CHAPTER 3.1.23.

VESICULAR STOMATITIS

SUMMARY

Vesicular stomatitis (*VS*) *is a vesicular disease of horses, cattle and pigs caused by vesiculoviruses of the family* Rhabdoviridae*. This disease is clinically indistinguishable in relevant susceptible species from foot and mouth disease* (*FMD*)*, vesicular exanthema of swine* (*VES*)*, or swine vesicular disease* (*SVD*)*. Sheep, goats and many other wild species can be infected. Humans are also susceptible. The disease is endemic in the Americas, but has occasionally spread to other continents.*

Virus is transmitted directly by the transcutaneous or transmucosal route and has been isolated from sandflies and mosquitoes. Experimental transmission has been shown from black flies to both pigs and cattle. There is seasonal variation in the occurrence of VS: it disappears at the end of the rainy season in tropical areas, and at the first frosts in temperate zones. The pathogenesis of the disease is unclear, and it has been observed that the specific circulating antibodies do not always prevent infection with different VS serogroups.

Although VS may be suspected when horses are involved as well as pigs and cattle, prompt differential diagnosis is essential because the clinical signs of VS are indistinguishable from FMD when cattle and pigs are affected, and from SVD, VES and senecavirus A when only pigs are affected.

Diagnosis of VS is by virus isolation or by the demonstration of VS viral antigen or nucleic acid in samples of tissue or fluid. Detection of virus-specific antibody against structural proteins, in paired serum, can also be used as an indicator of infection.

Detection of the agent: *Virus can be readily isolated by the inoculation of several tissue culture systems, or embryonated chicken eggs. Viral RNA can be detected from epithelial tissue and vesicular fluid by conventional and real-time reverse transcription polymerase chain reaction* (*RT-PCR*). *Viral antigen can be identified by an indirect sandwich enzyme-linked immunosorbent assay* (*IS-ELISA*) *– this is the least expensive and most rapid test. The complement fixation test* (*CFT*) *is also a good alternative. The virus neutralisation test* (*VNT*) *may be used, but it is elaborate and time-consuming.*

Serological tests: *Convalescent animals develop specific antibodies within 4–8 days of infection that are demonstrated by a liquid-phase blocking ELISA* (*LP-ELISA*), *a competitive ELISA* (*C-ELISA*) *and VNT. Other described tests are CFT, agar gel immunodiffusion and counter immuno-electrophoresis. The demonstration of specific antibodies to structural proteins in nonvaccinated animals is indicative of prior infection with VS virus.*

Requirements for vaccines: *Inactivated virus vaccines with aluminium hydroxide or oil as adjuvants have been tested in the United States of America and in Colombia, respectively. Both vaccines generated high levels of specific antibodies in the sera of vaccinated cattle. However, it is not yet clear if serum antibodies would prevent the disease. An attenuated virus vaccine has been used in the field with unknown efficacy.*

A. INTRODUCTION

Vesicular stomatitis (VS) was described in the United States of America (USA) by Oltsky *et al*. (1926) and Cotton (1927) as a vesicular disease of horses, and subsequently of cattle and pigs. Vesicles are caused by virus on the tongue, lips, buccal mucosa, teats, and in the coronary band epithelium of cattle, horses, pigs, and many other species of domestic and wild animals. Natural disease in sheep and goats is rare, although both species can be

experimentally infected. Mixed infections of foot and mouth disease (FMD) and VS viruses have occurred in the same herds of cattle and can be induced experimentally. Many species of laboratory animals are also susceptible. The disease is endemic in the Americas, but has occasionally spread to other continents.

Influenza-like signs, normally without vesicles, have been observed in humans who are in contact with animals with VS or who handle infective virus. Laboratory manipulations involving live virus, including handling infective materials from animals, should be carried out at an appropriate biosafety and containment level determined by risk analysis (see Chapter 1.1.4 *Biosafety and biosecurity: Standard for managing biological risk in the veterinary laboratory and animal facilities*).

