Annex 2
Requirements for haemorrhagic fever with renal syndrome (HFRS) vaccine (inactivated)
(Requirements for Biological Substances No. 44)

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General considerations

Haemorrhagic fever with renal syndrome (HFRS), an acute interstitial nephropathy characterized by high fever and varying degrees of renal insufficiency and haemorrhage, is caused by viruses belonging to the Hantavirus genus of the family Bunyaviridae. Approximately 200,000 cases of HFRS occur annually, with a case fatality rate ranging from less than 1% to more than 10% (1).

Numerous murid, arvicolid and cricetid rodents and other small mammals harbour hantaviruses, but Apodemus, Clethrionomys and Rattus species are the principal reservoir hosts.

Various haemorrhagic fevers with a very similar syndrome have been reported throughout Europe and Asia: (a) as haemorrhagic nephropathia epidemica in the area of the former Soviet Union, with several thousand cases reported annually since 1931; (b) as Songo fever or epidemic haemorrhagic fever, first recognized in 1931 in China, with more than 32,000 cases reported in 1981; (c) as nephropathia epidemica in Scandinavia, with several hundred cases reported annually since 1934; (d) as epidemic nephritis or epidemic haemorrhagic fever in Eastern Europe, since 1934; (e) as Korean haemorrhagic fever since 1951; and (f) as epidemic haemorrhagic fever in Japan, since 1960. Epidemics involving thousands of cases of a similar disease of unknown etiology occurred among soldiers stationed during wartime in Lapland and Manchuria (1).

During the Korean War more than 3200 United Nations troops stationed in the demilitarized zone developed a haemorrhagic disease terminating in shock, renal failure, and death in about 10–15% of cases. The causative agent of this outbreak was first isolated in 1976 from the rodent Apodemus agrarius and in 1978 from patients, and was propagated in a human cell culture line. It was named Hantaan virus after the Hantaan river, which runs near the 38th parallel across the Korean peninsula. A close etiological relationship was established by antibody studies between the Korean haemorrhagic disease and haemorrhagic fever with renal syndrome in the former Soviet Union, nephropathia epidemica in Scandinavia, and epidemic haemorrhagic fever in China and Japan. Recently, acute hantavirus-associated respiratory disease has been identified in the south-western United States, with death in some 60% of cases.

The WHO Working Group on Haemorrhagic Fever with Renal Syndrome, which met in Tokyo in 1982, recommended that the above-mentioned diseases with different names should all be referred to as “haemorrhagic fever with renal syndrome” (2).

Disease control has essentially depended upon reducing contact between humans and rodent excreta. In rural areas control measures are expensive and difficult to maintain since it is impossible to eradicate the rodent
reservoir of the virus, but in urban areas rodent control is feasible and should be encouraged. Now that a vaccine is available it is possible to protect high-risk population groups.

**Vaccine development**

The development of inactivated HFRS vaccines in several countries has made it desirable to prepare international requirements for HFRS vaccine. At present the following HFRS vaccines are available:

1. **In** the Republic of Korea, a vaccine has been developed from a strain of Hantaan virus originally isolated from the early-stage blood of an HFRS patient by direct inoculation of Vero E-6 cells and passaged in both suckling-mouse brains and suckling-rat brains. Experimental vaccines obtained by inactivation of approximately 10^7 LD_{50} (median lethal doses) of the mouse-brain or rat-brain preparation with 0.05% formalin have been extensively tested in rats, guinea-pigs, hamsters, rabbits and Apodemus species. Antibody responses measured by indirect fluorescent antibody tests, enzyme-linked immunosorbent assay and neutralization tests were higher after subcutaneous than after intramuscular administration. Human trials have been conducted with formalin-inactivated mouse- and rat-brain vaccines, with aluminium hydroxide adjuvant (3). In one trial, in which 336 people received a second dose of vaccine after a one-month interval, vaccination resulted in 99% seroconversion according to indirect fluorescent antibody tests. The vaccine was considered safe and only minor side-effects were observed. The efficacy of this vaccine against HFRS in the endemic areas of HFRS remains to be determined (4).

2. **The** B-1 strain of Seoul virus isolated from rat tumour cells has been used to prepare vaccines in Japan. Ten per cent suspensions of infected suckling-mouse brain are treated with protamine sulfate, inactivated with 0.025% formalin, purified on sucrose gradients, adjusted to a standard antigen concentration and injected with aluminium hydroxide as an adjuvant. Laboratory mice and guinea-pigs show antibody responses to vaccination, as measured by indirect fluorescent antibody tests, neutralization tests and inhibition of haemagglutination, and the vaccine has a protective effect in mice, indicated by reduced yields of virus in lung or spleen suspensions prepared five days after a high-titre viral challenge. Vaccines diluted 1:40 were effective in both homologous and heterologous protection tests in animals (3).

