

RIFT VALLEY FEVER

SUMMARY

Rift Valley fever (RVF) is a peracute or acute zoonotic disease of domestic ruminants in Africa. It is caused by a single serotype of a mosquito-borne bunyavirus of the genus Phlebovirus. The disease occurs in climatic conditions favouring the breeding of mosquito vectors and is characterised by liver damage. The disease is most severe in sheep, goats and cattle, in which it produces abortions in pregnant animals and a high mortality rate in the newborn. Older nonpregnant animals, although susceptible to infection, are more resistant to clinical disease. There is considerable variation in the susceptibility to RVF of animals of different breeds. Those breeds or strains that are exotic to Africa or are from areas where RVF is not endemic, tend to be more susceptible. Camels suffer an inapparent infection with RVF, but abortion rates can be as high as in cattle. Among ruminant game, buffalo also abort during an inapparent RVF infection.

Humans are susceptible to infection through contact with infected material or mosquito bites. Infection of humans by vectors is a striking feature in countries with a relatively small population of animal hosts. In such areas, RVF may be recognised first in humans. It has caused serious disease in laboratory workers and must be handled with high level biosecurity. It is recommended that laboratory workers be vaccinated.

Identification of the agent: *RVF virus consists of a single serotype of a bunyavirus of the genus Phlebovirus and has morphological and physicochemical properties typical of bunyaviruses.*

The virus can be isolated from blood, preferably collected in an anticoagulant, during the febrile stage of the disease, or from liver, spleen and brain tissues of animals that have died and from the organs of aborted fetuses. Primary isolations are usually made on cell cultures of various types, such as African green monkey kidney (Vero) cells, baby hamster kidney cells, chicken embryo reticulum, or primary cells of sheep or cattle origin. Alternatively, hamsters, adult or suckling mice, embryonated chicken eggs or 2-day-old lambs may be used for primary virus isolation.

A rapid diagnosis can be achieved by using the supernatant of homogenised samples as antigen in virus neutralisation (VN) tests; immunofluorescent staining of impression smears of liver, spleen, brain or infected cell cultures; or by the demonstration of virus in serum, taken during the febrile stage of the disease, by enzyme immunoassay or immunodiffusion.

The presence of characteristic histopathological lesions in the liver assists in the diagnosis.

Serological tests: *Infected animals develop specific antibodies that may become demonstrable by VN as early as 3 days following infection and after 6–7 days by enzyme-linked immunosorbent assay, and by haemagglutination inhibition. Serological tests used less often include immunofluorescence, complement fixation and immunodiffusion.*

Requirements for vaccines and diagnostic biologicals: *Live virus vaccines and antigens for use either in countries where RVF is endemic or during outbreaks, should be prepared from nonpathogenic mouse- or mutagen-attenuated strains of RVF virus grown in cell cultures. The mutagen-attenuated strain of RVF is not yet at a stage where it can be recommended for use.*

In RVF-free countries, vaccines and diagnostic tests should be limited to those using inactivated virus. Suitable virus strains can be obtained from the OIE Reference Laboratory for RVF (see Table given in Part 3 of this Terrestrial Manual).

A. INTRODUCTION

RVF virus consists of a single serotype of a bunyavirus of the genus *Phlebovirus* and has morphological and physicochemical properties typical of bunyaviruses. The virus is enveloped, spherical and 80–120 nm in diameter. Short glycoprotein spikes project through a bilayered lipid envelope and the virus is readily inactivated by lipid solvents and acid conditions below pH 6. The virus has a three-segmented, single-stranded, negative-sense RNA genome. and consists of the three segments: L (large), M (medium) and S (small), each of which is contained in a separate nucleocapsid within the virion. The S segment is ambisense RNA, i.e. has bi-directional coding (12).

