

## CHAPTER 2.1.19.

# RINDERPEST (INFECTION WITH RINDERPEST VIRUS)

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### SUMMARY

*In the past, classical rinderpest was an acute, viral disease of domestic cattle, yaks and wild African buffaloes (*Syncerus caffer*) and Asian water buffaloes (*Bubalus bubalis*). It was characterised by high morbidity and mortality rates. Sheep, goats, pigs and wild ungulates might also be affected. Between 2002 and 2011, there were no reported field cases of rinderpest. The eradication campaign concluded in 2011 with an international declaration of global freedom from rinderpest.*

*Existing collections of virulent and attenuated rinderpest viruses will remain under sequestration in research and approved vaccine manufacturing laboratories. To guard against the accidental release of virus from laboratory sources, the FAO<sup>1</sup> and the OIE are collaborating in establishing the principle of international oversight and regulation of facilities holding rinderpest virus. All diagnostic testing, vaccine production and research activities that use live rinderpest virus or rinderpest virus-containing materials should be performed in an OIE/FAO regulated rinderpest holding facility.*

*Rinderpest remains a notifiable disease and adequate surveillance systems must be maintained for the early detection of clinical cases should there be any accidental escape of the virus. The OIE (with FAO) will ensure the permanent availability of educational materials demonstrating the range of signs associated with rinderpest cases in live animals.*

**Description of the disease:** *Clinical recognition of classical rinderpest is based on the finding of an individual dead animal or small groups of extremely sick animals showing one or more of the following signs: pyrexia, inappetance, depression, emaciation, shallow erosions of the upper and lower lip and gum, erosions or blunting of the cheek papillae, serous or mucopurulent ocular discharges and/or nasal discharges, diarrhoea, and terminal recumbency. It is more than likely that the group will contain a number of dead animals with such lesions. The introductory section of this chapter provides a more detailed description.*

**Identification of the agent:** *Laboratory confirmation is based on demonstrating the presence of the virus, virus-specific RNA or precipitating antigens in samples from the spleen, lymph nodes, or ocular or nasal secretions of acutely infected animals.*

**Serological tests:** *A competitive enzyme-linked immunosorbent assay (ELISA) can be used to determine the presence of rinderpest antibodies in animals that have been infected with field virus or received rinderpest vaccine. The test used should be sensitive with respect to the lineage of virus likely to be present and be highly specific. Neutralising antibody estimations may be used for the same purpose. As with the virus, serum samples from rinderpest-suspected cases, and that may contain the virus or viral sequences, may only be examined in OIE approved high security laboratories.*

**Requirements for vaccines:** *A live attenuated cell culture rinderpest vaccine is available. At this time no animal outside a biosecure facility will be inoculated with a rinderpest vaccine. In compliance with international oversight and regulation of facilities holding rinderpest virus, the retention and further manipulation of vaccine seed viruses will be internationally regulated.*

*In order to prepare for the possibility of a rinderpest virus release, under the terms of the international sequestration agreement, FAO and OIE, in collaboration with member countries, have developed a strategic plan for the post-eradication era that includes an international contingency*

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1 FAO: Food and Agriculture Organization of the United Nations

*plan, designation of a minimum number of Reference Centres/Reference Laboratories and creation of emergency vaccine repositories to maintain preparedness.*

## A. INTRODUCTION

In the past, classical rinderpest was an acute, viral disease of domestic cattle, yaks and wild African buffaloes (*Syncerus caffer*) and Asian water buffaloes (*Bubalus bubalis*). It was characterised by high morbidity and mortality rates. Sheep, goats, pigs and wild ungulates might also be affected. Between 2002 and 2011 there were no reported field cases of rinderpest. Further, in the period leading up to January 2011, the OIE Scientific Commission for Animal Diseases scrutinised a comprehensive world-wide list of applications (evidence-based and historical) for national recognition of rinderpest-freedom. This process concluded in 2011 with an international declaration of global freedom from rinderpest.

For the immediate future, existing collections of virulent and attenuated rinderpest viruses will remain under sequestration in research and approved vaccine manufacturing laboratories. To guard against the accidental release of virus from laboratory sources, FAO and the OIE are collaborating in establishing the principle of international oversight and regulation of facilities holding rinderpest virus based on minimising the number of repositories. All diagnostic testing, vaccine development and research activities that use live rinderpest virus or rinderpest virus-containing materials should be performed in an OIE/FAO regulated rinderpest holding facility.

Rinderpest remains a notifiable disease and adequate surveillance systems must be maintained for the early detection of clinical cases should there be any accidental escape of the virus. The OIE (with FAO) will ensure the permanent availability of educational materials demonstrating the range of signs associated with rinderpest cases in live animals. A recent account of the history of rinderpest, its eradication and its socio-economic impact is available (Roeder & Rich, 2009).

Rinderpest is caused by a negative-strand RNA virus of the *Morbillivirus* genus within the family *Paramyxoviridae*. The virus exists as three geographically restricted clades, described as African Lineages 1 and 2 and Asian Lineage 3, which cross-protect fully and are only differentiated by molecular characterisation. The tissue culture rinderpest vaccine virus was derived from another genetically distinct virus which was introduced into Africa from Asia in the 19<sup>th</sup> Century. Classic descriptions of rinderpest refer to it as a highly fatal disease of domestic cattle, yaks and wild African and Asian water buffaloes. The virus also affects swine and a very large variety of wildlife species within the order *Artiodactyla*, although not always in a clinically apparent form; a recent review identifies sheep and goats as susceptible but largely epidemiologically unimportant hosts of rinderpest (Taylor & Barrett, 2007).

Although some strains of rinderpest evolved into a mild, nonfatal, infectious disease of cattle, all strains retain two very dangerous attributes. The first is an almost certain ability to undergo virulence modulations. The second is an ability to infect wild animal species and, in African buffaloes, eland, giraffe, lesser kudu and warthog, to cause an acute infection associated with high mortality.

Rinderpest is not a zoonotic disease, but the virus or virus-containing materials must be handled in accordance with strict biocontainment procedures as described in Chapter 1.1.4 *Biosafety and biosecurity: Standard for managing biological risk in the veterinary laboratory and animal facilities* and in conformity with the Guidelines for Rinderpest Virus Sequestration.