3. **In** China, experimental vaccines have been developed using preparations of infected suckling-mouse brain and virus propagated in cell cultures derived from golden hamster kidney (GHK) or Mongolian gerbil kidney (MGK). The cell-culture preparations, containing more than 10^5 median cell-culture infective doses (CCID_{50}) per ml before inactivation, have been used to prepare monovalent GHK and MGK vaccines as well as GHK-propagated bivalent
vaccines based on both Seoul and Hantaan virus strains. The GHK vaccine virus is inactivated with formalin and the MGK vaccine virus with β-propioloactone. These cell-culture vaccines appear effective in protection assays, as shown by reduced replication of challenge virus in hamsters or Mongolian gerbils and reduced yields of virus antigen in lung and spleen samples taken from immunized animals after challenge.

In human trials, MGK vaccine was reported to be safe, and 1100 volunteers given the vaccine responded by producing neutralizing and haemagglutination-inhibiting antibodies (3). The monovalent GHK vaccine has been administered to 12 people (ten received three inoculations and two received two inoculations), all of whom showed seroconversion, as determined by measurement of IgG (by indirect fluorescent antibody tests and enzyme-linked immunosorbent assay) and neutralizing antibody; ten of them were still seropositive 360 days after the first inoculation (5). In 206 volunteers given a bivalent GHK vaccine prepared with GHK-adapted Hantaan virus JR strain and Seoul virus L99 strain, seroconversion rates were similar to those observed with the monovalent GHK vaccine mentioned above. In addition, specific and non-specific T-cell transformation indices increased, by 96% and 48% respectively (6).

4. In the Democratic People’s Republic of Korea, a purified inactivated HFRS vaccine has been produced from the virus-infected brains of suckling rats and hamsters, by a method based on that used to prepare purified inactivated Japanese encephalitis vaccine from mouse brain.¹ The vaccine induced antibody production in human volunteers immunized twice within a 10–21-day interval, and when volunteers were revaccinated 5–12 months after primary vaccination, the proportion showing positive antibody responses increased. WHO has received information that, during the period 1986–1991, an epidemiological study was conducted to assess the protective effect of the vaccine in people living in several HFRS endemic areas in the Democratic People’s Republic of Korea. A total of 1.2 million people living in these endemic areas have been immunized with the vaccine.

**Vaccine requirements**

The vaccine requirements presented here take into account the recommendations of a scientific group convened by WHO’s Regional Office for the Western Pacific in 1989 to discuss the development of HFRS vaccines (3).

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Each of the following sections constitutes a recommendation. The parts of each section printed in normal type have been written in the form of requirements, so that, if a national control authority so desires, they may be adopted as they stand as definitive national requirements. The parts of each section printed in small type are comments or recommendations for guidance. To facilitate the international distribution of vaccine made in accordance with these Requirements, a summary protocol for recording the results of tests is provided as Appendix 1. A general scheme for the preparation of HFRS vaccine is given as Appendix 2.

Should individual countries wish to adopt these Requirements as the basis for their national regulations concerning inactivated HFRS vaccine, it is recommended that modifications be made only on condition that the modified requirements ensure at least an equal degree of safety and potency of the vaccine. It is desirable that the World Health Organization be informed of any such changes.

**Part A. Manufacturing requirements**

**A.1 Definitions**

**A.1.1 International name and proper name**

The international name of the vaccine shall be “Vaccinum haemorrhagia febris cum renis sindronum”. The proper name shall be the equivalent of the international name in the language of the country of origin.

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

**A.1.2 Descriptive definition**

“Vaccinum haemorrhagia febris cum renis sindronum” is a fluid or freeze-dried preparation of a suitable hantavirus grown in the neural tissue of suckling rodents or in cell cultures and inactivated by a suitable method. Preparations for human use shall satisfy all the requirements formulated below.

**A.1.3 International reference materials**

No international reference materials are available.

**A.1.4 Terminology**

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

*Master virus seed lot:* A quantity of virus processed as a single lot to ensure a uniform composition and fully characterized. It is used for the
preparation of working seed lots. The master seed lot and its passage level are approved by the national control authority.

**Working virus seed lot:** A quantity of fully characterized virus suspension of uniform composition, derived from a master seed lot by a number of passages that does not exceed the maximum approved by the national control authority. The working seed lot is used for the production of vaccine.

