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PROPOSED REVISION
RECOMMENDATIONS FOR JAPANESE ENCEPHALITIS VACCINE (INACTIVATED) FOR HUMAN USE

Adopted by the 58th meeting of the WHO Expert Committee on Biological Standardization, October 2007. A definitive version of this document, which will differ from this version in editorial but not scientific details, will be published in the WHO Technical Report Series.

CONTENTS

Recommendations published by WHO are intended to be scientific and advisory in nature. The parts of each section printed in type of normal size have been written in such a form that, should a national regulatory authority desire, they may be adopted as they stand as definitive national requirements or used as the basis of such requirements. Those parts of each section printed in small type are comments and additional guidance. It is recommended that modifications be made only on condition that the modifications ensure that the vaccine is at least as safe and efficacious as that prepared in accordance with the recommendations set out below. In order to facilitate the international distribution of vaccine made in accordance with these recommendations, a summary protocol for the recording of results of the tests is given in Appendix.

Introduction

These Recommendations are intended to provide national regulatory authorities and vaccine manufacturers with background and guidance on the production, quality control and evaluation of the safety and efficacy of inactivated Japanese encephalitis (JE) vaccines in order to facilitate their international licensure and use.

Since the adoption of the World Health Organization (WHO) Requirements (now termed as Recommendations) for inactivated JE vaccines for human use in 1987 (***1***) by the WHO Expert Committee on Biological Standardization, alternative modes of production have been introduced that use continuous cell lines as a substrate for production instead of mouse brain.

The Committee at its 56th meeting in 2005 recommended that the guidance for inactivated JE vaccines be revised and that sections on nonclinical and clinical evaluation should be added. To facilitate this process, WHO convened two meetings (in Geneva, 1-2 June 2006 (***2***) and in Bangkok, 7-9 February 2007) at which scientific experts, regulatory professionals and other stakeholders met to develop a revised document for inactivated JE vaccines for human use.

The scope of the present Recommendations encompasses inactivated JE vaccines produced in mouse brain and in cell substrates (e.g. primary hamster kidney cells and a continuous Vero cell line).

This document sets out the recommendations for manufacture and quality assessment in Part A. Guidance specific to the nonclinical and clinical evaluation of inactivated Japanese encephalitis vaccines is provided in Parts B and C, respectively. Part D

provides recommendations for national regulatory agencies. This document should be read in conjunction with all relevant WHO guidelines including those on nonclinical (***3***) and clinical evaluation (***4***) of vaccines.

These Recommendations are based on experience gained from the inactivated JE vaccines that have been developed so far, as described below, and may need to be updated in accordance with any important future developments.

General considerations

JE virus belongs to the family *Flaviviridae* and is included in the genus *Flavivirus*. The flaviviruses are enveloped RNA viruses and include yellow fever and dengue viruses, among others, that are serologically related to JE virus. JE virus strains are grouped into 5 genotypes, based on the nucleotide sequence and deduced amino acid sequence of the part of genome, but there is only one known serotype.

The principal vectors of JE virus are mosquitoes of the genus *Culex*. In Asia transmission is mainly via the bites of *Culex tritaeniorhynchus*. In endemic regions, JE virus is maintained in nature between vector mosquitoes and vertebrate animals, especially pigs. Pigs are also considered to act as an amplifier for JE virus. Infected humans do not transmit virus to biting mosquitoes (i.e. humans are considered as dead-end hosts) because viraemia is transient with low level of virus concentrations.

Based on serological studies in endemic areas and medical histories JE virus infection may be asymptomatic in humans. It has been estimated that between 1 and 3 per 1,000 infected humans may have a clinically manifest illness that includes evidence of virus-induced inflammation in the cerebrum, cerebellum and spinal cord. The incubation period of JE is 5-15 days and the illness usually starts with fever and headache, with or without vomiting, diarrhoea and myalgia. If meningeal irritation occurs it becomes apparent on the second day and then other cerebral symptoms may develop rapidly, including altered consciousness, apathy or coma. The case fatality rate ranges from 5-30% but approximately 30-50% of the surviving patients have permanent neuropsychiatric sequelae and complete recovery occurs in only one-third of patients.

In public health terms, JE is the most important viral encephalitis encountered in South-East Asia and the Western Pacific countries where it is endemic or occurs in epidemics (***5***). During the last 25 years, incidence of JE has intensified in certain countries. Further, the disease has extended its geographical borders to previously unaffected areas of Asia and to northern Australia, where cases were reported in the Torres Strait in 1995 and in the York peninsula of the subcontinent in 1998. There is a year-round transmission in tropical countries but the transmission pattern in temperate and subtropical zones is seasonally defined.

Nearly 3 billion people (i.e. about 60% of the world's population) are believed to be at risk for JEV infection and approximately 20,000 clinical cases with 6,000 deaths are reported annually (***5***). However, implementation of a surveillance system specifically for JE is incomplete as the etiology of encephalitis is not differentiated in many Asian countries. In the countries where JE virus is hyper-endemic, most of the patients are children under 4 years of age and almost all are less than 10 years of age. However, in

some countries where routine childhood immunization has been implemented for many years, JE now occurs mainly in adults and especially in the elderly.

Vaccination of humans is the most effective means of preventing JE. There are 3 types of inactivated vaccines currently used in the world: i) mouse brain-derived, purified vaccine, which is based on either the Nakayama-NIH or Beijing-1 [P-1] strains; ii) primary hamster kidney (PHK) cell-derived, purified vaccine, based on the Beijing-3 [P-3] strain; and iii) Vero-cell derived purified vaccine based on the Beijing-3 [P-3] strain. Newer Vero cell-derived inactivated JE vaccines under development use either Beijing-1 or SA14-14-2 strains as virus seeds. All these strains belong to genotype 3.

A mouse brain-derived inactivated JE vaccine was first licensed in Japan in 1954. This type of vaccine is manufactured in a similar fashion and using the Nakayama-NIH strain in Japan (for export only) and also in South Korea, Taiwan, Thailand, India and Vietnam. Since 1989 the JE vaccine that is actually used in Japan has contained the Beijing-1 strain. The efficacy of mouse brain-derived, inactivated JE vaccines was evaluated in two field trials in endemic areas. A study in Taiwan in 1966 showed that efficacy of the Nakayama-NIH strain vaccine was 80% after two doses. A later study in Thailand demonstrated that the efficacy of each of the monovalent Nakayama-NIH vaccine and a Nakayama-NIH and Beijing-1 bivalent vaccine was 91% over two transmission seasons. The CDC, USA later pointed out that the level of protective efficacy observed in this study might in part reflect past exposure to JE virus and/or other flaviviruses. It was considered that the regimen of two doses given 7 days apart could not be assumed to give similar results in non-immune travellers. Therefore, the US license for the Nakayama-NIH strain vaccine (approved in the US in 1992) recommends a 3-dose schedule based on immunogenicity data from non-immune US soldiers administered 2 or 3 doses. The duration of protection in non-immunes after a 3-dose primary regimen remains unknown. Booster doses of the US-licensed Nakayama-NIH strain vaccine are recommended after 2 years although some studies undertaken in Japan indicate that protective antibody levels persist for at least 4 years.

There is considerable information available on the adverse events associated with use of mouse brain-derived inactivated JE vaccines. Local reactions at the injection sites and fevers each occur in approximately 10% of vaccinated children in Japan. Severe allergic reactions characterized by generalized urticaria, respiratory symptoms and cardiovascular symptoms have been reported. Severe neurological disorders including acute disseminated encephalomyelitis (ADEM) have been reported following vaccination with mouse brain-derived JE vaccine. Eighteen cases of ADEM were reported in Japan after vaccination with mouse brain-derived inactivated JE vaccines from 1996 to 2005, which corresponds to approximately 2 cases per year following about 3 million inoculations. It is, however, assumed that there are 60–120 cases of ADEM per year whatever the cause is in children in Japan (***2***).

Inactivated JE vaccine prepared from the Beijing-3 strain in primary hamster kidney (PHK) cells has been produced exclusively in China since 1968. Approximately 75 million doses were distributed annually in China up to 1988. Randomized field trials in China estimated that protection against JE was about 85% (***5***).

A Vero cell-derived inactivated JE vaccine using the Beijing-3 strain has been licensed in China since 1998 where approximately 10 million doses were distributed up to 2006 and a clinical trial showed that the seroconversion rate (based on measurement of neutralizing antibody to JEV) in school-age children was 92% (data presented at WHO informal consultation held in Geneva, 1-2 June 2006).

The mouse-brain derived, inactivated vaccine has been used successfully to reduce the incidence of JE in a number of countries and is likely to be used nationally and internationally for some more years. Because of the high benefit-to-risk ratio of routine vaccination, immunization against JE in public health programs should continue using available vaccines (***5***). Nevertheless, reduction of animals for production of vaccines, potential risks relating to residual neural substances in mouse brain-derived vaccines and technology advances in vaccine production are major driving forces to move away from the conventional mouse brain-derived vaccines towards cell culture-derived vaccines.

In addition, a PHK cell-derived, live attenuated vaccine based on the SA14-14-2 strain has been produced in China since 1988 where it has been reported that more than 100 million children have received the vaccine in a regimen of two doses given 1 year apart. This vaccine has been also licensed in South Korea, Nepal, India, and Sri Lanka.

Other JE vaccines in various stages of development include a chimeric live yellow fever-JE vaccine, DNA vaccines, poxvirus-based vaccines and virus-like particle vaccines. These products as well as any other live attenuated JE vaccines are outside the scope of these Recommendations.

The presence of neutralizing antibody provides the best evidence available that protective immunity has likely been established. Epitope mapping studies have indicated that there are at least 8 functional epitopes on JEV although not all of these elicit neutralizing antibody. There are several methodologies for determining functional antibody responses to the virus (see *C.2.1*). The neutralizing antibody assay methodology most often used is the plaque reduction neutralization test (PRNT). The cut-off for seroprotection is defined as a $PRNT_{50}$ of at least 1:10 based on studies in mice that led to a conclusion that a titre of at least 1:10 protected against challenge with a JE virus dose higher than the maximum titre estimated to be transmitted by a mosquito.