The pathogenic agents causing vesicular stomatitis belong to the *Rhabdoviridae* family, genus *Vesiculovirus* with four species as follows: *Indiana vesiculovirus* – VSIV (formerly IND-1), *Cocal vesiculovirus* – COCV (formerly IND-2), *Alagoas vesiculovirus* – VSAV (formerly IND-3) and *New Jersey vesiculovirus* – VSNJV. They have been extensively studied at the molecular level (Fowler *et al.,* 2016; ICTV, 2020; Pauszek *et al.,* 2011). Several other closely related rhabdoviruses have been isolated from sick animals over the past decades. The VSIV, Cocal virusand Alagoas virus are serologically related (Federer *et al.,* 1967). Strains of the VSNJV and VSIV are endemic in livestock in areas of southern Mexico, Central America, Chile, Venezuela, Colombia, Ecuador and Peru, with VSVNJ causing the majority of the clinical cases. Sporadic activity of VSNJV and VSIV has been reported in northern Mexico and the western United States. *Cocal vesiculovirus* was isolated from domestic animals only in Argentina and Brazil and only from horses (Salto-Argentina/63, Maipú-Argentina/86, Rancharia-Brazil/66, Ribeirão-Brazil/79) (Alonso *et al.,* 1991; Alonso Fernandez & Sondahl, 1985). This finding confirms the first descriptions, in 1926 and 1927 (Cotton, 1927; Oltsky *et al.,* 1926) of the VSNJV and VSIV, COCV, VSAV in horses, and subsequently in cattle and pigs; this same predilection has been observed in other VS outbreaks. The Alagoas virus, (Alagoas-Brazil/64), has been identified, endemically in North-eastern Brazil, in cattle, horses, swine, sheep and goats (Alonso *et al.,* 1991; Alonso Fernandez & Sondahl, 1985; Cargnelutti *et al.,* 2014; PANAFTOSA-OPS/OMS, 2019; Rocha *et al.,* 2020).

The mechanism of transmission of the virus is unclear. The viruses have been isolated from sandflies, mosquitoes, and other insects (Comer *et al*., 1992; Francy *et al*., 1988; Mason, 1978). Experimental transmission of VSNJV has been demonstrated to occur from black flies (*Simulium vittatum*) to domestic swine and cattle (Mead *et al*., 2004; 2009). During the 1982 epizootic in the western USA, there were a number of cases where there was direct transmission from animal to animal (Sellers & Maarouf, 1990). VSV has historically been considered to be endemic in feral pigs on Ossabaw Island, Georgia, USA (Boring & Smith, 1962), but subsequently may have disappeared from the island (Killmaster *et al*., 2011).

The incidence of disease can vary widely among affected herds. Usually 10–15% of the animals show clinical signs. Clinical cases are mainly seen in adult animals. Cattle and horses under 1 year of age are rarely affected. Mortality is close to zero in both species. However, high mortality rates in pigs affected by VSNJV have been observed. Sick animals recover in about 2 weeks. The most common complications of economic importance are mastitis and loss of production in dairy herds (Lauerman *et al*., 1962). Recent VS outbreaks in the USA have been associated primarily with horses and VSNJV.

B. DIAGNOSTIC TECHNIQUES

VS cannot be reliably differentiated clinically from the other vesicular diseases in the relevant susceptible species, such as FMD, vesicular exanthema of swine (VES), and swine vesicular disease (SVD). An early laboratory diagnosis of any suspected VS case is therefore a matter of urgency.

The sample collection and technology used for the diagnosis of VS must be in concordance with the methodology used for the diagnosis of FMD (chapter 3.1.8), VES and SVD, in order to facilitate the differential diagnosis of these vesicular diseases.

Vesicle fluid, epithelium covering unruptured vesicles, epithelial flaps of freshly ruptured vesicles, or swabs of the ruptured vesicles are the best diagnostic samples. These samples can be collected from mouth lesions, as well as from the feet and any other sites of vesicle development. It is recommended to sedate the animal before collection of samples to avoid injury to animals and people Tissue and fluid samples, including swabs from all species should be placed in containers of Tris-buffered tryptose broth (TBTB) minimal essential medium (MEM) or 0.08 M phosphate buffer (with phenol red and antibiotics [1000 units/ml penicillin, 100 units/ml nystatin, 100 units/ml neomycin, and 50 units/ml polymyxin B], and adjusted to pH 7.2–7.6. Tissue samples can also be placed in glycerol/phosphate buffer with phenol red pH 7.2–7.6. (Note: glycerol is toxic to cell cultures and decreases the sensitivity of virus isolation.) Samples should be sent to the laboratory on ice packs if they can arrive at the laboratory within 48 hours after collection. If samples require more than 48 hours transit time, they should be sent frozen on dry ice with precautions to protect the sample from direct contact with CO_2. There are special packaging requirements for shipping samples with dry ice (see Chapter 1.1.2 *Collection, submission and storage of diagnostic specimens* for further information on shipping of diagnostic samples).