Rift Valley fever (RVF) is a peracute or acute, febrile, mosquito-borne, zoonotic disease caused by a virus of the family *Bunyaviridae*, genus *Phlebovirus*. It usually presents in epizootic form over large areas of a country following heavy rains and flooding, and is characterised by high rates of abortion and neonatal mortality, primarily in sheep, goats and cattle. The susceptibility of different breeds to RVF varies considerably. Some indigenous African animals may have only inapparent infections, while exotic or other breeds suffer severe clinical disease with mortality and abortion. Susceptible, older nonpregnant animals and some other species usually do not show signs of disease. Camels have been regularly involved in the RVF epidemics in East Africa and Egypt. Clinical disease is not seen in adult camels, but abortion occurs and some early post-natal deaths have been observed.

Signs of the disease tend to be nonspecific, rendering it difficult to recognise individual cases (8–11, 13, 22, 34) and during epidemics; however, the occurrence of numerous abortions and mortalities among young animals, together with disease in humans, is characteristic. RVF has a short incubation period: 12–36 hours in lambs. A biphasic fever of up to 41°C may develop, and the fever remains high until shortly before death. Affected animals are listless, disinclined to move or feed, and may show enlarged superficial lymph nodes and evidence of abdominal pain. Lambs rarely survive longer than 36 hours after the onset of signs of illness. Animals older than 2 weeks may die peracutely, acutely or may develop an inapparent infection. Some animals may regurgitate ingesta and may show melaena or bloody, foul-smelling diarrhoea and bloodstained mucopurulent nasal discharge. Icterus may sometimes be observed, particularly in cattle. In addition to these signs, adult cattle may show lachrymation, salivation and dysgalactia. In pregnant sheep, the mortality and abortion rates vary from 5% to almost 100% in different outbreaks and between different flocks. The death rate in cattle is usually less than 10%.

The hepatic lesions of RVF are very similar in all species, varying mainly with the age of the infected individual (9). The most severe lesion occurring in aborted fetuses and newborn lambs is a moderately to greatly enlarged, soft, friable liver with a yellowish-brown to dark reddish-brown colour with irregular congested patches. Numerous greyish-white necrotic foci are invariably present in the parenchyma, but may not be clearly discernible. In adult sheep, the lesions are less severe and pinpoint reddish to greyish-white necrotic foci are distributed throughout the parenchyma. Haemorrhage and oedema of the wall of the gallbladder are common. Hepatic lesions in lambs are almost invariably accompanied by numerous small haemorrhages in the mucosa of the abomasum. The contents of the small intestine and abomasum are dark chocolate-brown as a result of the presence of partially digested blood. In all animals, the spleen and peripheral lymph nodes are enlarged, oedematous and may have petechiae.

Microscopically, hepatic necrosis is the most obvious lesion of RVF in both animals and humans. In fetuses and neonates of cattle and sheep, foci of necrosis consist of dense aggregates of cellular and nuclear debris, some fibrin and a few inflammatory cells. There is a severe lytic necrosis of most hepatocytes and the normal architecture of the liver is lost. In about 50% of affected livers, intranuclear inclusion bodies that are eosinophilic and oval or rod-shaped are found. Mineralisation of necrotic hepatocytes is also seen. In adult animals, hepatic necrosis is less diffuse and in sheep, icterus is more common than in lambs (32).

In humans, RVF infections are usually inapparent or associated with a moderate to severe, nonfatal, influenza-like illness (19, 21). A minority of patients may develop ocular lesions, encephalitis, or severe hepatic disease with haemorrhagic manifestations, which is generally fatal. RVF virus has caused serious human infection in laboratory workers. Staff should either be vaccinated and work under containment level 3, work under containment level 4 conditions, or wear respiratory protection. Particular care needs to be exercised when working with infected animals or when performing post-mortem examinations (see Chapter 1.1.2 Biosafety and biosecurity in the veterinary microbiology laboratory and animal facilities).

No significant antigenic differences have been demonstrated between RVF isolates and laboratory-passaged strains from many countries, but differences in pathogenicity have been shown (5, 33).

Infection of humans by mosquito vectors is a striking feature in countries, such as Egypt, with a relatively small population of animal hosts and a large population of mosquitoes.