Mouse data have indicated that immune responses against the JE strain in a vaccine can result in cross-neutralizing antibody against different strains of JE virus (***6,7,8***). However, neutralizing antibody titers are usually higher against homologous virus strains than against the strains belonging to other genotypes. Recent studies with a candidate JE vaccine and with the US-licensed Nakayama-NIH strain vaccine showed differential results in neutralizing antibody and passive protection tests in mice according to the viral genotype used in the assays and for challenge (***9***). The degree of clinical cross-protection that might be afforded by vaccine strains against a range of wild-type viruses merits further investigation.

Part A. Manufacturing recommendations

A.1 Definitions

A.1.1 International name and proper name

The international name should be *Japanese encephalitis vaccine (inactivated) for human use.* The proper name should 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 recommendations formulated below.

A.1.2 Descriptive definition

Japanese encephalitis vaccine (inactivated) for human use is a liquid or freeze-dried preparation of virus grown in mouse brains or in cell cultures and inactivated by a suitable method. The preparations for human use should satisfy all the recommendations formulated below.

A.1.3 International standards and reference reagents

At the time that these Recommendations were prepared, no international reference standard preparations are available.

A.1.4 Terminology

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

Cell bank: A collection of ampoules containing aliquots of a suspension of cells from a single pool of cells of uniform composition, stored frozen under defined conditions (ideally in liquid nitrogen for mammalian cell lines).

Master cell bank (MCB): A quantity of fully characterized cells of human or animal origin stored frozen under defined conditions in aliquots of uniform composition derived from the cell seed, one or more of which may be used for the production of a manufacturer's working cell bank.

Working cell bank (WCB): A quantity of cells of uniform composition derived from one or more ampoules of the master cell bank, which may be used for the production cell culture. In normal practice, a cell bank is expanded by serial subculture up to passage number (or population doubling, as appropriate) selected by the manufacturer, at which point the cells are combined to give a single pool and preserved cryogenically to form the WCB. One or more of the cryotubes from such a pool may be used for the production of cell culture.

Production cell culture: A cell culture derived from one or more containers of the WCB used for the production of vaccines.

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 virus seed 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 regulatory 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 regulatory authority. Material is drawn from working virus seed lots for inoculating cell cultures or mouse brain for the production of vaccine.

Adventitious agents: Contaminating microorganisms of the virus, or cell substrate or materials used in their cultures, that may include bacteria, fungi, mycoplasmas, and endogenous and exogenous viruses that have been unintentionally introduced.

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.

Purified bulk: A pool of inactivated/purified 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.

Adjuvant: A vaccine adjuvant is a component that potentiates the immune response to an antigen and/or modulates it towards the desired immune responses

Final bulk: The formulated bulk present in the container from which the final containers are filled. The final bulk may be prepared from one or more purified bulks, which may or may not be adsorbed on an aluminium containing adjuvant.

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 should therefore consist of containers that have been filled in one working session and, if freeze-dried, have been freeze-dried together in the same chamber at the same time.

A.2 General manufacturing recommendations

The general manufacturing recommendations for manufacturing establishments contained in the *Good manufacturing practices for pharmaceutical products: main principles* (***10***) and the *Good manufacturing practices for biological products* (***11***) should apply to establishments manufacturing Japanese encephalitis vaccine for human use, with the addition of the following directives:

The assignment of a virus to a biosafety level for production and quality control facilities should be based on a risk assessment. Such an assessment will take the risk group, as well as other factors, into consideration in establishing the appropriate biosafety level. For example, a virus assigned to risk group 2 may generally require biosafety level 2 facilities, equipment, practices and procedures for safe conduct of work. The biosafety level assigned for the specific work is based on a risk assessment rather than by automatic assignment of a laboratory biosafety level according to the particular risk group designation of the pathogenic agent to be used. Further guidance on the risk assessment and assignment of appropriate biosafety level are available in the WHO laboratory biosafety manual (***12***). However, countries should draw up a national policy for the manufacture of JE vaccines based on risk assessment and by risk group.

All personnel working in the production and control areas should have a serum neutralizing antibody titre of at least 1:10 against Japanese encephalitis virus.

In the production area, only mouse brains and cell cultures approved by the national regulatory authority for the production of Japanese encephalitis vaccine should be introduced into or handled.

A.3 Control of source materials

A.3.1 Animals and cells for vaccine production

A.3.1.1 Mice

When mice are used for the propagation of Japanese encephalitis vaccine virus in the brain, only animals less than 5 weeks of age should be used, and they should be free from any signs of diseases.

Animal colonies should be shown to be healthy. Only animal stocks approved by the national regulatory authority should be used for virus propagation.

When an animal colony is established, animals should be screened for ectoparasites, endoparasites, fungi, protozoa, bacteria, and viruses either for which evidence exists of a capacity to infect man or primates, or for which there is no evidence of infection in man but which could nevertheless pose a potential danger, for example in immunocompromised individuals. These may include hantavirus (haemorrhagic fever with renal syndrome), lymphocytic choriomeningitis virus (LCMV), reovirus type 3, Sendai virus, ectromelia virus, K virus, lactate dehydrogenase-elevating virus (LDV), minute virus of mice (MVM), mouse adenovirus (MAV), mouse cytomegalovirus (MCMV), Theiler's mouse encephalomyelitis virus (TMEV, GDVII strain), mouse hepatitis virus (MHV), mouse rotavirus (EDIM), pneumonia virus of mice (PVM), polyoma virus, retrovirus, thymic virus.

The colony should be monitored for zoonotic viruses and markers for contamination at regular intervals. Sera of the animals are screened for antibodies against viruses. The choice of tests and testing procedures as well as appropriate number of animals should be approved by the national regulatory authority. For instance, enzyme-linked immunosorbent assay (ELISA), haemagglutination inhibition (HAI), indirect fluorescent antibody (IFA) assay or any other suitable method can be used for estimation of theses antibodies. For validity of these tests a suitable positive and negative control should always be included in all tests.

After the colony is established, it should be monitored by testing a representative group of animals. The choice of tests and testing procedures for monitoring as well as appropriate number of animals should be approved by the national regulatory authority. In addition, the colony should be screened for the presence of pathogenic bacteria, including mycobacteria, fungi and mycoplasma. This should be performed in 100% of the animals over a defined period of time. The screening programme should be approved by the national regulatory authority.

Any animal that dies should be investigated to determine the cause of death. If the presence of an infectious agent is demonstrated in the colony, the national regulatory authority should be informed and the manufacture of vaccine should be discontinued. In this case, manufacture should not be resumed until a thorough investigation has been completed and precautions have been taken against the infectious agent being present in the product, and only with the approval of the national regulatory authority.

If the vaccine is produced in mouse brain, methods for intracerebral inoculation and harvesting should be approved by the national regulatory authority.

A.3.1.2 Primary hamster kidney cells

When primary hamster kidney cells are used for the propagation of Japanese encephalitis vaccine virus, animals and the primary cells should be approved by the national regulatory authority.

A.3.1.2.1 Hamsters

Hamsters, 10-14 days old, may be used as the source of kidneys for cell culture. Only hamster stock approved by the NRA should be used as the source of tissue and should be derived from a closed, healthy colony. A closed colony is a group of animals sharing a common environment and having their own caretakers who have no contact with other animal colonies. The animals are tested according to a defined programme to ensure freedom from specified pathogens, including the absence of antibodies to these pathogens. When new animals are introduced into the colony, they should be maintained in quarantine in vermin-proof quarters for a minimum of two months and shown to be free from these specified pathogens. The parents of animals to be used as a source of tissue should be maintained in vermin-proof quarters. Neither parent hamsters nor their progeny should previously have been used for experimental purposes, especially those involving infectious agents. The colony should be monitored for zoonotic viruses and markers for contamination at regular intervals.

At the time the colony is established, all founder animals should be tested to determine freedom from antibodies to the following pathogens: microorganisms pathogenic for hamsters (e.g. *Mycobacterium tuberculosis,* lymphoma virus, papilloma virus, polyomavirus, adenoviruses and retroviruses), lymphocytic choriomeningitis virus, pneumonia virus of mice, reovirus type-3, minute virus of mice, Sendai virus, hantavirus, SV-5, Toolans H-a virus, mouse poliovirus, mouse hepatitis virus, lactate dehydrogenase-elevating virus, and Kilham rat virus. Antibody production tests in mouse (MAP), hamster (HAP), and rat (RAP) should also be performed. A test for retroviruses using a sensitive PCR based reverse transcriptase (Rtase) assay also should be included. The results of such assays need to be interpreted with caution because Rtase activity is not unique to retroviruses and may derive from other sources, such as retrovirus-like elements that do not encode a complete genome (***13***). Nucleic acid amplification tests for retrovirus may also be used. A PCR test for hamster polyoma virus should be used on a selected number of hamster tissues, especially kidneys, to qualify the colony, and at intervals thereafter.

After the colony is established, it should be monitored by testing a representative group of animals. The choice of tests and testing procedures for monitoring as well as

appropriate number of animals should be approved by the national regulatory authority. In addition, the colony should be screened for the presence of pathogenic bacteria, including mycobacteria, fungi and mycoplasma. This should be performed in 100% of the animals over a defined period of time. The screening programme should be approved by the national regulatory authority.

Any animal that dies should be investigated to determine the cause of death. If the presence of an infectious agent is demonstrated in the colony, the national regulatory authority should be informed and the manufacture of vaccine should be discontinued. In this case, manufacture should not be resumed until a thorough investigation has been completed and precautions have been taken against the infectious agent being present in the product, and only then with the approval of the national regulatory authority.

At the time of kidney harvest, the animals should be examined for the presence of any abnormalities and if kidney abnormalities or other evidence of pathology is found, those animals are not to be used for JE vaccine production.

Each group of control cultures derived from a single group of animals used to produce a single virus harvest should remain identifiable as such until all testing, especially for adventitious agents, is completed.

A.3.1.2.2 PHK cell cultures

Kidneys derived from animals which comply with the guidelines set out in section *A.3.1.2.1* should be dissected and minced under conditions approved by the national regulatory authority. A primary cell suspension is obtained after trypsin digestion and this is distributed into cell culture vessels with growth medium.