An illustrated description of the disease is given in the OIE Atlas of Transboundary Animal Diseases (Fernandez & White, 2010). Classical rinderpest has an incubation period of between 1 and 2 weeks, the clinical disease is characterised by an acute febrile attack within which prodromal and erosive phases can be distinguished. The prodromal period lasts approximately 3 days, during which affected animals develop a pyrexia of between 40 and 41.5°C together with partial anorexia, constipation, congestion of visible mucosae, serous ocular and nasal discharges, depression and drying of the muzzle. However, it is not until the onset of the erosive phase, and the development of necrotic mouth lesions, that a tentative clinical diagnosis of rinderpest can be made. At the height of fever, flecks of necrotic epithelium appear on the lower lip and gum and in rapid succession may appear on the upper gum and dental pad, on the underside of the tongue, on the cheeks and cheek papillae and on the hard palate. Through the enlargement of existing lesions and the development of new foci, the extent of the oral necrosis can increase dramatically over the following 2–3 days. Much of the necrotic material works loose giving rise to shallow, non-haemorrhagic mucosal erosions.

Diarrhoea is another characteristic feature of rinderpest and develops 1–2 days after the onset of mouth lesions. The diarrhoea is usually copious and watery at first, but later on may contain mucus, blood and shreds of epithelium and it may be accompanied, in severe cases, by tenesmus. During the erosive phase, necrosis may be observed in the nares, in the vulva and vagina, and on the preputial sheath. Anorexia develops, the muzzle dries

out completely, the animal is depressed and emaciated, the breath is fetid and mucopurulent ocular and nasal discharges develop.

Deaths will occur but depending on the strain involved, the breed of cattle infected and environmental conditions, the mortality rate may vary from 100% (peracute strains in European breeds), to 20–30% (acute strains in zebu cattle), to zero (mild strains in zebu cattle). With both acute and mild strains, the mortality rate may be expected to rise as the virus gains progressive access to large numbers of susceptible animals. In the terminal stages of the illness, animals may become recumbent for 24–48 hours prior to death. Some animals die while showing severe necrotic lesions, high fever, emaciation and diarrhoea, others after a sharp fall in body temperature, often to subnormal values. In survivors, the pyrexia may remit slightly in the middle of the erosive period and then, 2–3 days later, return rapidly to normal accompanied by a quick resolution of the mouth lesions, a halt to the diarrhoea and an uncomplicated convalescence.

In cases where rinderpest is suspected, post-mortem examinations should pay particular attention to the abomasum, which may be highly engorged or show a grey discoloration; to the Peyer's patches, which may show lymphoid necrosis; and to the development of linear engorgement and blackening of the crests of the folds of the caecum, colon and rectum. The principal differential diagnoses in cattle are bovine viral diarrhoea/mucosal disease and malignant catarrhal fever; differentiation of these diseases requires the use of appropriate laboratory methods. Diagnosis (and differential diagnosis) of rinderpest suspect material will only be undertaken in OIE approved high security laboratories.

Typically the carcass of the dead animal is dehydrated, emaciated and soiled. The nose and cheeks bear evidence of mucopurulent discharges, the eye is sunken and the conjunctiva congested. In the oral cavity, there is often extensive desquamation of necrotic epithelium, which always appears sharply demarcated from adjacent areas of healthy mucosa. The lesions frequently extend to the soft palate and may also involve the pharynx and the upper portion of the oesophagus; the rumen, reticulum and omasum are usually unaffected, although necrotic plaques are occasionally encountered on the pillars of the rumen. The abomasum, especially the pyloric region, is severely affected and shows congestion, petechiation and oedema of the submucosa. Epithelial necrosis gives the mucous membrane a grey colour. The small intestine is not commonly involved except for striking changes to the Peyer's patches where lymphoid necrosis and sloughing leaves the supporting architecture engorged or blackened. In the large intestine changes involve the ileocaecal valve, the caecal tonsil and the crests of the longitudinal folds of the caecal, colonic and rectal mucosae. The folds appear highly engorged in acute deaths or darkly discoloured in long-standing cases; in either event the lesions are referred to as 'zebra striping'.

Taking the mild expression of rinderpest, which was associated with African lineage 2 rinderpest virus in endemic areas of eastern Africa, as an example, the incubation period could be between 1 and 2 weeks and the ensuing clinical disease little more than a subacute febrile attack in cattle. The fever was not invariable; it was short-lived (3–4 days) and low (38–40°C). The depression that characterised more acute forms of rinderpest was absent from mildly affected animals and, as a result cattle often did not lose their appetite, and continued to graze, water and trek as well as unaffected animals. Diarrhoea, if present, was not marked. On close examination there might be some slight congestion of the visible mucous membranes and small, focal areas of raised, whitish epithelial necrosis might be found on the lower gum – sometimes no larger than a pin head – along with a few eroded cheek papillae. Some animals totally escaped the development of such erosions, the appearance of which was fleeting. Other animals might show a slight, serous, ocular or nasal secretion but, in contrast to the more severe forms of the disease, these did not progress to become mucopurulent.

Even though infections with mild rinderpest could pass unnoticed in cattle, the virus remained highly infectious for wildlife species, and among those generally regarded as highly susceptible (tragelaphine species, such as lesser kudu and eland, African buffalo, and giraffe) it caused fever, a nasal discharge, typical erosive stomatitis, gastroenteritis, and death. Kock (2006) observed that in addition, African buffaloes infected with lineage 2 showed enlarged peripheral lymph nodes, plaque-like keratinised skin lesions and keratoconjunctivitis. Lesser kudus were similarly affected, but whereas blindness – caused by a severe keratoconjunctivitis – was common, diarrhoea was unusual. Eland also showed necrosis and erosions of the buccal mucosa together with dehydration and emaciation. Therefore, in these circumstances, a diagnosis of rinderpest in any of these species points to the likelihood of the simultaneous transmission of the virus, even at a subclinical level, in neighbouring cattle and possible dissemination of infection through live animal trade.

