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

Sterility (2.6.1). Carry out the test for sterility using 10 ml for each medium.

Absence of toxin and irreversibility of toxoid. Using the same buffer solution as for the final vaccine, without adsorbent, prepare a solution of bulk purified toxoid at the same concentration as in the final vaccine. Divide the dilution into 2 equal parts. Keep one of them at 5 ± 3 °C and the other at 37 °C for 6 weeks. Test both dilutions as described below. Use 15 guinea-pigs, each weighing 250-350 g and that have not previously been treated with any material that will interfere with the test. Inject subcutaneously into each of 5 guinea-pigs 5 ml of the dilution incubated at 5 ± 3 °C. Inject subcutaneously into each of 5 other guinea-pigs 5 ml of the dilution incubated at 37 °C. Inject subcutaneously into each of 5 guinea-pigs at least 500 Lf of the non-incubated bulk purified toxoid in a volume of 1 ml. The bulk purified toxoid complies with the test if during the 21 days following the injection no animal shows signs of or dies from tetanus. If more than 1 animal dies from non-specific causes, repeat the test; if more than 1 animal dies in the second test, the toxoid does not comply with the test.

Antigenic purity. Not less than 1000 Lf per milligram of protein nitrogen.

FINAL BULK VACCINE

The final bulk vaccine is prepared by adsorption of a suitable quantity of bulk purified toxoid onto a mineral carrier such as hydrated aluminium phosphate or aluminium hydroxide; the resulting mixture is approximately isotonic with blood. Suitable antimicrobial preservatives may be added. Certain antimicrobial preservatives, particularly those of the phenolic type, adversely affect the antigenic activity and must not be used.

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

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than the minimum amount shown to be effective and is not greater than 115 per cent of the quantity stated on the label.

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

ASSAY

The virus is propagated in chick embryo cells prepared out with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

Provided the free formaldehyde content has been determined on the bulk purified toxoid or on the final bulk and it has been shown that the content in the final lot will not exceed 0.2 g/l, the test for free formaldehyde may be omitted on the final lot.

IDENTIFICATION

Tetanus toxoid is identified by a suitable immunochemical method (2.7.1). The following method, applicable to certain vaccines, is given as an example. Dissolve in the vaccine to be examined sufficient sodium citrate R to give a 100 g/l solution. Maintain at 37 °C for about 16 h and centrifuge until a clear supernatant liquid is obtained. The clear supernatant liquid reacts with a suitable tetanus antitoxin, giving a precipitate.

TESTS

Aluminium (2.5.13): maximum 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

Free formaldehyde (2.4.18): maximum 0.2 g/l.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than the minimum amount shown to be effective and is not greater than 115 per cent of the quantity stated on the label.

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

ASSAY

Carry out one of the prescribed methods for the assay of tetanus vaccine (adsorbed) (2.7.8).

The lower confidence limit (P = 0.95) of the estimated potency is not less than 40 IU per single human dose.

LABELLING

The label states:

– the minimum number of International Units per single human dose,
– the name and the amount of the adsorbent,
– that the vaccine must be shaken before use,
– that the vaccine is not to be frozen.

01/2005:1375

TICK-BORNE ENCEPHALITIS VACCINE (INACTIVATED)

Definitio

Tick-borne encephalitis vaccine (inactivated) is a liquid preparation of a suitable strain of tick-borne encephalitis virus grown in cultures of chick-embryo cells or other suitable cell cultures and inactivated by a suitable, validated method.

Production

Production of the vaccine is based on a virus seed-lot system. The production method shall have been shown to yield consistently vaccines comparable with the vaccine of proven clinical efficacy and safety in man. Unless otherwise justified and authorised, the virus in the final vaccine shall not have undergone more passages from the master seed lot than the virus in the vaccine used in clinical trials.

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 chick embryo cells prepared from eggs derived from a chicken flock free from specified pathogens (5.2.2) or in other suitable cell cultures (5.2.3).
**SEED LOTS**

The strain of virus used is identified by historical records that include information on the origin of the strain and its subsequent manipulation. Virus seed lots are stored at or below −60 °C.

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

**Identification.** Each seed lot is identified as containing the vaccine strain of tick-borne encephalitis virus by a suitable immunochemical method (2.7.1), preferably using monoclonal antibodies.

**Virus concentration.** The virus concentration of each seed lot is determined by titration in suitable cell cultures to monitor consistency of production.

**Extraneous agents** (2.6.16). Each seed lot complies with the requirements for extraneous agents in viral vaccines for human use; the tests in cell cultures are carried out in human and simian cells only. For neutralisation of the vaccine virus, the use of monoclonal antibodies is preferable.

**VIRUS PROPAGATION AND HARVEST**

All processing of the cell cultures is performed under aseptic conditions in an area where no other cells are being handled. Serum and trypsin used in the preparation of cell suspensions and media used must be 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. At least 500 ml of the cell cultures employed for vaccine production is set aside as uninfected cell cultures (control cells).

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

**Identification.** The single harvest is shown to contain tick-borne encephalitis virus by a suitable immunochemical method (2.7.1), preferably using monoclonal antibodies, or by virus neutralisation in cell cultures.

**Bacterial and fungal contamination** (2.6.1). The single harvest complies with the test for sterility, carried out using 10 ml for each medium.

**Mycoplasmas** (2.6.7). The single harvest complies with the test for mycoplasmas carried out using 1 ml for each medium.

**Control cells.** The control cells comply with the tests for extraneous agents (2.6.16). If the vaccine is produced using a cell-bank system, the control cells comply with a test for identification.

**Virus concentration.** Determine the virus concentration by titration in suitable cell cultures to monitor consistency of production.