Considering the need for differential diagnosis with FMD, if it is not possible to collect epithelium from unruptured or freshly ruptured vesicles or vesicular fluid, oesophageal–pharyngeal (OP) fluid samples, can be collected by probang cups from ruminants only, as an alternative source of virus. Mix probang fluid with an equal volume of transport fluid (see Chapter 3.1.8 *Foot and mouth disease*). The container should be capable of withstanding freezing above dry ice (solid carbon dioxide) or liquid nitrogen (Kitching & Donaldson, 1987).

When it is not possible to collect samples for identification of the agent, serum samples can be used for detecting and quantifying specific antibodies. Paired sera from the same animals, collected 7–14 days apart, may be needed depending on the serological assay being used and prior history of vesicular stomatitis in the animals.

Specific reagents for VS diagnosis are not commercially available and each laboratory must produce its own or obtain them from a Reference Laboratory. The OIE Reference Laboratories for vesicular stomatitis (see Table given in Part 4 of this *Terrestrial Manual*), produce and distribute diagnostic reagents on request.

Table 1. *Test methods available for the diagnosis of vesicular stomatitis and their purpose*

Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination***
Detection of the agent[1]						
Virus isolation*	–	+	–	+++	–	–
IS-ELISA*	–	+	–	+++	–	–
CFT*	–	+	–	++	–	–
RT-PCR*	–	+	–	++	–	–
Detection of immune response[2]						
LP-ELISA**	++	++	++	++	++	++
C-ELISA**	+++	++	++	–	+++	++
VNT**	+++	+++	+++	++	++	+++
CFT**	–	+	+	++	+	–

Key: +++ = recommended for this purpose; ++ recommended but has limitations;
+ = suitable in very limited circumstances; – = not appropriate for this purpose.
IS-ELISA = indirect sandwich enzyme-linked immunosorbent assay; CFT = complement fixation test;
RT-PCR = reverse transcription polymerase chain reaction; LP-ELISA = liquid-phase blocking ELISA;
C-ELISA = competitive ELISA; VNT = virus neutralisation test.
*Should only be used on animals demonstrating clinical signs compatible with VSV. A positive result is meaningful. A negative result could mean the animal is no longer shedding virus, the virus level is too low to detect, or, for virus isolation samples that the samples were not maintained at appropriate temperatures and received in an appropriate time period following collection for virus isolation (virus inactivated).
**The presence of VSV antibodies only indicates prior exposure to VSV. It does not determine whether the antibodies are due to current or past infection. Paired sera from the same animals, collected at least 7–14 days apart, may be needed to evaluate seroconversion depending on the serological assay being used and prior history of VS. Interpretation of results needs to be based on serological results, clinical presentation, and epidemiology. CF antibody duration in an animal is generally less than 1 year. Antibodies detected by the VNT and competitive ELISAs can be detected for years following infection. The difference in sensitivity of the serological assays has an effect on detection during the acute phase of infection; combination testing, such as C-ELISA and CF or paired sampling showing four-fold titre change (CFT, VNT, LP-ELISA), is therefore necessary when an animal presents with acute clinical signs of VS.
***Indicates the presence of antibodies only; does not indicate protection from infection.

1 A combination of agent identification methods applied on the same clinical sample is recommended.
2 One of the listed serological tests is sufficient.

1. Detection of the agent

1.1. Direct visualisation

Due to the different morphological characteristics of the rhabdovirus (VS serogroup viruses), picornavirus (FMD virus, SVD virus and senecavirus A), calicivirus (VES) and the large number of virus particles present in vesicular fluids and epithelial tissues, electron microscopy can be a useful diagnostic tool for differentiating the virus family involved.

