Rabies vaccine for human use prepared in cell cultures

**FINAL BULK VACCINE**

The final bulk vaccine is prepared from one or more satisfactorily monovalent pooled harvests and may contain more than one virus type. Suitable flavouring substances and stabilisers may be added.

Only a final bulk vaccine that complies with the following requirement may be used in the preparation of the final lot.

**Bacterial and fungal contamination.** Carry out the test for sterility (2.6.1), using 10 ml for each medium.

**FINAL LOT**

Only a final lot that complies with the following requirement for thermal stability and is satisfactory with respect to each of the requirements given below under Identification, Tests and Assay may be released for use.

**IDENTIFICATION**

The vaccine is shown to contain poliovirus of each type stated on the label, using specific antibodies.

**TESTS**

**Bacterial and fungal contamination.** The vaccine complies with the test for sterility (2.6.1).

**Thermal stability.** Maintain not fewer than 3 vials of the final lot at 37 ± 1 °C for 48 h. Determine the total virus concentration as described under Assay in parallel for the heated vaccine and for vaccine maintained at the temperature recommended for storage. The estimated difference between the total virus concentration of the unheated and heated vaccines is not greater than 0.5 log_{10} infectious virus units (CCID_{50}) per single human dose.

The test is not valid if:

- the confidence interval \( (P = 0.95) \) of the logarithm of the virus concentration of the reference preparation is greater than ± 0.3;
- the virus concentration of the reference preparation differs by more than 0.5 log CCID_{50} from the assigned value;
- the range of virus concentrations found for the replicates of any sample is greater than 0.8 log CCID_{50}.

**LABELLING**

The label states:

- the types of poliovirus contained in the vaccine,
- the minimum amount of virus of each type contained in 1 single human dose,
- the cell substrate used for the preparation of the vaccine,
- that the vaccine is not to be injected.

01/2008:0216

**RABIES VACCINE FOR HUMAN USE PREPARED IN CELL CULTURES**

**Vaccinum rabiei ex cellulis ad usum humanum**

**DEFINITION**

Rabies vaccine for human use prepared in cell cultures is a freeze-dried preparation of a suitable strain of fixed rabies virus grown in cell cultures and inactivated by a validated method.

The vaccine is reconstituted immediately before use as stated on the label to give a clear liquid that may be coloured owing to the presence of a pH indicator.

**PRODUCTION**

**GENERAL PROVISIONS**

The production of the vaccine is based on a virus seed lot and, if a cell line is used for virus propagation, a cell-bank system. The production method shall have been shown to yield consistently vaccines that comply with the requirements for immunogenicity, safety and stability. Unless otherwise justified and authorised, the virus in the final vaccine must not have undergone more passages from the master seed lot than were used to prepare the vaccine shown in clinical studies to be satisfactory with respect to safety and efficacy; even with authorised exceptions, the number of passages beyond the level used for clinical studies must not exceed 5.
The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for immunosera and vaccines for human use (2.6.9).

**SUBSTRATE FOR VIRUS PROPAGATION**

The virus is propagated in a human diploid cell line (5.2.3), in a continuous cell line approved by the competent authority, or in cultures of chick-embryo cells derived from a flock free from specified pathogens (5.2.2).

**SEED LOTS**

The strain of rabies virus used shall be identified by historical records that include information on the origin of the strain and its subsequent manipulation.

Working seed lots are prepared by not more than 5 passages from the master seed lot.

Only a working seed lot that complies with the following tests may be used for virus propagation.

**Identification**. Each working seed lot is identified as rabies virus using specific antibodies.

**Virus concentration**. The virus concentration of each working seed lot is determined by a cell culture method using immunofluorescence, to ensure consistency of production.

**Extraneous agents** (2.6.16). The working seed lot complies with the requirements for virus seed lots. If the virus has been passaged in mouse brain, specific tests for murine viruses are carried out.