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

**Cell seed:** A quantity of fully characterized cells of human, animal or other origin stored frozen at \(-70^\circ\text{C}\) or below in aliquots of uniform composition, one or more of which are used for the production of a manufacturer’s working cell bank.

**Manufacturer’s working cell bank (MWCB):** A quantity of cells of uniform composition derived from one or more ampoules of the cell seed and stored frozen at \(-70^\circ\text{C}\) or below in aliquots, one or more of which are used for production purposes.

In normal practice a cell seed is expanded by serial subculture up to a passage number (or population doubling, as appropriate) selected by the manufacturer and approved by the national control authority. The cells are combined in a single pool, distributed into ampoules and preserved cryogenically to form the MWCB.

**Production cell culture:** A group of cell cultures derived from the same pool of cells and processed together.

**Single harvest:** A quantity of virus suspension derived from a batch of production cell cultures or group of rodents that were inoculated with the same working seed lot and processed together in a single production run.

**Virus pool:** A homogeneous pool of single harvests collected into a single vessel before clarification.

**Final bulk:** The homogeneous finished virus suspension prepared from one or more clarified virus pools in the vessel from which the final containers are filled.

**Filling lot (final lot):** A collection of sealed final containers of finished vaccine that are homogeneous with respect to the risk of contamination during filling and freeze-drying. All the final containers must, therefore, have been filled from a single vessel of final bulk in one working session and, if appropriate, lyophilized under standardized conditions in a common chamber.

### A.2 General manufacturing requirements

The general requirements for manufacturing establishments contained in Good Manufacturing Practices for Pharmaceutical (7) and Biological (8)
Products shall apply to establishments manufacturing HFRS vaccine for human use, with the addition of the following directives.

HFRS vaccine shall be produced in premises that meet the requirements for Biosafety Level 3 (9). Staff must not handle other infectious microorganisms, animals or tissue cultures in the same working day. The staff shall consist of persons who are found to be healthy on medical examination. Steps shall be taken to ensure that all personnel in the production and control areas have demonstratable serum neutralizing antibody to the strain of hantavirus used in vaccine production.

Only suckling-rat rodent brain tissue and cell cultures approved by the national control authority for the production of HFRS vaccine shall be introduced into or handled in the production area.

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

Particular attention shall be given to the recommendations contained in section 3 of Good Manufacturing Practices for Biological products (8) regarding the training and experience of the persons in charge of production and testing and of those assigned to various areas of responsibility in the manufacturing establishment, as well as to the registration of such personnel with the national control authority.

A.3 Control of source materials

The general production precautions formulated in Good Manufacturing Practices for Biological Products (8, sections 4 and 8) shall apply to the manufacture of HFRS vaccine.

A.3.1 Animals and cell cultures used for production

A.3.1.1 Hamsters and gerbils for cell cultures

When cell cultures derived from hamster or Mongolian gerbil kidneys are used for the propagation of hantavirus, only hamster or gerbil stock approved by the national control authority shall be used as a source of tissue.

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

A.3.1.2 Rodents for virus propagation in neural tissue

Rodents used for the propagation of hantavirus in neural tissue shall be younger than five days of age and free from all signs of disease. The
methods used for intracerebral inoculation and harvesting of virus shall be approved by the national control authority. The animal stock should be free from relevant pathogenic microorganisms.

A.3.1.3 Continuous cell lines
Continuous cell lines used for the propagation of hantavirus shall be approved by and registered with the national control authority. They shall be characterized according to the Requirements for Continuous Cell Lines Used for Biologicals Production (10).

The use of continuous cell lines shall be based on the cell seed lot system, and the maximum number of passages (or population doublings) of the cell seed shall be established by the national control authority.

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

Suitable methods include biochemical tests (e.g. isoenzyme analyses), immunological tests (e.g. histocompatibility antigen assays) and cytogenetic marker tests.

A.3.1.4 Serum used in cell-culture medium
Serum used for the propagation of cells for vaccine production shall be tested to demonstrate freedom from bacteria, fungi and mycoplasmas as specified in Part A, sections 5.2 and 5.3, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (11). Serum of bovine origin must come from herds or countries certified to be free of bovine spongiform encephalopathy.

Some countries require that bovine serum should come from herds that have not been given feed derived from ruminant protein.

Suitable tests for detecting viruses in calf or newborn-calf serum are given in Appendix 1 of the revised Requirements for Biological Substances No. 7 (Requirements for Poliomyelitis Vaccine (Oral)) (12).

