilution), shake manually, allow to stand in an atmosphere of carbon dioxide at 37 °C for 90 min, add 0.2 ml of the cell suspension, shake manually and allow to stand in an atmosphere of carbon dioxide at 37 °C for 24 h.

After 24 h, discard the medium and remove the plastic walls. Wash the cell monolayer with phosphate buffered saline pH 7.4 R and then with a mixture of 20 volumes of water R and 80 volumes of acetone R and fix in a mixture of 20 volumes of water R and 80 volumes of acetone R at −20 °C for 3 min. Spread on the slides fluorescein-conjugated rabies antiserum R ready for use. Allow to stand in an atmosphere with a high level of moisture at 37 °C for 30 min. Wash with phosphate buffered saline pH 7.4 R and dry. Examine twenty fields in each chamber at a magnification of 250 ×, using a microscope equipped for fluorescence readings. Note the number of fields with at least one fluorescent cell. Check the test dose used in the virus titration slide and determine the dilution of the reference preparation and the dilution of the preparation to be examined that reduce the number of fluorescent fields by 50 per cent, calculating the two or three dilutions together using probit analysis. The test is not valid unless the statistical analysis shows a significant slope of the dose-response curve and no evidence of deviation from linearity or parallelism.

The stated potency is not less than 150 IU/ml. The estimated potency is not less than the stated potency and is not greater than twice the stated potency. The confidence limits (P = 0.95) of the estimated potency are not less than 80 per cent and not more than 125 per cent.