A.3.1.3 Continuous cell lines

The use of a continuous cell line for the propagation of Japanese encephalitis vaccine virus should be based on cell bank system and tests on master and manufacturer's working cell banks should conform with the *Requirements for use of animal cells as in vitro substrates for the production of biologicals* (***13,14***) where appropriate, and should be approved by the national regulatory authority.

> WHO has established a cell bank of Vero cells characterized in accordance with the recommendations in the report of the WHO Expert Committee on Biological Standardization (***13,14***), which is available as a well characterized starting material to manufacturers for preparation of their own master and working cell bank on request to the Coordinator, Quality, Safety and Standards Team, WHO, Geneva, Switzerland.

The maximum number of passages (or population doublings) allowable between the MCB, the WCB and the production cells should be approved by the national regulatory authority. Additionally, the MCB or WCB cells should be propagated to or beyond the maximum production level and be examined for tumorigenicity in an animal test system and for the presence of bacteria, fungi, mycoplasmas, retroviruses and other adventitious agents.

The MCB is made in sufficient quantities and stored in a secure environment and is used as the source material to make manufacturer's working cell bank. In normal practice, an

MCB is expanded by serial subculture up to a passage number (or population doubling, as appropriate) selected by the manufacturer and approved by the national regulatory authority, at which point the cells are combined to give a single pool distributed into ampoules and preserved cryogenically to form the WCB.

Tests on the master and working cell banks are performed in accordance with the *Requirements for use of animal cells as in vitro substrates for the production of biologicals* (***13,14***).

Full characterization may be performed on either the master cell bank or on the working cell bank.

The manufacturer's WCB is used for the preparation of production cell culture, and thus for production of Japanese encephalitis vaccine virus batches.

The manufacturer's WCB should be identified by means of, for example, biochemical (e.g. isoenzyme analysis), immunological, and cytogenetic marker tests, approved by the national regulatory authority.

A.3.1.4 Cell culture medium

If serum is used for the propagation of cells, it should be tested to demonstrate freedom from bacteria, fungi and mycoplasmas, according to the recommendations given in Part A, sections 5.2 and 5.3 of the revised *Requirements for biological substances no. 6* (***15,16***), and from infectious viruses. Suitable tests for detecting viruses in bovine serum are given in *Appendix 1* of the *Recommendations for production and control of poliomyelitis vaccine (oral)* (***17***).

> Validated molecular tests for bovine viruses may replace the cell culture tests of bovine sera. As an additional monitor of quality, sera may be examined for freedom from phage, endotoxin and antibodies to JE virus. Gamma-irradiation may be used to inactivate potential contaminant viruses.

The acceptability of the source(s) of any components of bovine, porcine, sheep or goat origin used should be approved by the national regulatory authority. These components should comply with the *Guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products* (***18***) and *WHO guidelines on tissue infectivity distribution in transmissible spongiform encephalopathies* (***19***) .

If trypsin is used for preparing cell cultures and aiding in virus infection, it should be tested and found free of bacteria, fungi, mycoplasmas and infectious viruses, especially bovine or porcine parvoviruses, as appropriate. The methods used to ensure this should be approved by the national regulatory authority. The trypsin should be gamma irradiated if possible.

Human serum should not be used. However, human serum albumin may be used. If used, it should meet the revised *Requirements for the collection, processing and quality control of blood, blood components and plasma derivatives (requirements for biological substances no. 27)* (***20***), as well as WHO guidelines on transmissible spongiform encephalopathies (***18,19***).

Penicillin and other beta-lactams should not be used at any stage of the manufacture because of their nature as highly sensitizing substances. Other antibiotics may be used in the manufacture provided that the quantity present in the final product is acceptable to the national regulatory authority.

> Minimal concentrations of suitable antibiotics such as kanamycin and neomycin may be used if approved by the national regulatory authority.

Any other added substances should approved by the national regulatory authority.

> Non-toxic pH indicators may be added, e.g. phenol red in a concentration of 0.002%.

A.3.2 Virus seed

Different virus seed strains are used for production of inactivated JE Vaccine.
A seed lot system should be followed in preparation of master and working seed. Passage number of the working seed and final product is similar to the batch which has been found effective in clinical efficacy studies.

A.3.2.1 Strain of virus

The strains of virus used in the production of all seed lots should be approved by the national regulatory authority and should yield safe and immunogenic vaccines when the virus has been inactivated. They should be identified by historical records which include passage history. It should be shown to be free of adventitious agents by infectivity tests, serological or molecular biological tests, and animal inoculation.

A.3.2.2 Virus seed lot system

The preparation of Japanese encephalitis vaccine should be based on the use of a virus seed lot system. The national regulatory authority should 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 should be made from a working virus seed lot without further intervening passage. Virus seed lots should be freeze-dried or frozen. The dried seed should be kept at or below -20 °C, while the frozen seed should be kept at or below -60 °C.

Seed lots should have been shown, to the satisfaction of the national regulatory authority, to be capable of yielding vaccine that meets all these Recommendations.

A.3.2.3 Tests on the master virus seed lots

A.3.2.3.1 Test for identity

The master virus seed lot should be identified as Japanese encephalitis virus strain by methods approved by the national regulatory authority.

A.3.2.3.2 Tests for bacteria, fungi and mycoplasmas

Each master virus seed lot should be tested for bacterial, fungal, and mycoplasmal contamination by appropriate tests according to Part A, sections 5.2 and 5.3 of the

revised *Requirements for biological substances no. 6* (*General requirements for sterility of biological substances*)(***15,16***).

A.3.2.3.3 Tests for adventitious agents

The master virus seed lot should be tested for adventitious agents. For these tests the virus should be neutralized by a specific anti-Japanese-encephalitis serum. The specificity and sensitivity of assays should be defined and approved by the national regulatory authority

A.3.2.3.4 Additional tests

Tests should be carried out to characterize the virus strain. Such tests should include the titration of virus. Additional tests should also take in to account the passages of the virus in different animal species.

A.3.2.4 Tests on the working virus seed lots

A.3.2.4.1 Test for identity

The working virus seed lot should be identified as Japanese encephalitis virus strain as in *A.3.2.3.1*

A.3.2.4.2 Tests for bacteria, fungi and mycoplasmas

Each working virus seed lot should be tested for bacterial, fungal, and mycoplasmal contamination as described in *A.3.2.3.2*.

A.3.2.4.3 Tests for adventitious agents

If the working virus seed lot is derived from mouse brain or primary cell cultures, it should be tested for adventitious agents as in *A.3.2.3.3*. If working virus seed lots are produced in cells derived from a validated cell bank where a master virus seed lot was tested for adventitious agents, these tests do not have to be repeated.

A.3.2.4.4 Additional tests

Each time a new working virus seed lot is prepared, tests should be carried out to characterize the virus strain as described in *A.3.2.3.4*.

A.4 Control of vaccine production

A.4.1 Mouse brain

The brains of mice inoculated intracerebrally with the virus strain for production should be harvested when the mice exhibit advanced signs of JE virus infection such as encephalitis. The harvested mouse brains should be homogenized in a suitable medium and processed to give a uniform virus suspension.

The harvested and processed virus suspension should be subjected to the control tests for single virus harvests given in *A.4.3* of these Recommendations.

A.4.2 Cell cultures

A.4.2.1 Preparation of control cell cultures

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

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

A.4.2.2 Tests on control cell cultures

The control cell cultures should be treated in a similar way to the production cell cultures, but they should remain uninoculated to serve as control cultures for the detection of extraneous viruses.

The control cell cultures should be incubated under the same conditions as the inoculated cultures for at least 14 days and should 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 should have had to be discarded for nonspecific, accidental reasons. At the end of the observation period, the control cell cultures should be examined for the presence of adventitious agents as described below (*A.4.2.2.2* and *A.4.2.2.3*).

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 should not be used for vaccine production.

Samples not tested immediately should be stored at –60 °C or below.

A.4.2.2.1 Identity test if continuous cell lines are used

At the production level, and for vaccines produced in continuous cell lines, the cells should be identified by using one of the methods specified in the *Requirements for the use of animal cells as in vitro substrates for production of biologicals* (***13,14***). The method (s) should be approved by the national regulatory authority.

> Methods for identity testing include, but are not limited to, biochemical (e.g. isoenzyme analysis), immunological (e.g. major histocompatibility antigens), cytogenetic tests (e.g. for chromosomal markers), and tests for genetic markers (DNA fingerprinting).

A.4.2.2.2 Tests for haemadsorbing viruses

At the end of the observation periods, haemadsorbing viruses should be tested. If multiple harvest pools are prepared at different times, the cultures should be observed and tested at the time of the collection of each pool.

> In some countries, 25% of the control cells are tested for the presence of haemadsorbing viruses by using guinea-pig erythrocytes. If the red blood cells have been stored, the duration of storage should not

have exceeded 7 days and the temperature of storage should have been in the range of 2-8 °C.

In tests for haemadsorbing viruses, calcium and magnesium ions should be absent from the medium.

In some countries the national regulatory authority requires that tests for haemadsorbing viruses should also be done with erythrocytes from other species, including human blood group O, monkeys and chickens (or other avian species).

The results of all tests should be noted after incubation of the erythrocytes with the cultured cells for 30 minutes at 0-4 °C and again after a further incubation for 30 minutes at 20-25 °C. For the test with monkey erythrocytes, the results should be noted a third time, after a final incubation for 30 minutes at 34-37 °C.

A.4.2.2.3 Tests for other adventitious agents on supernatant fluids

At the end of the observation period, a sample of the pooled fluids from each group of control cultures should be tested for adventitious agents. At least 10 ml of each pooled supernatant fluid from the control cultures should be tested in the same cell substrate, but not the same batch of cells, as that used for production. Additional samples of at least 10 ml should be tested in human cells and at least one other sensitive cell system.

The samples should be inoculated into bottles of these cell cultures, in such a way that the dilution of the supernatant fluid in the nutrient medium does not exceed 1 in 4. The area of the cell sheet should be at least 3 cm^2/ml of supernatant fluid. At least one bottle of each of the cell cultures should remain uninoculated and serve as a control.

