Annex 4

PROPOSED REQUIREMENTS FOR RIFT VALLEY FEVER VACCINE (INACTIVATED) FOR HUMAN USE

(Requirements for Biological Substances No. 32)

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GENERAL CONSIDERATIONS

Specific vaccines are probably the most commonly used tools for the control of Rift Valley fever (RVF). Evidence shows that effective vaccines will protect animals and humans against illness, protect pregnant female livestock populations against abortion, and will remove the vaccinated animal from the pool of potential amplifying hosts of the virus. It is reasonable to assume that the widespread use of effective vaccines in the susceptible animal populations would eliminate the possibility of occurrence of a major RVF epizootic by reducing the number of susceptible animals. Thus, there exists a vital role for veterinary vaccines in immunizing animals, both within and adjacent to known areas of RVF endemicity. Nevertheless, in the absence of a soliutely immunized livestock population, there may be a high level of virus transmission to man.

The need for immunization of humans against RVF in the classic areas of endemicity and epizootic activity is limited. Little or no exposure occurs except in persons closely associated with livestock. This would generally include herders, veterinarians, and slaughterhouse workers. However, the extension of RVF into Egypt in 1977 demonstrated the devastating public health potential of this virus when it
occurs in animal populations living in close association with man in relatively high density. Members of field epidemiological teams as well as medical staff of all hospitals may also benefit from the use of vaccine. In view of the potential need to immunize large numbers of people in such areas against this disease, requirements for inactivated RVF vaccine for use in humans have been formulated.

As with the requirements for vaccines that have been formulated or revised during the last five years, a protocol for reporting the results of all tests has been included (see Appendix). Completion of such a protocol, which is based on the present requirements, enables a control authority to check whether all the tests have been completed.

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

Should individual countries wish to adopt these requirements as the basis of their national regulations concerning RVF vaccine (inactivated), it is recommended that a clause should be included permitting modifications of manufacturing requirements on the condition that it can be demonstrated, to the satisfaction of the national control authority, that such modified requirements ensure that the degree of safety and the potency of the vaccine are at least equal to those provided by the requirements formulated below. The World Health Organization should then be informed of the action taken.

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

**PART A.**

**MANUFACTURING REQUIREMENTS**

1. **DEFINITIONS**

1.1 **International name and proper name**

The international name shall be “Vaccinum febris Rift Valley inactivatum (ad usum humanum)”. The proper name shall be the
equivalent of the international name in the language of the country of origin. The use of the international name should be limited to vaccines that satisfy the requirements formulated below.

1.2 Descriptive definition

Vaccinum febris Rift Valley inactivatum (ad usum humanum) shall consist of an aqueous suspension of Rift Valley fever virus grown in cell cultures and inactivated. The preparation shall satisfy all the requirements formulated below.

1.3 Terminology

Virus seed lot. A quantity of virus processed together and of uniform composition. In each manufacturing establishment a primary virus seed lot is that from which further virus seed lots are prepared. Vaccine is one passage removed from the seed lot, i.e., two passages from the primary seed lot.

Cell seed. A quantity of cells derived from normal tissue and stored frozen at −70°C or below in aliquots, one or more of which would be used for the production of a manufacturer’s working cell bank (MWCB).

Manufacturer’s working cell bank (MWCB). All of the cellular material derived by serial subculture from one or more ampoules of cell seed. After serial subculture the cells are combined into a pool and preserved cryogenically to form the MWCB. One or more ampoules from a pool would be used for the production of a single harvest.

Single harvest. A virus suspension harvested from cell cultures that has been derived from a single batch of cells and processed together.

Single pool. A virus suspension processed at the same time.

Purified and concentrated pool. A purified and concentrated virus suspension or vaccine processed at the same time.

Final bulk. The finished biological preparation present in the container from which the final containers are filled.

Filling lot (final lot). A collection of sealed final containers that are homogeneous with respect to the risk of contamination during filling. A filling lot must, therefore, have been filled in one working session from a single final bulk.
Tissue culture infective dose 50% (TCID₅₀). The smallest quantity of a virus suspension that will infect 50% of inoculated cell cultures.

Plaque-forming unit (PFU). The smallest quantity of a virus suspension that will produce a plaque in cell cultures.

2. GENERAL MANUFACTURING REQUIREMENTS

The general manufacturing requirements contained in the revised Requirements for Biological Substances No. 1 (General Requirement for Manufacturing Establishments and Control Laboratories) (1) shall apply to establishments manufacturing Rift Valley fever (RVF) vaccine (inactivated), with the addition of the following directives:

Production areas shall be decontaminated before they are used for the manufacture of RVF vaccine.

The production of RVF vaccine shall be conducted by staff who have not handled other infectious microorganisms or animals on the same working day. The staff shall consist of persons who shall be examined medically and found to be healthy. Steps shall be taken to ensure that all such persons in the production areas are immune to RVF virus.

No culture of any microorganisms or cells other than the cultures approved by the national control authority for the production of RVF vaccine shall be introduced or handled in the production area at any time during manufacture of the vaccine.

Persons not directly concerned with the production processes shall not be permitted to enter the production area.

3. PRODUCTION CONTROL

3.1 Control of source materials

3.1.1 Virus strains and seed lot system

The strains of RVF used in the production of vaccine shall be approved by the national control authority. They shall be identified by historical records, by infectivity tests, and by immunological methods. Any strain that will yield a vaccine meeting the requirements set forth in the present document may be used. Production of vaccine shall be based on the seed lot system; the seed virus used for vaccine

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production shall not have passed more than 10 subcultures, counted from a seed lot used for the production of the vaccine on which the original laboratory and field tests were done.

Samples of the strain used shall be deposited in the national control laboratory.

3.1.2. Cell cultures for virus propagation

3.1.2.1 Monkeys used for obtaining tissues for cell cultures. If monkey cell cultures, such as fetal lung cultures, are used, monkeys that are in good health and of a species approved by the national control authority, and that have not previously been used for experimental purposes, shall be used as the source of tissue for the production of virus.

The monkeys shall be kept in well-constructed animal rooms in adequately ventilated cages spaced as far apart as possible. Adequate precautions shall be taken to prevent cross-infection between cages. Not more than 2 monkeys shall be housed in each cage and cagemates shall not be interchanged. The monkeys shall be kept in the country of manufacture of the vaccine in quarantine groups\(^3\) for a period of not less than 6 weeks before use. If at any time during the quarantine period the overall death rate of a shipment consisting of one or more groups reaches 5% (excluding deaths from accidents or causes specifically determined not to be due to infectious diseases) monkeys from that entire shipment shall continue in quarantine from such time for a further period of not less than six weeks.

