

Annex 2

Requirements for tick-borne encephalitis vaccine (inactivated)

(Requirements for Biological Substances No. 51)

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Introduction

Tick-borne encephalitis (TBE) is an acute viral illness caused by two closely related viruses of the family *Flaviviridae*: the central European encephalitis (CEE) virus, found in many European countries, and the Russian spring–summer encephalitis (RSSE) virus, found predominantly in the Asian parts of the former Soviet Union. These viruses, which are endemic to forested areas, are transmitted by ticks. In addition to humans, they infect small mammals and, to a lesser extent, birds (1, 2). In Asia, the disease is characterized by abrupt onset of fever, severe headache, nausea and vomiting and severe back pain often associated with focal epilepsy and flaccid paralysis, especially of

the shoulder girdle. Such paralysis may be permanent. The central European form of the disease has a longer course, often with biphasic fever, but severe sequelae are less frequent. The initial febrile stage is normally not associated with disease of the central nervous system, but the second phase, following approximately 4–10 days after apparent recovery, is characterized by fever and meningoencephalitis. The case-fatality rate is approximately 20% for the Asian form of the disease and 1–5% for the European form.

TBE is endemic to most European countries, the Russian Federation and possibly China. It is the most important arthropod-transmitted viral disease in Europe, and in some countries it represents a major public-health problem. The disease has been known by several names, including RSSE, Far Eastern encephalitis and CEE.

CEE virus is found in every European country, with the exception of Belgium, Luxembourg, the Netherlands, Portugal, Spain and the United Kingdom, and is transmitted primarily by the tick *Ixodes ricinus*. RSSE virus is most prevalent in the eastern part of the former Soviet Union and is transmitted by the tick *Ixodes persulcatus* (3).

Flaviviruses are a large group of small, enveloped viruses responsible for a number of severe human diseases, including yellow fever, Japanese encephalitis, dengue haemorrhagic fever and TBE. TBE virus particles are roughly spherical in shape, 40–50nm in diameter, and contain a core, 20–30nm in diameter. The genome consists of single-stranded positive-sense RNA with a relative molecular mass of about 4×10^6 . Three structural proteins and the capsid, membrane and envelope proteins are all encoded by the viral genome (4). The envelope glycoprotein induces neutralizing and haemagglutination-inhibition antibodies and is the most important antigen for providing protection from disease.

The first vaccine against TBE was prepared in 1941 in the brains of mice (5). Some 20 years later TBE vaccines derived from cell cultures (chicken embryo fibroblast cells) were developed and used for active immunization in humans in the former Soviet Union (6–8). Later, a purified, inactivated virus vaccine was developed which proved to be more immunogenic than previous TBE vaccines (9, 10).

The efficacy of these vaccines has been well documented (11, 12). They have also been shown to protect mice from a lethal challenge with several TBE-virus isolates obtained over a period of more than 30 years from all over Europe and the Asian part of the former Soviet Union. In addition, it has been demonstrated that antibodies induced by vaccination of human volunteers neutralized all tested isolates (13).

Large outbreaks of TBE, sometimes involving thousands of cases, continue to occur in endemic areas. In addition, certain special groups, such as forest workers, geologists, travellers to endemic areas and laboratory workers, are also at risk. In view of the need to immunize large numbers of people, requirements for inactivated TBE vaccine have been formulated. In drafting these requirements, account has been taken of the regulations and requirements for the manufacture and control of TBE vaccines that have already been established in several countries.

General considerations

Inactivated TBE vaccines for human use are prepared from harvested virus propagated in appropriate cells. Harvest is followed by inactivation, purification and formulation. Inactivation is one of the critical production steps, and the inactivation process should be carefully monitored.

These requirements are scientific and advisory in nature. The parts of each section printed in normal type have been written in the form of requirements so that, should a national control authority so desire, they may be adopted as they stand as national requirements. If changes are made, they should be validated and approved by the national control authority. The parts of each section printed in small type are comments or recommendations for guidance.

To facilitate the international distribution of vaccine made in accordance with these requirements, a summary protocol is available (14). The summary protocol indicates the kind of information that should be included for viral vaccines in a routine batch-release document submitted to a national control laboratory, as well as that to be included in a release certificate provided by the national control authority.