The inoculated culture should be incubated at 35-37 °C and should be observed for cytopathic effects for a period of at least 14 days.

For the tests to be valid, at least 80% of the cell cultures should be available and suitable for evaluation at the end of the test period.

If any cytopathic changes due to adventitious agents occur in any of the cultures, the virus harvest produced from the batches of cells from which the control cells were taken should be discarded.

A.4.3 Control of single virus harvests

After inoculation of the production cells with the virus working seed lot, inoculated and control cell cultures should be kept within a temperature range approved by the national regulatory authority for the defined incubation periods. The optimal range for pH, multiplicity of infection, cell density and time of incubation should be established, and be approved by the national regulatory authority.

The appropriate time for harvest should be defined and approved by the national regulatory authority.

It is advisable that the inoculated cell cultures are processed in such a manner that each virus suspension harvested remains identifiable as a single harvest and is kept separate from other harvests until the results of all the tests described in *A.4.2* have been obtained.

Only the virus harvests satisfying the recommendations below should be pooled and used in the preparation of the inactivated virus harvest.

A.4.3.1 Sterility tests for bacteria and fungi

A sample removed from each virus harvest should be tested for bacterial and fungal contamination by appropriate tests recommended in Part A, section 5.2 of the revised *Requirements for biological substances no. 6* (*General requirements for the sterility of biological substances*) (***16***). Any single virus harvest in which contamination is detected should be discarded.

A.4.3.2 Identity test for vaccine virus

The single virus harvest should be identified as Japanese encephalitis vaccine virus using suitable methods approved by the national regulatory authority.

A.4.3.3 Test of virus content

A sample removed from each virus harvest should be tested for virus content using suitable methods approved by the national regulatory authority.

Both mice and cell culture of defined sensitivity are suitable for testing infectivity. Manufacturers should set an in-house specification for titer of each harvest.

A.4.3.4 Consistency of yield

Virus content mentioned above is an appropriate parameter for monitoring the consistency of yield. Therefore, internal specifications should be set.

A.4.4 Preparation and control of purified bulk

A.4.4.1 Preparation of purified bulk

Only virus harvests satisfying the recommendations for sterility and virus content in *A.4.3* should be pooled.

One or more single harvests may be purified and/or concentrated by methods demonstrated to yield safe, potent and immunogenic vaccine. The virus harvest or pools should be inactivated by a validated method at a defined stage of the process which may be before or after concentration and purification.

The process should be approved by the national regulatory authority and should be shown to give consistent results.

The bulk suspension derived from mouse brains should be purified by a process that should be designed to reduce the myelin content to the lowest possible level and have been approved by the national regulatory authority (see A.4.4.3.1).

A.4.4.2 Inactivation of virus

A.4.4.2.1 Treatment before inactivation

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

The importance of filtration or clarification using 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.

A.4.4.2.2 Inactivation

The process for the inactivation of the Japanese encephalitis virus should be approved by the national regulatory authority.

Inactivation should be commenced immediately after the preparation and sampling of single virus harvests when mouse brain 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 1:2,000 for 50-60 days at 4 °C.

A.4.4.2.3 Test for effective inactivation

Each bulk suspension should be tested in an appropriate test system for effective inactivation of the virus before the addition of preservatives and other substances. The sensitivity of the assay should be determined according to the JE virus used for production and the most sensitive assay should be used. Test should be performed immediately after inactivation.

If samples are not tested immediately after inactivation they should be stored frozen at –60 °C or below. The conditions of storage should be validated to confirm no loss of virus titre. If test is performed at a later stage of production, appropriate biosafety levels should be maintained.

The test should be approved by the national regulatory authority. The test should be performed with the undiluted bulk suspension. A test sample corresponding to no less than 25 human doses of the final bulk should be used.

In some countries the test involves direct inoculation intracerebrally into mice followed by three blind passages.

The total volume of the test sample should 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 should be used for 1 ml of the test material.

During the incubation period, no cytopathic change should be detected. At the completion of the observation, the cultured 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 bulk passes the test if the product has been shown to be free from residual live virus.

A.4.4.3 Tests on purified bulk

A.4.4.3.1 Test for myelin basic protein if mouse brain was used for production

Each purified bulk should be tested for myelin basic protein. The method and specification for myelin basic protein content should be approved by the national regulatory authority.

Some licensed JE vaccines have been reported to contain myelin basic protein lower than 2 ng per human dose.

A.4.4.3.2 Protein content

Each purified bulk should be tested for the total protein content using a suitable method such as the micro-Kjeldahl method, the Lowry technique or another suitable method.

A.4.4.3.3 Antigen content

The test for viral antigen content should be made on each bulk suspension. The method used should be approved by the national regulatory authority.

A.4.4.3.4 Test for residual DNA if continuous cell lines are used for production

For viruses grown in continuous cell lines, purified bulk should be tested for residual cellular DNA. If this test has not been carried out at this stage, it should be done on final bulk or final lot.

The removal process should be shown to consistently reduce the amount of cell DNA. It is expected that the levels of residual host cell DNA in a final dosage form will meet the maximum levels cited in the *Requirements for use of animal cells as in vitro substrates for the production of biologicals* (***13***).

A.4.4.3.5 Test for residual animal serum

If animal serum is used for production of cell culture vaccines, residual bovine serum albumin (BSA) content should be measured as an indicator of animal serum in the purified bulk. This should result in a level of no greater than 50 ng per human dose or its equivalent.

In some countries, tests are carried out to estimate the amount of residual animal serum in the final vaccine. Other serum proteins may also be measured.

A.4.4.3.6 Test for residual chemicals

The concentration of chemicals such as inactivating agent remaining in the final vaccine should be determined using methods approved by the national regulatory authority. These concentrations should not exceed the upper limits specified by the national regulatory authority. For preservatives, both the method of testing and the concentration should be approved by the national regulatory authority.

Alternatively, tests for residual chemicals may be performed on the final bulk.

A.4.5 Preparation and control of final bulk

A.4.5.1 Preservatives and other substances including adjuvants added

In the preparation of the final bulk, only adjuvant, preservatives or other substances such as human albumin approved by the national regulatory authority should be added. Such substances should 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 should be such that the amount of formaldehyde in the final bulk is no greater than 0.01%. The test method used should be approved by the national regulatory authority.

Additional antibiotics should not be added to the final bulk of Japanese encephalitis vaccine for human use.

Antigen produced in cell cultures may be adsorbed onto an adjuvant such as aluminium. In that case, the mineral vehicle and its concentration used should be approved by the national regulatory authority. Antigen produced in the mouse brain should not be adsorbed onto any adjuvant. Until the bulk is formulated into the final bulk, the suspension should be stored under conditions shown by the manufacturer to retain the desired biological activity.

A.4.5.2 Tests on final bulk

A.4.5.2.1 Sterility tests for bacteria and fungi

Each final bulk should be tested for sterility according to the recommendations in Part A, section 5.2 of the revised *Requirements for biological substances no. 6* (*General requirements for the sterility of biological substances*) (***16***).

A.4.5.2.2 Adjuvant content and degree of adsorption (where appropriate)

If an adjuvant has been added to the vaccine, its content should be determined by a method approved by the national regulatory authority. The amount and nature of the adjuvant should be within the range shown to be clinically effective and should be approved by the national regulatory authority. When aluminium compounds are used, the content of aluminium should not be greater than 1.25 mg per single human dose.

The formulation of adjuvant and antigen should be stable and consistent. The purity of the adjuvant should be demonstrated to be within the range found for vaccine lots shown to be clinically effective.

Adsorbed bulk may be assayed for the content of the adjuvant until production consistency is demonstrated.

The degree of adsorption (completeness of adsorption) of each adsorbed bulks should be assessed. This test may be omitted upon demonstration of process consistency.

A.4.5.2.3 Preservative content

If a preservative has been added to the vaccine, the content of preservative should be determined by a method approved by the national regulatory authority.
The amount of preservative in the vaccine dose should be shown not to have any deleterious effect on the antigen nor impair the safety of the product in humans. The preservative, its use at different stages of manufacturing process as well as the residual amount present in the product should be approved by the national regulatory authority.

A.4.5.2.4 Potency

This test may be performed on the final bulk. The method for detection of neutralizing antibody and the analysis of data should be approved by the national regulatory authority. The vaccine potency should be compared with that of a reference preparation and the national regulatory authority should determine limits of potency. The national regulatory authority should approve the reference preparation used.

This test may be conducted on each final lot derived from the final bulk.

A.5 Filling and containers

The recommendations concerning filling and containers in the *Good manufacturing practices for biological products* (***11***) should apply, with the addition of the following directive.

Containers of freeze-dried vaccine should 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 should be tested for leaks and all defective containers should be discarded.

Care should be taken to ensure that the materials of which the container and, if applicable, transference devices and closure are made do not adversely affect the quality of vaccine.

The manufacturers should provide the national regulatory authority with adequate data to prove the stability of the product under appropriate conditions of storage and shipping.

A.6 Control tests on final lot

A.6.1 Inspection of final containers

Every container in each final lot should be inspected visually, and those that show abnormality should be discarded.

A.6.2 Identity test

An identity test should be performed on at least one labeled container from each final lot by methods approved by the national regulatory authority.

> The test for potency, as described in section *A.6.6* of these Recommendations may serve as an identity test.

A.6.3 Sterility tests for bacteria and fungi

Each final lot should be tested for bacterial and fungal sterility according to the recommendations in Part A, section 5.2 of the revised *Requirements for biological substances no. 6* (*General requirements for the sterility of biological substances*) (***16***).

A.6.4 Tests of pH

The pH value of a pool of final containers should be tested. The freeze-dried vaccine is dissolved in the approved diluent. The pH value should be approved by the national regulatory authority, and be within the range of values found for vaccine lots shown to be clinically safe and effective.

A.6.5 Test of osmolarity

The osmolarity of a pool of final containers should be tested. The freeze-dried vaccine is dissolved in the approved diluent. The osmolarity should be approved by the national regulatory authority, and be within the range of values found for vaccine lots shown to be clinically safe and effective.