1.2. Virus isolation in cell culture

For identification of VS viruses and the differential diagnosis of vesicular diseases, clarified suspensions of field samples suspected to contain virus should be submitted for testing. For virus isolation, the samples are inoculated into appropriate cell cultures. The inoculation of African green monkey kidney (Vero), baby hamster kidney (BHK-21) and IB-RS-2 cell cultures with the same sample permits differentiation of the vesicular diseases: VS viruses cause a cytopathic effect (CPE) in all three cell lines; FMD virus causes a CPE in BHK-21 and in IB-RS-2, while SVD virus causes a CPE in IB-RS-2 only. Many other cell lines, as well as most primary cell cultures of animal origin, are susceptible to VS serogroup viruses.

1.2.1. Test procedure

i) Where tissue has been collected in phosphate-buffered saline (PBS)/glycerol solution, it should be blotted dry on absorbent paper to reduce the glycerol content, which is toxic for cell cultures, and weighed. A suspension is then prepared using a tissue grinder or by grinding the sample in sterile sand in a sterile pestle and mortar with a small volume of tissue culture medium and antibiotics. Further medium should be added until a final volume of nine times that of the original sample has been added, giving a 10% suspension. For swabs samples, a 10% solution is made using the cell culture medium. The sample is clarified on a bench centrifuge at 2000 ***g*** for at least 10 minutes.

ii) The clarified suspension of tissues, swab or vesicular fluid from field samples suspected to contain VSV are inoculated onto cell culture vessels (BHK-21, IB-RS-2 or Vero cells).

iii) Incubate inoculated cell cultures at 37°C for 1 hour (adsorption).

iv) Discard inoculum and wash cell cultures three times with cell culture medium and replace with cell culture medium containing 2.5% fetal bovine serum (FBS).

v) Incubate plates, plastic tubes or flasks cell cultures at 35–37°C and observe for cytopathic effect (CPE). The cell cultures should be examined for CPE for 48–72 hours. If, after 72 hours, no CPE has been detected, a blind passage must be made. The cell culture is freeze–thawed and clarified by centrifugation, and the supernatant is used for inoculation of fresh monolayers or cell suspension. The sample is considered to be negative if there is no evidence of a CPE after on average of three blind passages in cell cultures; longer passages of up to 7 days may be conducted using fewer passages.

vi Reverse-transcription polymerase chain reaction (RT-PCR) may be used to identify virus recovery, using appropriate sets of primers (Rainwater-Lovett *et al.,* 2007; Sepulveda *et al.,* 2007). Alternatively, immunological methods for the identification of the viral antigens can be used such as enzyme-linked immunosorbent assay (ELISA) (Alonso et al., 1991; Ferris & Donaldson, 1988), the complement fixation test (CFT) (Alonso *et al.,* 1991; Jenny *et al.,* 1958) or fluorescent antibody staining. The virus neutralisation test (VNT), with known positive antisera against VSNJV and VSIV, may be used in tissue cultures or embryonated eggs, but it is more time-consuming.

1.3. *In-ovo* testing

The virus replicates and can be isolated in 8- to 10-day-old chicken embryos by inoculation into the allantoic sac.

1.4. Enzyme-linked immunosorbent assay

The indirect sandwich ELISA (IS-ELISA) (Alonso *et al*., 1991; Ferris & Donaldson, 1988) is a common diagnostic method of choice for identification of VS and other vesicular diseases. Specifically, the ELISA procedure with a set of polyvalent rabbit/guinea-pig antisera, prepared against virions for representative

strains of VSIV, COCV, and VSAV (Alonso *et al*., 1991). For detection of VSNJV, a monovalent set of rabbit/guinea-pig antisera is suitable (Alonso *et al*., 1991; Ferris & Donaldson, 1988).