## B. DIAGNOSTIC TECHNIQUES

THIS SECTION WAS ADOPTED IN 2012  
AND IS CURRENTLY BEING CONSIDERED FOR REVISION

### 1. Identification of the agent

Any suspicion of rinderpest must be viewed as a potential threat to international biosecurity and must be rapidly confirmed or differentiated. If confirmed, back-tracing measures must be immediately instigated but based on an understanding that the virus has been isolated, its lineage identified and its virulence in experimental cattle assessed (Anderson *et al.*, 1996). A variety of suitable tests is available.

Blood in anticoagulant is the preferred sample wherever possible. On average, the onset of viraemia slightly precedes the onset of pyrexia, and may continue for 1–2 days after pyrexia begins to wane. Consequently, animals showing a pyrexia are probably viraemic and therefore the best source of blood with which to attempt virus isolation. However, as occasional febrile animals may no longer be viraemic, samples from several febrile animals should be collected for submission. It is important to ensure that there is adequate tissue available for at least two virus isolation attempts from the initial submission of a suspected outbreak. The other procedures described should only be attempted if there is extra tissue available.

#### 1.1. Virus isolation

Rinderpest virus can be cultured from the leukocyte fraction of whole blood that has been collected into heparin or EDTA (ethylene diamine tetra-acetic acid) at final concentrations of 10 international units (IU)/ml and 0.5 mg/ml, respectively. Samples should be thoroughly mixed and transferred to the laboratory on ice, but never frozen. Virus can also be isolated from samples of the spleen, prescapular or mesenteric lymph nodes of dead animals; these samples may be frozen for transportation; transportation must be under biosecure conditions in compliance with international transport regulations described in Chapter 1.1.2 *Collection, submission and storage of diagnostic specimens*, Chapter 1.1.3 *Transport of specimens of animal origin* and with the Guidelines for Rinderpest Virus Sequestration.

To isolate the virus from blood, uncoagulated blood is centrifuged at 2500 g for 15 minutes to produce a buffy coat layer at the boundary between the plasma and erythrocytes. This is removed as cleanly as possible, mixed in 20 ml physiological saline and recentrifuged in a washing procedure designed to remove any neutralising antibody present in the plasma. The resulting cell pellet is suspended in cell culture maintenance medium and 2 ml aliquots are distributed on to established roller tube monolayers of primary calf kidney, B95a marmoset lymphoblastoid transformed bovine T lymphoblast or African green monkey kidney (Vero) cells. The culture maintenance medium should be decanted and replaced every 2 or 3 days and the monolayer observed microscopically for the development of cytopathic effects (CPE). These are characterised by refractility, cell rounding, cell retraction with elongated cytoplasmic bridges (stellate cells) and/or syncytial formation. The speed with which the CPE develops varies by substrate and probably by strain of virus also. Up to 12 days should be allowed in primary cells, a week in Vero and 2–4 days in B95a cells. Blind passages may be attempted before declaring an important sample negative, but a preferable technique would be to inoculate the cell suspension, and any residue of the original sample, intravenously into a rinderpest-susceptible ox and attempt to re-isolate the virus from its blood. Isolates of virus can be partially identified by the demonstration of morbillivirus-specific precipitinogens in infected cell debris, or completely identified by the demonstration of specific immunofluorescence using a conjugated monoclonal antibody (MAb).

Alternatively, 20% suspensions (w/v) of lymph node or spleen may be used. These should be made by macerating the solid tissues in serum-free culture maintenance medium using standard grinding or shearing techniques and inoculating monolayers as before. The release of virus from solid tissue can be achieved in several ways. Perhaps the easiest is with a pestle and mortar, but this technique requires the use of sterile sand as an abrasive. Alternatively, tissue may be ground without an abrasive using all-glass grinders, for example, a Ten Broeck grinder. Shearing techniques are equally applicable using, for example, Silverson or Waring blenders. Virus-containing suspensions are clarified by low-speed centrifugation. The volume of the inoculum is not critical; a working volume is between 1 and 2 ml. Commonly used antibiotics are penicillin and streptomycin in combination, each at a concentration of 100 IU/ml. A similar broad-spectrum cover can be obtained using neomycin at 50 µl/ml. Fungizone should be included at 2.5 µg/ml.

### 1.3. Antigen detection by agar gel immunodiffusion

The agar gel immunodiffusion (AGID) tests may be conducted in Petri dishes or on glass microscope slides (Foreman *et al.*, 1983). In either instance the surface should be covered with agar to a depth of about 4 mm using a 1% aqueous solution of any high quality agar or agarose. Wells are usually cut in a hexagonal pattern of six peripheral wells around a single central well. For slides, wells should be 3 mm in diameter and 2 mm apart. For Petri dishes, the wells can be increased to 4 mm in diameter and the distance between wells to 3 mm. The closer the wells are placed from each other, the shorter the reaction time.

Using a small volume pipette, rinderpest hyperimmune rabbit serum should be placed in the central well. Similarly, control positive antigen, prepared from the macerated lymph nodes of rabbits infected with the Nakamura III lapinised strain of rinderpest, should be placed in alternate peripheral wells (i.e. one, three and five). Negative control antigen is placed in well four. Test antigens are obtained as exudates from the cut surface of spleen or lymph nodes submitted for testing; if no exudate can be obtained a small portion of the sample should be ground with a minimum of saline. Ocular exudates may be squeezed directly from the swabs or, alternatively, by compression in a microtip (the cotton wool should be cut off the swab and placed into the wide end of a plastic 50–250 µl pipette tip; the stem of the swab may then be used to compress the cotton wool and force a small volume of exudate out of the narrow end of the tip). Test samples are added to wells two and six. Tests are best developed at 4°C or low ambient temperatures. The reaction area should be inspected from 2 hours onwards for the appearance of clean, sharp lines of precipitation between the wells forming a line of identity with the controls. Tests should be discarded after 24 hours if no result has been obtained. The result is not acceptable unless precipitation reactions are also obtained giving a line of identity with the control positive antigen preparation.

Although the test is neither highly sensitive nor highly specific, it is robust and adaptable to field conditions. A positive reaction from a large domestic ruminant should be treated as if it were rinderpest. From a small ruminant, a positive result should be treated as having been derived from a case of rinderpest or peste des petits ruminants (PPR) and requiring further differentiation.