This test may be discontinued when consistency of production has been demonstrated

A.6.6 General safety (innocuity) tests

Each final lot should be tested for the absence of abnormal toxicity using a general safety (innocuity) test approved by the national regulatory authority.

> This test may be omitted for routine lot release once consistency of production has been well established to the satisfaction of the national regulatory authority and when good manufacturing practices are in place. Each lot, if tested, should pass a test for general safety.

A.6.7 Test for protein content

For mouse brain vaccine, the maximum protein content should not be greater than 80 µg/ml.

> Experience from production by some manufacturers indicates that that levels of 10-40 µg/ml are obtained.

The protein content of cell culture-derived vaccines should be approved by the national regulatory authority.

> If protein stabilizers such as gelatin are added to vaccine, the total protein content should reflect such additions.

A.6.8 Test for residual cellular DNA

When continuous cell lines are used for production, the cellular DNA content in a final dosage form should be determined. As recommended in the WHO *Requirements for the use of animal cells as in vitro substrates for the production of biologicals* (***13***), the amount of residual cell DNA should be less than 10 ng per purified human dose. The assay for determination of residual cell DNA with defined sensitivity for detection of specified levels should be approved by the national regulatory authority. If this test has already been carried out earlier stage of production, e.g. purified or final bulk, an estimate of the level of residual cellular DNA retained in the final lot should be presented and justified. The specification set for the level of residual DNA should comply with current WHO requirements for cell substrates (***13***). This test may be discontinued once consistency has been demonstrated.

A.6.9 Potency test

The potency test should be determined by titration of the neutralizing antibody produced in immunized mice by the plaque-reduction neutralization test. Neutralization antibody titers should be calculated as 50% plaque-reduction neutralization titer. The test should be made in parallel with a reference vaccine (standard) derived from a homologous virus strain. The challenge strain should be the virus strain homologous to that in the test vaccine. The mouse strain used should have been shown to give adequate responses following immunization with the JE vaccine on test.

Appropriate vaccine and challenge virus are approved by the national regulatory authority and should be homologous to the JE virus strain used for production.

The test procedure used, including the reference vaccine, should be approved by the national regulatory authority (see *D.1*).

The reference vaccine should be well characterized in respect of its immunogenic potential. The reference vaccine should, either, have been included and shown to be efficacious in clinical trials, or be traceable to such a batch of vaccine.

In some countries, a single dilution assay has been used. However, a multi-dose assay has been implemented in at least one country which facilitates statistical evaluation.

Briefly, the multi-dose test is as follows: the test vaccine and a reference are diluted to make appropriate serial dilutions. Five hundred microliter of each dilution is injected intraperitoneally into at least ten mice, of the same sex, approximately 4 weeks of age and typically 14-18 g body weight, on 2 occasions 7 days apart. Seven days after the second injection, each animal is bled. An equal volume of separated serum is pooled, and heat inactivated at 56 °C for 30 minutes. The pooled serum may be stored at –20 °C or below.

If pooled serum is tested for virus neutralizing antibodies, the following procedures are used:

A series of dilutions are prepared in Eagle's MEM containing fetal bovine serum or another appropriate medium. Equal volumes of the diluted serum and the challenge virus are mixed. The mixture is kept at 36±l °C for 90 minutes with intermittent shaking every 15 - 30 minutes. One hundred microliter of the serum-virus mixture is

inoculated onto at least three wells of appropriate cells such as Vero or chick embryo fibroblast cells in 6-well plates. The challenge virus is mixed with an equal volume of the medium dilution to serve as the virus control. All the inoculated cells are incubated at 36 ± 1 °C for 90 minutes in 5% CO_2, The infected cells are overlaid with the overlaying agar medium containing 1% agar or methyl cellulose.

After incubation for an appropriate time (5–8 days), the cells are stained and the number of plaques counted. The mean number of plaques of the control should be 50–150 per dish. Neutralizing antibody titers (based on initial serum dilutions before mixing with virus preparation) are calculated as 50% PRNT.

The 50% PRNTs induced by the tested vaccine are compared with those induced by the reference by the appropriate statistical methods approved by the national regulatory authority. The potency of the test sample should be no less than the reference vaccine.

A.6.10 Accelerated thermal stability test

The performance of accelerated thermal stability tests should be considered in the context of the overall stability evaluation of JE vaccine (***21***) (section A.11.1).

Previous experience indicated that the potency of liquid vaccine may be determined after storage of samples at 37 °C for 1 week and freeze-dried vaccines at 37 °C for 4 weeks

If accelerated stability data consistently meet requirements for potency and other stability indicating parameters, thermal stability test for the purpose of lot release could be performed at regular intervals instead of testing each lot as part of ongoing stability studies following licensing**.**

A.6.11 Residual moisture tests on freeze-dried vaccine

The residual moisture in a representative sample of each freeze-dried lot should be determined by a method approved by the national regulatory authority. The upper limit for the moisture content should be specified by the national regulatory authority.

Moisture levels of 3% or less are generally considered satisfactory.

A.6.12 Test for pyrogenic substances

Each final lot should be tested for pyrogenic substances. The test procedures should be approved by the national regulatory authority.

A.6.13 Test for residual animal serum

For cell culture-derived vaccines, the final lot should be tested to verify that the level of residual bovine serum albumin as an indicator of residual serum protein in the final vaccine is less than 50 ng per human dose.

This test may be performed on the purified bulk or on the final bulk. Tests for other residual serum proteins may also be used. (See *A.4.4.3.5)*

A.6.14 Test for preservatives

Each final lot should be tested for the presence of preservative, if added. The concentrations of preservatives should be approved by the national regulatory authority. Such substances should have been shown by appropriate tests not to impair the safety or immunogenicity of the vaccine.

If any modification of preservative content in already licensed vaccine is made, general principles for vaccine evaluation described in the WHO *Guidelines on regulatory expectations related to the elimination, reduction or replacement of thiomersal in vaccines (**22**)*, should be followed .

A.6.15 Adjuvant content and degree of adsorption (where appropriate)

If vaccines are adjuvanted, each final lot should be assayed for the adjuvant content. When aluminium compounds are used, the content of aluminium should not be greater than 1.25 mg per single human dose.

The degree of adsorption (completeness of adsorption) of the antigen in each final lot should be assessed and the limits should be approved by the national regulatory authority. This test may be omitted for routine lot release upon demonstration of the product consistency.

A.7 Records

The recommendations in the *Good manufacturing practices for biological products* (***11***) should apply.

A.8 Retained Samples

The recommendations in the *Good manufacturing practices for biological products* (***11***) should apply.

A.9 Labelling

The recommendations given in section 7 of the *Good manufacturing practices for biological products* (***11***) should apply, with the addition of the following information.

The label on the carton, the container or the leaflet accompanying the container should state:

- that the vaccine has been prepared from mouse brains, primary hamster kidney cells, or Vero cells;
- the strain of the vaccine virus present in the preparation;
- the number of doses, if the product is issued in a multiple-dose container;
- the name and maximum quantity of any antibiotic present in the vaccine;
- the name and concentration of any preservative added;
- the name and concentration of any adjuvant added;
- the temperature recommended during storage and transport;

- the expiry date; and
- any special dosing schedules.

A.10 Distribution and shipping

The recommendations in the *Good manufacturing practices for biological products* (***11***) should apply.

A.11 Stability, storage and expiry date

A.11.1 Stability testing

Stability testing should be performed at different stages of production, namely on single harvests, purified bulk, final bulk, and final lot. Stability-indicating parameters should be defined or selected appropriately according to the stage of production. It is advisable to assign a shelf-life to all in-process materials during vaccine production, in particular intermediates such as single harvests, purified ,and final bulk.

The stability of the vaccine in its final container and at the recommended storage temperatures should be demonstrated to the satisfaction of the national regulatory authorities on at least three lots of final product. Accelerated thermal stability tests may be undertaken on each final lot to give additional information on the overall stability of a vaccine (see *A.6.10*).

The formulation of vaccine and adjuvant (if used) should be stable throughout its shelf-life. Acceptable limits for stability should be agreed with national authorities.

A.11.2 Storage conditions

The final container vaccine should be kept at +2 to +8 °C. If other storage conditions are used, they should be fully validated and approved by the national regulatory authority. The vaccine should have been shown to meet the release specifications for a period equal to that between the date of release and the expiry date. During storage, liquid vaccines should not be frozen.

A.11.3 Expiry date

The expiry date should be fixed upon the approval of the national regulatory authority, and should take account of the experimental data on stability of the vaccine.

> In a country, the expiry dating period of a liquid JE vaccine was set no less than 3 months after the potency test and a freeze-dried vaccine no less than 2 years.
>
> For freeze-dried vaccines, the expiry date for the vaccine and the diluent may be different.

Part B. Nonclinical evaluation of new JE vaccines (inactivated)

Nonclinical evaluation of new JE vaccines should be based on the *Guidelines on nonclinical evaluation of vaccines* (***3***). The following specific issues should be

considered in the context of the development of an inactivated JE vaccine. In any event, the nonclinical experiments should be discussed with the national regulatory authorities.

B.1 Immunogenicity studies

For JE virus the role of antibody in protection is well studied and neutralization assays are considered more appropriate than the virus binding assays like ELISA (see *General considerations* and *C.2*). Nonclinical studies should normally be undertaken using the same formulation of the vaccine that is intended for use in clinical trials unless otherwise justified.

The first studies should involve immunization of animals with various doses of the candidate vaccine given at various regimens and evaluation of the kinetics of the neutralizing antibody response. The inclusion of at least one licensed vaccine as a comparator may provide useful supporting data but is optional. Studies are usually undertaken in mice as this species demonstrates an adequate immune response.

B.2 Active protection studies

The protective efficacy of the vaccine may be evaluated in challenge studies. The focus of these studies should be to demonstrate that prior vaccination protects against disease due to the homologous virus strain. These should be performed before the commencement of clinical studies. Protection studies that employ challenge should be undertaken with at least one other genotype 3 virus (e.g. another strain used for vaccine production). Similar studies using a non-genotype 3 virus are encouraged. The latter studies may be performed later on in the development programme. Issues regarding biocontainment should be taken into consideration.