Only monkeys that have reacted negatively to tuberculin at the start of the quarantine period and again within 2 weeks prior to use shall be used in the manufacture of vaccine. The groups shall be kept continually in isolation, as in quarantine, even after completion of the quarantine period, until the monkeys are used or discarded. After the last monkey of a group has been taken, the room that housed the group shall be thoroughly cleaned and decontaminated before being used for a fresh group.

In some countries the test for sensitivity to tuberculosis in monkeys may be omitted because the monovalent pools are subjected to 2 stages of filtration through 0.22-µm filters.

\(^3\) A quarantine group is a colony of selected, healthy monkeys kept in one room, with separate feeding and cleaning facilities, and having no contact with other monkeys during the quarantine period.
All actions of working personnel shall be based on the premise that there is a great potential hazard at all times in the quarantine area. Personnel shall be provided with protective clothing, including gloves, footwear, and masks or visors. Street clothes shall not be permitted in the animal rooms. Personnel shall be forbidden to smoke, eat, or drink while they are in the animal rooms.

Responsibility shall be assigned to a supervisor for reporting illness among employees and ensuring that all injuries are properly treated. No worker who has cuts or abrasions on exposed areas of the body shall enter the animal area. Any unexplained febrile illness while off duty shall be considered as potentially related to the employee's occupation.

Monkeys from which tissues are to be removed shall be thoroughly examined at necropsy, particularly for evidence of tuberculosis or simian herpesvirus (B virus) infection. Monkeys prepared for removal of tissues shall be anaesthetized.

A monkey that shows any pathological lesion relevant to the use of its tissues in the preparation of a seed lot or vaccine shall not be used, and none of the remaining monkeys of the quarantine group concerned shall be used unless it is evident that their use will not impair the safety of the product.

All operations described in this section shall be conducted outside the areas where vaccine is made.

If the monkey cells are passaged in series, the number of cell doublings permitted before the growth of the RVF virus shall be approved by the national control authority.

It has been shown that a "primary" cell culture may have undergone about 5 cell doublings, "secondary" cells about 10 cell doublings and "tertiary" cells about 15 doublings. Preferably, records of the cell doublings should be kept. It has been shown also that because of their decreased exposure to other animals, monkeys bred in captivity may have fewer extraneous agents than monkeys caught in the wild. If the cells are passaged in series, therefore, monkeys bred in captivity may be preferred.

In some countries karyology is also required. The extent of such tests should be determined by the national control authority.

3.1.2.2 Cell banks used for providing cell cultures. If human or monkey diploid cells are used for the propagation of RVF virus they shall be those approved by and registered with the national control authority. The cells shall have been characterized with respect to their genealogy, growth characteristics, viability during storage, and karyology.
They shall have been shown, by tests in animals and eggs, to be free from detectable adventitious agents and the requirements for human or monkey diploid cells used for the production of vaccine (Part C) shall apply.

If continuous cell lines are used for the propagation of RVF virus they shall be those approved by and registered with the national control authority. The requirements for the use of a non-tumorigenic continuous cell, such as VERO cells, (Part D) shall apply.

If the diploid cells are serially passaged, then the cells at the production level shall be tested for tumorigenicity (see Part C, section 3.1.2).

3.1.3 *Cell culture medium*

Serum used for the propagation of cells for vaccine production shall be tested to demonstrate freedom from bacteria, fungi, and mycoplasma, according to the requirements given in Part A, sections 5.2 and 5.3 of the revised Requirements for Biological Substances No. 6 (General Requirements for Sterility of Biological Substances) (2) as well as freedom from pathogens of the species of the origin of the serum by methods approved by the national control authority.

In some countries sera are examined for freedom from phage.

Penicillin shall not be used at any stage in the production.

Suitable other antibiotics in minimum concentrations required for sterility may be used. Nontoxic pH indicators may be added, e.g., phenol red in a concentration of 20 mg/l.

3.1.4 *Tests on virus seed lots*

Each virus seed lot shall be subjected to all tests applicable to a single pool (see part A, sections 3.2, 3.4.1, 3.4.2, 3.4.4, 3.4.5, and 3.4.6). The national control authority shall approve the virus seed lots.

If the virus for vaccine production has been isolated or passaged in mice, tests for murine viruses should be included.

3.2 *Control cells for vaccine prepared in monkey cells*

A cell sample equivalent to at least 1% of the cell suspension, at the concentration employed for vaccine production cultures, shall be used to prepare control cell cultures.
In some countries in which the technology of large-scale production has been developed the national control authority should determine the size of the sample of cells to be examined, the time at which the control cells should be taken from the production culture, and the monitoring of the control vessels.

These control cell cultures shall be incubated for at least 2 weeks and shall be examined during this period for evidence of cytopathic changes. For the test to be valid, not more than 20% of the control cell cultures may be discarded for nonspecific, accidental reasons.

At this time, the control cell cultures shall be examined for any evidence of degeneration caused by an extraneous agent. If human or monkey diploid cells or continuous cell lines are used, tests on control cells shall be carried out according to the requirements described in Part C, section 3.2 or Part D, section 3.2, respectively. If primary or subcultured monkey cells are used, the control cells and the supernatant fluid from such cells shall also be tested for extraneous agents by the tests described in Part A, sections 3.2.1, 3.2.2, and 3.2.3. If this examination or any of the tests required in this section show evidence of the presence in a control culture of any adventitious agent, the RVF virus grown in the corresponding inoculated cultures shall not be used for vaccine production.

In some countries in which primary cell cultures are prepared from the tissues of monkeys caught in the wild, "foamy virus" is an inherent contaminant. Such cultures may be used for vaccine manufacture at the discretion of the national control authority.

3.2.1 Tests for haemadsorbing viruses

At the end of the observation period or at the time the virus is harvested from the production cultures, whichever is the later, 25% of the control cells shall be tested for the presence of haemadsorbing viruses using guinea-pig red cells. If the guinea-pig red cells have been stored, the duration of storage shall not have exceeded 7 days, and the temperature of storage shall have been in the range of 2–8°C.

This test is usually made using guinea-pig red cells. In some countries the national control authority requires that tests for haemadsorbing viruses should be made in addition using other types of red cells, including those from humans (blood group O), monkeys, and chickens (or other avian species).