Part A. Manufacturing requirements

A.1 Definitions

A.1.1 *International name and proper name*

The international name shall be "*Vaccinum encephalitis ixodicae advectae inactivatum*". The proper name shall be the equivalent of the international name in the language of the country of origin.

The use of the international name should be limited to vaccines that satisfy the requirements formulated below.

A.1.2 **Descriptive definition**

Tick-borne encephalitis vaccine (inactivated) is a fluid or freeze-dried preparation of a suitable strain of TBE virus grown in cell cultures and inactivated by an appropriate and validated method. The preparation shall satisfy all the requirements formulated below.

A.1.3 **International reference materials**

An International Reference Reagent for Anti-tick-borne Encephalitis Serum (Russian spring-summer encephalitis (Sophyn and Absetarov) virus) is available on request from the National Institute of Biological Standards and Control, Potters Bar, England.

A.1.4 **Terminology**

The following definitions are given for the purposes of these requirements only.

Master cell bank. A quantity of adequately characterized cells stored in liquid nitrogen or the vapour phase of liquid nitrogen in aliquots of uniform composition, one or more of which may be used for the production of a manufacturer's working cell bank.

Manufacturer's working cell bank. A quantity of cells of uniform composition derived from one or more aliquots of the master cell bank. A master cell bank is expanded by serial subculture up to a passage number (or population doubling, as appropriate) selected by the manufacturer, at which point the cells are combined to yield a single pool, distributed into containers and preserved cryogenically to form the manufacturer's working cell bank. All containers are treated identically and, once removed from storage, are not returned to the stock. One or more of the containers from such a working cell bank may be used for inoculating the continuous-cell-line production culture.

Continuous-cell-line production cell culture. A cell culture, derived from one or more containers of the manufacturer's working cell bank, suspended in an appropriate culture medium and used for the production of TBE virus.

Chicken-fibroblast production cell culture. A number of cell cultures derived from a pool of cells and processed together.

Adventitious agents. Microorganisms contaminating the cell substrate or materials used in its culture, including bacteria, fungi, mycoplasmas and endogenous and exogenous viruses.

Master seed lot. A quantity of virus that has been prepared as a single lot and has a uniform composition. It is used for the preparation of working seed lots.

Working seed lot. A quantity of virus of uniform composition derived from the master seed lot by a method and at a passage level approved by the national control authority. The working seed lot is used to inoculate production cell cultures.

Single harvest. A quantity of virus suspension harvested from production cell cultures inoculated with the same working seed lot and incubated and harvested together in a single production run. Multiple harvests from the same production cell culture may be pooled and considered a single harvest.

Bulk material. A purified, inactivated single harvest or a pool of inactivated single harvests that have been purified at the same time.

Final bulk. The finished homogeneous material prepared from one or more batches of bulk material present in the vessel from which the final containers are filled.

Final lot. A collection of sealed final containers of vaccine that are homogeneous with respect to the risk of contamination during the preparation process, including filling and, where applicable, freeze-drying. All the final containers in a final lot must, therefore, have been filled from one container of final bulk in a single working session and freeze-dried together under standardized conditions in one chamber.

A.2 **General manufacturing requirements**

The general manufacturing requirements contained in Good Manufacturing Practices for Pharmaceutical (15) and Biological (16) Products shall apply to establishments manufacturing TBE vaccine with the following addition: the staff involved in the production and quality control of TBE vaccine shall be shown to be immune to TBE.

A.3 **Source materials**

A.3.1 **Cell cultures for virus propagation**

A.3.1.1 *Cells used for providing production cell cultures*

If chicken fibroblast cells are used for the manufacture of vaccine they shall meet the general requirements set out in Requirements for the Use of Animal Cells as *in vitro* Substrates for the Production of Biologicals (17). The tissues of chicken embryos used in the manufacture of vaccine shall be obtained from a healthy flock. Monitoring of the flock or embryos shall include tests for exclusion of infection by at least *Salmonella*, *Mycobacterium avium* and fowl pox virus. If the

flock has to be vaccinated against Newcastle disease virus, then inactivated Newcastle disease virus vaccine must be used.

Manufacturers are strongly encouraged to use only tissues from closed, specific-pathogen-free flocks of chickens, in particular those free from avian leukosis virus.

If continuous cell lines are used for the propagation of TBE virus, they shall be derived from a master cell bank and approved by and registered with the national control authority. They shall also meet the requirements for continuous-cell-line substrates set out in Requirements for the Use of Animal Cells as *in vitro* Substrates for the Production of Biologicals (17).

