World Health Organization Technical Report Series, No. 771, 1988

Annex 6

REQUIREMENTS FOR JAPANESE ENCEPHALITIS VACCINE (INACTIVATED) FOR HUMAN USE

(Requirements for Biological Substances No. 43)

GENERAL CONSIDERATIONS

Specific vaccines are the most commonly used means for the control of Japanese encephalitis. The causative virus is amplified in nature in a cycle involving *Culex* mosquitos and vertebrate animals,

especially pigs. Humans are susceptible at all ages unless immunized by natural infection or vaccination. Evidence shows that effective vaccines will protect animals and humans against illness and will remove the vaccinated animal from the pool of potential amplifying hosts of the virus. Although the control of mosquitos and the vaccination of pigs are effective in some circumstances in preventing Japanese encephalitis in humans, these measures have not proved effective in practice over much of the endemic area. It is also important to recognize that the infection is a zoonosis, that humans are incidental hosts, and that for protection, vaccine coverage must be maintained indefinitely in all persons exposed to the infection.

Two types of formalin-inactivated vaccines have each been used in millions of people. A vaccine produced in adult mouse brain is purified to remove myelin basic protein and is not associated with central nervous system damage in recipients. A vaccine produced in primary hamster tissue culture is also widely used.

Large outbreaks of Japanese encephalitis, sometimes involving thousands of cases, continue to occur in the classic areas of endemicity. In addition, certain special groups, such as travellers to endemic areas and laboratory workers, require immunization. In view of the need to immunize large numbers of people in such circumstances, requirements for Japanese encephalitis vaccine for human use have been formulated. In drafting them, account has been taken of the opinions of consultants, of the regulations and requirements for the manufacture and control of Japanese encephalitis vaccine that have been formulated in several countries, and of information from both published and unpublished sources.

Each of the following sections constitutes a recommendation. The parts of each section that are printed in large type have been written in the form of requirements, so that, if a health administration so desires, these parts as they appear may be included in definitive national requirements. The parts of each section that are printed in small type are comments and recommendations for guidance.

Should individual countries wish to adopt these requirements as the basis of their national regulations concerning Japanese encephalitis vaccine, it is recommended that a clause should be included that would permit modifications of manufacturing requirements on the condition that it can be demonstrated to the satisfaction of the national control authority that such modified requirements ensure that the degree of safety and the potency of the vaccine are at least equal to those provided by the requirements

formulated below. The World Health Organization should then be informed of the action taken.

The terms "national control authority" and "national control laboratory", as used in these requirements, always refer to the country in which the vaccine is manufactured.

PART A. MANUFACTURING REQUIREMENTS

1. Definitions

1.1 International name and proper name

The international name shall be *Vaccinum encephalitidis japonicae*. 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.

1.2 Descriptive definition

Vaccinum encephalitidis japonicae is a fluid or freeze-dried preparation of virus grown in neural tissue of mice or in cell cultures and inactivated by a suitable method. The preparations for human use shall satisfy all the requirements formulated below.

1.3 International standards and reference reagents

Preparations are currently under study.

1.4 Terminology

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

Master virus seed lot: a quantity of virus of uniform composition, processed at one time, and distributed into a number of containers. Seed lots are derived from a seed virus used in the preparation of inactivated vaccines shown to be immunogenic in man, and not more passages removed from it than a number approved by the national control authority. The master virus seed lot is used for the preparation of working virus seed lots.

Working virus seed lot: a quantity of virus suspension that has been processed together, is of uniform composition, and is not more passages removed from the master virus seed lot than a number approved by the national control authority. Material is drawn from working virus seed lots for inoculating cell cultures or mouse neural tissue for the production of vaccine.

Adventitious agents: contaminating microorganisms, including bacteria, fungi, mycoplasmas, and endogenous and exogenous viruses.

Single harvest: a virus suspension derived from one cell substrate lot, all the cultures having been inoculated at the same time with the same inoculum and harvested at the same time.

Bulk material: a pool of inactivated single harvests before preparation of the final bulk. It may be prepared from one single harvest or a number of single harvests and may yield one or more final bulks.

Final bulk: the finished biological material prepared from one or more purified bulks present in the container from which the final containers are filled.

Final lot: a collection of sealed final containers, filled from the same final bulk, that are homogeneous with respect to the risk of contamination during filling or drying. A final lot must therefore consist of containers that have been filled in one working session and (for lyophilized products) have been freeze-dried together in the same chamber at the same time.