All tests should be read after incubation for 30 min at 0–4°C and again after a further incubation for 30 min at 20–25°C. The test with monkey red cells should be read once more after yet another incubation for 30 min at 34–37°C.
3.2.2 Tests in rabbit kidney cell cultures

A sample of at least 10 ml of the pooled supernatant fluid from the control cultures shall be tested for the presence of simian herpesvirus (B virus) and other viruses in rabbit kidney cell cultures. Serum used in the nutrient medium of these cultures shall have been shown to be free from B virus inhibitors. The sample shall be inoculated into bottles of these cell cultures, in such a way that the dilution of the harvest suspension in the nutrient medium does not exceed 1 in 4. The area of the cell sheet shall be at least 3 cm²/ml of harvest suspension. At least one bottle of the cell cultures shall remain uninoculated and serve as a control.

The cultures shall be incubated at a temperature of 37°C and shall be observed for a period of at least 2 weeks.

For the test to be valid at least 80% of the culture vessels shall be available and suitable for evaluation at the end of the test period.

If the presence of B virus is demonstrated the manufacture of RVF vaccine shall be discontinued and the national control authority shall be informed. Manufacturing shall not be resumed until a thorough investigation has been completed and precautions have been taken against reappearance of the infection, and then only with the approval of the national control authority.

3.2.3 Test in Cercopithecus kidney cell cultures

A sample of at least 10 ml of the pooled supernatant fluid of the control cultures shall be tested for the presence of SV40 virus and other extraneous agents by inoculation on to cell cultures prepared from the kidneys of Cercopithecus monkeys by the method described in Part A, section 3.2.2. The cell cultures shall be incubated at 37°C and observed for a period of at least 2 weeks.

A cell culture shown to be equally sensitive to SV40 virus may be used.

For the test to be valid, at least 80% of the culture vessels shall be available and suitable for evaluation at the end of the observation period.

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2 On account of the danger of handling B virus, herpes simplex virus, has been used as the indicator for freedom from B virus inhibitors.
If there is any cytopathic effect attributable to the supernatant fluid, the virus grown on the same batch of cells shall not be used for vaccine production.

3.3 Production precautions

The general production precautions as formulated in Part A, section 3, of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) \(^1\) shall apply to the manufacture of RVF vaccine (inactivated). If animal serum is used for the growth of cell cultures, the serum concentration in the final vaccine shall be not more than 1 \(\mu l/l\). The serum concentration shall be reduced to this level by rinsing the cell cultures with serum-free medium and/or purification of the virus harvests.

In some countries control tests are carried out to detect the residual animal serum content in the final vaccine (see Part A, section 5.5).

3.4 Control at the single harvests

When the tests on the control cells have shown the cells to be satisfactory, the single virus harvest from such cells may be pooled and samples taken for the following tests.

3.4.1 Test in Cercopithecus cell cultures (applied to all seeds and to all harvests grown in monkey cells except for continuous cell lines)

A sample of at least 10 ml of each single pool produced in primary or serially passaged monkey cell culture shall be tested for the presence of SV40 virus or other adventitious agents.

The single pool shall be neutralized by a high-titred antiserum against RVF.

The immunizing antigen used for the preparation of the antiserum should be shown to be free from extraneous agents and grown in cell cultures free from extraneous microbial agents that might elicit antibodies that could inhibit the growth of any adventitious agents present in the RVF virus pool.

This sample shall be tested in primary Cercopithecus kidney cell cultures or cells that have been demonstrated to be of equal sus-
ceptibility to SV40 virus. The tissue cultures shall be incubated at
37°C and observed for 14 days. At the end of this observation period
at least one subculture of fluid shall be made in the same tissue culture
system and both primary cultures and subcultures observed for an
additional 14 days.

If necessary, serum may be added to the primary cultures at
this stage, provided that the serum does not contain SV40 anti-
body or other inhibitors.

The virus pool passes the test if there is no evidence of the presence
of SV40 virus or other adventitious agent attributable to the virus pool.

3.4.2 Test in rabbits (applied to all seeds and to all harvests grown
in monkey cells except for continuous cell lines)

A sample of at least 30 ml of each single pool shall be tested as
soon as possible after pooling by inoculation into three healthy rab-
bits, each weighing between 1.5 and 2.5 kg; proportionately larger
volumes shall be used if more animals are inoculated. The inocula-
tions shall be made at multiple sites, each rabbit being given a total
of 1 ml of the single pool by intradermal injection and 9 ml by sub-
cutaneous injection. The animals shall be observed for at least three
weeks. All rabbits that die after the first 24 h of the test or that show
signs of illness shall be examined by autopsy, with removal of the
brain and organs for detailed inspection.

The single pool passes the test if at least 2 of the rabbits remain
healthy and if none of the rabbits shows lesions of any kind at the
sites of inoculation or shows evidence of infection with B virus or with
any adventitious transmissible agent attributable to the single pool.

In some countries it is permissible to replace the test for B
virus in rabbits by the test in rabbit kidney cell cultures.

3.4.3 Treatment before inactivation

Prior to inactivation each RVF virus pool shall be filtered.

The importance of filtration or clarification of the crude virus
susensions as a means of improving the regularity of the inactiva-
tion process has been clearly established. Generally, filters are
used in series or filtration is performed stepwise through filters of
decreasing porosity. Satisfactory results have been reported with
several filter types but a final filtration of 0.22 μm should be
used.
Filters containing asbestos should not be used.
In some countries the virus suspension is concentrated and purified before inactivation.

For vaccines produced in continuous cell lines the RVF virus pool shall be purified before inactivation by a method approved by the national control authority (see Part D).

3.4.3.1 Purification of the single pools. Each filtered single pool of virus grown in a continuous cell line (see section D) shall be purified before inactivation. The purification process, which shall be shown to reduce consistently the level of cellular DNA from that of the initial virus harvest by a factor of at least $10^8$, shall be approved by the national control authority.

3.4.4 Identity test
The virus in the single pool shall be tested for identity by the neutralization of the virus with specific antisera.

3.4.5 Sterility tests
Each pool after filtration shall be tested for sterility according to the requirements given in Part A, sections 5.1, 5.2 and 5.3, of the Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (2).

3.4.6 Virus titration
Before any inactivating agent is added, a sample shall be taken of each pool for titration of infective RVF using tissue culture methods. This titration shall be carried out in not more than 10-fold dilution steps and using 10 cultures per dilution, or any other arrangement yielding equal precision.

Each pool should show a titre of not less than $10^7$ TCID$_{50}$ per ml using a batch of tissue culture of normal sensitivity.
The main purpose of determining the titre of virus pools destined for inactivation is to select pools which can be expected to meet potency requirements after inactivation.