β-Lactam antibiotics shall not be used at any stage of manufacture.

Minimal concentrations of suitable antibiotics such as kanamycin may be used when approved by the national control authority.

The cells in any cell bank shall have been characterized with respect to their genealogy, growth characteristics and viability during storage, and shall have been shown to be free from detectable adventitious agents (17).

A.3.1.2 Cell-culture medium

Serum used for the propagation of cells for TBE vaccine production shall demonstrate freedom from bacteria, fungi and mycoplasmas as specified in Part A, section 5.2, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (18) and the revised Sterility Test for Mycoplasmas (General Requirements for the Sterility of Biological Substances, amendment 1995) (19) and shall demonstrate freedom from viruses. Serum of bovine origin should comply with guidance given in Requirements for the Use of Animal Cells as *in vitro* Substrates for the Production of Biologicals (17) and *Report of a WHO Consultation on Medicinal and Other Products in Relation to Human and Animal Transmissible Spongiform Encephalopathies* (20) and shall be approved by the national control authority.

Suitable tests for detecting viruses in bovine serum are found in Appendix 1 of the revised Requirements for Biological Substances No. 7 (Requirements for Poliomyelitis Vaccine (Oral)) (21). Where appropriate, more sensitive methods may be used.

In some countries, sera are examined for freedom from certain phages.

In other countries, irradiation is used to inactivate potential viruses.

Trypsin. Trypsin used for preparing cell cultures shall be bacteriologically sterile and free from mycoplasmas and viruses, in particular porcine parvoviruses. If bovine trypsin is used, it shall comply with

guidance given in *Report of a WHO Consultation on Medicinal and Other Products in Relation to Human and Animal Transmissible Spongiform Encephalopathies* (20) and in Requirements for the Use of Animal Cells as *in vitro* Substrates for the Production of Biologicals (17). It shall be approved by the national control authority.

Human albumin. If human albumin is used in the cell-culture medium, it shall meet the requirements set out in Parts C and D of the revised Requirements for Biological Substances No. 27 (Requirements for the Collection, Processing and Quality Control of Blood, Blood Components and Plasma Derivatives) (22), as well as the guidance given in *Report of a WHO Consultation on Medicinal and Other Products in Relation to Human and Animal Transmissible Spongiform Encephalopathies* (20).

A.3.2 **Virus seed**

A.3.2.1 *Virus strain*

The virus strain used in the production of virus seed lots shall be approved by the national control authority and shall yield safe and immunogenic vaccines when the virus has been inactivated. Each strain shall be identified by historical records that include information on virus origin, infectivity tests, serological tests and animal responses to inoculation.

A.3.2.2 *Seed lot system*

The preparation of TBE vaccine shall be based on the use of a master and working seed lot system. The national control authority shall determine the maximum acceptable number of passages in mice between the master seed lot and any working seed lot. Vaccine shall be made from a working seed lot without additional passages in mice. Virus seed lots shall be maintained either in dried or in frozen form. The dried seed shall be kept at a temperature below -20°C , and the frozen seed at a temperature below -60°C .

Virus seed lots shall have been shown to the satisfaction of the national control authority to be capable of yielding vaccine that meets all the present requirements.

Mice used for production of master seed lots should ideally be specific-pathogen-free. In addition, suckling mice are preferred.

In some countries the national control authority distributes the master virus seed to manufacturers.

A.3.2.3 *Tests on master and working seed lots*

Identity test. Each virus seed lot shall be identified as TBE virus by an appropriate method.

Suitable serological methods include enzyme immunoassay or a TBE-virus-neutralization assay using a reference serum or a monoclonal antibody known to neutralize TBE virus. Sera should be characterized using the International Reference Reagent of Anti-tick-borne Encephalitis Serum.

Freedom from bacteria, fungi and mycoplasmas. Each virus seed lot shall be tested for bacterial, mycotic and mycoplasmal contamination by appropriate tests as specified in Part A, section 5.2, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (18, pp. 49–52) and the revised Sterility Test for Mycoplasmas (General Requirements for the Sterility of Biological Substances, amendment 1995) (19).

Tests for adventitious viruses. Each virus seed lot shall be tested for adventitious viruses. For the tests, the virus shall be neutralized by a specific anti-tick-borne-encephalitis-virus serum, which shall itself have been shown to be free from adventitious viruses.