RVF usually occurs in epizootics in Africa, which may involve several countries in a region at one and the same time. These follow the periodic cycles of exceptionally heavy rain, which may occur very rarely in semi-arid zones (25–35-year cycles), or more frequently (5–15-year cycles) in higher rainfall savannah grasslands. Low level

undetectable RVF activity may take place in inter-epizootic periods. RVF should be suspected when unusually heavy rains are followed by the occurrence of abortions together with fatal disease marked by necrosis and haemorrhages in the liver that particularly affect newborn lambs, kids and calves, concurrent with the occurrence of an influenza-like illness in farm workers and people handling raw meat.

Preventative measures to protect workers from infection should be employed when there are suspicions that RVF-virus-infected meat and tissue samples are to be handled.

B. DIAGNOSTIC TECHNIQUES

1. Identification of the agent

RVF virus may be isolated from serum and blood collected in an anticoagulant during the febrile stage of the disease, from liver, spleen and brain of animals that have died, or from aborted fetuses. Primary isolation is usually performed in hamsters, infant or adult mice, or on cell cultures of various types.

a) Culture

Approximately 5 ml of blood collected during the febrile stage of the disease or approximately 5 g of liver, spleen and brain collected after death should be presented for virus isolation. The samples should be kept at 0–4°C during transit. If transport to the laboratory is likely to take more than 24 hours, the samples should be frozen and sent on dry ice.

Approximately 1 g of homogenised tissue is suspended 1/10 in cell culture medium or buffered saline, pH 7.5, containing sodium penicillin (1000 International Units [IU]/ml), streptomycin sulphate (1 mg/ml), mycostatin (100 IU/ml), or fungizone (2.5 µg/ml). The suspension is centrifuged at 1000 *g* for 10 minutes and the supernatant fluid is injected intracerebrally into 1–5-day-old mice or intraperitoneally into hamsters or adult mice. Infant mice will either die or be obviously ill by day 2. Adult mice are affected 1–3 days later. Although mice or hamsters are the laboratory animal of choice, lambs and embryonated chicken eggs may also be used.

A variety of cell monolayers including African green monkey kidney (Vero), baby hamster kidney (BHK), chicken embryo reticulum (CER: cells developed by Tsunemasa Motohashi at the Nippon Institute for Biological Science, Tokyo, Japan; recharacterised as a hamster line) (4) and primary kidney or testis cells of calves and lambs may be inoculated with 1 ml of clarified sample supernates and incubated at 37°C for 1 hour. It is advisable to also inoculate some cultures with a further 1/100 dilution of the inoculum. This is to avoid the production of defective particles, which follows the use of very high virus inocula. Some tubes containing flying cover-slips should also be prepared. The cultures are washed with phosphate buffered saline at room temperature and covered with medium containing 2% serum free from antibodies against RVF. The cultures are observed microscopically for 5–6 days. RVF virus induces a cytopathic effect (CPE) characterised by slight rounding of cells followed by destruction of the whole cell sheet within 12–24 hours. Specific identification of RVF virus antigen may be made 18–24 hours after infection by immunofluorescent staining of the cover-slip preparations.

The virus may also be detected by immunofluorescence carried out on impression smears of liver, spleen and brain. A rapid diagnosis can sometimes be made by demonstrating viral antigen in tissues or in serum of febrile animals by a complement fixation or agar gel immunodiffusion (AGID) test. A rapid diagnosis can also be made by detection of viral RNA using a reverse-transcription polymerase chain reaction (RT-PCR).

b) Agar gel immunodiffusion

The AGID test is useful in laboratories without tissue-culture facilities. Approximately 1 gram of tissue, preferably liver, is homogenised and made up to a 10–20% suspension in borate saline buffer, pH 9.0. The material is centrifuged at 1000 *g* and the supernatant is used in the test. Micro-AGIDs are performed on standard microscope slides covered with 3 ml of 1% agarose in borate saline. Patterns of six peripheral wells and a central well are prepared and filled with reagents as follows: a positive, preferably hyperimmune serum in the central well, positive control antigen in wells 1 and 4, test tissues in wells 2 and 5 and negative tissues in wells 3 and 6. A precipitin line of continuity should be formed between control antigen and positive serum that extends to include a line between test tissue and serum for a case to be considered positive.