The optimal concentration of challenge virus and the route of inoculation which consistently result in disease and/or death in unvaccinated mice should be established. The intracerebral route is generally used for challenge but the intraperitoneal route may be appropriate for some virus strains. Mice are generally challenged with virus at the time of the maximum immune response.

B.3 Passive protection studies

Passive protection studies involve the administration of sera from vaccinated animals or humans to unvaccinated animals followed by virus challenge as described for active protection studies. By this means it may be possible to estimate titres of neutralizing antibody raised in response to vaccination that correlate with protection. While not necessarily mandatory, such studies could be undertaken in conjunction with phase 1 clinical studies when post-vaccination human sera become available.

B.4 Toxicology

Toxicology studies on vaccines should reflect the maximum clinical dose anticipated for use in man, the route of administration and the anticipated schedule.

If a vaccine is to be indicated for use in women of child bearing age reproductive and developmental toxicology studies are recommended. However, these are not required if the vaccine is to be recommended only for use in children under age 12 years.

The addition of any preservative and novel adjuvant requires additional toxicological analysis. The absence of detailed toxicology studies should be justified. Changes in the manufacturing procedures might require a nonclinical assessment.

Part C. Clinical evaluation of new JE vaccines (inactivated)

C.1 General considerations for clinical studies

C.1.1 Clinical development programme

Clinical trials should adhere to the principles described in the *Guidelines for good clinical practice (GCP) for trials on pharmaceutical products* (***23***) and to the *Guidelines on clinical evaluation of vaccines: regulatory expectations* (***4***). All clinical trials should be approved by the relevant national regulatory authorities.

Some of the issues that are specific to the clinical evaluation of JE vaccines are discussed in the following sections. These sections should be read in conjunction with the general guidance mentioned above. It is also recommended that manufacturers should consult with relevant NRAs regarding the overall clinical development programme and the plans for assessment of immune responses.

This guidance is intended to be applicable to all novel inactivated JE vaccines whatever the mode of production (i.e. including use of vectors to express viral antigens).

C.1.2 Range of clinical studies

The availability and widespread deployment of effective vaccines in JE endemic areas makes it unethical to conduct protective efficacy studies (i.e. with the endpoint of prevention of clinically apparent illness) that compare new JE vaccines against an unvaccinated group. In addition, the use of the available JE vaccines has reduced the incidence of clinically apparent infections to such an extent that a study with sufficient power to estimate the relative protective efficacy of a new vaccine compared with a licensed JE vaccine would require such large sample sizes that it would not be a feasible undertaking.

As a result, the evaluation of the likely protective efficacy of new JE vaccines should be based on evidence derived from active and passive protection in animal models (see section B) and on an immunological parameter that is a suitable correlate for clinical protection in humans (see *General considerations* and *C.2*).

It is important that the immunogenicity of a new JE vaccine should be assessed in accordance with the intended mode of use. Ideally, the clinical development programme should assess the safety and immunogenicity of the new vaccine in cohorts resident in non-endemic, endemic and hyper-endemic areas in order to enroll subjects with no and with varying degrees of pre-existing immunity to JE as a result of previous vaccination and/or natural exposure. Some important considerations include the following:

- The focus for use of JE vaccines in endemic and hyper-endemic areas is most likely to be the vaccination of residents from an early age. Therefore an adequate assessment of the immunogenicity of a new vaccine in children of various age groups is important. Studies in the youngest children should generally follow on after satisfactory assessments of safety and immunogenicity have been obtained in adults and older children;

- In endemic and hyper-endemic areas a substantial proportion of residents may have received JE vaccines in the past. Therefore it may be useful to evaluate the ability of a new JE vaccine to boost immunity in persons who were previously vaccinated with other types of JE vaccines;
- Another consideration for study design and location is the existence of cross-reactive immunity between flaviviruses, which can influence pre-vaccination and post-vaccination antibody levels to JE virus. For example, past natural infection with dengue or West Nile viruses (which may have been sub-clinical or not diagnosed) and/or past exposure to or vaccination against yellow fever may result in detectable antibody to JE virus before any vaccination is administered; and
- In contrast the use of JE vaccine in non-endemic areas is mainly to protect those traveling to endemic regions. Thus, although there is a potential for use across all age groups most recipients of JE vaccines in non-endemic areas are likely to be non-immune adults.

C.2 Immunogenicity

C.2.1 Methodology

It is recommended that the primary assessment of the immunogenicity of a new JE vaccine should be based on measurement of serum neutralizing antibody in pre- and post-vaccination sera. The plaque reduction neutralization test (PRNT) is the most commonly used method for measurement of neutralizing antibody. However, the PRNT is technically demanding and methods vary between laboratories especially regarding choice of cell substrate, incubation conditions, exogenous complement, the size of wells and the definition of endpoints. Therefore it is essential that the methodology that is employed for determining PRNT titres in clinical studies should be fully validated. It is also preferable that a single laboratory is used to perform these assays throughout a clinical development programme. If this is not possible cross-validation data between laboratories should be provided.

Expression of neutralizing antibody titres in terms of the highest dilutions of sera before mixing with virus preparation that accomplish a 50% reduction in viral plaques (i.e. $PRNT_{50}$) is preferred over the use of 90% reduction in plaques (i.e. $PRNT_{90}$).

Initial studies should seek to establish whether vaccination elicits adequate immune responses to the vaccine strain (i.e. homologous virus) and should evaluate antibody kinetics. Further studies should evaluate post-vaccination $PRNT_{50}$ titres against other (i.e. heterologous) strains of JEV in randomly chosen subsets of sera. There are five JE genotypes. Therefore use of heterologous strains of various genotypes of JEV in PRNT assays is encouraged.

Methods that assess total (i.e. including non-functional) antibody may also be used during the clinical development programme but the results of these tests should be regarded as secondary immunogenicity parameters. These methods include haemagglutination inhibition (HI), enzyme-linked immunosorbent assay (ELISA) or immunofluorescent antibody (IFA) tests. If such tests are performed any correlation between the results and those of $PRNT_{50}$ should be explored.

Consideration may also be given to the assessment of vaccine-induced cell-mediated immunity. Mouse studies have shown that adoptive transfer of T lymphocytes can confer passive protection against viral challenge. Also, human CD4 and CD8 cells harvested from vaccinated persons can be stimulated by JEV to proliferate in vitro.

However current uncertainties regarding the interpretation of these data mean that they would also be considered secondary immunogenicity parameters.

C.2.2 Endpoints and analyses

The primary assessment of immune responses should be based on proportions of previously seronegative subjects that reach a $PRNT_{50}$ titre of at least 1:10 after vaccination (see also *General considerations*).

The primary population should be pre-defined in the protocol and should be selected in accordance with the study objectives. The population to be used in the primary analysis of immune responses should usually be confined to those subjects who are seronegative for JE virus before vaccination (i.e. have $PRNT_{50}$ titres < 1:10). Therefore, before commencement of a study in a particular geographic area, there should be an estimate made of the likely percentage of subjects who will have pre-vaccination $PRNT_{50}$ titres ≥ 1:10. In some instances it may be appropriate to actively exclude those with a history of prior vaccination against JE in order to reduce the likelihood that subjects will already be seropositive. Alternatively or in addition studies could include a screening visit so that a subject's pre-vaccination serostatus can be determined before enrolment and administration of the vaccine.

In persons who are seronegative before vaccination the most appropriate primary parameter for assessment of the immune response will be the proportion reaching $PRNT_{50}$ titres ≥ 1:10 after vaccination, which will equal the seroconversion rate. Other immune parameters examined should include increases in titres after sequential doses, geometric mean titres and reverse cumulative distributions of titres. Inter-subject variability in the immune response should also be reported.

In endemic areas it will be important to obtain some data on safety and immunogenicity of the new JE vaccine in subjects who are already seropositive due to previous doses of other JE vaccines and/or due to natural exposure to JE virus. This is because routine or emergency (i.e. outbreak control) vaccination programs do not determine the serostatus of individuals before vaccination. Therefore some studies should plan to enroll and vaccinate subjects who are already seropositive. Analyses that include data from all vaccinated persons regardless of baseline serostatus and which compare responses between previously seronegative and seropositive cohorts should be planned. Depending on the study design and objectives immune responses may also be compared between subjects of various ages and/or with certain other demographic characteristics.

In persons who are seropositive at baseline (i.e. have $PRNT_{50}$ titres ≥ 1:10) the primary assessment of immune responses to vaccination would usually be based on proportions achieving substantial increases (e.g. at least a 4-fold rise) in titre after one or more doses.

After completion of what is considered to constitute a primary course of vaccination it is critical that assessment of antibody persistence is planned. Therefore protocols should include appropriate long-term serological follow-up in at least selected cohorts of subjects. Generally it would be expected that subjects should be followed for a minimum of 2 years and ideally up to five years after completion of the primary series. In endemic areas, antibody persistence may reflect past vaccination as well as natural boosting due to exposure to JE virus and/or other flaviviruses. Therefore, antibody persistence data should not be extrapolated to non-endemic areas or to other endemic areas with much lower or higher risk of exposure to flaviviruses.

Antibody persistence data should be used to guide the need for and response to booster doses. However, it may also be useful to pre-plan for administration of a booster dose to selected cohorts at specified times post-primary. The timing of booster doses may be based on currently approved vaccines. Pre- and post-boost antibody responses and post-boost follow-up are important elements of the overall assessment and will provide evidence of past priming with the new JE vaccine.

C.2.3 Dose and schedule

Based on past experience with inactivated JE vaccines it is anticipated that more than one dose will be needed to achieve and maintain protection. As with all vaccines it is important that sufficient immunogenicity data should be generated to support the dose of antigen chosen, number of doses and dose intervals. However, it is accepted that there are limitations on the number of possible regimens that can realistically be explored and so some degree of justification for the regimen chosen based on available vaccines may be acceptable.

As a minimum, it is important that an appropriate schedule is identified for children in endemic areas taking into account the recommended age from which vaccination should commence. If the vaccine is proposed for travelers from non-endemic areas, who are very likely to be non-immune, different primary vaccination schedules may have to be explored. For example, it may be important to study accelerated immunization schedules for persons who have to travel at very short notice.

