test if not fewer than 80 per cent of the guinea-pigs survive to the end of the observation period and remain in good health and no animal shows signs of infection with filoviruses.

**FINAL BULK VACCINE**

The final bulk vaccine is prepared from one or more satisfactory 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.

**Thermal stability.** Maintain samples of the final lot at 37 °C for 48 h. Determine the total virus concentration as described under Assay in parallel for the heated vaccine and for unheated vaccine. 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.

**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).

**ASSAY**

Titrate for infectious virus at least in triplicate using the method described below. Use an appropriate virus reference preparation to validate each assay. If the vaccine contains more than one poliovirus type, titrate each type separately, using appropriate type-specific antisemur (or preferably a monoclonal antibody) to neutralise each of the other types present.

For a trivalent vaccine, the estimated mean virus titres must be: not less than 1 x 10^{6.0} infectious virus units (CCID_{50}) per single human dose for type 1; not less than 1 x 10^{5.0} infectious virus units (CCID_{50}) for type 2; and not less than 1 x 10^{5.0} infectious virus units (CCID_{50}) for type 3.

For monovalent or divalent vaccine, the minimum virus titres are decided by the competent authority.

**Method.** Inoculate groups of 8 to 12 flat-bottomed wells in a microtitre plate with 0.1 ml of each of the selected dilutions of virus followed by a suitable cell suspension of the Hep-2 (Cincinnati) line. Incubate the plates at a suitable temperature. Examine the cultures on days 7-9. The assay is not valid if the confidence interval (P = 0.95) of the logarithm of the virus concentration is greater than ± 0.3.

**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.
infectious extraneous agents; trypsin complies with the
monograph on Trypsin (0694). 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 (2.6.10).

**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.

**Inactivation.** 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 doses of vaccine into cell
cultures of the same type as those used for production of the
vaccine. Make a passage after 7 days. Maintain the cultures
for a further 14 days and then examine the cell cultures for rabies virus using an
immunofluorescence test. No rabies virus is detected.

**Residual host-cell DNA.** If a continuous cell line is used for
virus propagation, 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
100 pg 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 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 inactivation 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**

**Inactivation.** 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. Make a passage
after 7 days. Maintain the cultures for a further 14 days
and then examine the cell cultures for rabies virus using an
immunofluorescence test. No rabies virus is detected.

**Bovine serum albumin.** Not more than 50 ng per single
human dose, determined by a suitable immunochemical
method (2.7.1).

**Sterility (2.6.1).** The vaccine complies with the test for
sterility.

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

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

**Water (2.5.12).** Not more than 3.0 per cent, determined by
the semi-micro determination of water.

**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 three 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 will 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
Selection and distribution of the test animals. Use in the test healthy female mice about 4 weeks old, each weighing 11 g to 15 g, and from the same stock. Distribute the mice into six groups of a size suitable to meet the requirements for validity of the test and, for titration of the challenge suspension, four groups of five.

Preparation of the challenge suspension. Inoculate mice intracerebrally with the CVS strain of rabies virus and when the mice show signs of rabies, but before they die, sacrifice them, 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. Distribute the suspension in small volumes in ampoules, seal and store at a temperature as the challenge suspension. Distribute the suspension in suitable diluent. Separate gross particulate matter by centrifugation and use the supernatant liquid.

Preparation of the vaccine.

Vaccination of monkeys. Inoculate monkeys intravenously with a high dose of rabies virus. The monkeys develop signs of rabies. The duration of the disease varies with the strain of virus, administered dose and the condition of the individual monkey. The monkeys are killed 10 days to 14 days after challenge. The experimental site must be free from all murine and human twitter. The challenge virus 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.

Identifying agents (2.6.16). The working seed lot complies with the requirements for seed lots.

Neurovirulence (2.6.18). The working seed lot complies with the test for neurovirulence of live virus vaccines. Macaca and Cercopithecus monkeys are suitable for the test.

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).

Proponents of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes.

Identification. The master and working seed lots are identified as rubella virus by serum neutralisation in cell culture, using specific antibodies.

Virus concentration. The virus concentration of the master and working seed lots is determined to ensure consistency of production.

Extraneous agents (2.6.16). The working seed lot complies with the requirements for seed lots.

Neurovirulence (2.6.18). The working seed lot complies with the test for neurovirulence of live virus vaccines. Macaca and Cercopithecus monkeys are suitable for the test.

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. Suitable animal (but not human) serum may be used in the growth medium, but the final medium for maintaining cell growth during virus multiplication does not contain animal serum. Serum and trypsin used in the preparation of cell suspensions and culture media are shown to be free from extraneous agents. The cell culture medium may contain a pH indicator such as phenol red and suitable antibiotics at the lowest effective concentration. It is preferable to have a substrate free from antibiotics during production. Not less than 500 ml of the production cell cultures is set aside as uninfected cell cultures (control cells). The temperature of incubation is controlled during the growth of the virus. The virus suspension is harvested.