c) Polymerase chain reaction

A rapid diagnosis can also be made by detection of viral RNA (30) using RT-PCR. The PCR was used, among other techniques, for antigen detection in two recent RVF virus outbreaks in Africa – one in Kenya in 1998 and a limited outbreak in South Africa in 1999. It may also be used to detect RVF virus in mosquito pools (18). RT-PCR followed by sequencing of the NS(S) protein-coding region has been used in phylogenetic analysis to characterise two distinct lineages of RVF virus – one Egyptian and the other sub-Saharan – making this technique a powerful molecular epidemiological tool (29).

d) Histopathology

Histopathological examination of the liver of affected animals will reveal characteristic cytopathology, and immunostaining will allow the specific identification of the RVF viral antigen in infected cells. This is an important diagnostic tool because liver or other tissue may be placed in formol saline in the field for diagnostic purposes, which facilitates handling and transport in areas remote from the laboratory.

2. Serological tests

Virus neutralisation (VN) tests including microneutralisation, plaque reduction neutralisation (PRN) and neutralisation in mice have been used to detect antibodies against RVF virus in the serum of a variety of species. Neutralisation tests are highly specific and will record the earliest response, but these tests can only be performed with live virus and are not recommended for use outside endemic areas or in laboratories without appropriate biosecurity facilities and vaccinated personnel.

Other available tests include enzyme-linked immunosorbent assay (ELISA), haemagglutination inhibition (HI), AGID, immunofluorescence, radioimmunoassay and complement fixation. In these tests, however, cross-reactions may occur between RVF virus and other phleboviruses. An advantage of these tests is the fact that they can be performed with inactivated antigen and can therefore be used in RVF-free countries.

The ELISA is a reliable and sensitive test that may be employed with several species to detect antibodies against RVF virus. An IgM-capture ELISA allows diagnosis of a recent infection to be made on a single serum sample.

The HI test can be employed with great confidence in nonendemic areas. However, sera from individuals that have had previous infections with phleboviruses other than RVF may react with RVF antigen to titres as high as 40 and, rarely, to titres of 320 (33). In suspected cases, the OIE Reference Laboratory for RVF (see Table given in Part 3 of this *Terrestrial Manual*) can be of assistance in carrying out neutralisation tests for specificity. The HI antibody titre after vaccination with RVF virus vaccine may be as high as 640 or, rarely, 1280, whereas titres following natural infections with RVF virus are usually significantly higher.

a) Virus neutralisation (the prescribed test for international trade)

The VN test may be employed to determine the presence of antibodies in naturally infected animals and in animals vaccinated with RVF vaccine. The test is highly specific and can be used to test serum of any species. It is generally used to measure vaccine efficacy. Factors other than neutralising antibodies may play a part in resistance to RVF. The Smithburn neurotropic mouse brain strain of highly attenuated RVF virus (31), also referred to as modified live virus and adapted to cell culture, is used as antigen. The antigen is stored at -80°C or 4°C in freeze-dried form. The stock is titrated to determine the dilution that will give 100 TCID₅₀ (50% tissue culture infective dose) in 25 μl under the conditions of the test.

o Test procedure

- i) Inactivate the test sera for 30 minutes in a water bath at 56°C .
- ii) Add 25 μl of cell culture medium with 5% RVF-negative serum and antibiotics to each well of a 96-well cell culture plate.
- iii) Add 25 μl of test serum to the first well of each row and make twofold dilutions. Titrate each serum in duplicate from 1/10 to 1/80 for screening purposes or in quadruplet and to higher dilutions for determination of end-point titres. Include known positive and negative control sera.
- iv) Add 25 μl per well of RVF virus antigen (diluted in cell culture medium and calculated to provide 100 TCID₅₀ per well) to each well that contains diluted test serum and to wells in rows containing negative and positive control serum. In addition, make twofold dilutions of antigen in at least two rows each containing cell culture medium only.
- v) Incubate for 30 minutes at 37°C .
- vi) Add 50 μl per well of Vero, CER or any other suitable cell suspension at 3×10^5 cells/ml or at a dilution known to produce a confluent monolayer within 12 hours.