Serum shall also be shown to be free from inhibitors of hantavirus. Human serum shall not be used. If human albumin is used, it shall meet the revised Requirements for Biological Substances No. 27 (Requirements for the Collection, Processing and Quality Control of Blood, Blood Components and Plasma Derivatives) (13).

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

A.3.2 Virus seed

A.3.2.1 Strain of virus
Vaccine production shall be based on hantavirus strains associated with human disease. The strains used to produce seed lots shall be approved by the national control authority and shall yield safe and immunogenic
vaccines when the virus has been inactivated. They shall be identified by historical records, infectivity tests, serological tests and animal responses to inoculation.

A.3.2.2 Virus seed lot system
The preparation of HFRS vaccine shall be based on a virus seed lot system. The national control authority shall determine the maximum acceptable number of passages between the master virus seed lot and any working virus seed lot. Vaccines shall be made from a working virus seed lot without any additional passages. Virus seed lots shall be maintained in dried form at a temperature below 10°C or frozen at a temperature below -60 °C.

Seed lots shall have been shown, to the satisfaction of the national control authority, to be capable of yielding vaccine that meets all the present Requirements.

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

A.3.2.3 Tests on virus seed lots
Identification of the virus. Each virus seed lot shall be identified as hantavirus by methods approved by the national control authority.

Freedom from bacteria, fungi and mycoplasmas. Each virus seed lot shall be shown to be free from bacterial, mycotic and mycoplasmal contamination by appropriate tests as specified in Part A, sections 5.2 and 5.3, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (II).

Tests for adventitious viruses. Each virus seed lot shall be tested for adventitious agents after neutralization with a specific anti-hantavirus serum.

The individual tests on the virus seed lots should be designed to satisfy the requirements of the national control authority. The anti-hantavirus serum should be free from antibody to known adventitious viruses.

Tests on working virus seed lots. Whenever a new working virus seed lot is prepared, tests shall be carried out to characterize the virus strain. These tests shall include virus titration and an identity test using standard serum provided by the national control authority.

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

A.4 Control of vaccine production
A.4.1 Rodent brains
The brains of rodents inoculated intracerebrally with the virus strain for vaccine production shall be harvested immediately before the expected
time of death. The harvested brains shall be trituated in buffered isotonic sodium chloride solution, or any other suitable medium, and centrifuged. The supernatant shall be collected and treated by alcohol precipitation, with protamine sulfate, by ultracentrifugation, or by any other appropriate methods to achieve an initial purification of the virus suspension.

A.4.2 Cell cultures

Penicillin and other β-lactam antibiotics shall not be used at any stage of manufacture.

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

At least 5% or 500 ml of the cell suspension employed to prepare the production cell cultures shall be used to prepare control cell cultures.

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

The treatment of control and production cell cultures shall be similar, but control cells shall remain uninoculated. The control cultures shall be examined for evidence of cytopathic changes for at least two weeks or until the time of the last viral harvest from the production cultures. For the test to be valid, no more than 20% of the control cell cultures shall have been discarded for non-specific 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 shows evidence of the presence in a control culture of any adventitious agent, the hantavirus grown in the corresponding production cultures shall not be used for vaccine production.

A.4.3 Single harvests

A.4.3.1 Sterility tests

A sample of each single harvest shall be tested for bacterial and fungal contamination by appropriate tests as specified in Part A, section 5.2, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (II). Any single harvest in which contamination is detected shall be discarded.

A.4.3.2 Virus content

The virus content of each single harvest shall be determined, before any purification, by intracerebral inoculation of suckling mice of two days of age or in sensitive cell cultures. The minimum virus content shall be established by the national control authority.

In some countries, the minimum virus content is $10^{7.5} \text{ LD}_{50}/0.02$ ml when neural tissue is used and $10^{7.0} \text{ CCID}_{50}/\text{ml}$ when cell culture is used.
A.4.4 Virus pools

A.4.4.1 Pooling of single harvests
Only virus harvests satisfying the requirements for sterility and virus content of sections A.4.3.1 and A.4.3.2 shall be pooled.

A.4.4.2 Inactivation of virus
The process for inactivating the hantavirus shall be approved by the national control authority.

*Treatment before inactivation.* When cell cultures are used, the virus pool shall be clarified by filtration or continuous centrifugation to remove cell debris before the virus is inactivated.

Clarification by filtration or continuous centrifugation of the crude virus suspension has been clearly established as an important means of improving the regularity of the inactivation process.

Generally, similar filters are used in series or the virus suspension is filtered sequentially through filters of decreasing porosity. Satisfactory results have been reported with several filter types, but the final filtration step should be approved by the national control authority.