1.4.1. Test procedure

i) *Solid phase:* ELISA plates are coated either for 1 hour at 37°C or overnight at 4°C with rabbit antisera and normal rabbit serum (as described in Alonso *et al*., 1991), and optimally diluted in carbonate/bicarbonate buffer, pH 9.6. Subsequently, the plates are washed once with PBS and blocked for 1 hour at room temperature with 1% ovalbumin Grade V (grade of purification) in PBS. After washing, the plates can be used immediately or stored at –20°C for future use.

ii) *Test samples:* Antigen suspensions of test samples (10–20% epithelial tissue suspension, in PBS or MEM or undiluted clarified cell culture supernatant fluid) are deposited in the corresponding wells and the plates are incubated for 1 hour at 37°C on an orbital shaker.

iii) *Detector:* Monovalent guinea-pig antisera to VSNJV and polyvalent guinea-pig antisera to VSIV, COCV and VSAV, that are homologous to coated rabbit serum and that have been diluted appropriately in PBS containing 0.05% Tween 20, 1% ovalbumin Grade II, 2% normal rabbit serum, and 2% normal bovine serum (PBSTB) are added to the corresponding wells and left to react for 30–60 minutes at 37°C on an orbital shaker.

iv) *Conjugate:* Peroxidase/rabbit or goat IgG anti-guinea-pig Ig conjugate, diluted in PBSTB, is added and left to react for 30–60 minutes at 37°C on an orbital shaker.

v) *Substrate:* H_2O_2-activated substrate is added and left to react at room temperature for 15 minutes, followed by the addition of sulphuric acid to stop the reaction. Absorbance values are measured using an ELISA reader.

Throughout the test, 50 µl reagent volumes are used. The plates are washed three–five times between each stage with physiological saline solution or PBS containing 0.05% Tween 20. Controls for the reagents used are included.

vi) *Interpretation of the results:* Absorbance values of positive and negative antigen control wells should be within specified values for acceptance. Sample wells giving an absorbance ≥0.3 are considered to be positive for the corresponding virus subtype. Absorbance values <0.3–0.2 are considered suspicious and values <0.2 are considered negative for the corresponding virus subtype. Suspicious and negative samples should be inoculated in cell culture and passages re-tested in ELISA.

1.5. Complement fixation test

The IS-ELISA is preferable to the CFT because it is more sensitive and it is not affected by pro- or anti-complementary factors. When ELISA reagents are not available, however, the CFT may be performed. The CFT in U-bottomed microtitre plates, using the reagents titrated by CF50% test, is described.

1.5.1. Test procedure

i) *Antisera:* Monovalent guinea-pig antisera to VSNJV and polyvalent guinea-pig antisera to VSIV, COCV and VSAV, diluted in barbital buffer or an alternative CF buffer at a dilution containing 2.5 CFU_{50} (50% complement fixation units) against homologous virus, are deposited in plate wells. Those antisera are the detectors used in ELISA.

ii) *Test samples:* The antigen suspension of test samples, prepared as described for IS-ELISA, is added to the wells with serum.

iii) *Complement:* 4 CHU_{50} (50% complement haemolytic units) are added to the serum and antigen. (An alternative is to use 7.5, 10 and 20 CHU_{50} with the goal of reaching 4 CHU_{50} in the test.) The mixture of antisera, test samples and complement is incubated at 37°C for 60 minutes.

iv) *Haemolytic system:* A suspension of sheep red blood cells (SRBC) in CF buffer, sensitised with 10 HU_{50} (50% haemolytic units) of rabbit anti-SRBC serum, is added to the wells. The haemolytic system has an absorbance of 0.66 read at 545 nm, in the proportion of two volumes of haemolytic system + three volumes of distilled water. The mixture is incubated for 30 minutes at 37°C. Subsequently, the plates are centrifuged and the reaction is observed visually.

Volumes of 25 µl for antisera, test samples and complement, and 50 µl of haemolytic system, are required. Appropriate controls for the antisera, antigens, complement and haemolytic system are included.

It is possible to perform the CF50% test in tubes (Alonso *et al*., 1991) using reagent volumes of 200 µl (eight times greater than those indicated for the CFT in microtitre plates). With the CF50% test, the reaction can be expressed as absorbance read spectrophotometrically at 545 nm.

v) *Interpretation of the results:* When controls react as expected, samples with haemolysis <20% for one antiserum in comparison with the other antiserum and controls are considered to be positive for the corresponding type.