**VIRUS PROPAGATION AND HARVEST**

All processing of the cell bank and subsequent cell cultures is done under aseptic conditions in an area where no other cells are handled. Approved animal (but not human) serum may be used in the media, but the final medium for maintaining cell growth during virus multiplication does not contain animal serum; the media may contain human albumin. Serum and trypsin used in the preparation of cell suspensions and media are shown to be free from extraneous agents. The cell culture media may contain a pH indicator such as phenol red and approved antibiotics at the lowest effective concentration. Not less than 500 ml of the cell cultures employed for vaccine production are set aside as uninfected cell cultures (control cells). The virus suspension is harvested on one or more occasions during incubation. Multiple harvests from the same production cell culture may be pooled and considered as a single harvest.

Only a single harvest that complies with the following requirements may be used in the preparation of the inactivated viral harvest.

**Identification**. The single harvest contains virus that is identified as rabies virus using specific antibodies.

**Virus concentration**. Titrate for infective virus in cell cultures; the titre is used to monitor consistency of production.

**Control cells**. The control cells of the production cell culture from which the single harvest is derived comply with a test for identification and with the requirements for extraneous agents (2.6.16).

**PURIFICATION AND INACTIVATION**

The virus harvest may be concentrated and/or purified by suitable methods; the virus harvest is inactivated by a validated method at a fixed, well-defined stage of the process, which may be before, during or after any concentration or purification. The method shall have been shown to be capable of inactivating rabies virus without destruction of the immunogenic activity. If betapropiolactone is used, the concentration shall at no time exceed 1:3500.

Only an inactivated viral suspension that complies with the following requirements may be used in the preparation of the final bulk vaccine.

**Residual infectious virus**. Carry out an amplification test for residual infectious rabies virus immediately after inactivation or using a sample frozen immediately after inactivation and stored at –70 °C. Inoculate a quantity of inactivated viral suspension equivalent to not less than 25 human doses of vaccine into cell cultures of the same type as those used for production of the vaccine. A passage may be made after 7 days. Maintain the cultures for a total of 21 days and then examine the cell cultures for rabies virus using an immunofluorescence test. The inactivated virus harvest complies with the test if no rabies virus is detected.

**Residual host-cell DNA**. The content of residual host-cell DNA, determined using a suitable method as described in *Products of recombinant DNA technology* (0784), is not greater than 10 ng per single human dose.

**FINAL BULK VACCINE**

The final bulk vaccine is prepared from one or more inactivated viral suspensions. An approved stabiliser may be added to maintain the activity of the product during and after freeze-drying.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

**Glycoprotein content**. Determine the glycoprotein content by a suitable immunochemical method (2.7.1), for example, single-radial immunodiffusion, enzyme-linked immunosorbent assay or an antibody-binding test. The content is within the limits approved for the particular product.

**Sterility** (2.6.1). The final bulk vaccine complies with the test for sterility, carried out using 10 ml for each medium.

**FINAL LOT**

The final bulk vaccine is distributed aseptically into sterile containers and freeze-dried to a moisture content shown to be favourable to the stability of the vaccine. The containers are then closed so as to avoid contamination and the introduction of moisture.

Only a final lot that complies with each of the requirements given below under Identification, Tests and Assay may be released for use. Provided that the test for residual infectious virus has been carried out with satisfactory results on the inactivated viral suspension and the test for bovine serum albumin has been carried out with satisfactory results on the final bulk vaccine, these tests may be omitted on the final lot.

**IDENTIFICATION**

The vaccine is shown to contain rabies virus antigen by a suitable immunochemical method (2.7.1) using specific antibodies, preferably monoclonal; alternatively, the assay serves also to identify the vaccine.

**TESTS**

**Residual infectious virus**. Inoculate a quantity equivalent to not less than 25 human doses of vaccine into cell cultures of the same type as those used for production of the vaccine. A passage may be made after 7 days. Maintain the cultures for a total of 21 days and then examine the cell cultures for rabies virus using an immunofluorescence test. The vaccine complies with the test if no rabies virus is detected.

**Bovine serum albumin**: maximum 50 ng per single human dose, determined by a suitable immunochemical method (2.7.1).

**Sterility** (2.6.1). It complies with the test.
Rubella vaccine (live)

Bacterial endotoxins (2.6.14): less than 25 IU per single human dose.

Pyrogens (2.6.8). It complies with the test. Unless otherwise justified and authorised, inject into each rabbit a single human dose of the vaccine diluted to 10 times its volume.