### 1.4. Histopathology and immunohistochemistry

At post-mortem examination, tissues should be collected and placed in 10% neutral buffered formalin for histopathology and immunohistochemistry; the base of the tongue, retropharyngeal lymph node and third eyelid are suitable tissues. Sections stained with haematoxylin and eosin should be examined for the presence of syncytial cell formation, and cells with intranuclear viral inclusion bodies. The presence of rinderpest antigens can be demonstrated in the same formalin-fixed tissues by immunoperoxidase staining following the quenching of endogenous peroxidase activity. If a polyclonal antiserum is used, this test will fail to differentiate between rinderpest and PPR. However, this problem can be circumvented by using monoclonal antibodies specific for rinderpest and PPR in duplicate tests (Brown, 1997).

### 1.5. Lineage identification using the reverse-transcription polymerase chain reaction

The reverse-transcription polymerase chain reaction (RT-PCR) (Forsyth & Barrett, 1995) produces DNA suitable for gene sequence analysis. Viral RNA can be purified from spleen (not ideal because of its high blood content), lymph node and tonsil (ideal), peripheral blood lymphocytes (PBLs), or swabs from eyes or mouth lesions (contingent). Solid tissues (0.5–1.0 g) are minced and homogenised with 4.0 ml denaturing solution, eye and mouth swabs are treated with 1.0 ml, and purified PBLs (from 5 to 10 ml whole blood) are treated with 0.4 ml according to the published procedure. Solution D (disruption solution): the procedure is that recommended to minimise the hazard of handling poisonous guanidium thiocyanate. It should be carried out in a chemical safety hood. The following are the amounts of guanidium thiocyanate for a 250 g bottle, but the volumes can be adjusted for other quantities. Do not attempt to weigh out the guanidium thiocyanate, but dissolve it in the manufacturer's bottle by adding 293 ml sterile distilled water, 17.6 ml 0.75 M sodium citrate, pH 7.0, and 26.4 ml 10% sarcosyl, then heat to 65°C in a water bath to dissolve. This solution can be kept for several months in the dark at room temperature in a chemical safety cabinet. The final solution D is made by the addition of 0.36 ml 2-mercaptoethanol to 50 ml of the stock solution. This solution should not be kept for more than 1 month.

In the past few years, RNA extraction spin columns have become widely used for fast purification of high quality RNA (RNeasy kit, Qiagen). The resulting RNA is precipitated with 2.5 volumes of ethanol, washed in 70% ethanol, dissolved in sterile water, or TE buffer (Tris/EDTA, 10 mM, pH 7.5, 1 mM EDTA) and stored at –70°C or –20°C until required. The cDNA synthesis is carried out using random

hexanucleotide primers to enable several different specific primer sets to be used in the PCR amplification step. Aliquots of the resulting cDNA are amplified using at least three primer sets that can detect and differentiate between PPR and rinderpest. These primer sets include two 'universal' sets based on highly conserved regions in the phosphoprotein and nucleoprotein genes that should detect all morbilliviruses, and rinderpest virus-specific sets based on sequences in the fusion protein genes of the virus. The PCR products are analysed on a 1.5% (w/v) agarose gel along with a suitable DNA marker to identify the specific DNA product. A positive control such as measles or canine distemper virus RNA, and a negative control using sterile distilled water instead of RNA, must be included in each RT-PCR. Positive reactions should be confirmed either by using 'nested' primer sets based on the F gene sequences or by sequence analysis of the DNA product. It is important to use more than one set of primers for the PCR step when testing for the presence of RNA viruses, as their nucleotide sequences can vary significantly and one change at the 3'-end of the primer sequence may result in failure of the primers to amplify the DNA. The World Reference Laboratory in the United Kingdom (UK), which is also an OIE Reference Laboratory for rinderpest, and the OIE Reference Laboratory in France (see Table given in Part 4 of this *Terrestrial Manual*), can advise on use of the technique for field sample analysis.

Most recently, a simple Taqman real-time RT-PCR assay for RPV diagnostic has been described. This real-time RT-PCR assay for rinderpest virus has been validated to be highly sensitive in infected tissue culture supernatant and clinical samples from experimentally infected cattle. The assay has proved to be able to detect isolates representative of all known phylogenetic lineages of the virus and clearly differentiate from PPR virus and other look-alike diseases (foot and mouth disease virus, bovine viral diarrhoea virus, bovine herpesvirus, vesicular stomatitis virus). The analytical sensitivity of the L10 primer-probe system exceeded 1–100 TCID<sub>50</sub> (50% tissue culture infective dose)/ml, depending on the rinderpest virus strain. Comparison of samples from experimentally infected animals showed that white blood cells and conjunctival swabs are the sample of choice for epidemiological surveillance of the disease, allowing the preclinical detection of the disease by 2–4 days. In the event of a rinderpest virus outbreak, this portable, single-tube format, real-time RT-PCR has the capability of preclinical diagnosis, thus aiding efforts to prevent further transmission of disease.

### 1.6. Differential immunocapture ELISA

Neither clinical observations nor AGID tests can differentiate between rinderpest and PPR; consequently, if either disease is suspected in sheep or goats in countries where both diseases occur, other tests like the real-time PCR must be used. Rapid differentiation can be achieved using a differential immunocapture ELISA test (Libeau *et al.*, 1994). This test employs MAbs directed against the N protein of the two viruses. One MAb, with a reactivity against both viruses, is used as a capture antibody, while a second biotinylated MAb specific for a nonoverlapping antigenic N protein site, and specific against either rinderpest or PPR, is used to determine which N protein has been captured.

High protein-binding ELISA plates (or strips) are coated with 100 µl/well of capture antibody. After three washes, the wells are loaded with 50 µl of test sample diluted 1/10 in a lysis buffer, 25 µl of the manufacturer's recommended dilution of the virus-specific MAb and 25 µl of streptavidin peroxidase at a final dilution of 1/3000. The wells are then placed on an orbital shaker for 1 hour at 37°C, after which time they are again washed; following the addition of 100 µl of ortho-phenylenediamine (OPD), the wells are re-incubated at room temperature for 10 minutes. Reactions are halted by the addition of 100 µl of 1 N sulphuric acid, and the results, measured at 492 nm with an automated ELISA reader, are expressed as absorbance values.

