tetanus toxin with the quantity of a reference preparation of human tetanus immunoglobulin, calibrated in International Units, necessary to give the same protection.

The International Unit of antitoxin is the specific neutralising activity for tetanus toxin contained in a stated amount of the International Standard, which consists of freeze-dried human immunoglobulin. The equivalence in International Units of the International Standard is stated by the World Health Organisation.

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

Selection of animals. Use mice weighing 16 g to 20 g.

Preparation of the test toxin. Prepare the test toxin by a suitable method from the sterile filtrate of a culture in liquid medium of *C. tetani*. The two methods shown below are given as examples and any other suitable method may be used.

(1) To the filtrate of an approximately 9-day culture add 1 to 2 volumes of *glycerol R* and store the mixture in the liquid state at a temperature slightly below 0 °C.

(2) Precipitate the toxin by addition to the filtrate of *ammonium sulphate R*, dry the precipitate *in vacuo* over *diphosphorus pentoxide R*, reduce to a powder and store dry, either in sealed ampoules or *in vacuo* over *diphosphorus pentoxide R*.

Determination of test dose of toxin (Lp/10 dose). Prepare a solution of the reference preparation in a suitable liquid such that it contains 0.5 IU of antitoxin per millilitre. If the test toxin is stored dry, reconstitute it using a suitable liquid. Prepare mixtures of the solution of the reference preparation and the test toxin such that each contains 2.0 ml of the solution of the reference preparation, one of a graded series of volumes of the test toxin and sufficient of a suitable liquid to bring the volume to 5.0 ml. Allow the mixtures to stand, protected from light, for 60 min. Using six mice for each mixture, inject a dose of 0.5 ml subcutaneously into each mouse. Observe the mice for 96 h. Mice that become paralysed may be euthanised. The test dose of toxin is the quantity in 0.5 ml of the mixture made with the smallest amount of toxin capable of causing, despite partial neutralisation by the reference preparation, paralysis in all six mice injected with the mixture, within the observation period.

Determination of potency of the immunoglobulin. Prepare a solution of the reference preparation in a suitable liquid such that it contains 0.5 IU of antitoxin per millilitre. Prepare a solution of the test toxin in a suitable liquid such that it contains five test doses per millilitre. Prepare mixtures of the solution of the test toxin and the immunoglobulin to be examined such that each contains 2.0 ml of the solution of the test toxin, one of a graded series of volumes of the immunoglobulin to be examined and sufficient of a suitable liquid to bring the total volume to 5.0 ml. Also prepare mixtures of the solution of the test toxin and the solution of the reference preparation such that each contains 2.0 ml of the solution of the test toxin, one of a graded series of volumes of the solution of the reference preparation centred on that volume (2.0 ml) that contains 1 IU and sufficient of a suitable liquid to bring the total volume to 5.0 ml. Allow the mixtures to stand, protected from light, for 60 min. Using six mice for each mixture, inject subcutaneously a dose of 0.5 ml into each mouse. Observe the mice for 96 h. Mice that become paralysed may be euthanised. The mixture that contains the largest volume of immunoglobulin that fails to protect the mice from paralysis contains 1 IU. This quantity is used to calculate the potency of the immunoglobulin in International Units per millilitre.

The test is not valid unless all the mice injected with mixtures containing 2.0 ml or less of the solution of the reference preparation show paralysis and all those injected with mixtures containing more do not.

POTENCY

The potency is determined by comparing the antibody titre of the immunoglobulin to be examined with that of a reference preparation calibrated in International Units, using an immunoassay of suitable sensitivity and specificity (*2.7.1*).

The International Unit is the activity contained in a stated amount of the International Standard for anti-tetanus immunoglobulin. The equivalence in International Units of the International Standard is stated by the World Health Organisation.

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

The stated potency is not less than 100 IU of tetanus antitoxin per millilitre. The estimated potency is not less than 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.