As mentioned in *C.2.2* the assessment of the need for an optimal timing of booster doses should be built into the overall clinical development plan. However, as with other vaccines, it is commonly possible to gain an initial marketing authorization without specific data on antibody persistence and responses to booster doses and to modify the prescribing information at a later date whenever sufficient data become available.

C.2.4 Comparative immunogenicity studies

The clinical development programme for a novel JE vaccine should include at least one study in which the immune response is compared between the candidate and a licensed and widely used JE vaccine. Preferably, these comparisons should be made in seronegative persons since such studies would be more sensitive for detection of any real differences between vaccines.

In some instances it may be useful or necessary to perform studies against more than one licensed product depending on the regions where subjects are enrolled and the JE vaccines that are available. If more than one comparative vaccine is employed in the same study then the protocol should pre-determine whether the primary analysis should compare the new vaccine with the pooled comparative vaccines or with individual comparative vaccines. Each of these study designs raises some potentially complex statistical issues and expert advice is warranted before finalizing the protocol and analysis plan.

The comparison between immune responses to the candidate and to the licensed vaccine should be assessed against the respective vaccine strains. Immune responses to heterologous strains should also be assessed. The selection of the primary immune parameter should take into consideration the points made in section *C.2.2*. Whatever is chosen as the primary parameter the margin of non-inferiority will need very careful justification and published guidance should be consulted along with expert statistical

input. In addition, protocols should plan for secondary analyses based on examination of a full range of immune response parameters.

Although provision of at least one comparative study would be expected it is recognized that in some countries there is no licensed JE vaccine and in others the comparative vaccine or vaccines that are chosen for study may not be licensed. Therefore in these countries the regulatory approach to the data from such studies may not be the same as in the countries in which at least one of the selected comparative vaccines is licensed. As a result, regulators may place less emphasis on the demonstration of non-inferiority and relatively more reliance on the immune response to the new vaccine especially in relation to $PRNT_{50}$ titres.

C.2.5 Concomitant vaccinations

As with all vaccines a specific endorsement in the prescribing information for co-administration with another vaccine should be supported by clinical data (see the WHO guidelines on regulatory expectations for the clinical evaluation of vaccines (***4***).

However, special considerations would arise if it is proposed that a new JE vaccine could be co-administered with a vaccine against another flavivirus. Yellow fever vaccines are widely available and used and vaccines against dengue and West Nile fever are in development. There are some overlaps in endemic areas between each of these diseases and JE. The effects of co-administration of antigens from closely related flaviviruses on safety and immunogenicity cannot easily be predicted. Therefore special care may be needed when considering such investigations.

C.3 Safety

The general approach to the assessment of safety of a new JE vaccine during clinical studies should be in accordance with the WHO guidelines on regulatory expectations for the clinical evaluation of vaccines (***4***).

Matters that require particular attention for JE vaccines include:

- Reactogenicity with sequential doses in the primary series and with boosters administered at different intervals;
- Comparisons of reactogenicity between sub-populations with or without pre-existing antibody to JE virus and/or other flaviviruses as a result of natural exposure; and
- Comparisons of reactogenicity between persons who have or have not been exposed to other JE vaccines and/or other flavivirus vaccines in the past.

The latter two issues are especially important for actual use since pre-vaccination serostatus and the vaccination history may be unknown or uncertain.

C.4 Post-licensure investigations

C.4.1 Effectiveness

Because it is not feasible to study the protective efficacy of a new JE vaccine before initial licensure, it is highly desirable that plans should be made to assess its effectiveness by disease surveillance after its introduction into a vaccination programme. However, the following issues need to be taken into consideration:

- Unless a specific JE vaccine were to be the only such product used in a country or region then the overall effectiveness measured will not be product-specific but "campaign-specific";
- The effectiveness of JE vaccines in a country or region may be heavily influenced by pre-existing immunity in the population whether from natural exposure or previous vaccination. Therefore the findings may not necessarily be extrapolated between regions;
- It is not likely possible or appropriate for manufacturers to conduct studies to estimate vaccine effectiveness since coordinated national or regional public health networks and infrastructures are necessary to ensure that cases are reliably detected. However, manufacturers should discuss arrangements for ongoing disease surveillance and the potential for estimating effectiveness with the relevant NRAs in the countries where the new vaccine is to be used and where reliable surveillance systems are in place; and
- Effectiveness data should be used in conjunction with data on antibody persistence to identify the need for and timing of booster doses

C.4.2 Safety

The general considerations for safety surveillance and for development of a pharmacovigilance plan are the same as for all other types of medicinal products. It is particularly important that data are collected on any vaccine failures.

If particular issues arise during pre-licensure studies or during post-licensure safety surveillance then it may be necessary to conduct specific post-licensure safety studies and/or to put in place a scheme for enhanced surveillance of specific adverse events.

C.4.3 Studies to support change in manufacturing process

Changes in production methods and/or vaccine formulation may sometimes require the provision of a comparative clinical study. Such studies would usually compare the safety and immunogenicity of the "new" versus ″previous" vaccine. The need for, and the design of, a clinical study intended to support the proposed change should be evaluated on a case by case basis after a careful assessment of the data provided by a manufacturer. For this reason, it is recommended that relevant national regulatory authorities should be consulted regarding all changes prior to their implementation since this would enable an early appraisal of the likely need for clinical data to be generated. The design of a clinical study to support a change will depend on the primary objective. In most instances it is likely that the primary or co-primary objective would be to demonstrate that the immune responses to the "new" vaccine are non-inferior to those elicited by the "previous" vaccine. Further details on demonstrating non-inferiority are described in the *Guidelines on clinical evaluation of vaccines: regulatory expectations* (***4***).

C.4.4 Studies to support new dosing schedules and a new population

In general any proposed modifications of the mode of use of a vaccine after initial licensure would require provision of suitable clinical data. Examples include endorsements for use in immunosuppressed, elderly and premature populations. In these cases it is usual to perform a comparative safety and immunogenicity study to compare vaccination of the population of interest with vaccination of the population in which the vaccine is already approved. In the case of adding recommendations for booster dose(s)

antibody persistence data and post-licensure effectiveness data may indicate the need for and optimal timing of additional doses.

Part D. Recommendations for national regulatory authorities

D.1 General

The general recommendations for national regulatory authorities provided in the *Guidelines for national authorities on quality assurance for biological products* (***24***) should be followed. These specify that no new biological substance should be licensed until consistency of production has been established.
The detailed production and control procedures as well as any change in them that may affect quality, safety and efficacy of JE vaccine should be discussed with and approved by the national regulatory authority.

D.2 Release and certification

A vaccine lot should be released only if it fulfils Part A of these Recommendations. Before any vaccine lot is released from a manufacturing establishment, the recommendations for consistency of production provided in the *Guidelines for national authorities on quality assurance for biological products* (***24***) should be met.

A statement signed by the appropriate official of the national regulatory authority/national control laboratory should be provided and should certify whether or not the lot of vaccine in question meets all national requirements, as well as Part A of these Recommendations. The certificate should also state the lot number, the number under which the lot was released, and the number appearing on the labels of the containers. In addition, date of the last satisfactory potency test as well as assigned expiry date on the basis of shelf life should be stated. A copy of the official national release document should be attached.

> The purpose of the certificate is to facilitate the exchange of JE vaccine between countries.

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Appendix 1: Model summary protocol for Japanese encephalitis vaccine (inactivated) for human use

The following protocol is intended to provide general guidance, and indicates the information that should be provided as a minimum by the manufacturer to the national regulatory authority. The protocol should be accompanied by a lot release certificate from the licensing authority which may or may not be the country of manufacturing origin. Information and tests may be added or deleted as required by the national regulatory authority of the importing country, if applicable.

It is thus possible that a protocol for a specific product may differ in detail from the model provided. The essential point is that all relevant details demonstrating compliance with the license and with the relevant WHO guidance of a particular product should be given in the protocol submitted.

The section concerning the final product should be accompanied by a sample of the label and a copy of the leaflet that accompanies the vaccine container. If the protocol is being submitted in support of a request to permit importation, it should also be accompanied by a lot release certificate from the national regulatory authority of the country in which the vaccine was produced stating that the product meets national requirements as well as Part A of these WHO Recommendations.

It is important to note that satisfactory test results do not necessarily imply that the vaccine is safe and effective, since many other factors should be taken into account, including the characteristics of the manufacturing facility.

1. Summary information on the finished product (final lot)

International name: _____
Trade name: _____
Batch number(s): _____
Type of container:
Total number of containers in this batch: _____
Number of doses per container: _____
Composition (antigen concentration) /
Volume of single human dose: _____
Target group: _____
Date of expiry: _____
Storage temperature: _____
Product license number: _____
Name and address of manufacturer: _____
Name and address of product license holder
(if different): _____

2. Production information

Purified bulk
Site of manufacture: _____
Date of manufacture _____

Final bulk
Site of manufacture: _____
Date of manufacture _____

Finished product
Site of manufacture: _____
Date of manufacture: _____

3. Cell banks and virus seeds

The information requested below is to be presented on each submission. Full details on cell banks and virus seed lots should be provided upon first submission only and whenever a change has been introduced.

3.1 Cell banks

Origin of cell substrate _____

Master cell bank (MCB)
Lot number: _____
Date of preparation: _____
Population doubling level: _____

Manufacturer's working cell bank (MCWB)
Lot number: _____
Date of preparation: _____
Population doubling level: _____

Identification of cell substrate
Method: _____
Specification: _____
Date: _____
Result: _____

Nature and concentration of antibiotics or selecting agent (s) used in production cell culture maintenance medium: _____

Identification and source of starting materials used in preparing production cells including excipients and preservatives (particularly any materials of human or animal origin e.g. albumin; serum): _____

3.2. Virus seed

Strain name and short description of history: _____

Master seed lot
Lot number: _____
Date of preparation: _____

Working seed lot

Lot number: _____
Date of preparation: _____

Number of passages between master and working virus seed lots: _____

Number of subcultures between working seed lot and production _____

3.2.1 Test for each seed lot

Identity
Method: _____
Specification: _____
Date: _____
Result: _____

Bacteria and fungi
Method: _____
Media: _____
Volume inoculated: _____
Date (test on –off): _____
Result: _____

Mycoplasmas
Method: _____
Media: _____
Volume inoculated: _____
Date (test on – off): _____
Result: _____

Adventitious agents
Method: _____
Specification: _____
Date: _____
Result: _____

Additional tests e.g. virus titration
Method: _____
Specification: _____
Date: _____
Result: _____

4. Control cell cultures

Provide information on control cells corresponding to each single harvest.