Field samples that are negative by the ELISA or CFT should be inoculated into cell culture. If there is no evidence of viral infection after three passages, the specimen is considered to be negative for virus.

1.6. Molecular methods

The RT-PCR can be used to amplify small genomic areas of the VS virus (Hofner *et al*., 1994; Hole *et al.,* 2010; Rodriguez *et al*., 1993; Wilson *et al*., 2009). This technique will detect the presence of virus RNA in tissue and vesicular fluid samples and cell culture, but cannot determine if the virus is infectious.

The diagnosis of VS in samples of epithelium, vesicular fluid and cell culture by molecular methods is the most suitable for confirmation of the disease. No screening protocol that allows the detection of all species of vesiculovirus of economic interest has been described. However, there are some multiplex tests described below that allow the detection of more than one viral species. Therefore, for a differential diagnosis, different protocols are needed to detect all VS viruses.

Extraction of nucleic acid from the sample must be done according to the manufacturer's instructions.

Methods for detecting and typing the four VS viruses have been described using both real-time and conventional RT-PCR.

1.6.1. Real-time RT-PCR detection and typing

VSNJV, VSIV, COCV and VSAV can be detected and typed using the real-time RT-PCR methods described by Hole *et al.* (2010) with adaptations, de Oliveira *et al.* (2018) and Sales *et al.* (2020), and the primers and probes described in Table 2.

Hole *et al.* (2010) developed a real-time RT-PCR assay that allows differentiation between VSIV and VSNJV with good performance. Primer concentrations must be 0.2 mM except the reverse VSNJV, which is 0.8 mM, and probe concentrations are 0.2 mM for VSNJV and 0.1 mM for VSIV. The real-time RT-PCR protocol consists of reverse transcription cycles at 50°C for 30 minutes and denaturation at 95°C for 1 minute, followed by 45 cycles of 95°C for 15 seconds, 54°C for 30 seconds and 72°C for 60 seconds. The reaction is performed according to the kit manufacturer's instructions, adding $MgSO_4$ (4 mM).

Due to the extent of genetic variation of this virus in some regions, de Oliveira *et al.* (2018) developed a method for detecting VSAV by multiplex real-time RT-PCR with excellent sensitivity and specificity. The test is performed according to the kit manufacturer's instructions and the concentration of the primers in a final volume of 25 µl was 0.4 µM. The real-time RT-PCR protocol consists of reverse transcription cycles at 50°C for 10 minutes and denaturation at 95°C for 5 minutes followed by 45 cycles of 95°C for 10 seconds and 60°C for 1 minute.

Sales *et al.* (2020) described a real-time RT-PCR protocol for the diagnosis of COCV. The test is carried out according to the kit manufacturer's instructions, with the concentration of each primer 0.1 µM and probe 0.4 µM in a final volume of 25 µl. The real-time RT-PCR protocol consists of reverse transcription cycles of 45°C for 10 minutes and denaturation of 95°C for 5 minutes, followed by 45 cycles of 95°C for 10 seconds and 60°C for 1 minute.