3.4.7 Time of inactivation
Inactivation shall be initiated as soon as possible and not later than 72 h after filtration.
It is preferable to start inactivation within 24 h of filtration. Since the purpose of the filtration step is to remove particulate matter and other interfering substances that may diminish the effectiveness of the inactivation process, and since aggregates tend to increase on standing after filtration, efforts should be made to keep within this time limit.

3.4.8 Inactivation procedure

The virus in the single pools shall be inactivated through the use of an agent such as formaldehyde or some other method that has been demonstrated to be consistently effective in the hands of the manufacturer. The method shall be approved by the national control authority.

The time of inactivation should be at least 3 times that taken to reduce the virus titre to undetectable levels.

The progress of inactivation shall be followed by suitably spaced determinations of virus titres.

A second filtration during the process of inactivation shall be made.

This step is made after the virus titre has fallen below detectable levels but before the first sample for the safety test is taken.

The method of inactivation shall be shown to give consistent inactivation for the production of acceptable vaccine. A record of consistency shall be established by the production of 5 consecutive lots and if broken a further 5 monovalent lots shall be prepared and shown to be satisfactory to re-establish this record.

3.4.9 Test for effective inactivation

A sample of at least 200 ml or an equivalent volume of concentrated pool diluted to 200 ml shall be taken and, after removal or neutralization of the formaldehyde, if appropriate, shall be tested by inoculation into tissue cultures for the absence of infective RVF virus. The kinetics of inactivation shall be established by each manufacturer and approved by the national control authority. One sample shall be taken at the end of the inactivation period and the other not later than three-quarters of the way through this period. The two samples shall be inoculated into bottles of tissue cultures derived from different batches of cells. The dilution of the vaccine in the nutrient fluid shall not exceed 1:4 and the area of the cell sheet shall be at
least 3 cm²/ml of vaccine. One or more bottles of each batch of cultures shall be set aside to serve as uninoculated control bottles with the same medium.

If formaldehyde is present in samples of vaccine for tissue culture tests, it is usually neutralized at the time of sampling by the addition of bisulfite. Usually, the samples are subsequently dialysed.

It is possible to conduct tissue culture tests on nondialysed material; however, this is often found to be toxic to cells even with a dilution of 1:4. If in such tests non-specific degeneration of cells occurs, or if the sensitivity of the tissue culture system is reduced, the test should be repeated on dialysed material.

In some countries this test is made using tissue cultures that are also sensitive to SV40 virus, as an additional measure for detecting this extraneous agent.

Tissue culture bottles shall be observed for at least 2 weeks. At that time, at least 0.5 ml shall be harvested from each cell culture flask, and this may be pooled with fluids from no more than 4 other flasks. At least one-third of the individual or pooled fluid harvest shall be inoculated by intraperitoneal injection into at least 5 adult mice. The mice shall be observed daily and their condition recorded for a total of 14 days. Mice that become ill or die shall be tested for the presence of RVF virus by inoculating samples from the serum and liver into cell cultures and suckling mice.

At the end of the observation period the cell cultures used for the detection of residual live virus shall be challenged with live RVF virus. The required number of virus particles in the challenge shall be determined by the national control authority.

3.5 Control of bulk products

Only those pools that have been shown to be satisfactory shall be blended to form a bulk.

In some countries a pool from which live virus has been isolated may be subjected to reactivation and tested again for the presence of live virus. In such cases the national control authority should approve the reactivation procedure.

3.5.1 Tests for absence of RVF virus

Before the addition of preservatives, a sample of at least 200 ml or, if purified and concentrated vaccine is prepared, the equivalent of
200 doses shall be tested in cell cultures for the absence of infective virus by the procedure described in Part A, section 3.4.9 of these requirements. If infectious RVF is isolated, this batch of bulk product shall not be used.

3.5.2 Sterility test

The bulk shall be tested for sterility according to the requirements given in Part A, sections 5.1 and 5.2 of the Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (2).

3.5.3 Preservatives and other substances added

Preservatives or other substances that might be added to or combined with vaccine shall have been shown to the satisfaction of the national control authority to have no deleterious effect on the immunizing potency of the product.

4. FILLING AND CONTAINERS

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

The containers shall be of colourless and fully transparent glass so as to permit the detection of visible changes in the vaccine.

Single- and multiple-dose containers may be used.

5. CONTROL TESTS ON FINAL PRODUCT

5.1 Identity test

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

The potency test referred to in Part A, section 5.4, of these requirements may serve as the identity test.
5.2 Sterility tests

Each filling lot shall be tested for sterility according to the requirements given in Part A, sections 5.1 and 5.2, of the Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (2).

5.3 Innocuity test

Each final lot shall be tested for abnormal toxicity by appropriate tests involving injection into mice and guinea-pigs. The tests shall be approved by the national control authority.

5.4 Potency test

Each vaccine shall be tested for immunizing potency by tests approved by the national control authority. Such tests shall include an in vitro assay for antigen content and an in vivo assay for immune response. The potency of the vaccine shall be approved by the national control authority.

5.5 Protein nitrogen content

Rift Valley fever vaccine (inactivated) shall not contain more than 10 mg of protein nitrogen per human dose.

In some countries in vitro tests for serum proteins are included. Counter-current electrophoresis is useful for such tests.

6. RECORDS

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

7. SAMPLES

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

8. LABELLING

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

The leaflet accompanying the package shall include the following information:
(i) the nature of the cell cultures used;
(ii) the virus strain used for the production of the vaccine; and
(iii) the method used for inactivating the virus.

9. DISTRIBUTION AND SHIPPING

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

10. STORAGE AND EXPIRY DATE

10.1 Storage conditions

RVF vaccine (inactivated) shall be stored at all times at a temperature between 2 °C and 8 °C, if in liquid form; the freeze-dried product may alternatively be stored at −20 °C.

10.2 Expiry date

The expiry date shall be fixed with the approval of the national control authority and shall be not more than 18 months after the date of the last satisfactory potency test, the date of a potency test being that date on which the test animals were inoculated with the vaccine. The expiry date shall not, however, be more than 12 months from the date at which the vaccine was issued by the manufacturer.
In some cases the national control authority may decide, on the basis of experimental evidence, to leave the expiry date at 18 months after the date of the last satisfactory potency test, irrespective of the date of issue by the manufacturer.

PART B.
NATIONAL CONTROL REQUIREMENTS

1. GENERAL

The general requirements for control laboratories contained in Part B of the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (I) shall apply with the addition of the following directives:

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

In some countries the potency is tested by measuring the antibody response in man.