**INACTIVATION**

To avoid interference, viral aggregates are removed by filtration immediately before the inactivation process. The virus suspension is inactivated by a validated method; the method shall have been shown to be consistently capable of inactivating tick-borne encephalitis virus without destroying the antigenic and immunogenic activity; as part of the validation studies, an inactivation curve is plotted representing residual live virus concentration measured on not fewer than three occasions. If formaldehyde is used for inactivation, the presence of an excess of free formaldehyde is verified at the end of the inactivation process.

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

**Residual infective virus.** Inoculate a quantity of the inactivated harvest equivalent to not less than ten human doses of vaccine in the final lot into primary chicken fibroblast cell cultures, or other cells shown to be at least as sensitive to tick-borne encephalitis virus, with not less than 3 cm² of cell sheet per millilitre of inoculum. Incubate at 37 ± 1 °C for 14 days. No cytopathic effect is detected at the end of the incubation period. Collect the culture fluid and inoculate 0.03 ml intracerebrally into each of not fewer than ten mice about 4 weeks old. Observe the mice for 14 days. They show no evidence of tick-borne encephalitis virus infection.

**PURIFICATION**

Several inactivated single harvests may be pooled before concentration and purification by suitable methods, preferably by continuous-flow, sucrose density-gradient centrifugation.

Only a purified, inactivated harvest that complies with the following requirements may be used in the preparation of the final bulk vaccine.

**Sterility** (2.6.7). The purified, inactivated harvest complies with the test for sterility carried out using 10 ml for each medium.

**Specific activity.** Determine the antigen content of the purified, inactivated harvest by a suitable immunochemical method (2.7.1). Determine the total protein content by a suitable method. The specific activity, calculated as the antigen content per unit mass of protein, is within the limits approved for the specific product.

**FINAL BULK VACCINE**

The final bulk vaccine is prepared from one or more purified, inactivated harvests.

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

**Sterility** (2.6.7). The final bulk vaccine complies with the test for sterility, carried out using 10 ml for each medium.

**FINAL LOT**

Only a final lot that is satisfactory with respect to each of the requirements given below under Identification, Tests and Assay may be released for use. Provided that the tests for free formaldehyde, bovine serum albumin (where applicable) and pyrogens and the assay have been carried out with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

**IDENTIFICATION**

The vaccine is shown to contain tick-borne encephalitis virus antigen by a suitable immunochemical method (2.7.1) using specific antibodies or by the mouse immunogenicity test described under Assay.

**TESTS**

**Aluminium** (2.5.13): maximum 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

**Free formaldehyde** (2.4.18): maximum of 0.1 g/l.

**Bovine serum albumin.** If bovine serum albumin has been used during production, the vaccine contains not more than 50 ng per single human dose, determined by a suitable immunochemical method (2.7.1).

**Sterility** (2.6.7). The vaccine complies with the test for sterility.

**Pyrogens** (2.6.8). The vaccine complies with the test for pyrogens. Inject into each rabbit, per kilogram of body mass, one dose of vaccine.
ASSAY
The potency is determined by comparing the dose necessary to protect a given proportion of mice against the effects of a lethal dose of tick-borne encephalitis virus, administered intraperitoneally, with the quantity of a reference preparation of tick-borne encephalitis vaccine necessary to provide the same protection. For this comparison an approved reference preparation and a suitable preparation of tick-borne encephalitis virus from an approved strain for use as the challenge preparation are necessary.

The following is cited as an example of a method that has been found suitable for a given vaccine.

Selection and distribution of test animals. Use healthy mice weighing 11 g to 17 g and derived from the same stock. Distribute the mice into not fewer than six groups of a suitable size to meet the requirements for validity of the test; for titration of the challenge suspension, use not fewer than four groups of ten mice. Use mice of the same sex or distribute males and females equally between groups.

Determination of potency of the vaccine. Prepare not fewer than three suitable dilutions of the vaccine to be examined and of the reference preparation; in order to comply with validity criteria four to five dilutions will usually be necessary. Prepare dilutions such that the most concentrated suspension is expected to protect more than 50 per cent of the animals and the least concentrated suspension less than 50 per cent. Allocate each dilution to a different group of mice and inject subcutaneously into each mouse 0.2 ml of the dilution allocated to its group. 7 days later make a second injection using the same dilution scale. 14 days after the second injection prepare a suspension of the challenge virus containing not less than 100 LD_{so} in 0.2 ml. Inject 0.2 ml of this virus suspension intraperitoneally into each vaccinated mouse. To verify the challenge dose, prepare a series of not fewer than three dilutions of the challenge virus suspension at not greater than one-hundredfold intervals. Allocate the challenge suspension and the four dilutions, one to each of the five groups of ten mice, and inject intraperitoneally into each mouse 0.2 ml of the challenge suspension or the dilution allocated to its group. Observe the animals for 21 days after the challenge and record the number of mice that die in the period between 7 days and 21 days after the challenge.

Calculations. Calculate the results by the usual statistical methods for an assay with quantal responses (for example, 5.3).

Validity criteria. The test is not valid unless:
- the concentration of the challenge virus is not less than 100 LD_{so}.
- for both the vaccine to be examined and the reference preparation the 50 per cent protective dose (PD_{50}) lies between the largest and smallest doses given to the mice,
- the statistical analysis shows a significant slope and no significant deviation from linearity and parallelism of the dose-response lines,
- the confidence limits (P = 0.95) are not less than 33 per cent and not more than 300 per cent of the estimated potency.

Potency requirement. Include all valid tests to estimate the mean potency and the confidence limits (P = 0.95) for the mean potency; compute weighted means with the inverse of the squared standard error as weights. The vaccine complies with the test if the estimated potency is not less than that approved by the competent authority, based on data from clinical efficacy trials.

LABELLING
The label states:
- the strain of virus used in preparation,
- the type of cells used for production of the vaccine.