*Method of inactivation.* The method and agents used for inactivation shall be approved by the national control authority. The method used shall be demonstrated to be consistently effective in the hands of the manufacturer. Inactivation shall be commenced immediately after the initial purification (see section A.4.1) and sampling of single harvests when neural tissue is used, or immediately after clarification when cell cultures are used.

Formalin or β-propiolactone may be used to inactivate the hantavirus.

*Test for effective inactivation.* Each undiluted bulk suspension shall be tested for inactivation of the virus by a method approved by the national control authority. The test sample shall correspond to at least 25 human doses of the final product.

In one country, the test sample is dialysed at about 5 °C for at least 24 hours against a sufficient volume of buffered isotonic sodium chloride solution, and diluted if necessary to minimize any cytopathogenic effect due to residual inactivating agent or other substances.

Several methods have been found suitable for detecting residual live virus: (a) Primary or continuous cell cultures known to be susceptible to hantaviruses are inoculated with the test sample and examined periodically by an indirect fluorescent antibody test for hantaviral antigen during serial blind passage of cells for at least 60 days.

(b) Each of at least 10 mice of two days of age is inoculated intracerebrally with 0.03 ml of the culture fluid obtained after serial blind passage as described in (a) above. The animals are observed for 21 days, and the lungs of those that die after the first 24 hours of the test are examined by indirect fluorescent antibody assay or an equivalent sensitive assay for the presence of hantaviral antigen.
(c) Vero E-6 cells are inoculated with the test sample and culture fluid is collected after two or three passages at 10-day intervals. Suckling mice and hamsters are inoculated with the culture fluid and observed for 21 days. An indirect fluorescent antibody test is used to examine the animals' brains for hantavirus antigen and to test their serum for antibodies against homologous virus antigen.

Other sensitive methods for detecting live hantavirus, for example based on the polymerase chain reaction, could also be applied.

The bulk passes the test if it is shown to be free from residual live virus.

A.4.4.3 Purification of inactivated virus suspension
After virus inactivation, the bulk suspension derived from brains (see section A.4.1) shall be purified by a process that has been approved by the national control authority and shown to give consistent results.

The purification process for brain-derived material should be designed to reduce the myelin content to the lowest possible level.

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

The routine method used for purifying inactivated virus pool from both rodent brains and cell culture is ultrafiltration. The filter should not pass substances of the relative molecular mass of hantaviruses.

A.4.4.4 Antigen content of bulk suspension
A test for antigen content shall be made on each bulk suspension by a method approved by the national control authority.

The results of enzyme-linked immunosorbent and other assays should be shown to be related to immunogenicity in animals before such assays are used instead of the animal immunogenicity test.

A.4.5 Final bulk

A.4.5.1 Added substances
In the preparation of the final bulk, only preservatives, adjuvants and other substances approved by the national control authority shall be added. Such substances shall have been shown by appropriate tests not to impair the safety or effectiveness of the product in the amounts used.

When the final bulk contains an adjuvant, the concentration used shall be approved by the national control authority. The concentration of aluminium shall not exceed 1.25 mg per single human dose.

If formalin has been used for inactivation, the amount of formaldehyde in the final bulk shall not be greater than 0.01%. The test method used shall be approved by the national control authority.
If β-propiolactone has been used for inactivation, this chemical shall not be detectable in the final bulk. The test used for β-propiolactone shall be approved by the national control authority.

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

A.4.5.2 Sterility tests
Each final bulk shall be tested for bacterial and mycotic sterility as specified in Part A, section 5.2, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (II).

A.4.5.3 Animal serum
For cell-culture-derived vaccines, the serum albumin concentration in the final bulk shall be less than 50 ng per single human dose.

In some countries, the final vaccine is tested to determine the concentration of residual animal serum proteins.

A.5 Filling and containers
The requirements concerning filling and containers in Good Manufacturing Practices for Biological Products (8, section 4) shall apply.

A.6 Control tests on final product
A.6.1 Identity test
The virus in at least one labelled container from each final lot shall be identified by an appropriate method.

The test for potency described in section A.6.5 may serve as an identity test.

A.6.2 Sterility tests
Each final lot shall be tested for bacterial and mycotic sterility as specified in Part A, section 5.2, of the revised Requirements for Biological Substances No. 6 (General Requirements for the Sterility of Biological Substances) (II).