### 1.7. Chromatographic strip test

A rapid chromatographic strip test (Bruning-Richardson *et al.*, 2011a) has been developed for assisting field personnel in investigating suspected outbreaks of rinderpest. Any positive result should be treated as indicating a highly suspicious rinderpest case that must immediately be subjected to a thorough investigation. The test strip itself should be sent to the appropriate OIE/FAO Reference Laboratory along with other samples as viral nucleic acid can be harvested from used strips for characterisation (Bruning-Richardson *et al.*, 2011b).

## 2. Serological tests

### 2.1. The competitive enzyme-linked immunosorbent assay

A competitive ELISA is available for the detection of rinderpest antibodies in the serum of animals of any species previously exposed to the virus. The test is based on the ability of positive test sera to compete with a rinderpest anti-H protein MAb for binding to rinderpest antigen. The presence of such

antibodies in the test sample will block binding of the MAb, producing a reduction in the expected colour reaction following the addition of enzyme-labelled anti-mouse IgG conjugate and a substrate/chromogen solution. As this is a solid-phase assay, wash steps are required to ensure the removal of unbound reagents.

The rinderpest antigen is prepared from Madin–Darby bovine kidney cell cultures infected with the attenuated Kabete 'O' strain of rinderpest virus *and inactivated at 56°C for 2 hours*. The viral antigen is extracted from the infected cells by repeated cycles of sonication and centrifugation. The MAb was obtained by fusing the splenocytes of hyperimmunised mice with the NSO myeloma cell line, and then shown to be rinderpest H protein specific (Anderson *et al.*, 1991); this MAb has now been designated as C1. Both C1 and standardised rinderpest antigen are directly available from the OIE Reference Laboratory for Rinderpest in the UK (see Table given in Part 4 of this *Terrestrial Manual*). Kits will continue to be available commercially.

### 2.1.1. Test procedure

- i) Reconstitute the freeze dried rinderpest antigen with 1 ml of sterile water and further dilute it to the manufacturer's recommended working dilution using 0.01 M phosphate buffered saline (PBS), pH 7.4.
- ii) Immediately dispense 50 µl volumes of the diluted antigen into an appropriate number of wells of a flat-bottomed, high protein-binding ELISA microplate using two wells per test serum. Tap the sides of the microplate to ensure that the antigen is evenly distributed over the bottom of each well and, having sealed the plate, incubate it on an orbital shaker for 1 hour at 37°C. Wash the wells three times with 0.002 M PBS, pH 7.4.
- iii) Add 40 µl of blocking buffer (0.01 M PBS, 0.1% [v/v] Tween 20 and 0.3% [v/v] normal bovine serum) to each test well followed by 10 µl volumes of all test sera.
- iv) Follow the manufacturer's recommendations to prepare a working dilution of the MAb in blocking buffer, and add 50 µl of this to each test well. Seal the plates and re-incubate on an orbital shaker for 1 hour at 37°C.
- v) Follow the manufacturer's recommendations to prepare a working dilution of rabbit anti-mouse immunoglobulin horseradish peroxidase conjugate in blocking buffer and add 50 µl to each test well. Seal the plates and re-incubate on an orbital shaker for 1 hour at 37°C.
- vi) At the end of this period the plates are washed as before and immediately refilled with 50 µl volumes of substrate/chromogen mixture (1 part 3% H<sub>2</sub>O<sub>2</sub> to 250 parts OPD), and incubate at room temperature for 10 minutes without shaking. Then add 50 µl of a stopping solution consisting of 1 M sulphuric acid.
- vii) The test system must include known rinderpest positive and negative serum samples, a MAb control and a conjugate control.
- viii) Measure the resulting absorbance values on an ELISA reader with a 492 nm interference filter and express the test results as percentage inhibition values compared with the value obtained using the MAb control. Inhibition values of 50% or more are considered to be positive and values below 50% are considered to be negative.

Lowering the positive/negative threshold to 40% or less increases the sensitivity of the test, but inevitably affects specificity by increasing the proportion of false-positive test results encountered. In practise, the 50% value is recommended by GREP at which level sensitivity is at least 70% and specificity exceeds 99%. The sensitivity needs to be taken into account when designing sampling frames for serosurveillance.

### 2.2. Virus neutralisation

The 'gold standard' virus neutralisation (VN) test is performed in roller-tube cultures of primary calf kidney cells following the method of Plowright & Ferris (1961); the test has been validated in experimentally infected cattle. In the roller tube procedure, sera, that has not been inactivated, are diluted at intervals of 1 in 10 and then, starting with undiluted serum, mixed with an equal volume of 10<sup>3.0</sup> TCID<sub>50</sub> per ml of the attenuated Kabete 'O' vaccine strain virus. Mixtures are held overnight at 4°C, after which 0.2 ml volumes are inoculated into each of five roller tubes, immediately followed by 1 ml of dispersed indicator cells suspended in growth medium at a rate of 2 × 10<sup>5</sup> cells per ml. Tubes are incubated at 37°C, sloped for the first 3 days, after which they are replenished with maintenance medium and placed on a roller apparatus. They are examined regularly for virus-specific cytopathology and positive tubes recorded and discarded; the final examination takes place on day 10.

For calculating end-points, the virus dose is regarded as satisfactory if the final dilution falls within the range  $10^{1.8}$  to  $10^{2.8}$  TCID<sub>50</sub>/tube. This test should be used to examine the sera of ELISA reactors during national serosurveillance programmes designed to demonstrate freedom from infection, or to qualify susceptible cattle for vaccine testing. Under these circumstances, the presence of any detectable antibody in the 1/2 final serum dilution is considered to indicate previous infection with rinderpest virus. The VN test is the test of choice for the examination of wildlife serum samples.