The individual tests on the virus seed lots should be designed so that they satisfy the requirements of the national control authority.

A.4 Production control

A.4.1 Cell cultures

At least 5% or 500 ml but no more than 1000 ml of the cell suspension employed to prepare the production cell cultures shall be used to prepare control cultures of uninfected cells.

The treatment of control and production cell cultures shall be similar. The control cultures shall be maintained for at least 2 weeks or until the time of final viral harvest from the production cultures if this is longer.

At the time of virus harvest, control cell cultures shall be examined for degeneration caused by adventitious viruses. In addition, samples of fluid collected from control cell cultures shall be pooled and tested for the presence of adventitious viruses as described below in sections A.4.1.2 and A.4.1.3. Samples not tested immediately shall be stored at -60°C or below.

If multiple harvests are made from the same production cell culture, the samples of fluid collected from control cell cultures at the time of each harvest shall be frozen and stored at -60°C or below until the last harvest has been completed. The samples shall then be pooled in proportion to the respective amounts of each harvest and submitted to the required tests.

If any test shows evidence of the presence of any adventitious viruses in a control cell culture, the TBE virus grown in the corresponding production cultures shall not be used for vaccine production.

For a test to be valid, no more than 20% of the control vessels shall have been discarded for non-specific reasons by the end of the test period.

In some countries, the national control authority may permit reduced testing for adventitious viruses where the manufacturer has demonstrated that:

- all materials of animal origin used in the cell culture process, the manufacturer's working cell bank and the virus seed lots are free from detectable adventitious viruses;
- at the end of the cell culture process, the cell culture system is free from bacterial, fungal and mycoplasmal contamination as specified in Part A, section 5.2, of the revised Requirements for Biological Substances No. 6 (General Requirements for Sterility of Biological Substances) (18) and the revised Sterility Test for Mycoplasmas (General Requirements for the Sterility of Biological Substances, amendment 1995) (19);
- the manufacturing process is consistent and has been validated to remove or inactivate a panel of representative adventitious agents.

A.4.1.1 Identity test

If a continuous cell line is used in TBE vaccine production, an identity test shall be performed on the control cell cultures by a method approved by the national control authority.

Methods for identity testing include, but are not limited to, biochemical (e.g. isoenzyme analysis), immunological (e.g. histocompatibility antigen assays), cytogenic (e.g. chromosomal) marker and genetic marker (DNA-fingerprinting) tests.

If continuous cell lines are used in TBE vaccine production the cells shall be documented as being derived from the characterized manufacturer's working cell bank.

A.4.1.2 Test for haemadsorbing adventitious viruses

At the end of the observation period, an amount equivalent to 25% of the control cell-culture suspension shall be tested for the presence of haemadsorbing viruses. If the erythrocytes have been stored, the duration of storage shall not have exceeded 7 days, and the temperature of storage shall have been in the range of 2–8°C.

Some national control authorities require production cell cultures to be tested for the presence of haemadsorbing viruses at the end of the incubation period(s) as described in section A.4.1.3. If so, the test on control cell cultures described here may not be required.

The test has usually been performed using guinea-pig erythrocytes. In some countries, the national control authority requires that alternative or additional tests for haemadsorbing viruses are made on control cultures between 3–5 days and 12 days after inoculation of the production cultures, and that other types of erythrocytes, including those from humans (blood group O), monkeys and chickens (or other avian species), should be used. In all tests, readings should be taken after incubation for 30 minutes at 0–4°C, and again after a further incubation for 30 minutes at 20–25°C. For

the test using monkey erythrocytes, readings should also be taken after a final incubation for 30 minutes at 34–37°C.

For a test to be valid, no more than 20% of the control culture vessels shall have been discarded for non-specific reasons by the end of the test period.

A.4.1.3 Tests for non-haemadsorbing adventitious viruses

At 14 days after the day of inoculation of the production cultures or at the time of final virus harvest, a sample of cell-culture fluid shall be taken from each control cell culture and pooled. A sample of 10 ml of the pool shall be tested in the same cell culture, but not the same batch, as that used for the production cell culture. Additional 10-ml samples of the pool shall be tested in both human and monkey cells.