- vii) Incubate the plates in an atmosphere of 3–5% CO₂ for 3–5 days.
- viii) Using an inverted microscope, the monolayers are examined daily for evidence of CPE. There should be no CPE in rows containing positive control serum and clear evidence of CPE in rows containing negative control serum indicating the presence of virus. Determine the results by the Spearman–Kärber method.

b) Enzyme-linked immunosorbent assay

For the serodiagnosis of RVFV a number of ELISAs using different formats have been published and are commercially available (1, 28). The use of inactivated whole virus or mouse liver antigens has recently been replaced by recombinant nucleocapsid (N) protein as antigen.

These ELISAs are at present in an indirect format and apart from the very important safety consideration also have the advantage of antigen stability and the ability to test 40 sera in duplicate per plate instead of only 20.

An indirect ELISA with pre-coated plates using a nucleocapsid protein (NC) recombinant antigen and Protein G peroxidase conjugate is described below (17).

• **Test procedure**

Unless otherwise stated, all dilutions are made with 10% (w/v) dried milk buffer and all washes performed three times with volumes of 250–300 µl/well.

- i) Using pre-coated plates add 50 µl of diluted (1/100) serum in duplicate wells
- ii) Add control sera at predetermined dilutions in duplicate wells. Incubate for 60 minutes at 37°C. Wash the plate.
- iii) Add Protein G/horseradish peroxidase conjugate at a working dilution to all wells of the plate. Incubate for 60 minutes at 37°C. Wash the plate
- iv) Add 50 µl of ready-to-use TMB Substrate to all wells of the plate. Cover the plate and incubate at room temperature in the darkness for 20–30 minutes.
- v) Add 50 µl of ready-to-use Stop solution to all wells of the plate. Tap plate gently to allow contents to mix. Wait 5 minutes and read plate using a spectrophotometer equipped with a 450 nm filter.
- vi) Suggested plate layout.

	1	2	3	4	5	6	7	8	9	10	11	12
A	CC	CC	1	2	3	4	5	6	7	8	9	10
B	CC	CC	1	2	3	4	5	6	7	8	9	10
C	C++	C++	11	12	13	14	15	16	17	18	19	20
D	C++	C++	11	12	13	14	15	16	17	18	19	20
E	C+	C+	21	22	23	24	25	26	27	28	29	30
F	C+	C+	21	22	23	24	25	26	27	28	29	30
G	C–	C–	31	32	33	34	35	36	37	38	39	40
H	C–	C–	31	32	33	34	35	36	37	38	39	40

CC: Conjugate control; C++: High positive control serum; C+: low positive control serum;
C–: negative control serum; 1–40: test samples.

Newer ELISA formats are being introduced, including formats that are more specific for IgG and IgM (27).

c) Haemagglutination inhibition

The HI test adapted to a microtechnique is based on Clarke & Casals (7). A sucrose/acetone-extracted hamster liver antigen is used in a 96-well U-bottomed plate test and antigen is diluted so that 4 haemagglutinating units are used in the test. Nonspecific inhibitors of haemagglutinin are removed by kaolin extraction of sera followed by adsorption with packed goose erythrocytes (RBC) prior to testing. Doubling dilutions of sera made in borate saline buffer, pH 9, are tested against equal volumes of antigen.

Plates are held overnight at 4°C before the addition of 50 µl of 0.5% RBC to each of the wells. Plates are read after 30 minutes at room temperature and end-points are recorded as the reciprocal of the highest serum dilution producing complete inhibition of agglutination.

Positive and negative control sera are incorporated into each test. A test is considered to be valid only if the control sera give the expected results. Sera with titres below 1/40 are considered to be negative.

HI is an appropriate screening test for surveys although it is not specific. Marked cross-reactions do occur between the phleboviruses, but homologous titres exceed heterologous titres. Experimentally, African phleboviruses other than RVF have been shown to be nonpathogenic for ruminants, and antibodies that they might induce are unlikely to cause confusion in RVF diagnosis (33).