A microplate method may be used as a screening test. In this procedure, an initial serum dilution of 1/5 is further diluted at twofold intervals. Thereafter, 50 µl volumes of serum are incubated with 50 µl volumes of virus diluted to contain between  $10^{1.8}$  and  $10^{2.8}$  TCID<sub>50</sub> (Taylor & Rowe, 1984). Following a 45-minute or an overnight incubation period, between 1 and  $2 \times 10^5$  calf kidney, lamb kidney or Vero cells are added as indicators. Tests are terminated after 6 or 7 days. Such tests may give indications of nonspecific neutralisation at high serum concentrations. There appear to be factors in some normal (with respect to prior rinderpest exposure) sera that bring about the failure of the virus to penetrate and replicate in indicator cells. In the tube test, these factors were probably removed during changes in maintenance medium; in the microplate method, they remain present the whole time. If the most concentrated final serum dilution is limited to 1/10, the effect disappears.

## C. REQUIREMENTS FOR VACCINES

### 1. Background

#### 1.1. Rationale and intended use of the product

The live attenuated tissue culture rinderpest vaccine (TCRV) described in previous editions of the *Terrestrial Manual* (Plowright, 1962) was developed in Kenya through the serial passage in primary bovine calf kidney cells of RBOK (rinderpest bovine old Kabete, or "Kabete O"), a virulent bovine rinderpest field strain isolated in 1911. While the modern division of rinderpest viruses into four lineages (Africa 1 and 2 and an old African one which includes Kabete O, and Asian) was unknown until 1995 (Wamwayi *et al.*, 1995) (the RBOK virus undoubtedly cross-protects against all strains of all lineages. Since its development, the RBOK vaccine seed was widely distributed and hundreds of millions of doses of it have been used on the Indian subcontinent, the Middle and Near East, and Africa in the control and eradication of rinderpest.

Other currently active vaccine strains, LA (Nakamura & Miyamoto, 1953) and LA-AKO (Furutani *et al.*, 1957a), were established from a previously developed lapinised vaccine strain, Nakamura III (alternatively, L strain; Nakamura *et al.*, 1938), by repeated passages in rabbits and chicken embryos. The parental Nakamura III had been widely used to control the disease in East and South-East Asia. LA and LA-AKO are reported to be far less virulent than the parental strain, especially to highly susceptible cattle in Eastern Asia such as Japanese black and Korean yellow. Currently, LA-AKO has been used for cell culture-based production of rinderpest vaccine for emergency use at a biologically safe and secure facility approved by OIE/FAO.

### 2. Outline of production and minimum requirements for conventional vaccines

#### 2.1. Characteristics of the seed

##### 2.1.1. Biological characteristics

##### i) RBOK

Seed lots used in the manufacture of TCRV must produce a cell-culture vaccine that is safe, that confers an immunity in cattle lasting at least 5 years. The immunogenicity of seed virus was demonstrated up to the 122<sup>nd</sup> BK passage level, which should not be exceeded.

Vaccine seed must be maintained in a seed-lot system between passage levels 90 and 120. Seed-lot virus must be preserved in a freeze-dried state at a temperature of  $-20^{\circ}\text{C}$  or lower. The virus must be cultured in Vero cells or primary or serially cultivated kidney cells derived from a normal bovine foetus or a very young calf. Serially cultivated cells may not be more than ten passages removed from the primary cultivation.



The seed virus produces a vaccine that is safe to use in a variety of European, African and Indian cattle breeds. Its safety and efficacy have never been assessed in Chinese or Japanese cattle breeds.

ii) LA-AKO

The seed virus (LA-AKO) was established from Nakamura III (at the 897th rabbit passage level) by repeated passages in rabbits (29 passages) and chick embryos (456 passages). LA-AKO does not cause any clinical signs except slight hyperthermia in highly susceptible animals such as Japanese black cattle. It should be noted however that the virus induces marked enlargement of the spleen in inoculated chick embryos (Furutani *et al.*, 1957b). Recently, the whole genome sequence of LA-AKO, and its strain of origin, Nakamura III, have been registered in the public database (Fukai *et al.*, 2011; Takamatsu *et al.*, 2015).

Seed lots were lyophilised and stored at a temperature of –20°C or lower until use.

### 2.1.2. Quality criteria

i) RBOK and LA-AKO

For both RBOK and LA-AKO strains, seed lots must be shown to be:

a) **Pure**

Free from contamination with viruses, bacteria, fungi or mycoplasmas.

b) **Safe**

Inducing no abnormal clinical reaction on inoculation into rinderpest-susceptible cattle.

c) **Efficacious**

Inducing an immunity to rinderpest in rinderpest-susceptible cattle.

## 2.2. Method of manufacture

### 2.2.1. Procedure

i) RBOK

Individual vaccine batches are prepared by infecting cell cultures and, after an appropriate incubation period, harvesting the overlying media into which large numbers of live virus particles have been released. Virus may be grown in primary kidney cells from bovine embryos or calves, or cells derived in a homogeneous manner by up to ten serial subcultures from either of these sources. In addition, vaccine may be manufactured in approved continuous cell lines provided the cells are known not to be infected with adventitious viruses including bovine viral diarrhoea (BVD) virus and are maintained in a seed lot system; Vero cells have been used for this purpose. To constitute a batch, infected cultures must have been inoculated with the same seed virus and incubated and harvested together.

Virus should be harvested from cultures maintained in roller bottles not more than 10 days after the date that these cultures were infected. The decision to harvest should be based on the development of extensive characteristic CPEs within the cell monolayer. To form a bulk suspension the harvest should be clarified by low-speed centrifugation before mixing with cryoprotectant.

Two harvests are permissible from the same set of cultures and may be pooled to form a single bulk suspension. Written records must accompany all stages of vaccine manufacture.

For long-term storage and cold-chain distribution, bulk suspensions are freeze dried.

ii) LA-AKO

Individual vaccine batches are prepared by infecting Vero cell cultures with reconstituted seeds and, after an appropriate incubation period, harvesting the overlying media and infected cells in which large numbers of live virus particles exist. A batch may be prepared

from a bulk suspension containing virus that has been maintained by up to ten serial subcultures from the vaccine seed. In addition, vaccine may be manufactured under sterile culture conditions free from adventitious viruses including BVD virus, bovine leukaemia virus (BLV) and bovine rotavirus.

Virus should be harvested from cultures maintained in roller bottles not more than 7 days after the date that these cultures were infected. The decision to harvest should be based on the development of extensive characteristic CPEs within the cell monolayer. To form a bulk suspension the harvest should be clarified by low-speed centrifugation before mixing with cryoprotectant.