Each sample shall be inoculated into bottles of cell culture in such a way that the dilution of the pooled fluid in the nutrient medium does not exceed 1:4. The area of the cell sheet shall be at least 3 cm² per ml of pooled fluid. At least one bottle of the cell culture shall remain uninoculated as a control.

The inoculated cultures shall be incubated at a temperature of 35–37°C for a period of at least 14 days, after which the cells shall be examined for abnormal morphology.

The tests are satisfactory if no cytopathic changes attributable to adventitious viruses are detected.

Some national control authorities require that, at the end of the observation period, a subculture is made in the same culture system and observed for at least 7 days. Furthermore, some national control authorities require that the subcultures are tested for the presence of haemadsorbing viruses.

For the tests to be valid, no more than 20% of the control culture vessels shall have been discarded for non-specific reasons by the end of the test.

A.4.2 Control of single virus harvests

A.4.2.1 Cells used for vaccine production

On the day of inoculation with the seed virus, each production cell culture and control cell culture shall be examined for degeneration caused by infective agents. If such examination shows evidence of the presence in a cell culture of any adventitious agent, the whole group of cultures concerned shall not be used for vaccine production.

After virus inoculation, cell cultures for vaccine production shall be incubated under controlled temperature conditions approved by the national control authority.

If animal serum is used in the growth medium for cell cultures, the serum shall be removed from the cell cultures either before or after inoculation with seed virus. Before the virus is harvested, the cell cultures shall be rinsed and the growth medium replaced with serum-free maintenance medium.

A.4.2.2 Sterility tests

A sample removed from each single harvest shall be tested for bacterial, fungal and mycoplasmal contamination by appropriate tests as specified in Part A, section 5.2, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (18) and in the revised Sterility Test for Mycoplasmas (General Requirements for the Sterility of Biological Substances, amendment 1995) (19). Any single virus harvest in which contamination is detected shall be discarded.

A.4.2.3 Virus content

A sample removed from each single harvest shall be tested for virus content by intracerebral inoculation of mice or by plaque assay.

Experience suggests that a virus content of not less than $10^{7.0}$ LD₅₀/ml (median lethal dose for mice per ml) results in satisfactory vaccine.

If a plaque assay is used, a laboratory shall establish a correlation between the plaque assay and the mouse-LD₅₀ assay.

A.4.2.4 Inactivation of virus

The virus in each single harvest shall be inactivated by a validated method. The kinetics of inactivation shall be suitably monitored and demonstrated by the manufacturer to be consistently effective. Each harvest shall be processed to remove virus aggregates immediately before inactivation.

The method and agents used for inactivation shall be approved by the national control authority.

In some countries, the virus is inactivated by the addition of formaldehyde to a final concentration of 0.05% followed by continuous stirring for 5 days at a temperature of 22°C.

A.4.2.5 Test for effective inactivation

Each single harvest shall be tested for virus inactivation. The test shall be performed prior to pooling with a sample of undiluted virus suspension by a method approved by the national control authority.

The test sample should correspond to at least 20 human doses of the final bulk. The total volume of the test sample should be inoculated into a primary chicken fibroblast cell culture, or a culture of any other cells with no less susceptibility to the virus than chicken embryo cells, and incubated at a temperature of $37 \pm 1^\circ\text{C}$ for a period of 14 days. A cell culture sheet of

not less than 3cm² should be used for each 1 ml of the test material. No cytopathic change should be detected at the end of the incubation period.

At the completion of the observation period, the culture fluid should be collected and inoculated intracerebrally at a dose of 0.03 ml into at least 10 mice of about 4 weeks of age. The animals should be observed for 14 days. The single harvest passes the test if the product has been shown to be free from residual live virus.

Other methods to assess inactivation may be approved by the national control authority.

A.4.3 Preparation and control of purified bulk material

A.4.3.1 Pooling of inactivated single harvests

Only harvests satisfying the requirements of section A.4.2 shall be pooled.

In some countries the pooled material is retested for inactivation of virus content (see section A.4.2.4). If so, the test should be approved by the national control authority.

A.4.3.2 Purification procedures

Each pool of inactivated virus shall be purified by suitable methods approved by the national control authority.

The inactivated TBE virus can be purified and concentrated by continuous-flow zonal ultracentrifugation or by ultrafiltration.

Each manufacturer shall demonstrate, by testing of each lot, or by validation of the purification process, that all residual contaminants are consistently reduced to a level acceptable to the national control authority.