2. RELEASE AND CERTIFICATION

RVF vaccine (inactivated) shall be released only if it fulfils Part A of these requirements.

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

The purpose of the certificate is to facilitate the exchange of biological substances between countries.
PART C.
REQUIREMENTS FOR HUMAN OR MONKEY DIPLOID CELLS USED FOR VIRUS VACCINE PRODUCTION

The following requirements are applicable to the cell substrate for virus vaccine production where production is based on a cell seed system. The tests so far have been formulated for human diploid cells but any cell bank and cell seed system shall comply with similar requirements as appropriate.

1. DEFINITIONS

1.1 Terminology

*Cell seed.* A quantity of cells derived from a single human or monkey tissue stored frozen at \(-70\, ^{\circ}\)C or below in aliquots, one or more of which would be used for the production of a manufacturer's working cell bank.

*Manufacturer's working cell bank (MWCB).* A quantity of cells derived from a single ampoule of the cell seed and of uniform composition stored frozen at \(-70\, ^{\circ}\)C or below in aliquots, one or more of which would be used for the production of each single harvest.

In normal practice for human cells such a seed culture (or ampoule) is issued to manufacturers at or near the eighth population doubling level (PDL). This is expanded by serial subculture up to a PDL selected by the manufacturer, at which point the cells are combined into one or more pools and preserved in the frozen state to form the MWCB. One or more of such ampoules from a pool would be used for the production of a single harvest.

*Production cell culture.* A collection of cell cultures at the population doubling used for virus growth that have been derived from a single ampoule of the MWCB.

2. GENERAL MANUFACTURING REQUIREMENTS

The general requirements contained in the revised Requirements for Biological Substances No. 1 (General Requirements for Manufac-
turing Establishments and Control Laboratories) (I, p. 11) shall apply, with the addition of the following directive:

No cell cultures other than those approved by the national control authority for the production of appropriate vaccine shall be introduced or handled in the production area.

3. PRODUCTION CONTROL

3.1 Cell seed

The utilization of human or monkey diploid cell cultures for vaccine manufacture shall be based on the cell seed system. Early population doubling of diploid cell cultures shall be subcultured to a population doubling that is convenient for the preparation of a cell seed.

The cell seed used for the production of virus vaccine shall be that approved by and registered with the national control authority. The accepted cell strain from which the cell seed has been derived shall have been characterized with respect to genealogy, growth characteristics, genetic markers (HLA), virus susceptibility, storage conditions, and karyology, and it shall have been shown, by tests in animals, eggs, and cell culture to be free from detectable adventitious agents.

These data shall be made available to the national control authority. Each manufacturer shall show, to the satisfaction of the national control authority, that the cell substrate propagated from the accepted cell strain and laid down as a working cell bank conforms with the test outlined in this section for freedom from extraneous agents by tests in animals and eggs, for lack of tumorigenicity, for normal karyology throughout approximately the first two-thirds of its normal life-span, and for identity.

In some countries the cells are examined also by ultrathin sections and negative staining under the electron microscope.

3.1.1 Tests in animals and eggs for extraneous agents

The tests in animals for extraneous agents shall include the inoculation of each of the following groups of animals with cells by the intramuscular route, using at least $10^7$ viable cells divided equally between the animals in each group:
2 litters of suckling mice, comprising at least 10 animals, less than 24 h old; 
10 adult mice; 
5 guinea-pigs; and 
5 rabbits.

At least \(10^6\) viable cells shall be injected also into the allantoic cavity of each of 10 embryonated chicken eggs 9–11 days old.

The animals shall be observed for at least 4 weeks and the embryonated chicken eggs shall be examined after not less than 3 days’ incubation. Any animals that are sick or show any abnormality shall be investigated to establish the cause of illness. The allantoic fluids shall be tested with guinea-pig and chick or other avian red cells for the presence of haemagglutinins.

The cells are suitable for vaccine production if at least 80% of the animals or eggs inoculated with the cells remain healthy and survive the observation period and none of the animals or eggs shows evidence of the presence in the cell cultures of any extraneous agent.

3.1.2 Tumorigenicity

The cells at the production level shall also be shown in animals to be free from tumorigenicity by appropriate tests approved by the national control authority.

Particularly for new non-continuous cell strains some control authorities may wish to consider applying the tests for tumorigenicity as outlined in Part D, section 3.1.2. For the diploid cell strains that have been in use for many years, however, the tests suggested below have been shown to be satisfactory.

Suitable tests using immunosuppressed animals may be made as follows. Approximately \(10^4\) viable cells obtained from cultures at the same passage level as those used for vaccine production are injected into (a) newborn mice or hamsters treated with antilymphocyte serum; or (b) athymic mice (nude-nu/nu genotype); or (c) thymectomized mice irradiated and bone-marrow reconstituted (T-B-). Some of the same group of animals should be inoculated with a similar dose of HeLa or KB cells and it should be shown that tumour formation is caused by the inoculation of the neoplastic tissue, thus demonstrating the ability of the strain of animals to give rise to tumours. The animals should be observed for not less than three weeks. Any other test using animals treated with immunosuppressive agents and with equal sensitivity to neoplastic cells may be used.

Only those cell seeds shown not to be tumorigenic shall be used.
3.1.3 Chromosomal characterization and monitoring

3.1.3.1 Chromosomal characterization. At least four samples shall be examined as described in Part C, section 3.1.3.2, at approximately equal intervals over the life-span of the cell line during serial cultivation. Each sample shall consist of 1000 metaphase cells.

It is also recommended that photographic reconstruction should be employed in the preparation of chromosome-banded karyotypes of 50 metaphase cells per 1000 cell sample using either G-banding or Q-banding techniques. This constitutes 5% of the sample and no specific limits for acceptability are yet recommended. The incidence of karyotypic abnormalities (pseudodiploidy, inversions, translocations, etc.) that are detectable with the greater resolution provided by banding should be evaluated when a larger data base than is at present available has been accumulated.

3.1.3.2 Chromosomal monitoring—preparation and testing. For the determination of the general character of each pool in the MWCN, a minimum of 500 cells in metaphase shall be examined at the production level or at any passage thereafter for frequency of polyploidy and for exact counts of chromosomes, frequency of breaks, structural abnormalities, and other abnormalities such as despiralization or marked attenuations of the primary or secondary constrictions.

For vaccine production, examination of the cells is usually made between the twenty-seventh and thirty-third population doubling. The national control authority should determine the level of cell population doubling allowable.