A.6.3 General safety tests
Each final lot shall be tested for abnormal toxicity by appropriate tests in mice and guinea-pigs, using parenteral injections. The test procedures shall be approved by the national control authority.
A.6.4 Protein content
The protein content of a sample from each final lot shall be determined. The maximum permissible protein content shall be set by the national control authority. It shall be no greater than 80 µg/ml when neural tissue is used for vaccine production. When human albumin is used as a stabilizer for cell-culture vaccine, the protein content shall be no higher than 200 µg/ml.

A.6.5 Potency test
A sample from each final lot shall be tested for potency.

The challenge strain, reference vaccine and test procedure shall be approved by the national control authority (see section B.1), which shall also set the minimum acceptable potency.

The potency of the test sample should be no less than that of the reference vaccine.

A.6.6 Stability test
The method of vaccine production shall be such that stable vaccine is produced. A sample from each final lot shall be tested for stability by a method approved by the national control authority.

In some countries, stability assessment is based on the testing of samples of several vaccine lots throughout the proposed shelf-life of the vaccine.

A suitable method is to test the potency of liquid vaccine after storage for one week at 37°C and that of freeze-dried vaccine after storage for four weeks at 37°C. In order to pass the test, the lot should retain minimum potency, as defined by the national control authority.

A.6.7 Residual moisture in freeze-dried vaccine
The residual moisture in a representative sample of each freeze-dried lot shall be determined by a method approved by the national control authority. The upper limit for the moisture content shall be specified by the national control authority.

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

A.6.8 Test for pyrogenic substances
Each final lot shall be shown to be free from pyrogenic substances. The test shall be approved by the national control authority.
A.6.9 **Residual bovine serum albumin**
If bovine serum has been used in the cell-culture system, and the final bulk has not been tested for residual albumin, a sample from each final lot shall be tested to verify that the residual amount of bovine serum albumin is less than 50 ng per single human dose.

Alternatively the test may be performed on the final bulk.

A.6.10 **Adjuvant content**
Each final lot shall be assayed for the content of adjuvant. The method used shall be approved by the national control authority.

A.6.11 **Test for residual live virus**
In some countries, a test for virus inactivation is carried out by inoculating 10 mice intracerebrally with 0.03 ml of the final product. If this test is used, it should be conducted as described in small-type paragraph (b) of section A.4.4.2.

A.6.12 **Inspection of final containers**
Every container in each final lot shall be inspected, and those showing abnormalities shall be discarded.

A.7 **Records**
The requirements in section 8 of Good Manufacturing Practices for Biological Products (8, pages 27–28) shall apply.

A.8 **Samples**
The requirements in section 9 of Good Manufacturing Practices for Biological Products (8, page 29) shall apply.

A.9 **Labelling**
The requirements in section 7 of Good Manufacturing Practices for Biological Products (8, pages 26–27) shall apply, with the addition of the following directive.
The leaflet accompanying the package shall:
- specify the substrate used for vaccine production (suckling-rodent brain or a named cell-culture system);
- state the method used for inactivating the virus; and
if the vaccine is in freeze-dried form, state that, after its reconstitution, it shall be used immediately, unless data have been provided to the licensing authority to show that it may be stored for a limited time without loss of potency.

A.10 Distribution and shipping

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

A.11 Storage and expiry date

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

A.11.1 Storage conditions

HFRS vaccine shall be stored at a temperature of 5 ± 3 °C, without freezing, if in liquid form, and below 10 °C if in lyophilized form.

A.11.2 Expiry date

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

Part B. National control requirements

B.1 General

The general requirements for control laboratories in the Guidelines for National Authorities on Quality Assurance for Biological Products (14) shall apply.

The national control authority shall approve the strain of hantavirus used for vaccine production. It shall also provide or approve the challenge strain and reference vaccine for use in the potency test (see section A.6.5).

B.2 Release and certification

A vaccine lot shall be released only if it fulfils the national requirements and/or part A of the present Requirements. A protocol based on the model given in Appendix 1, signed by the responsible official of the manufacturing establishment, shall be prepared and submitted to the national control authority in support of a request for release of vaccine for use.
At the request of the manufacturing establishment, the national control
authority shall provide a certificate that states whether the vaccine meets
all national requirements and/or Part A of the present Requirements. The
certificate shall be based on the model given in Appendix 3.

The purpose of the certificate is to facilitate the exchange of HFRS vaccines
among countries.

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Western Pacific, Manila, Philippines).