Written records must accompany all stages of vaccine manufacture.

For long-term storage and cold-chain distribution, bulk suspensions are freeze dried.

### 2.2.2. Requirements for substrates and media

#### i) RBOK

##### a) Cells

Primary cells, serially cultivated primary cells or continuous cell lines must have been derived from normal looking animals or embryos, and must retain a normal morphology during cultivation. They must be shown to be free of contamination with adventitious viruses, particularly BVD virus (see below).

##### b) Media

Calf kidney are grown and maintained in Earle's Balanced Salts Solution or Eagle's Minimum Essential Medium [MEM] supplemented with 0.5% lactalbumin hydrolysate and 0.1% yeast extract together with 5% new-born calf serum that must come from rinderpest-susceptible animals and originate from countries with negligible risk of bovine spongiform encephalopathy.

Vero cells are grown and maintained under Glasgow Modified Eagle's medium (GMEM) supplemented with 14% TPB and 6% (rinderpest antibody-free) unheated bovine serum and antibiotics.

##### c) Cryoprotectant

The freeze-drying stabiliser should consist of a solution containing an equal volume of 5% lactalbumin hydrolysate and 10% sucrose.

#### ii) LA-AKO

##### a) Cells

Continuous cultures of Vero cells must retain a normal morphology during cultivation. They must be shown to be free from contamination with adventitious viruses.

##### b) Media

Vero cells are grown in Eagle's MEM supplemented with 10% heated fetal calf serum, 0.295% TPB and antibiotics.

##### c) Cryoprotectant

The freeze-drying stabiliser should consist of a solution containing an equal volume of 1% sodium glutamate, 0.3% polyvinylpyrrolidone and 10% sucrose.

### 2.2.3. In-process controls

#### i) RBOK

A virus titration must be undertaken on the seed lot using tenfold virus dilutions in a microplate or roller tube system and employing ten replicates per dilution.

Prior to lyophilisation the final bulk may be held for not more than 5 days at 4°C but considerably longer storage is permitted if frozen at –20°C to –60°C.

Checks for adventitious viral contamination should be undertaken on two uninfected control cell cultures prepared from the cell suspension used in batch production, after having been maintained using the same media and incubation conditions as the rinderpest-infected cells. They must be subjected to frequent in-process microscopic observations with negative results. After virus harvesting, the control cultures should be washed to remove bovine serum and re-incubated for 10 days in media containing bovine serum substitutes during which period they are again subject to frequent microscopic observations for evidence of cytopathic change. At the end of this period one culture should be examined for the presence of noncytopathic BVD virus using an immunofluorescence or immunoperoxidase test or RT-PCR.

The potency of the final bulk must be assessed by the same titration method used for the seed virus.

ii) LA-AKO

A virus titration must be undertaken on each batch of a final bulk suspension using tenfold virus dilutions in a microplate or roller tube system and employing at least four replicates per dilution.

To ensure the titre and the properties of a final bulk suspension, a marker test should be undertaken. LA-AKO induces marked increase in size of the spleen in inoculated chick embryos. 15 µl of 10- and 100-fold dilutions of a sample from a final bulk suspension are inoculated into a blood vessel of more than ten eggs each on day 11 to 12 after laying. Inoculated eggs are incubated at 38°C for 5 days. Spleens of inoculated chick embryos, which are still alive after incubation, are collected and weighed. These spleens become heavier than 15 mg in weight.

Checks for adventitious viral contamination should be undertaken on at least 1% of uninfected control cell cultures prepared from the cell suspension used for bulk production, after having been maintained using the same media and incubation conditions as the rinderpest-infected cells. They must be subjected to frequent in-process microscopic observations with negative results. On the day of virus harvesting, the control cultures should be examined for haemadsorption activity. The rinderpest-uninfected cultures should be washed to remove fetal calf serum, and divided into two groups. Each group is overlaid with 0.1% suspension of guinea-pig or goose red blood cells (RBCs) for 1 hour, then subjected to microscopic observation. The control cultures must not adsorb RBCs from either of those species.

Each batch of the final bulk suspension should also be examined for viral contamination by *in-vitro* and *in-vivo* assays. For *in-vitro* assays, samples from a final bulk are mixed with a neutralising titre of rabbit rinderpest antiserum, added to continuous cultures of bovine kidney and testicular cells, and incubated at 37°C for 7 days. These cells must not develop any CPE within the incubation period. The same samples are also inoculated to an African monkey-derived embryonic kidney cell line, MA-104, which is reported to be highly susceptible to Simian rotavirus (Smith *et al.*, 1979). Inoculated MA-104 cells must not develop CPE. For *in-vivo* assay 10 ml of the sample from the final bulk suspension is mixed with a neutralising titre of rabbit rinderpest antiserum and inoculated into a BLV susceptible sheep via an intramuscular route. The sera obtained from the sheep at 2 and 3 months after inoculation should be examined for the presence of BLV antibodies by an agarose gel immunodiffusion test.

The final bulk suspension is mixed with cryoprotectant and dispensed into vials on the day of lyophilisation. Longer storage of the final bulk suspension prior to the addition of the cryoprotectant may be acceptable if frozen at –20°C or lower.

#### 2.2.4. Final product batch tests

i) RBOK

a) Sterility and purity

Tests of biological materials for sterility and freedom of contamination may be found in chapter 1.1.9.

The final batch product consists of the freeze dried vials produced from a single bulk harvest; a batch may contain several filling lots. The contents of one container from

each filling lot must be exposed to neutralisation by rabbit rinderpest antiserum, using a varying virus/constant serum method, and inoculated into bovine kidney cells. The identity of the product is established if no rinderpest-specific CPE develop.

**b) Safety and efficacy**

Animals used in these procedures should be kept in isolation from other rinderpest-susceptible animals. At the end of the procedures they must be killed and the carcasses disposed of securely. Using rinderpest susceptible cattle, the contents of five randomly selected vials are pooled and used to inoculate one bovine with a volume equivalent to 100 cattle field doses (where a field dose is taken to be  $\geq 300$  TCID<sub>50</sub>) and each of three cattle with a volume equivalent to a single cattle field dose. These animals are maintained in close contact with a contact-control bovine for the following 3 weeks. During this period the animals are subjected to daily temperature recording and frequent clinical inspections. At the end of the 3 weeks, the cattle are examined for rinderpest neutralising antibodies using the microplate method. The vaccine is considered safe and efficacious if it does not induce any abnormal clinical reaction, if all vaccinated animals show a rinderpest virus-neutralising titre of 1/10 or greater, and if there is no evidence that the vaccine virus has been transmitted. Each vaccine lot must also be tested for innocuity in small animals.