For human cells examined in metaphase the upper limits\(^3\) of acceptability (upper fiducial limits at 95% (Poisson)) for abnormalities are, for a 1000- and 500-cell sample, as follows:

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>1000 cells</th>
<th>500 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromatid and chromosome breaks</td>
<td>47/1000</td>
<td>26/500</td>
</tr>
<tr>
<td>Structural abnormalities</td>
<td>17/1000</td>
<td>10/500</td>
</tr>
<tr>
<td>Hypertetraploidy</td>
<td>8/1000</td>
<td>5/500</td>
</tr>
<tr>
<td>Hypotetraploidy</td>
<td>180/1000</td>
<td>90/500</td>
</tr>
<tr>
<td>Polyploidy</td>
<td>30/1000</td>
<td>17/500</td>
</tr>
</tbody>
</table>

\(^3\) These upper limits for human cells are based on extensive experience with the examination of WI-38 and MRC-5 cells reported to and examined by the ad hoc Committee on Cytological Controls of Human Cell Substrates, which met in 1978 at Lake Placid, NY, USA. These values will not necessarily be applicable if another human cell strain or a monkey cell strain is used.

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All cells showing abnormalities shall be subjected to detailed examination, and records shall be maintained of the detailed criteria applied to particular abnormalities evaluated in the karyotype analysis.

Permanent stained slide preparations of the chromosome monitoring of the working cell bank pool, or photographs of these, shall be maintained as part of the record of the batch of vaccine for monitoring successive batches made from that cell pool.

It is desirable that a portion of the sample of pooled cell substrate removed from the culture vessels should be stored frozen so as to retain viability. This would be available for future reference for karyology or for any other purpose relating to the batch of vaccine.

Only those cell pools of the MWCB that have normal karyology shall be used for vaccine production.

3.1.4 *Identity test of the cells*

In some countries tests for characterizing HLA surface antigens are carried out in addition to chromosome monitoring.

3.2 *Production of cell cultures*

A cell sample equivalent to at least 1% of the cell suspension of the concentration employed for seeding the vaccine production cultures shall be used to prepare control cell cultures.

In some countries in which the technology of large-scale production has been developed, the national control authority should determine the size of the sample of cells to be examined, the time at which the control cells should be taken from the production cultures, and the monitoring of the control vessels.

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

These control cell cultures shall be incubated under similar conditions to the inoculated cultures for at least 2 weeks or until the time of the last harvest of the production cultures, whichever is the later, and shall be examined during this period for evidence of cytopathic changes. For the test to be valid, not more than 20% of the control cell cultures may be discarded for nonspecific, accidental reasons.

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

3.2.1 Test for haemadsorbing viruses

At the end of the observation period, cells comprising 25% of the control cells shall be tested for the presence of haemadsorbing viruses using guinea-pig red cells. If the guinea-pig red cells have been stored, the duration of storage shall not have exceeded 7 days and the temperature of storage shall have been in the range of 2–8 °C.

In some countries the national control authority requires that tests for haemadsorbing viruses be made in addition, using other types of red cells including those from humans (blood group O), monkeys, and chickens (or other avian species). The cultures should be examined at 3–5 days and again at 12 days. All tests should be read after incubation for 30 min at 0–4 °C and again after a further incubation for 30 min at 20–25 °C. The test with monkey red cells should be read once more after yet another incubation for 30 min at 34–37 °C.

3.2.2 Tests for other extraneous agents

At the time of each harvest of the production cultures and 14 days after the day of inoculation of the production cultures with seed lot virus, a sample of the pooled fluids shall be taken at each period of collection from each group of control cultures. 10 ml of each pool shall be tested in the same cells, but not the same batch of cells as that used for the production of virus growth, and additional 10-ml samples of each pool shall be tested in human cells and at least one other sensitive cell system.

The inoculated cultures shall be incubated at a temperature of 35–37 °C and shall be observed for a period of at least 14 days.

Some national control authorities require that at the end of this observation period a subculture should be made in the same cell culture system and observed for at least seven days. Furthermore, some national control authorities require that these cells should be tested for the presence of haemadsorbing viruses.

For the tests to be valid, at least 80% of the culture vessels should be available and suitable for evaluation at the end of the respective test periods.

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If any cytopathogenic changes occur due to extraneous agents in any of the cultures the virus harvests produced from the batch of cells from which the control cells were taken shall be discarded.

3.2.3 Identity test

At the production level the cells shall be identified as human or monkey by tests approved by the national control authority.

Suitable tests are isozymes, HLA, or other immunological tests or karyotyping, or at least one metaphase spread of chromosomes.

**PART D.**

**REQUIREMENTS FOR CONTINUOUS CELL LINES USED FOR INACTIVATED VIRUS VACCINE PRODUCTION**

The following requirements are applicable to the cell substrate for virus vaccine production where production is based on a cell seed system from a continuous cell line.

**1. DEFINITIONS**

1.1 Terminology

*Cell seed.* A quantity of cells derived from a normal tissue and stored frozen at −70 °C or below in aliquots, one or more of which would be used for the production of a manufacturer’s working cell bank.

*Manufacturer’s working cell bank (MWCB).* A quantity of cells derived from one or more ampoules of the cell seed and of uniform composition stored frozen at −70 °C or below in aliquots, one or more of which would be used for the production of each single harvest.

In normal practice such a seed culture (or ampoules) is expanded by serial subculture up to a passage number selected by the manufacturer, at which point the cells are combined into one pool and preserved cryogenically to form the MWCB. One or more of the ampoules from such a pool would be used for the production of a single harvest.
Production cell culture. A collection of cell cultures at the passage number used for virus growth that have been derived from one or more ampoules of the MWCB.

2. GENERAL MANUFACTURING REQUIREMENTS

The general requirements contained in the revised Requirements for Biological Substances No. 1 (General Requirements for Manufacturing Establishments and Control Laboratories) (I, p. 11) shall apply with the addition of the following directive:

No cell cultures other than those approved by the national control authority for the production of appropriate vaccine shall be introduced or handled in the production area.

3. PRODUCTION CONTROL

3.1 Cell seed and/or manufacturer’s working cell bank

The utilization of continuous cell lines for vaccine manufacture shall be based on the cell seed system. A passage of a continuous cell line shall be subcultured to a passage number which is convenient for the preparation of a cell seed.

The cell seed used for the production of virus vaccine shall be that approved by and registered with the national control authority. The accepted continuous cell line from which the cell seed and/or the MWCB have been derived shall have been characterized with respect to genealogy, growth characteristics, immunological markers, virus susceptibility, and storage conditions and it shall have been shown, by tests in animals, eggs, and cell culture to be free from detectable adventitious agents.