4. Lee HW et al. Field trial of an inactivated vaccine against haemorrhagic fever with


Appendix 1

Summary protocol for production and testing of HFRS vaccine (inactivated)

Identification of final lot

Name and address of manufacturer

Lot no. of vaccine

Date of manufacture of final lot

Expiry date

Total volume of final lot

Control of source materials (A.3)

Animals and cell cultures used for production (A.3.1)

Hamsters and gerbils for cell cultures (A.3.1.1)

Animal species and type of cells

Control tests performed on animals from which the cells originated

Rodents for virus propagation in neural tissue (A.3.1.2)

Species

Characteristics of the animal stock and methods used to show freedom from microorganisms pathogenic for rodents, including mycoplasmas

Continuous cell lines (A.3.1.3)

Origin and short history of MWCB

Authority that approved the cell seed

Characteristics of MWCB, according to the Requirements for Continuous Cell Lines Used for Biologicals Production


Identity test for MWCB
  Methods
  Results

Serum used in cell-culture medium (A.3.1.4)
Origin of serum
Tests performed on serum
  Methods
  Results

Virus seed (A.3.2)
Strain of virus (A.3.2.1)
Virus seed lot system (A.3.2.2)
Short history
Date of preparation of master virus seed lot
Date of preparation of working virus seed lot
Number of passages between master and working virus seed lots

Tests on virus seed lots (A.3.2.3)
Identification of the virus
  Methods used
  Results

Freedom from bacteria, fungi and mycoplasmas
  bacteria  fungi  mycoplasmas

Date of inoculation
Media used
Observation period
Results

Tests for adventitious viruses
Tests in animals:
  Methods used
  Results

72
Tests in cell cultures:
Methods used
Results

Tests on working virus seed lots
Virus titration:
Method
Results
Identity test:
Method
Results

Control of vaccine production (A.4)

Cell cultures (A.4.2)
Reference no. of control cell cultures
Quantity of cell cultures used as control cultures
Period of observation of control cells
Results

Single harvests (A.4.3)
Sterility tests (A.4.3.1)

<table>
<thead>
<tr>
<th>bacteria</th>
<th>fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of inoculation</td>
<td></td>
</tr>
<tr>
<td>Media used</td>
<td></td>
</tr>
<tr>
<td>Observation period</td>
<td></td>
</tr>
<tr>
<td>Results</td>
<td></td>
</tr>
</tbody>
</table>

Virus content (A.4.3.2)
Method
Results

Virus pools (A.4.4)
Pooling of single harvests (A.4.4.1)
Number of single harvests pooled
Volume of virus pool
**Inactivation of virus (A.4.4.2)**

<table>
<thead>
<tr>
<th>Treatment before inactivation</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Method of inactivation</td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td></td>
</tr>
<tr>
<td>Date of start of inactivation</td>
<td></td>
</tr>
<tr>
<td>Date of completion of inactivation</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Test for effective inactivation</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Method</td>
<td></td>
</tr>
<tr>
<td>Results</td>
<td></td>
</tr>
</tbody>
</table>

**Purification of inactivated virus suspension (A.4.4.3)**

<table>
<thead>
<tr>
<th>Method of purification</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Results</td>
<td></td>
</tr>
</tbody>
</table>

**Antigen content of bulk suspension (A.4.4.4)**

<table>
<thead>
<tr>
<th>Method</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Results</td>
<td></td>
</tr>
</tbody>
</table>

**Final bulk (A.4.5)**

**Added substances (A.4.5.1)**

<table>
<thead>
<tr>
<th>Name(s) of substance(s)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Method</td>
<td></td>
</tr>
<tr>
<td>Results (concentrations)</td>
<td></td>
</tr>
</tbody>
</table>

**Sterility tests (A.4.5.2)**

<table>
<thead>
<tr>
<th>Date of inoculation</th>
<th>bacteria</th>
<th>fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Media used</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Observation period</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Results</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Animal serum (A.4.5.3)**

<table>
<thead>
<tr>
<th>Method to determine concentration</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Result</td>
<td></td>
</tr>
</tbody>
</table>
Control tests on final product (A.6)

**Identity test (A.6.1)**
Method
Results

**Sterility tests (A.6.2)**

<table>
<thead>
<tr>
<th></th>
<th>bacteria</th>
<th>fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of inoculation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Media used</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Observation period</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Results</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**General safety tests (A.6.3)**

*Test in mice*

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of inoculation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of mice tested</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume and route of injection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Observation period</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Results (give details of deaths)</td>
<td></td>
<td></td>
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</tbody>
</table>

*Test in guinea-pigs*

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Date of inoculation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of guinea-pigs tested</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume and route of injection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Observation period</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Results (give details of deaths)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Protein content (A.6.4)**