In general terms the safety of the RBOK vaccine has been widely demonstrated in both European and Indian breeds of cattle and Dwarf West African breeds. It has not been tested in Japanese or Chinese breeds and its safety in such animals cannot be guaranteed.

**c) Batch potency**

The close relationship between immunising potency and infectivity allows the latter to be used as the basis for potency estimations. Three infectivity titrations are undertaken using cells of an approved continuous line or cells grown from each of three different bovine calf or embryonic kidneys. For the first titration, the pool of vials used for the safety test may be employed. The second and third estimates are made on further pools, each of three final containers. The sensitivity of the cells used in each working session must be measured using a standard laboratory rinderpest virus preparation. The final titre is the geometric mean of the three estimates, each undertaken using tenfold dilutions and ten observations per dilution. Potent vaccine should contain 100 field doses per vial.

**ii) LA-AKO**

**a) Sterility and purity**

Tests of biological materials for sterility and freedom from contamination may be found in chapter 1.1.9.

The final batch product consists of the freeze dried vials produced from a single bulk harvest. The contents of one container must be exposed to neutralisation by rabbit rinderpest antiserum, using a varying virus/constant serum method, and inoculated into bovine kidney cells and testicular cells. The inoculated cells must not develop CPE.

**b) Safety and efficacy**

Using two rinderpest-susceptible Japanese black breed cattle, the contents of a randomly selected vial is used to inoculate these cattle with a volume equivalent to one cattle field dose each (where a field dose is taken to be  $\geq 1000$  TCID<sub>50</sub>). The animals are maintained in a biologically secure animal facility for the following 2 weeks. During this period the animals are subjected to daily temperature recording and frequent clinical inspections. Serum samples are collected from the cattle at a sufficient period of time after inoculation and examined for the presence of rinderpest neutralising antibodies in Vero cell cultures. The vaccine is considered safe and efficacious if it does not induce any abnormal clinical reaction except slight pyrexia and if the titres of neutralising antibodies in both inoculated cattle sera ten times higher.

c) **Batch potency**

The close relationship between immunising potency and infectivity allows the latter to be used as the basis for potency estimations. Infectivity titration should be performed by the same titration method used for a final batch.

## 2.3. Requirements for authorisation

### 2.3.1. Safety requirements

i) Target and non-target animal safety

a) **RBOK and LA-AKO**

RBOK vaccine causes no clinical signs in rinderpest susceptible cattle or Asian water buffaloes. LA-AKO vaccine causes no clinical signs except slight pyrexia in rinderpest susceptible cattle. Neither spreads by contact transmission to rinderpest susceptible cattle housed in close proximity to vaccinates.

ii) Reversion to virulence

a) **RBOK and LA-AKO**

RBOK vaccine virus retains its attenuated characteristics during at least five back passages in cattle and lacks the ability to spread by contact. Any sub-strain of RBOK or LA-AKO used in the manufacture of TCRV must be identifiable by written historical records that trace its origins to either of these sub-strains.

iii) Environmental considerations

a) **RBOK and LA-AKO**

There are no environmental considerations with respect to either the manufacture or application of rinderpest vaccine.

### 2.3.2. Efficacy requirements

i) For animal production

a) **RBOK and LA-AKO**

Both vaccines protect vaccinated animals from clinical disease caused by virulent rinderpest virus infection.

ii) For control and eradication

a) **RBOK and LA-AKO**

For eradication purposes the object should be to use vaccine to immunise all susceptible animals in and around the vicinity of an outbreak in as short a period of time as possible (Taylor *et al.*, 2002).

### 2.3.3. Stability

i) **RBOK**

TCRV is highly stable when correctly freeze-dried and will keep for long periods at either +4 or –20°C provided the product retains a vacuum. The rate of degradation of lyophilised TCRV can be altered by the choice of stabiliser and by variations in the drying cycle. The most advantageous results were associated with the use of a 5% lactalbumin hydrolysate/10% sucrose stabiliser, a 72–74 hour drying cycle under reduced vacuum (100 milliTorr), initial drying for 16 hours at –30°C, and a final shelf temperature of 35°C. With high release titres, such vaccine can be used in the field for 30 days without refrigeration. Following reconstitution in either normal saline or 1M magnesium sulphate, the virus becomes much more thermolabile. The period for field distribution of reconstituted vaccine should not exceed its half-life, but as this parameter is temperature dependent and varies between 8 and 24 hours over a range from 4°C to 37°C, a common sense limit must be applied; a universal period of 4 hours can be recommended.

## ii) LA-AKO

TCRV is highly stable when correctly freeze-dried and will keep for long periods at either +4 or –20°C provided the product is filled with nitrogen gas. The rate of degradation of lyophilised TCRV can be altered by the choice of stabiliser and by variations in the drying cycle. The optimum results were associated with the use of the cryoprotectant mentioned above, a 48 hour drying cycle under reduced vacuum (approximately 10 Pa or less), initial drying for 24 hours at –45°C, a final shelf temperature of 22°C, and filling the vial with nitrogen gas. With high release titres, such vaccine may be usable in the field for several days without refrigeration. Following reconstitution in PBS, the virus may become much more thermolabile, hence, reconstituted vaccine should be delivered to and consumed by users as soon as possible.

### 3. Vaccines based on biotechnology

No biotechnology-based vaccines have so far been approved.

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**NB:** There are OIE Reference Laboratories for Rinderpest  
(see Table in Part 4 of this *Terrestrial Manual* or consult the OIE Web site for the most up-to-date list:  
<http://www.oie.int/en/our-scientific-expertise/reference-laboratories/list-of-laboratories/> ).  
Please contact the OIE Reference Laboratories for any further information on  
diagnostic tests, reagents and vaccines for Rinderpest