In some countries karyology is also required.

These data shall be made available to the national control authority.

Each manufacturer shall show, to the satisfaction of the national control authority, that the cells intended as the virus substrate, propagated from the accepted continuous cell line and laid down as the MWCB conform with the test outlined in this section for freedom from extraneous agents, by tests in animals and eggs (see Part D,
section 3.1.1), and for lack of tumorigenicity. In addition, the test for tumorigenicity shall be repeated on the cells at the passage level used for vaccine production.

In some countries the cells are examined by electron microscopy to establish their ultrastructural characteristics. The cells may also be tested for the presence of retroviruses after activation with agents such as bromodeoxyuridine (BUDR), by examining electron micrographs for virus particles and/or by performing assays for viral reverse transcriptase.

3.1.1 Tests in animals and eggs for extraneous agents

The tests in animals for extraneous agents shall include the inoculation of each of the following groups of animals with cells by the intramuscular route, using at least $10^7$ viable cells divided equally between the animals in each group:

- 2 litters of suckling mice, comprising at least 10 animals, less than 24 h old;
- 10 adult mice;
- 5 guinea-pigs; and
- 5 rabbits.

At least $10^6$ viable cells shall be injected also into the allantoic cavity of each of 10 embryonated chicken eggs 9–11 days old.

The animals shall be observed for at least 4 weeks and the embryonated chicken eggs shall be examined after not less than 3 days' incubation. Any animals that are sick or show any abnormality shall be investigated to establish the cause of illness. The allantoic fluids shall be tested with guinea-pig and chick or other avian red cells for the presence of haemagglutinins.

The cells are suitable for vaccine production if at least 80% of the animals or eggs inoculated with the cells remain healthy and survive the observation period and none of the animals or eggs shows evidence of the presence in the cell cultures of any extraneous agent.

3.1.2 Tests for tumorigenicity

Cells from the MWCB at the passage used for the virus production, or up to 10 passages thereafter, shall be shown to be non-tumorigenic in a test approved by the national control authority. Such a test must show a clear difference between the continuous cell line and a
reference preparation of HeLa cells. In an appropriate in vivo test 10^6 viable HeLa cells when given by the subcutaneous or intramuscular route shall produce progressively growing tumours in at least 9 out of 10 animals, of which one or more must show evidence of metastases, while the 10^6 cells of an acceptable continuous cell line shall produce neither progressively growing tumours nor metastases.

The systems shown to be suitable for this test include:

(a) newborn mice, rats or hamsters that have been treated with antithymocyte serum or globulin,

(b) thymectomized and irradiated mice that have been reconstituted with bone marrow from healthy mice,

(c) chick embryo skin organ cultures.

A suitable test using newborn animals treated with antithymocyte serum is to inoculate at least 20 animals with 0.1 ml of potent serum within 24 h of birth. The injection is given either by the intramuscular or subcutaneous route and is repeated on days 2, 7, and 14 of life. A potent antithymocyte serum or globulin is one that suppresses the immune mechanisms of the growing animals to the extent that the subsequent inoculum of 10^6 HeLa cells regularly produces tumours and metastases.

Also on the day of birth the two groups of 10 newborn animals that have been given the antithymocyte serum are given either 10^6 viable HeLa cells or 10^6 viable cells of the continuous cell line by the subcutaneous route at any site at which developing tumours can be palpated (the base of the neck or the abdomen are suitable sites). The animals are observed for 21 days for the evidence of nodule formation at the site of injection and measurements are made at suitable times to determine whether there has been progressive growth.

At the end of the observation period all animals from both groups are sacrificed and examined for gross evidence of tumour formation at the site of injection and in other organs such as the lymph nodes, lungs, kidneys, and liver. All tumour-like lesions are examined histopathologically. In addition, since some cell lines may form metastases without evidence of local tumour growth, the lungs and regional lymph nodes of all animals shall be examined histopathologically.

For the purposes of this requirement, a progressively growing tumour is defined as a palpable nodule that increases in diameter over the 21-day observation period and that shows viable and mitotically active inoculated cells when examined histopathologically. The presence of microscopically visible cells without gross nodule formation shall not be considered a progressively growing tumour; in addition, the presence of microscopically visible

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1 Information concerning the sources of suitable HeLa cells may be obtained from Chief, Biologicals, WHO, 1211 Geneva 27, Switzerland.
cells in association with a stationary or regressing nodule shall not be considered a progressively growing tumour.

In addition, some countries test the cells for tumorigenicity in animals of the homologous species from which the cells were derived. Such tests would include immunosuppression of the animals with species-specific antithymocyte serum, inoculation of candidate cells and control tumour cells, observation for at least three weeks, and the histopathological examination of the inoculation sites as well as any metastatic lesions.

In some countries an in vitro test is permitted to demonstrate the freedom from tumorigenicity provided that the test has been shown to be as sensitive as a test in animals.

A suitable test using organ cultures of chick embryonic skin is to inoculate $10^6$ HeLa cells or $10^6$ cells from an acceptable continuous cell line on to organ cultures of chick embryonic skin for 3 days. At the end of this period each culture is processed for histologic evaluation, and scored for cell growth and invasion. The reference HeLa cells should show extensive mitotic activity as well as extensive invasion into the chick substrate, while the continuous cell lines will show little or no invasion. In addition, secondary cell cultures derived from the same tissue as the continuous cell line may be tested in this system to provide guidance in interpreting invasive and mitotic activity.

3.1.3 Identity test of the cells

The MWCB shall be identified by a method approved by the national control authority.

The tests that may be used are karyology, isoenzymes analysis and/or immunological markers.

3.2 Production of cell cultures

A cell sample equivalent to at least 1% of the cell suspension at the concentration employed for seeding the vaccine production cultures shall be used to prepare control cultures.

In some countries in which the technology of large-scale production has been developed the national control authority should determine the size of the sample of cells to be examined, the time at which the control cells should be taken from the production cultures, and the monitoring of the control vessels.

The treatment of cells set aside as control material shall be similar to that of the production cell cultures but they shall remain uninoculated as control cultures for the detection of extraneous viruses.
These control cell cultures shall be incubated under similar conditions to the inoculated cultures for at least two weeks or until the time of the last harvest of the production cultures, whichever is the later, and shall be examined during this period for evidence of cytopathic changes. For the test to be valid, not more than 20% of the control cell cultures may be discarded for nonspecific, accidental reasons.