C. REQUIREMENTS FOR VACCINES AND DIAGNOSTIC BIOLOGICALS

A live vaccine prepared from Smithburn's attenuated strain of RVF virus has been used for the control of RVF in nonpregnant cattle and sheep in endemic areas and during outbreaks (6), while inactivated vaccines for use in pregnant animals and in RVF-free countries are prepared from virulent field strains (2, 3). Inactivated virus vaccines should be prepared from highly immunogenic strains of RVF virus produced in cell culture. The virus should be inactivated with formaldehyde and mixed with an adjuvant to enhance immunogenicity. The inactivated vaccine should be carefully safety tested to ensure that there is no residual live virus.

Guidelines for the production of veterinary vaccines are given in Chapter 1.1.8 Principles of veterinary vaccine production. The guidelines given here and in Chapter 1.1.8 are intended to be general in nature and may be supplemented by national and regional requirements.

In humans, an inactivated experimental RVF vaccine has been used for 25 years with considerable success to protect persons at risk. This vaccine is currently produced on diploid cells. However, the limited availability of the vaccine precludes its use in the general population.

Two new vaccine candidates produced from human RVF virus isolates are undergoing extensive testing with a view to replacing existing vaccines.

The first, MV P12, is a mutagen-derived strain of virus passaged in the presence of 5-fluorouracil with serial mutagenesis resulting in attenuation for mice. Immunogenicity and pathogenicity have been tested in sheep and the virus found to be non-abortogenic in pregnant ewes (23). MV P12 was protective in young lambs (14, 20) and in cattle (24, 25). In further testing in sheep, the vaccine, when used after 28 days of pregnancy, i.e. in the first trimester, resulted in abortion and severe fetal teratology (16).

The second candidate, Clone 13, a small plaque variant that did not react with two specific monoclonal antibodies, was found to be avirulent in mice and hamsters and highly immunogenic. Immunogenicity and pathogenicity have been tested in lambs, sheep, young and adult goats (26). In further trials it was non-abortogenic in pregnant sheep and gave more than 80% protection from virulent challenge (15). Clone 13 possesses a large deletion in the portion of the sRNA segment coding for the nonstructural proteins, which should result in a stable vaccine candidate.

In the following description of vaccine production, information is given on live vaccine production adjacent to information on inactivated vaccine production. It must be stressed that live and inactivated vaccines must never be produced in the same facility at the same time, because of the risk of contaminating the attenuated live vaccine with a virulent strain of virus before it is inactivated. Staff handling live RVF virus should be vaccinated and work at containment level 3 to minimise the risk of self infection.

1. Seed management

a) Characteristics of the seed virus

Live vaccine: The stock antigen is derived from Smithburn's original neurotropic strain. This strain is not lethal to adult mice inoculated intraperitoneally and is safe for use in all breeds of cattle, sheep and goats. However, it may cause fetal abnormalities or abortion in pregnant animals.

Inactivated vaccine: For seed virus, a highly immunogenic strain of RVF virus adapted to growth in cell culture may be used. It differs from the attenuated strain in that it is lethal to adult mice when injected intraperitoneally.

b) Method of culture

Both attenuated and inactivated virus strains are produced on BHK, Vero or CER cell cultures. The viruses are stored in a lyophilised form in vials containing 1 ml of a cell culture suspension. The virus titre (following intracerebral inoculation of infant mice) should be at least $10^{6.5}$ mouse LD₅₀ (50% lethal dose) per ml.

c) Validation as a vaccine

Seed virus must be shown to be free from adventitious agents, safe for use and able to stimulate effective immunity in species and breeds for which it is intended.

o Tests

The lyophilised seed virus is reconstituted in sterile cell culture medium without antibiotics and tested for freedom from bacteria and fungi. The contents of a reconstituted vial are inoculated into two tubes of thioglycollate and two tubes of soybean casein digest medium. The thioglycollate cultures are incubated at 37°C for 7 days and the soybean casein digest medium cultures at 20°C for 14 days. The cultures should remain negative.