The protein content per human dose and the minimum purity shall be approved by the national control authority. The upper limit for residual DNA for vaccine produced in continuous cells is 10ng/dose (17).

Serum albumin is an appropriate marker substance which can be monitored, though other components may be more appropriate depending on the specific manufacturing process employed.

A.4.3.3 Potency test

A potency test shall be carried out on each batch of purified bulk material. The method used and specification set shall be approved by the national control authority.

A potency test in mice may be used for this purpose. Groups of 10 mice are vaccinated subcutaneously with 0.2ml of serial dilutions of TBE vaccine containing 0.2% aluminium hydroxide.

Animals are revaccinated 14 or 21 days later. They are challenged 7 or 14 days following revaccination by intraperitoneal inoculation of 100–1000 mouse LD₅₀ of a suitable TBE virus strain. The median mouse protective dose is calculated after observation for 20 days. In some countries different immunization schedules are used.

A.4.3.4 Test for avian leukosis virus

If production is based on chicken fibroblast cells obtained from chicken flocks which are not demonstrated to be free from avian leukosis virus, each batch of bulk material shall be tested for infectious avian leukosis virus by a method approved by the national control authority.

A.4.4 Preparation and control of the final bulk

A.4.4.1 Preservatives and other added substances

In the preparation of the final bulk only preservatives or other substances approved by the national control authority shall be added. Such substances shall have been shown by appropriate tests not to impair the safety or effectiveness of the product in the amounts used.

No antibiotics shall be added to TBE vaccine intended for human use after the virus has been harvested.

A.4.4.2 Addition of adjuvant

If the final bulk contains adjuvant, the adjuvant and its concentration shall be approved by the national control authority.

If aluminium compounds are used, the concentration of aluminium shall not exceed 1.25 mg per single human dose.

A.4.4.3 Test for completeness of adsorption to adjuvant

If an aluminium compound is used as adjuvant, tests shall be carried out to confirm that TBE antigen is adsorbed to the adjuvant. The tests and acceptance limits shall be approved by the national control authority. Consistency of adsorption is important, and the degree of adsorption of production lots shall be within the specifications of lots shown to be clinically effective.

A.4.4.4 Sterility tests

Each final bulk shall be tested for bacterial and fungal contamination as specified in Part A, section 5.2, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (18) or by a method approved by the national control authority.

A.4.4.5 Potency test

A potency test may be performed on the formulated final bulk instead of on the purified bulk material (section A.4.3.3), if approved by the national control authority. The acceptance limits for potency of the vaccine and the assay method used shall be based on evidence submit-

ted to prove efficacy in clinical trials and shall be approved by the national control authority.

The test shall be performed in parallel with a test on a local reference vaccine. The challenge strain, the reference vaccine, and the test procedure used shall be approved by the national control authority.

A potency test in mice may be used for this purpose. Groups of 10 mice are vaccinated subcutaneously with 0.2ml of serial dilutions of TBE vaccine containing 0.2% aluminium hydroxide.

Animals are revaccinated 14 or 21 days later. They are challenged 7 or 14 days following revaccination by intraperitoneal inoculation of 100–1000 mouse LD₅₀ of a suitable TBE virus strain. The median mouse protective dose is calculated after observation for 20 days. In some countries different immunization schedules are used.

A.4.4.6 Tests for chemicals used in production

The concentration in the final vaccine of any organic solvent or inactivating agent added during production shall be determined by methods approved by the national control authority. The concentration shall not exceed the upper limits specified by the national control authority.

If formaldehyde has been used for inactivation, the concentration in the final bulk shall be no greater than 0.02%. The test method used shall be approved by the national control authority.

A.5 Filling and containers

The requirements concerning filling and containers given in Good Manufacturing Practices for Biological Products (16) shall apply.

A.6 Control tests on final lot

The national control authority may permit tests for pyrogenic substances, protein content, preservatives and adjuvants to be performed on the final bulk rather than on the final lot.

A.6.1 Identity

An identity test shall be performed by an appropriate method to identify the virus in at least one labelled container from each final lot.

The potency test described in section A.6.2 may serve as an identity test.

A.6.2 Potency

A potency test on the vaccine in the final containers shall be performed if it has not already been performed on the formulated final bulk (see section A.4.4.5).