Ratio or proportion of control to production cell cultures: _____
Volume of control cells: _____
Period of observation of cultures: _____
Percentage rejected for non-specific reasons: _____

Result: _____

Identity test by DNA finger printing (if applicable)
Method: _____
Probe: _____
Reference cells: _____
Restriction enzymes: _____
Date (test on – off): _____
Result: _____

Test for haemadsorbing viruses
Type(s) of RBC: _____
Storage time and temperature of RBC: _____
Incubation time and temperature of RBC: _____
% cultures tested: _____
Date (test on – off): _____
Result: _____

Tests on supernatant fluids for other adventitious agents (if relevant)
Date of sampling from production cell cultures: _____

- Name of production cell: _____
  Quantity of sample inoculated: _____
  Incubation temperature: _____
  Date (test on – off): _____
  % of viable culture at the end: _____
  Result: _____

- Name of human cells: _____
  Quantity of sample inoculated: _____
  Incubation temperature: _____
  Date (test on – off): _____
  % of viable culture at the end: _____
  Result: _____

- Name of other sensitive cells: _____
  Quantity of sample inoculated: _____
  Incubation temperature: _____
  Date (test on – off): _____
  % of viable culture at the end: _____
  Result: _____

5. Single harvests (or pools)

Batch Number(s): _____
Date of inoculation: _____
Date of harvesting: _____
Volume(s), storage temperature, storage time and approved storage period: _____

Pooling of single virus harvest
Number of single harvests pooled: _____

Volume of pooled bulk material: _____

Bacteria and fungi
Method: _____
Media: _____
Volume inoculated: _____
Date (test on –off): _____
Result: _____

Identity of vaccine virus
Method: _____
Specification: _____
Date: _____
Result: _____

Virus content
Method: _____
Specification: _____
Date: _____
Result: _____

Consistency of yield
Method: _____
Reference preparation: _____
Specification: _____
Date: _____
Result: _____

6. Purified bulk

Batch number(s) of inactivated, purified bulk: _____
Date(s) of purification(s) and/or inactivation: _____
Volume(s), storage temperature, storage time
and approved storage period: _____

6.1 Inactivation
Agent and concentration: _____
Temperature: _____
Period of inactivation _____
Date of start of inactivation: _____
Date of completion of inactivation: _____

Test for effective inactivation
Method: _____
Specification: _____
Date: _____
Result: _____

6.2 Purification of virus
Method of purification: _____
Concentration: _____

6.3 Tests on purified bulk

Myelin basic protein content (if applicable)
Method: _____
Specification: _____
Date: _____
Result: _____

Protein content
Method: _____
Specification: _____
Date: _____
Result: _____

Antigen content
Method: _____
Specification: _____
Date: _____
Result: _____

Ratio of antigen : protein content
Specification: _____
Result: _____

Residual DNA (if applicable)
Method: _____
Specification: _____
Date: _____
Result: _____

Residual animal serum
Method: _____
Specification: _____
Date: _____
Result: _____

Residual chemical(s)
Method: _____
Specification: _____
Date: _____
Result: _____

7. Final bulk

Batch number: _____
Date of manufacture: _____

Batch numbers and volumes of purified bulk vaccines used for the formulation of the final bulk vaccine: _____

Batch number(s) and volume(s) of bulk alum diluent (if applicable): _____

Volume, storage temperature, storage time and approved storage period: _____

Preservatives and other substances – name and concentrations: _____

7.1 Tests on final bulk

Bacteria and fungi
Method: _____
Media: _____
Volume inoculated: _____
Date (test on –off): _____
Result: _____

Adjuvants (if applicable)
Method: _____
Specification: _____
Date: _____
Result: _____

Degree of adsorption (if applicable)
Method: _____
Specification: _____
Date: _____
Result: _____

Preservative content
Method: _____
Specification: _____
Date: _____
Result: _____

Potency
Species, strain, sex and weight specifications: _____
Dates of vaccination, bleeding: _____
Date of assay of each type: _____
Batch number of reference vaccine and assigned potency: _____
Vaccine doses (dilutions) and number of animals responding at each dose for each type: _____
Specification: _____
$PRNT_{50}$ or ED_{50} of reference and test vaccine for each type: _____
Potency of test vaccine versus reference vaccine for each type with 95% fiducial limits of mean: _____

8. Final lot

Batch number: _____

Date of filling: _____
Type of container: _____
Filling volume: _____
Number of containers after inspection: _____

Inspection of final container (appearance)
Method: _____
Specification: _____
Date: _____
Result: _____

Identity
Method: _____
Specification: _____
Date: _____
Result: _____

Bacteria and fungi
Method: _____
Media: _____
Volume inoculated: _____
Date (test on –off): _____
Result: _____

pH
Method: _____
Specification: _____
Date: _____
Result: _____

Osmolarity
Method: _____
Specification: _____
Date: _____
Result: _____

General safety (abnormal toxicity)
Method: _____
Specification: _____
Date: _____
Result: _____

Protein content
Method: _____
Specification: _____
Date: _____
Result: _____

Residual DNA (if applicable)
Method: _____
Specification: _____
Date: _____
Result: _____

Potency

Species, strain, sex and weight specifications: _____
Dates of vaccination, bleeding: _____
Date of assay of each type: _____
Batch number of reference vaccine and assigned potency: _____
Vaccine doses (dilutions) and number of animals responding at each dose for each type: _____
Specification: _____
$PRNT_{50}$ or ED_{50} of reference and test vaccine for each type: _____
Potency of test vaccine versus reference vaccine for each type with 95% fiducial limits of mean: _____

Accelerated thermal stability

Method: _____
Specification: _____
Date: _____
Result: _____

Residual moisture (if applicable)

Method: _____
Specification: _____
Date: _____
Result: _____

Pyrogenic substances

Method: _____
Specification: _____
Date: _____
Result: _____

Residual animal serum albumin

Method: _____
Specification: _____
Date: _____
Result: _____

Preservatives

Method: _____
Specification: _____
Date: _____
Result: _____

Adjuvant content (if applicable)

Method: _____
Specification: _____
Date: _____
Result: _____

Degree of adsorption (if applicable)
Method: _____
Specification: _____
Date: _____
Result: _____

Extractable volume (if applicable)
Method: _____
Specification: _____
Date: _____
Result: _____

Freezing point (if applicable)
Method: _____
Specification: _____
Date: _____
Result: _____

Other tests

Additional comments (if any)

A sample of a completed final container label and package insert should be attached.

9. Certification

9.1 Certification by producer

Certification by head of the quality assurance department taking overall responsibility for production and control of the final vaccine:

I certify that lot no . . . of Japanese encephalitis vaccine (inactivated) for human use, whose number appears on the label of the final container, meets all national requirements[1] and satisfies Part A of the WHO *Recommendations for Inactivated Japanese Encephalitis Vaccine for Human Use.*

Signature: _____
Name (typed): _____
Date: _____

9.2 Certification by the national controller

If the vaccine is to be exported, please provide a copy of the certificate from the national regulatory authority as described in section D.2 and by referring to the model certificate in Appendix 2, together with a label of a final container, and a leaflet of instructions to users.

[1] If any national requirement(s) is (are) not met, specify which one(s) and indicate why release of the lot has nevertheless been authorized.

Appendix 2: Model certificate for the release of Japanese encephalitis vaccine (inactivated) for human use

Certificate Nº _____

LOT RELEASE CERTIFICATE

The following lot(s) of Japanese encephalitis vaccine (inactivated) for human use produced by _____[1] in _____[2], whose numbers appear on the labels of the final containers, meet all national requirements[3] and Part A[4] of the WHO *Recommendations for Inactivated Japanese Encephalitis Vaccine for Human Use* (_____)[5], and comply with *Good Manufacturing Practices for Pharmaceutical Products: Main Principles*[6] and *Good Manufacturing Practices for Biological Products*[7].

As a minimum, this certificate is based on examination of the summary protocol of manufacturing and control.

Final Lot No.	No. of released human doses in this final lot	Expiry date
_____	_____	_____

The Director of the National Regulatory Authority (or Authority as appropriate):

Name (Typed): _____
Signature: _____
Date: _____

[1] Name of manufacturer
[2] Country of origin
[3] If any national requirements are not met, specify which one(s) and indicate why release of the lot(s) has nevertheless been authorized by the national regulatory authority
[4] With the exception of provisions on distribution and shipping, which the national regulatory authority may not be in a position to assess.
[5] WHO Technical Report Series, No.____, YYYY, Annex__.
[6] WHO Technical Report Series, No. 908, 2003, Annex 4.
[7] WHO Technical Report Series, No. 822, 1992, Annex 1.

Appendix 3: General scheme for the preparation of Japanese encephalitis vaccines (inactivated)

Stage	Procedures	Tests
Single harvest (1 production run) • Cell culture vaccine • Mouse brain vaccine	— Inactivation	• Sterility • Identity • Virus content
Purified bulk (1 or more pooled harvests) • Cell culture vaccine (&) • Mouse brain vaccine (*)	Filtration or continuous centrifugation Inactivation Purification Purification	• Inactivation • Myelin basic protein* • Protein content • Antigen content • Residual DNA& • Residual animal serum& • Residual chemicals
Final bulk (1 or more pooled purified bulks)	Addition of preservatives and stabilizers	• Sterility • Adjuvant content if applicable • Preservative content • Potency
Final lot	Filling	• Inspection of final containers • Identity • Sterility • pH • Osmolarity • General safety • Protein content • Residual DNA& • Potency • Accelerated stability • Residual moisture • Pyrogenicity • Residual animal serum& • Preservative content • Adjuvant content if applicable

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The following pages are not the essential part of the document for publication.

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