In addition, 5 ml of reconstituted seed virus is mixed with an equal volume of specific RVF antiserum produced in rabbits. After incubation of the serum/virus mixture at 37°C for 30 minutes, the virus suspensions are tested before and after neutralisation on cell cultures, as well as in adult and infant mice, embryonated eggs, and guinea-pigs. The neutralised virus is:

- i) Seeded on to six roller tube cultures of primary lamb kidney cells and six roller tube cultures of BHK cells. The cell cultures are incubated at 37°C and observed daily for 7 days for CPE, after which they are subjected to the haemadsorption test with guinea-pig RBCs at 4°C and 37°C. There should be no evidence of CPE or haemadsorption. If cultures degenerate or show suspicious CPE, the material from these cultures should again be mixed with antiserum and subinoculated into new cell cultures, which are observed for a further period of 14 days. The presence of specific CPE or haemadsorption disqualifies the seed virus pool.
- ii) Inoculated intraperitoneally (0.2 ml) into groups of at least six adult and six 2–5-day-old mice. The mice should remain healthy for 14 days. If any mice should die, appropriate tissue should be emulsified, mixed with antiserum and subinoculated into further groups of mice, which should again be observed for a further period of 14 days. If there is any evidence of specific mortality, the seed virus pool is disqualified.
- iii) Inoculated into at least ten 8-day-old embryonated chicken eggs by means of the 'stab' method (combination of chorioallantoic membrane and allantoic sac route). The eggs are incubated at 37°C for 8 days and are candled daily. Embryos that die within 24 hours are discarded. However, the test should be repeated if <70% of the embryos are alive after 24 hours. The cause of embryo mortality during the subsequent observation period should be determined by setting up appropriate sterility and HI tests, and by examination of yolk-sac smears. If these tests are negative, subinoculation of embryo suspensions mixed with antiserum should be set up as before. On day 4 of incubation, at least four eggs are opened and allantoic fluid is collected. The remaining eggs are opened on day 8 of incubation. The membranes of both groups are examined for lesions and abnormalities of the embryos. The allantoic fluids are subjected to the HI test with guinea-pig and chicken RBCs at 4°C and 37°C. Specific embryo mortality, haemagglutinating activity of the allantoic fluids or any lesions on the membranes or embryo abnormalities disqualifies the seed virus pool.
- iv) Injected intraperitoneally with 1.0 ml of seed virus into each of two guinea-pigs. The guinea-pigs should remain healthy over an observation period of 14 days.

Failure to pass any test disqualifies the antigen for use as seed virus.

2. Method of manufacture

A vial of lyophilised seed virus is reconstituted and diluted 1/100 to 1/1000 with sterile Eagle's medium for the attenuated vaccine and 1/1000 for the inactivated vaccine. To prepare a working suspension, the diluted virus is seeded on to confluent BHK cell cultures in roller bottles and incubated at 37°C. When 70% of cells is affected (CPE), the medium and cells are harvested and the material is diluted 1/100 to 1/1000, after which 10 ml is again seeded on to roller bottles with confluent BHK cells and again incubated. As soon as 70% CPE is observed, the medium and cells are harvested and pooled.

Virus suspensions for both attenuated and inactivated vaccines are titrated intracerebrally in infant mice and should have a titre of at least $10^{6.5}$ mouse LD₅₀/ml. Alternatively, a plaque titration on CER cells may be performed.

Attenuated vaccine is lyophilised immediately after completion of titration and testing for bacteria and fungi.

A stabiliser should be used, such as 5% peptone in 0.3 M phosphate buffer. The volume of inactivated vaccine is adjusted so that the final vaccine will contain at least $10^{6.5}$ mouse LD_{50}/ml . The adjusted virus suspension is then inactivated at 37°C for 24 hours with formaldehyde at a final concentration of 0.2%. After inactivation, an equal volume of aluminium hydroxide gel is added to the cell suspension. The vaccine should have a final pH of 7–7.5.

3. In-process control

Prior to inoculation of cell cultures, seed virus is subjected to tests for bacteria and fungi in thioglycollate and soybean casein digest medium (see Section C.1.c and Chapter 1.1.9 Tests for sterility and freedom from contamination of biological materials).

A representative sample from each batch of vaccine is selected and the contents of each are reconstituted with 5 ml sterile distilled water and tested for freedom from bacteria and fungi.

For inactivated vaccines, inactivation must be checked using two passages in the same type of cell culture as used in the production of the vaccine.