2. General Manufacturing Requirements

The general requirements for manufacturing establishments contained in the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (*1*) shall apply to establishments manufacturing Japanese encephalitis vaccine for human use, with the addition of the following directives.

The production of Japanese encephalitis vaccine shall be conducted by staff who have not handled other infectious microorganisms, animals, or tissue cultures in the same working day. The staff shall consist of persons who shall be examined medically and found to be healthy. Steps shall be taken to ensure that all such persons in the production and control areas have a serum

neutralizing antibody titre of at least 1:10 from either immunization against or natural infection with Japanese encephalitis.

Only mouse brain tissue and cell cultures approved by the national control authority for the production of Japanese encephalitis vaccine shall be introduced into or handled in the production area.

Persons not directly concerned with the production processes, other than official inspectors, shall not be permitted to enter the production area without valid reason and specific authorization.

Particular attention shall be given to the recommendations contained in Part A, section 1, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (*1*, p. 13) regarding the training and experience of the persons in charge of production and testing and of those assigned to various areas of responsibility in the manufacturing establishment, as well as to the registration of such personnel with the national control authority.

3. Control of Source Materials

The general production precautions formulated in Part A, section 3, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (*1*, p. 15) shall apply to the manufacture of Japanese encephalitis vaccine.

3.1 Animals and cell cultures used for production

3.1.1 *Hamsters*

When hamster-kidney tissue is used for the propagation of Japanese encephalitis virus, only hamster stock approved by the national control authority shall be used as a source of tissue.

> The animal stock should be free from infection with mycoplasmas and from microorganisms pathogenic for hamsters. The parents of animals to be used as a source of tissue should be maintained in quarantine in vermin-proof quarters for a minimum of 3 months. Neither the parent hamsters nor their progeny should previously have been used for experimental purposes involving infectious agents.

3.1.2 *Mice*

When mice are used for the propagation of Japanese encephalitis virus in neural tissue, only animals less than 5 weeks of age shall be used, and they shall be free from all signs of disease. Methods for intracerebral inoculation and harvesting shall be approved by the national control authority.

The animal stock should be free from microorganisms pathogenic for mice.

3.1.3 *Serum used in cell culture medium*

Serum used for the growth of cells shall be tested to demonstrate freedom from bacteria, fungi, and mycoplasmas according to the requirements in Part A, sections 5.2 and 5.3, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (*2*, pp. 49–52), as well as freedom from pathogens, such as viruses of the species of origin of the serum, by methods approved by the national control authority (*3*, p. 99).

In some countries sera are examined for freedom from phage and endotoxin.

3.1.4 *Human albumin used in cell culture medium*

If human albumin is used, it shall meet the requirements in Parts C and D of the Requirements for Biological Substances No. 27 (Requirements for the Collection, Processing and Quality Control of Human Blood and Blood Products) (*4*).

3.2 Virus seed

3.2.1 *Strain of virus*

The strains of virus used in the production of all seed lots shall be approved by the national control authority and shall yield safe and immunogenic vaccines when the virus has been inactivated. They shall be identified by historical records and by infectivity tests, serological tests, and animal inoculation.

3.2.2 *Virus seed lot system*

The preparation of Japanese encephalitis vaccine shall be based on the use of a virus seed lot system. The national control authority shall determine the acceptable number of passages from the master virus seed lot to produce working virus seed lots. If mice are used for the passages, suckling mice are preferred. Vaccines shall be made from a working virus seed lot without further intervening passage. Virus seed lots shall be maintained either in the dried or in the frozen form. The dried seed shall be kept at a temperature below 10 °C, while the frozen seed shall be kept at a temperature below −60 °C.

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.

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

3.2.3 *Tests on virus seed lots*

Each virus seed lot shall be identified as Japanese encephalitis virus by methods approved by the national control authority.

3.2.3.1 *Freedom from bacteria, fungi and mycoplasmas*

Each virus seed lot shall be tested for bacterial, mycotic, and mycoplasmal contamination by appropriate tests according to the requirements in Part A, sections 5.2 and 5.3, of the revised Requirements for Biological Substances No. 6 (General Requirements for Sterility of Biological Substances) (*2*, pp. 49–52).

3.2.3.2 *Tests for adventitious agents*

Each virus seed lot shall be tested for adventitious agents. For these tests the virus shall be neutralized by a specific anti-Japanese-encephalitis serum.