STORAGE

See *Human normal immunoglobulin (0338).*

LABELLING

See *Human normal immunoglobulin (0338).*

The label states the number of International Units per container.

01/2008:0724

HUMAN VARICELLA IMMUNOGLOBULIN

Immunoglobulinum humanum varicellae

DEFINITION

Human varicella 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 selected donors having antibodies against *Herpesvirus varicellae*. *Human normal immunoglobulin (0338)* may be added.

It complies with the monograph on *Human normal immunoglobulin (0338)* except for the minimum number of donors, the minimum total protein content and, where authorised, the test for antibody to hepatitis B surface antigen.

POTENCY

The potency is determined by comparing the antibody titre of the immunoglobulin to be examined with that of a reference preparation calibrated in International Units, using an immunoassay of suitable sensitivity and specificity (*2.7.1*).

The International Unit is the activity contained in a stated amount of the International Standard for anti varicella-zoster. The equivalence in International Units of the International Standard is stated by the World Health Organisation.

The stated potency is not less than 100 IU/ml. The estimated potency is not less than 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.

STORAGE

See *Human normal immunoglobulin (0338)*.

LABELLING

See *Human normal immunoglobulin (0338)*.

The label states the number of International Units per container.

01/2008:1528

HUMAN VARICELLA IMMUNOGLOBULIN FOR INTRAVENOUS ADMINISTRATION

Immunoglobulinum humanum varicellae ad usum intravenosum

DEFINITION

Human varicella immunoglobulin for intravenous administration is a liquid or freeze-dried preparation containing immunoglobulins, mainly immunoglobulin G. It is obtained from plasma from selected donors having antibodies against human herpesvirus 3 (varicella-zoster virus 1). *Human normal immunoglobulin for intravenous administration (0918)* may be added.

It complies with the monograph on *Human normal immunoglobulin for intravenous administration (0918)*, except for the minimum number of donors, the minimum total protein content and the limit for osmolality.

POTENCY

The potency is determined by comparing the antibody titre of the immunoglobulin to be examined with that of a reference preparation calibrated in International Units, using an immunoassay of suitable sensitivity and specificity (*2.7.1*).

The International Unit is the activity contained in a stated amount of the International Standard for anti varicella-zoster immunoglobulin. The equivalence in International Units of the International Standard is stated by the World Health Organisation.

The stated potency is not less than 25 IU/ml. The estimated potency is not less than 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.

STORAGE

See *Human normal immunoglobulin for intravenous administration (0918)*.

LABELLING

See *Human normal immunoglobulin for intravenous administration (0918)*.

The label states the number of International Units per container.

01/2008:2298

HUMAN VON WILLEBRAND FACTOR

Factor humanus von Willebrandi

DEFINITION

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

This monograph applies to preparations formulated according to the von Willebrand factor activity.

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

PRODUCTION

The method of preparation includes 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 von Willebrand factor per milligram of total protein before the addition of any protein stabiliser.

The von Willebrand factor 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

COMPOSITION. It shall be demonstrated that the manufacturing process yields a product having a consistent composition with respect to von Willebrand factor, factor VIII and the proportions of von Willebrand factor and factor VIII.

von Willebrand factor multimers. The distribution of the different von Willebrand factor multimers is determined by a suitable method such as sodium dodecyl sulphate (SDS) agarose gel electrophoresis with or without Western blot analysis, using a suitable normal human plasma as standard. Visualisation of the multimeric pattern may be performed using, for example, an immunoenzymatic technique and quantitative evaluation may be carried out by densitometric analysis.

von Willebrand factor activity (*2.7.21*). The von Willebrand factor activity is estimated by determining the ristocetin cofactor activity and by one or more other suitable assays such as determination of collagen-binding activity using a suitable reference preparation.

von Willebrand factor activity/antigen ratio. Consistency of the manufacturing process with respect to the ratio of von Willebrand factor activity to von Willebrand factor antigen content is demonstrated.