***Table 2**. Oligonucleotides target for real-time RT-PCR of VSV*

Real-time RT-PCR for vesicular stomatitis		
Multiplex New Jersey vesiculovirus and *Indiana vesiculovirus*		
Name	**Sequence (5'–3')**	**Reference**
VSNJV 7230-7254	Forward: TGA-TTC-AAT-ATA-ATT-ATT-TTG-GGA-C	Hole *et al.,* 2010
VSNJV 7476-7495 R	Reverse: AGG-CTC-AGA-GGC-ATG-TTC-AT	
VSNJV 7274-7296 P 1	Probe: FAM-TTT-ATG-CAT-GAC-CCW-GCA-ATA-AG-NFQ-MGB	
VSNJV 7334-7353 P 2	Probe: FAM-TTG-CAC-ACC-AGA-ACA-TTC-AA-BHQ1	
VSIV 7230-7254 IN F	Forward: TGA-TAC-AGT-ACA-ATT-ATT-TTG-GGA-C	
VSIV 7433-7456 IN R	Reverse: GAG-ACT-TTC-TGT-TAC-GGG-ATC-TGG	
VSIV 7274-7290 P	Probe: VIC-ATG-ATG-CAT-GAT-CCA-GC-NFQ-MGB	
Alagoas vesiculovirus		
Name	**Sequence (5'–3')**	**Reference**
VSAV-3.GP.95.F	Forward: GGG-TWA-ACA-TCC-GTG-CTA	de Oliveira *et al.,* 2018
VSAV-3.GP.95.R	Reverse: GTC-ACA-AGT-GGT-GAT-CCA	
VSAV-3.GP.95.S	Probe: FAM-cac+Atc+Cat+Cca+Tcagc-IowaBlack	
VSAV.LP.78.F	Forward: GTC-CAT-CAA-CCC-ATT-GTT-CC	
VSAV.LP.78.R	Reverse: ATC-AAT-CCA-TCT-GCG-ACT-CC	
VSAV.LP.78.S	Probe: FAM-CGC-GAT-TCT-TAA-GTG-AGT-TCA-AAT-CAG-GA-IowaBlack	
VSAV.GP.87.F	Forward: GAG-TGT-GGA-TCA-ACC-CAG	
VSAV.GP.87.R	Reverse: CTG-TGG-CTT-GAA-CRA-TCA	
VSAV.GP.87.S	Probe: FAM-CTGC+GGTTATG+CC+TCCA-IowaBlack	
Cocal vesiculovirus		
Name	**Sequence (5'–3')**	**Reference**
COCV.GP.81.F	Forward: CGT-TGC-TGT-GAT-TGT-YCA	Sales *et al.,* 2020
COCV.GP.81.R	Reverse: GGG-AAC-TGG-GAG-TCA-ATC	
COCV.GP.81.S	Probe: FAM-ctc+Atc+Cac+Caa+Cacat -BHQ1	

1.6.2. Conventional RT-PCR detection and typing

VSNJV, VSIV, COCV and VSAV can also be detected and typed using the RT-PCR methods described by Rodriguez *et al.* (1993) and Pauszek *et al.* (2008; 2011), and the primers described in Table 3. After obtaining the reverse transcription cDNA, the conventional PCR is undertaken. The protocol for all RT-PCR described consists of cycles of denaturation at 94°C for 3 minutes followed by 40 cycles of 94°C for 1 minute, 50°C for 1 minute and 72°C for 1 minute, and the extension step should be 72°C for 5 minutes.

de Oliveira *et al.* (2018) developed an RT-PCR to detect Cocal vesiculovirus. The test is performed according to the kit manufacturer's instructions, with the concentration of each primer being 1 µM in a final volume of 20 µl. The RT-PCR protocol consists of reverse transcription cycles at 50°C for 30 minutes and denaturation at 95°C for 15 minutes followed by 40 cycles of 94°C for 1 minute, 54°C for 1 minute and 72°C for 1 minute.

Table 3. *Primers and references for conventional RT-PCR of VSV*

Conventional RT-PCR for vesicular stomatitis virus		
New Jersey vesiculovirus and Indiana vesiculovirus		
Name	**Sequence (5'–3')**	**Reference**
VSNJV P102	Forward: GAG-AGG-ATA-AAT-ATC-TCC	Rodriguez *et al.,* 1993
VSNJV P744	Reverse: GGG-CAT-ACT-GAA-GAA-TA	
VSNJV P179	Forward: GCA-GAT-GAT-TCT-GAC-AC	
VSNJV P793	Reverse: GAC-TCT-(C/T)GC-CTG-(A/G)TT-GTA	
Cocal vesiculovirus and Alagoas vesiculovirus		
Name	**Sequence (5'–3')**	**Reference**
COCV P66	Forward: AAT-TGG-ATG-ACG-CMG-TCC-A	Pauszek *et al.,* 2008
COCV P711	Reverse: CCT-CCD-ACH-GAR-ATG-AAY-TCT-CC	
VSAV PJX	Forward: TAT-GAA-AAA-AAI-TAA-CAG-IIA-TC	
VSAV P711	Reverse: CCT-CCD-ACH-GAR-ATG-AAY-TCT-CC	
Nested PCR for *Cocal vesiculovirus and Alagoas vesiculovirus*		
Name	**Sequence (5'–3')**	**Reference**
COCV P169	Forward: TTA-CCA-AAA-TCA-GGA-GGA-TGA	Pauszek *et al.,* 2011
COCV P686	Reverse: GCC-TCC-CAC-CGA-GAT-G	
VSAV P163	Forward: AGA-GCA-GCT-CCY-TCT-TAT-TAT	
VSAV P691	Reverse: TCA-TCA-TTC-CAT-TTC-CTC	
One step RT-PCR for *Alagoas vesiculovirus*		
Name	**Sequence (5'–3')**	**Reference**
VASV - P.722 F	Forward: GGG-GCC-ATT-CAA-GAG-ATA-GA	de Oliveira *et al.,* 2018
VASV -P.722 R	Reverse: TGA-TAT-CTC-ACT-CTG-GCC-TGA-TTA-T	