The individual tests on the virus seed lots should be so designed that they satisfy the requirements of the national control authority. The anti-Japanese-encephalitis serum should be free from known adventitious viruses.

3.2.3.3 *Tests on working virus seed lots*

Each time a new working virus seed lot is prepared, tests shall be carried out to characterize the virus strain. Such tests shall include

the titration of virus and an identity test using standard serum provided by the national control authority.

When an international standard becomes available, the national standard serum should be calibrated against it.

4. Control of Vaccine Production

4.1 Mouse brains

The brains of the mice inoculated intracerebrally with the virus strain for production shall be harvested immediately before death when the animals show typical signs of encephalitis. The harvested brains shall be triturated in buffered isotonic sodium chloride solution, or any other suitable medium, and centrifuged. The supernatant shall be collected and treated by alcohol precipitation, with protamine sulfate, by ultracentrifugation, or by any other appropriate methods to serve as the virus suspension.

The virus suspension shall be subjected to the tests given in Part A. sections 4.3.1 and 4.3.2, of these Requirements.

4.2 Cell cultures

Beta-lactam antibiotics shall not be used at any stage of manufacture.

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

At least 5% of the cell suspension (not less than 500 ml) at the concentration employed for seeding vaccine production cultures shall be used to prepare control cultures.

In some countries in which the technology of large-scale production has been developed the national control authority should determine the size of the cell sample to be examined and the control methods to be applied.

The treatment of cells set aside as control material shall be similar to that of the production cell cultures, but they shall remain uninoculated to serve as control cultures for the detection of extraneous viruses.

These control cell cultures shall be incubated under the same conditions as the inoculated cultures for at least 2 weeks or until the

time of the last harvest of the production cultures and shall be examined during this period for evidence of cytopathic changes. For the test to be valid, not more than 20% of the control cell cultures shall have had to be discarded for nonspecific, accidental reasons.

At the end of the observation period, the control cell cultures shall be examined for degeneration caused by an infectious agent. If this examination, or any of the tests specified in this section, shows evidence of the presence in a control culture of any adventitious agent, the Japanese encephalitis virus grown in the corresponding inoculated cultures shall not be used for vaccine production.

4.3 Control of single virus harvests

4.3.1 *Sterilitiy test of single virus harvests*

A sample removed from each virus harvest shall be tested for bacterial and fungal contamination by appropriate tests according to the requirements in Part A, section 5.2, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (*2*, p. 49). Any single virus harvest in which contamination is detected shall be discarded.

4.3.2 *Test of virus content*

A sample removed from each virus harvest shall be tested for virus content using the method of intracerebral inoculation of mice. The animals shall be observed for 14 days and the LD_{50} then calculated.

4.3.2.1 The virus content shall be not less than $10^{7.5}$ LD_{50}/0.03 ml when neural tissue is used.

4.3.2.2 The virus content shall be not less than $10^{7.0}$ LD_{50}/0.03 ml when cell culture is used.

4.4 Control of bulk material

4.4.1 *Pooling of single virus harvests*

Only virus harvests satisfying the requirements for sterility and virus content in Part A, sections 4.3.1 and 4.3.2, of these Requirements shall be pooled.

4.4.2 *Animal serum*

For cell-culture-derived vaccines, the serum concentration in the bulk vaccine shall not be more than 1 part per million (1 µl/litre).

In some countries, control tests are carried out to detect the residual animal serum content in the final vaccine.

4.4.3 *Inactivation of virus*

The process for the inactivation of the Japanese encephalitis virus and any adventitious agents shall be approved by the national control authority.

4.4.3.1 *Treatment before inactivation*

When cell cultures are used the bulk material shall be filtered or clarified by continuous centrifugation prior to inactivation.

The importance of filtration or clarification using continuous centrifugation of the crude virus suspensions as a means of improving the regularity of the inactivation process has been clearly established. Generally, filters are used in series or filtration is performed step-wise through filters of decreasing porosity. Satisfactory results have been reported with several filter types, but a final filtration using a 0.22 µm filter should be used.

4.4.3.2 *Method and agents*

The method and agents used for inactivation shall be approved by the national control authority. The method used shall be demonstrated to be consistently effective in the hands of the manufacturer. Inactivation should be commenced immediately after the preparation and sampling of single virus harvests when neural tissue is used, or immediately after filtration when cell cultures are used.