Water (2.5.12): maximum 3.0 per cent.

ASSAY

The potency of rabies vaccine is determined by comparing the dose necessary to protect mice against the effects of a lethal dose of rabies virus, administered intracerebrally, with the quantity of a reference preparation of rabies vaccine necessary to provide the same protection. For this comparison a reference preparation of rabies vaccine, calibrated in International Units, and a suitable preparation of rabies virus for use as the challenge preparation are necessary.

The International Unit is the activity contained in a stated quantity of the International Standard. The equivalence in International Units of the International Standard is stated by the World Health Organisation.

The test described below uses a parallel-line model with at least 3 points for the vaccine to be examined and the reference preparation. Once the analyst has experience with the method for a given vaccine, it is possible to carry out a simplified test using a single dilution of the vaccine to be examined. Such a test enables the analyst to determine that the vaccine has a potency significantly higher than the required minimum, but does not give full information on the validity of each individual potency determination.

The use of a single dilution allows a considerable reduction in the number of animals required for the test and must be considered by each laboratory in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes.

Selection and distribution of the test animals. Use healthy female mice, about 4 weeks old, each weighing 11-15 g, and from the same stock. Distribute the mice into 6 groups of a size suitable to meet the requirements for validity of the test and, for titration of the challenge suspension, 4 groups of 5.

Preparation of the challenge suspension. Inoculate mice intracerebrally with the Challenge Virus Standard (CVS) strain of rabies virus and when the mice show signs of rabies, but before they die, euthanise them, then remove the brains and prepare a homogenate of the brain tissue in a suitable diluent. Separate gross particulate matter by centrifugation and use the supernatant liquid as the challenge suspension.

Determine the potency of the vaccine. Prepare 3 fivefold serial dilutions of the vaccine to be examined and of the reference preparation and repeat the injections. 7 days after the second injection, prepare a suspension of the challenge virus such that, on the basis of the preliminary titration, 0.03 ml contains about 50 LD50. Inject intracerebrally into each vaccinated mouse 0.03 ml of this suspension. Prepare 3 suitable serial dilutions of the challenge suspension.

Allocate the challenge suspension and the 3 dilutions, 1 to each of the 4 groups of 5 control mice, and inject intracerebrally into each mouse 0.03 ml of the suspension or dilution allocated to its group. Observe the animals in each group for 14 days and record the number in each group that die or show signs of rabies in the period 5-14 days after challenge.

The test is not valid unless:

- for both the vaccine to be examined and the reference preparation the potency 50 per cent protective dose lies between the largest and smallest doses given to the mice;
- the titration of the challenge suspension shows that 0.03 ml of the suspension contained not less than 10 LD50;
- the statistical analysis shows a significant slope and no significant deviations from linearity or parallelism of the dose-response curves;
- the confidence limits (P = 0.95) are not less than 25 per cent and not more than 400 per cent of the estimated potency.

The vaccine complies with the test if the estimated potency is not less than 2.5 IU per human dose.

LABELLING

The label states the biological origin of the cells used for the preparation of the vaccine.

01/2008:0162

RUBELLA VACCINE (LIVE)

Vaccinum rubellae vivum

DEFINITION

Rubella vaccine (live) is a freeze-dried preparation of a suitable attenuated strain of rubella virus. The vaccine is reconstituted immediately before use, as stated on the label, to give a clear liquid that may be coloured owing to the presence of a pH indicator.

PRODUCTION

The production of vaccine is based on a virus seed-lot system and a cell-bank system. The production method shall have been shown to yield consistently live rubella vaccines of adequate immunogenicity and safety in man. Unless otherwise justified and authorised, the virus in the final vaccine shall have undergone no more passages from the master seed lot than were used to prepare the vaccine shown in clinical studies to be satisfactory with respect to safety and efficacy.

The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for immunosera and vaccines for human use (2.6.9).

SUBSTRATE FOR VIRUS PROPAGATION

The virus is propagated in human diploid cells (5.2.5).

SEED LOT

The strain of rubella virus used shall be identified by historical records that include information on the origin of the strain and its subsequent manipulation. To avoid the unnecessary use of monkeys in the test for neurovirulence,