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

3.2.1 Test for haemadsorbing viruses

At the end of the observation period cells comprising 25% of the control cells shall be tested for the presence of haemadsorbing viruses using guinea-pig red cells. If the guinea-pig red cells have been stored, the duration of storage shall not have exceeded 7 days and the temperature of storage shall have been in the range of 2–8 °C.

In some countries the national control authority requires that tests for haemadsorbing viruses be made in addition using other types of red cells including those from humans (blood group O), monkeys, and chickens (or other avian species). The cultures should be examined at 3–5 days and again at 12 days. All tests should be read after incubation for 30 min at 0–4 °C and again after a further incubation for 30 min at 20–25 °C. The test with monkey red cells should be read once more after yet another incubation for 30 min at 34–37 °C.

3.2.2 Tests for other extraneous agents

At the end of the observation period a sample of the pooled fluids from each group of control cultures shall be tested for extraneous agents. 10 ml of each pool shall be tested in the same cells, but not the same batch of cells, as that used for the production of virus growth, and additional 10-ml samples of each pool shall be tested in human cells and at least one other sensitive cell system.

The inoculated cultures shall be incubated at a temperature of 35–37 °C and shall be observed for a period of at least 14 days.
For the tests to be valid, at least 80% of the culture vessels shall be available and suitable for evaluation at the end of the respective test periods.

If any cytopathic changes occur due to extraneous agents in any of the cultures, the virus harvests produced from the batch of cells from which the control cells were taken shall be discarded.

3.2.3 Identity test

At the production level the cells shall be identified as the species of origin by tests approved by the national control authority.

Suitable tests are isoenzymes analysis, other immunological tests, or karyology.

3.2.4 Purity of virus prepared on a continuous cell line

The virus grown in a continuous cell line shall be purified by a process that has been approved by the national control authority and that has been shown to give consistent results.

Tests used to demonstrate the degree of purity achieved shall also be approved by the national control authority.

For RVF vaccine (inactivated) the purification process shall be shown to reduce consistently the level of cellular DNA from that of the initial virus harvest by a factor of at least 10⁶ (see Part A, section 3.4.3.1).

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REFERENCES


Appendix

SUMMARY PROTOCOL
OF RIFT VALLEY FEVER VACCINE (INACTIVATED)
PRODUCTION AND TESTING

(Based on Requirements for Biological Substances No. 32)
(Requirements for Rift Valley Fever Vaccine (inactivated)
for human use)

Identification of Final Lot

Name and address of manufacturer

Lot number of final product

Date of manufacture of final lot
(namely, date of last potency test)

Expiry date

Total volume of final lot

137
Cell cultures

Type of cell cultures used:

(Complete only the relevant part—e.g., A, B or C)

A. Cell cultures/monkey primary/secondary/tertiary

Type of cells (kidney, fetal kidney, lung, etc.)

Monkey species of cells

Controls performed on animals from which the cells originated

Number of cell doublings

Result

Amount of cell culture inoculated

Amount of control cell culture investigated

Tests performed on control cultures

Result

B. Cell cultures/human or monkey diploid

Origin and short history of cell seed

Authority that approved cell seed

Amount of cell culture inoculated

Amount of control cell culture investigated

Tests performed on control cultures

Result

C. Cell cultures/other cells

Origin and short history of cell seed

Authority that approved cell seed

Amount of cell culture inoculated

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<table>
<thead>
<tr>
<th>Amount of control cell culture investigated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tests performed on control cultures</td>
</tr>
<tr>
<td>Result</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Serum for Cell Cultures</td>
</tr>
<tr>
<td>Origin of serum used</td>
</tr>
<tr>
<td>Tests performed on serum</td>
</tr>
<tr>
<td>Result</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Seed Lots of Virus</td>
</tr>
<tr>
<td>Strain used</td>
</tr>
<tr>
<td>Origin and source of strain</td>
</tr>
<tr>
<td>Number of subcultures between primary seed lot and production</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Information on Manufacture</td>
</tr>
<tr>
<td>1. Single harvests</td>
</tr>
<tr>
<td>Date of inoculation of cells with virus</td>
</tr>
<tr>
<td>Date of virus harvest</td>
</tr>
<tr>
<td>Special observations on single harvests</td>
</tr>
<tr>
<td>Date of pooling</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Tests on pooled harvests</td>
</tr>
<tr>
<td>Tests in Cercopithecus cell cultures</td>
</tr>
<tr>
<td>Method used</td>
</tr>
<tr>
<td>Result of test</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Test in rabbits or kidney cell cultures</td>
</tr>
<tr>
<td>Number of rabbits (or cell cultures)</td>
</tr>
<tr>
<td>Total volume injected</td>
</tr>
</tbody>
</table>
Observation period

Result

**Product before inactivation**

Details on filtration and/or clarification and/or purification (if applied)

Date

**Tests on pool**

**Identity test**

Method

Result

**Sterility tests**

Method (media)

Result

**Virus titration**

TCID₅₀ per ml

Details on filtration and/or clarification and/or purification (if applied)

Date(s)

**Inactivation of product**

Agent(s) and concentration

Date of start of inactivation

Date of taking first sample

Date of completion of inactivation

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**Test for effective inactivation**
(after removal/neutralization of inactivating agent)

- Sample size tested
- Date of first sample
- Date of second sample
- Details of testing procedure

- Period of observation of cell cultures
- Period of observation of subcultures
- Result
- Result of challenge of used culture with live virus

2. **Bulk product**
   (single pools incorporated)

- Date of preparation
- Preservative (if added, type and concentration)

**Tests on bulk**

**Test for absence of infective RVF virus**

- Sample size tested
- Details of testing procedure

- Period of observation of cell cultures
- Period of observation of subcultures
- Result

**Sterility tests**

- Method (media)
- Result
3. **Tests on final product**

*Identity tests*

- Method used
- Result

*Sterility tests*

- Method (media)
- Result

*Innocuity test*

- Number of animals
- Dose
- Observation period
- Result

*Potency test*

- Method
- Result
- Date

*Protein nitrogen*

- Content of protein nitrogen in μg per human dose

*Serum protein tests (if done)*

- Result

Signature of head of laboratory
Certification by person taking overall responsibility for production of the vaccine.

I certify that lot No. ...... of Rift Valley fever vaccine (inactivated) satisfies the WHO Requirements for Rift Valley fever Vaccine (inactivated) for human use.

Date

Signature

Name typed

The protocol must be accompanied by a sample of the vaccine and a copy of the leaflet.