One method that has been successfully used to inactivate Japanese encephalitis virus is the treatment of the virus harvest with formalin at a final concentration of 1:2000 for 50–60 days at 4 °C.

The suspension containing inactivated virus shall serve as the bulk material when neural tissue is used, and as the final bulk when cell cultures are used.

4.4.3.3 *Test for effective inactivation*

Each bulk suspension shall be tested for inactivation of virus. The test shall be approved by the national control authority. The test

shall be performed with the undiluted bulk suspension. A test sample corresponding to no less than 25 human doses of the final bulk shall be used.

> In one country the test sample is dialysed at about 5 °C for not less than 24 hours against a sufficient volume of buffered isotonic sodium chloride solution—and diluted if necessary—to remove any cytopathogenic effect due to the residual inactivating agent or other substances.

The total volume of the test sample shall be inoculated into the primary culture of hamster-kidney cells, or any other cell cultures with no less susceptibility to the virus than hamster-kidney cells, and incubated at 35 ± 1 °C for a period of 14 days. A cell culture sheet not less than 3 cm^2 shall be used for 1 ml of the test material. During the incubation period, no cytopathic change shall be detected.

At the completion of the observation, the cultured fluid shall 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 shall be observed for 14 days. The bulk passes the test if the product has been shown to be free from residual live virus.

4.4.4 *Purification of inactivated virus suspension*

4.4.4.1 The bulk suspension derived from mouse brains (see part A, section 4.1, of these Requirements) shall be purified by a process that has been approved by the national control authority and has been shown to give consistent results.

> The purification process should be designed to reduce the myelin content to the lowest possible level.

4.4.4.2 The bulk suspension derived from cell culture shall be purified and concentrated by a process approved by the national control authority.

4.4.5 *Potency test of bulk suspension*

The test for potency shall be made on each bulk suspension by detecting the neutralizing antibody produced in immunized mice. The method used shall be approved by the national control authority.

4.5 Preparation and control of final bulk

4.5.1 *Preservatives and other substances added*

In the preparation of the final bulk only the 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.

If formalin has been used for inactivation, the procedure shall be such that the amount of formaldehyde in the final bulk is no greater than 0.01%. The test method used shall be approved by the national control authority.

No antibiotics shall be added to Japanese encephalitis vaccine for human use after virus harvest.

4.5.2 *Sterility tests*

Each final bulk shall be tested for sterility according to the requirements in Part A, section 5.2, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (*2*, p. 49).

5. Filling and Containers

The requirements concerning filling and containers in Part A, section 4, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (*1*, p. 16) shall apply, with the addition of the following directive.

Containers of dried vaccine shall be hermetically sealed under vacuum or after filling with pure, dry, oxygen-free nitrogen or any other gas not deleterious to the vaccine. All containers sealed under vacuum shall be tested for leaks and all defective containers shall be discarded.

6. Control Tests on Final Product

6.1 Identity test

An identity test shall be performed on at least one labelled container from each final lot by an appropriate method.

The test for potency, as described in Part A, section 6.5, of these Requirements may serve as an identity test.

6.2 Sterility tests

Each final lot shall be tested for bacterial and mycotic sterility according to the requirements in Part A, section 5.2, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (*2*, p. 49).

6.3 Innocuity tests

Each final lot shall be tested for abnormal toxicity by appropriate tests in mice and guinea-pigs, using parenteral injections. The test procedures shall be approved by the national control authority.

6.4 Protein content

6.4.1 The protein content shall be no greater than 80 µg/ml when neural tissue is used.

6.4.2 The protein content shall be no higher than 200 µg/ml when human albumin is added in cell culture.

6.5 Potency test

The potency test shall be determined by titration of the neutralizing antibody produced in immunized mice by the plaque-reduction method, using primary chick-embryo cells or BHK-21 cells. The test shall be made in parallel with a reference vaccine. The challenge strain and reference vaccine, as well as the test procedure used, shall be approved by the national control authority (see Part B, section 1, of these Requirements).

The test vaccine and the reference vaccine are diluted appropriately and each dilution is injected intraperitoneally in 2 doses of 0.5 ml each at 7-day intervals into at least 10 mice of 4 weeks of age. Seven days following the second injection, each animal is bled. The separated serum is pooled at each dilution of vaccine and then inactivated at 56 °C for 30 min; it may then be stored at −20 °C.