Method
Results

**Potency test (A.6.5)**

Method
Results

**Stability test (A.6.6)**

Method
Results
Residual moisture in freeze-dried vaccine (A.6.7)
Method
Results

Test for pyrogenic substances (A.6.8)
Method
Results

Residual bovine serum albumin (A.6.9)
Method
Results

Adjuvant content (A.6.10)
Method
Results

Test for residual live virus (A.6.11)
Method
Results

Inspection of final containers (A.6.12)
Date and result

Submission addressed to national control authority for batch release
Name (typed) and signature of head of production laboratory

Date

Certification by person taking overall responsibility for production and control of the vaccine:
I certify that lot no. ____ of HFRS vaccine (inactivated) satisfies national requirements and/or Part A of the Requirements for Biological Substances No. 44 (Requirements for Haemorrhagic Fever with Renal Syndrome (HFRS) Vaccine (Inactivated)).
Signature
Name (typed)
Date
## Appendix 2
### General scheme for the preparation of HFRS vaccine

<table>
<thead>
<tr>
<th>Stage in vaccine production</th>
<th>Material to be tested</th>
<th>Tests required</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control of source materials</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hamsters and gerbils for cell cultures</td>
<td>Freedom of animal stock from</td>
<td>Freedom of animal stock from pathogenic microorganisms</td>
</tr>
<tr>
<td>Rodents for virus propagation in neural tissue</td>
<td>pathogenic microorganisms</td>
<td></td>
</tr>
<tr>
<td>Continuous cell lines</td>
<td>Identity of MWCB</td>
<td></td>
</tr>
<tr>
<td>Serum used in cell-culture medium</td>
<td>Sterility and freedom from inhibitors of hantavirus</td>
<td></td>
</tr>
<tr>
<td>Virus strain</td>
<td>Identity, virus titration</td>
<td></td>
</tr>
<tr>
<td>Virus seed lots</td>
<td>Identity, sterility, freedom from adventitious viruses, virus titration</td>
<td></td>
</tr>
<tr>
<td><strong>Control of vaccine production</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control rodent brains</td>
<td>Freedom from specific pathological changes on autopsy</td>
<td></td>
</tr>
<tr>
<td>Control cell cultures</td>
<td>Freedom from adventitious agents</td>
<td></td>
</tr>
<tr>
<td>Virus harvest and initial purification of</td>
<td>Sterility, virus content</td>
<td></td>
</tr>
<tr>
<td>brain-derived material</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pooling of single harvests and clarification</td>
<td></td>
<td></td>
</tr>
<tr>
<td>of virus pool</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage in vaccine production</td>
<td>Material to be tested</td>
<td>Tests required</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>--------------------------------------</td>
<td>-------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Virus inactivation</td>
<td>Inactivated virus pool</td>
<td>Residual live virus</td>
</tr>
<tr>
<td>Purification</td>
<td>Purified, inactivated virus pool</td>
<td>Antigen content</td>
</tr>
<tr>
<td>Preparation of final bulk</td>
<td>Final bulk</td>
<td>Added substances, sterility and (for cell-culture-derived material) serum albumin concentration</td>
</tr>
</tbody>
</table>

**Control of final product**

| Filling of final containers and freeze-drying (if appropriate) | Final product | Identity, sterility, general safety, protein content, potency, stability, residual moisture (in freeze-dried vaccine), pyrogenicity, residual bovine serum albumin (in cell-culture vaccine), adjuvant content, [residual live virus,] inspection of final containers |
Appendix 3

Model certificate for the release of HFRS vaccine (inactivated) by national control authorities

The following lots of HFRS vaccine (inactivated) produced by _______ in _______ whose numbers appear on the labels of the final containers, meet all national requirements and Part A of Requirements for Biological Substances No. 44 (Requirements for Haemorrhagic Fever with Renal Syndrome (HFRS) Vaccine (Inactivated)), and comply with Good Manufacturing Practices for Pharmaceutical and Biological products.

<table>
<thead>
<tr>
<th>Lot no.</th>
<th>Date of last potency test</th>
<th>Expiry date</th>
<th>Lot no.</th>
<th>Date of last potency test</th>
<th>Expiry date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tbody>
</table>

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

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

Signature __________________________________________

Name (typed) __________________________________________

Date __________________________________________

---

1 To be completed by the national control authority of the country where the vaccine has been manufactured, and to be provided by the vaccine manufacturer to importers.
2 Name of manufacturer.
3 Country.
4 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 control authority.
5 With the exception of provisions on distribution and shipping, which the national control authority may not be in a position to assess.
9 Or his or her representative.