A.6.3 Sterility

Each final lot shall be tested for sterility as specified in Part A, sections 5.1 and 5.2, of the revised General Requirements for the Sterility of Biological Substances (18).

A.6.4 General safety

Each final lot shall be tested for abnormal toxicity. The test procedures shall be approved by the national control authority.

A.6.5 Pyrogenic substances

Each final lot shall be tested for pyrogenic substances. The test shall be approved by the national control authority.

A.6.6 Preservatives

Each final lot shall be tested for the presence of preservatives, if used. The test used and the permitted concentrations shall be approved by the national control authority.

A.6.7 Protein content

Limits shall be defined for the protein content of the finished product. Each final lot shall be tested for protein content by a method approved by the national control authority.

A.6.8 Adjuvant content

Each final lot shall be assayed for the content of adjuvant. The method used and the permitted concentration shall be approved by the national control authority.

The formulation shall be such that a homogeneous suspension is administered to the recipient.

A.6.9 Residual moisture in freeze-dried vaccine

The residual moisture in a representative sample of each freeze-dried lot shall be determined by a method approved by the national control authority.

Moisture levels of less than 3% are usually considered satisfactory.

A.6.10 Inspection of final containers

Every container in each final lot shall be inspected, and those showing any abnormalities shall be discarded.

A.7 Records

The requirements in section 8 of Good Manufacturing Practices for Biological Products (16) shall apply.

A.8 **Samples**

The requirements in section 9 of Good Manufacturing Practices for Biological Products (16) shall apply.

A.9 **Labelling**

The requirements in section 7 of Good Manufacturing Practices for Biological Products (16) shall apply, with the addition of the following:

The leaflet accompanying the package shall:

- specify the cell substrate(s) used for production;
- state the name of the virus strain used;
- state the method used for inactivating the virus;
- specify the nature and amount of adjuvant and preservative present, if used;
- if the vaccine is in freeze-dried form, state that, after its reconstitution, it shall be used immediately (unless data have been provided to the licensing authority that it may be stored for a limited time without loss of potency).

A.10 **Distribution and shipping**

The requirements in section 8 of Good Manufacturing Practices for Biological Products (16) shall apply.

A.11 **Storage and expiry date**

The requirements in section 8 of Good Manufacturing Practices for Biological Products (16) shall apply.

A.11.1 ***Storage conditions***

Inactivated TBE vaccine in liquid form shall be stored at all times at a temperature of 2–8°C, without freezing.

Inactivated TBE vaccine in freeze-dried form shall be stored below 10°C.

Alternative storage temperatures must be justified to and approved by the national control authority.

A.11.2 ***Expiry date***

The expiry date shall be based on data submitted by the manufacturer. Adequate stability studies form an essential part of vaccine development studies. The stability of the vaccine in its final form, stored at the recommended storage temperatures, shall be demonstrated to the satisfaction of the national control authority

with final containers from at least three consecutive lots of final product.

The desorption of antigen from aluminium-based adjuvants, if used, may take place over time and shall be demonstrated to be within limits agreed by the national control authority.

Accelerated stability studies may provide additional supporting evidence of the stability of the product but cannot replace real-time studies.

Part B. National control requirements

B.1 General

The general requirements for control laboratories contained in the Guidelines for National Authorities on Quality Assurance for Biological Products (23) shall apply, with the following additions:

- The national control authority shall approve the virus strain and the cell substrate used in vaccine production, and shall specify potency requirements.
- The national control authority shall be satisfied that the results of all tests, including those done on pools during the manufacturing process, are satisfactory and that consistency has been established.

B.2 Release and certification

A vaccine lot shall be released only if it fulfils the national requirements and/or part A of these requirements.

A certificate signed by the responsible official of the national control authority shall be provided at the request of the manufacturing establishment and shall certify whether the lot of vaccine in question meets all national requirements and/or Part A of the present requirements. The certificate shall state the date of the last satisfactory potency test by the manufacturer and the final lot number, which must also appear on the labels of the containers.

The purpose of the certificate is to facilitate the exchange of TBE vaccine between countries. An example of a suitable certificate is given in the forty-sixth report of the Expert Committee on Biological Standardization (14).

Authors

The first draft of these requirements was prepared by Dr V. Grachev, Scientist, Biologicals, and Dr E. Griffiths, Chief, Biologicals, World Health Organization, Geneva, Switzerland.

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