The serum is appropriately diluted and mixed with an equal volume of challenge virus, containing about 200 PFU/0.4 ml. The mixture is kept at 37 °C for 90 min for neutralization.

The virus suspension is then diluted and inoculated on to chick-embryo or BHK-21 cells. The infected cells are overlaid with 1% agar or methyl cellulose.

After incubation for an appropriate time, the cells are stained and the number of plaques formed on the cultures counted to obtain the plaque-reduction rates for the test and the reference vaccines. From the rates, the neutralizing antibody titres are calculated for each group.

The mean number of plaques of the control should be 50–150 per dish.

The potency of the test sample should be no less than that of the reference vaccine upon statistical comparison of the results.

6.6 Stability test

The method of production of vaccine shall be such that stable vaccine is produced. The test used shall be approved by the national control authority.

In some countries stability is ascertained by testing samples throughout the shelf-life of the vaccine.

The test for potency of liquid vaccine made after the storage of samples for 1 week at 37 °C is suitable. The test for potency of freeze-dried vaccine is made after the storage of samples for 4 weeks at 37 °C. In order to pass the test the lot should retain minimum potency, as defined in Part A, section 6.5, of these Requirements.

In some countries each lot of vaccine must be subjected to the stability test; in others the test is required only for the initial licensing lots to show consistency of production.

6.7 Residual moisture tests on 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. The upper limit for the moisture content shall be specified by the national control authority.

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

6.8 Inspection of final containers

Each container in each final lot shall be inspected, and any that show any abnormality shall be discarded.

6.9 Test for pyrogenic substances

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

6.10 Animal serum

The serum concentration in the final product shall be not more than 1 part per million (ppm).

6.11 Inactivation of virus

In some countries a test for virus inactivation is carried out by inoculating 10 mice intracerebrally with 0.03 ml of the final product.

7. Records

The requirements in Part A, section 6, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (*1*, p. 17) shall apply.

8. Samples

The requirements in Part A, section 7, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (*1*, p. 18) shall apply.

9. Labelling

The requirements in Part A, section 8, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (*1*, p. 18) shall apply, with the addition of the following directive.

The leaflet accompanying the package shall include the following information.

(*a*) whether the vaccine was prepared by an *in vivo* or an *in vitro* method;

(*b*) the method used for inactivating the virus; and

(*c*) if the vaccine is in freeze-dried form, a statement that, after its reconstitution, it shall be used immediately unless data are provided to show that it may be stored for a limited time without loss of potency.

10. Distribution and Shipping

The requirements in Part A, section 9, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (*1*, p. 18) shall apply.

11. Storage and Expiry Date

The requirements in Part A, section 10, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (*1*, p. 19) shall apply.

11.1 Storage conditions

Japanese encephalitis vaccine shall be stored at a temperature of 5 ± 3 °C avoiding freezing if in liquid form, and at less than 10 °C if in lyophilized form.

11.2 Expiry date

The expiry date shall be based on data submitted by the manufacturer and shall be determined by the national control authority.

PART B. NATIONAL CONTROL REQUIREMENTS

1. General

The general requirements for control laboratories in Part B of the revised Requirements for Biological Substances No. 1 (General

Requirements for Manufacturing Establishments and Control Laboratories) (*1*, p. 19) shall apply.

The national control authority shall aprove the strain of Japanese encephalitis virus used in the production of vaccine.

The national control authority shall provide or approve the strain for challenge and the reference vaccine for use in the potency test (see Part A, section 6.5, of the present Requirements).

2. Release and Certification

A vaccine lot shall be released only if it fulfils Part A of these Requirements.

A statement signed by the appropriate official of the national control laboratory shall be provided at the request of the manufacturing establishment and shall certify whether or not the lot of vaccine in question meets all national requirements as well as Part A of these Requirements. The certificate shall further state the date of the last satisfactory potency test, the lot number, the number under which the lot was released, and the number appearing on the labels of the containers. In addition, a copy of the official national release document shall be attached.

The purpose of the certificate is to facilitate the exchange of Japanese encephalitis vaccine between countries.