4. Batch control

a) Sterility

Prior to freeze-drying or inactivation, each container of pooled vaccine, and thereafter representative samples of the batch, are tested for sterility in thioglycollate and soybean casein digest medium (see also Chapter 1.1.9 of this *Terrestrial Manual*).

b) Safety

Live vaccine: Final containers of lyophilised attenuated vaccine are selected at random, and each is reconstituted in distilled water as for vaccination. Four susceptible sheep are injected subcutaneously with one dose of vaccine. The sheep are observed daily for 14 days and the rectal temperatures are recorded. The sheep must remain healthy.

Vaccine is also injected intraperitoneally into six adult mice (0.25 ml each), two hamsters and two guinea-pigs (1 ml each). The animals are observed for a period of 14 days during which they should remain healthy. Mortality attributed to the vaccine disqualifies the batch.

Inactivated vaccine: In the case of inactivated RVF vaccine, each of four susceptible sheep is injected subcutaneously with 2.0 ml of vaccine, observed daily for 3 weeks and rectal temperatures are recorded. The sheep should remain healthy.

In addition, safety is also determined by intracerebral injection of six adult mice and two litters of at least six infant mice per litter, and by intraperitoneal injection of two guinea-pigs and two hamsters. The mice, hamsters and guinea-pigs are observed for a period of 14 days. They should remain healthy. Mortality attributed to the vaccine disqualifies the batch.

c) Potency

Live vaccine: Lyophilised attenuated vaccine from two final containers is reconstituted and titrated intracerebrally in infant mice. The final vaccine should contain at least $10^{4.4}$ mouse $LD_{50}/dose$. Alternatively, titrations may be done on cell cultures.

Two final containers are kept at 37°C for 1 week, reconstituted and titrated as before. Each should contain at least $10^{3.4}$ mouse $LD_{50}/dose$. Alternatively, titrations may be done on cell cultures.

Inoculated sheep (see Section C.4.b) are bled 2 and 3 weeks after vaccination, and their antibody response is determined by PRN. A virus neutralising antibody titre of 100 or more is regarded as satisfactory.

Inactivated vaccine: The sheep, injected subcutaneously to determine safety (Section C.4.b), are bled after 3 weeks and their antibody response is determined by VN test. A virus neutralising antibody titre of 100 or more is regarded as satisfactory.

d) Duration of immunity

Both the live attenuated and the inactivated vaccines have had extensive field use. The live vaccine is considered to induce lifelong immunity against clinical disease, although controversy exists over the immunogenicity of the Smithburn vaccine. Nevertheless, cattle can be immunised with the live virus vaccine

using this strain. Experience of the field efficacy of inactivated vaccines is limited because they are used in areas where RVF is not endemic, consequently natural field challenge of the vaccine does not occur. However, in South Africa, during the outbreak of RVF in 1976–1978, observations by State Veterinarians supported the efficacy of the vaccine. In more recent epizootics elsewhere, the inactivated vaccine failed to protect animals against abortion, following two vaccinations. When using the inactivated vaccine, a booster dose should be given 3–6 months after the initial vaccination and thereafter vaccination should be repeated yearly (2, 3).

e) Stability

When stored at 4°C, lyophilised attenuated vaccines are stable for at least 4 years, while inactivated vaccine may be stored for many years. Storage at higher temperatures is not recommended.

f) Preservatives

No preservatives are used.

g) Precautions (hazards)

Although humans can be infected by handling infected material, no case of disease is known to have occurred in humans infected with attenuated vaccine virus, but seroconversion often occurs. However, the strains used to prepare inactivated vaccine may cause disease. Therefore, all staff likely to be exposed to vaccine virus should be vaccinated with the human formalin-inactivated vaccine.

5. Tests on the final product

a) Sterility

Representative samples of the final product are collected and tested as in Section C.4.a.

b) Moisture content

The moisture content of the lyophilised attenuated vaccine should not exceed 3%.

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NB: There is an OIE Reference Laboratory for Rift Valley fever (see Table in Part 3 of this *Terrestrial Manual* or consult the OIE Web site for the most up-to-date list: www.oie.int).