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The second draft of these Requirements was formulated in March 1987 by the following WHO staff, taking into consideration the first draft and the need for

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ACKNOWLEDGEMENTS

Acknowledgements are due to the following experts for their comments and advice on the second draft and for supplying additional data relevant to these Requirements: Dr M. Abe, Deputy Director, Biologics and Antibiotics Division, Ministry of Health and Welfare, Tokyo, Japan; Dr Ao Jian, Chief, First Division for Viral Vaccines, National Institute for the Control of Pharmaceutical and Biological Products, Ministry of Public Health, Beijing, China; Dr J. Blok, Queensland Institute of Medical Research, Brisbane, Queensland, Australia; Dr S. Huilan, Health Laboratory Technology, WHO Regional Office for the Western Pacific, Manila, Philippines; Dr Li He-min, Honorary Director, National Institute for the Control of Pharmaceutical and Biological Products, Ministry of Public Health, Beijing, China; Dr A. Oya, Director, Department of Virology and Rickettsiology, National Institute of Health, Tokyo, Japan; Dr K. Pavri, Director, National Institute of Virology, Pune, India.

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Appendix 1

SUMMARY PROTOCOL FOR PRODUCTION AND TESTING OF JAPANESE ENCEPHALITIS VACCINE (INACTIVATED) FOR HUMAN USE

Identification of Final Lot

Name and address of manufacturer _____

_____

_____

Lot number of vaccine _____

Date of manufacture of final lot _____

Expiry date _____

Total volume of final lot _____

Control of Source Materials

Serum for cell cultures

Origin of serum used _____

Tests performed on serum _____

Results _____

Virus seed

Strain of virus

Name and short description of history _____

_____

Date of preparation of master virus seed lot _____

Date of preparation of working virus seed lot _____

Number of passages between master and working virus seed lots _____

Number of subcultures between working seed lot and production _____

Tests for identification of the virus seed lot

Method used _____

Results _____

Tests for freedom from bacteria, fungi, and mycoplasmas

Method used _____

Results _____

Tests for adventitious agents

Tests in animals:

Methods used _____

_____

Results _____

Tests in cell cultures:

Methods used _____

_____

Results _____

Control of Vaccine Production

Control of single virus harvests

Sterility test

Method _____

Results _____

Test of virus content

Method _____

Results _____

Bulk material

Pooling of single virus harvests

Number of single harvests pooled _____

Volume of bulk material _____

Animal serum test

Method _____

Result _____

Inactivation

Agent and concentration _____

Temperature _____

Date of start of inactivation _____

Date of completion of inactivation _____

Test for effective inactivation

Method _____

Results _____

Purification of virus

Method of purification _____

Concentration _____

Potency test of bulk suspension

Method _____

Results _____

Final bulk

Preservatives and other substances _____

Concentrations _____

Sterility test

Method _____

Results _____

Final product

Potency test

Method _____

Results _____

Identity test

Method _____

Results _____

Sterility test

Method _____

Results _____

Innocuity test

Number of animals _____

Route of injection _____

Volume of injection _____

Date of injection _____

Date of end of test _____

Results _____

Protein content

Method _____

Results _____

Stability test

Method _____

Results _____

Pyrogenicity test

Method _____

Results _____

Residual moisture test (for freeze-dried vaccine)

Method _____

Results _____

Content of inactivating agent

Method _____

Results _____

Content of preservatives _____

Inactivation of virus

Method _____

Results _____

Certification

Name (typed) and signature of head of laboratory _____

Date _____

Certification by person taking overall responsibility for production and control of the vaccine:

I certify that lot No. ..... of Japanese encephalitis vaccine (inactivated) meets the requirements of Part A of the WHO Requirements for Biological Substances No. 43 (Requirements for Japanese Encephalitis Vaccine (Inactivated) for Human Use).

Name (typed) _____

Signature _____

Date _____

Appendix 2

GENERAL SCHEME FOR THE PREPARATION OF JAPANESE ENCEPHALITIS VACCINES

Stage	Procedures	Tests
Single harvest (1 production run)		
Cell culture vaccine	—	Virus content
Mouse brain vaccine	Inactivation	Sterility
Bulk material (1 or more pooled harvests)		
Cell culture vaccine	Filtration or continuous centrifugation Inactivation Purification	Inactivation Potency
Mouse brain vaccine	Purification	
Final bulk (1 or more pooled purified bulks)	Addition of preservatives and stabilizers	Sterility
Final product	Filling	Potency Identity Sterility Innocuity Protein content Stability Pyrogenicity Residual moisture Content of inactivating agent Content of preservative Inactivation of virus Animal serum Inspection of final containers