2. Serological tests

For the identification and quantification of specific antibodies in serum, the ELISA and the VNT are preferable. The CFT may be used for quantification of early antibodies. Antibody can usually be detected between 5 and 8 days post-infection; the length of time antibody persists has not been accurately determined for the three tests but is thought to be relatively short for the CFT and for extended periods for the VNT and ELISA (Katz *et al*., 1997).

The competitive ELISA (C-ELISA) may be preferable to the CFT because it is more sensitive and it is not affected by pro- or anti-complementary factors; however during an outbreak where there may be previously exposed animals, appropriate assay selection, and paired serum samples collected at least 7–14 days apart are important to ensure ability to distinguish recent from past exposure. When C-ELISA reagents are not available however, the CFT may be performed. The CFT in U-bottomed microtitre plates, using the reagents titrated by CF50% test, is described.

2.1. Liquid phase blocking enzyme-linked immunosorbent assay

The liquid-phase blocking ELISA (LP-ELISA) is a method for the detection and quantification of antibodies to VS serogroup viruses. The use of viral glycoproteins as antigen is recommended because they are not infectious, allow the detection of neutralising antibodies, and give fewer false-positive results than the VNT (Allende *et al*., 1992).

2.1.1. Test procedure

i) *Solid phase:* As described above in Section B.1.5 for the IS-ELISA.

ii) *Liquid phase:* Duplicate, two- to five-fold dilution series of each test serum, starting at 1/4, are prepared in U-bottomed microtitre plates. An equal volume of VSNJV or VSIV glycoprotein, in a predetermined dilution, is added to each well and the plates are incubated for 1 hour at 37°C. 50 µl of these mixtures is then transferred to the ELISA plates with the solid phase and left to react for 30 minutes at 37°C on an orbital shaker.

iii) *Detector, conjugate and substrate:* The same steps described for the IS-ELISA are performed using monovalent antisera homologous to the test antigen, as detectors

iv) *Interpretation of the results:* 50% end-point titres are expressed in $\log_{10}$ in reference to the 50% OD of the antigen control, according to the Spearmann–Kärber method. Titres of >1.0 (1/10) are considered to be positive.

2.2. Competitive enzyme-linked immunosorbent assay

A C-ELISA for detection of antibodies has also been developed. The procedure described here is based on a procedure described by Afshar *et al.* (1993). It uses vesicular stomatitis VSNJV and VSIV recombinant antigens as described by Katz *et al.* (1995).

2.2.1. Test procedure

i) *Solid phase:* Antigens are diluted in carbonate/bicarbonate buffer, pH 9.6, and 75 µl is added to each well of a 96-well ELISA plate. The plates are incubated overnight at 4°C; coated plates can be frozen, with antigen *in situ*, at –70°C for up to 30 days. The plates are thawed, antigen is decanted, and 100 µl of blocking solution (5% nonfat dry milk powder solution in PBS [for example, 5 g dry milk powder dissolved in 95 ml PBS) is added. The plates are then incubated at 25°C for 15–30 minutes and blocking solution is decanted. The plates are washed three times with PBS/0.05% Tween 20 solution.

ii) *Liquid phase:* 50 µl of serum diluted 1/8 in 1% nonfat dry milk in PBS is added to each of the duplicate wells for each sample. A positive and negative control serum for each virus species should be included on each ELISA plate. The plates are incubated at 37°C for 30 minutes. Without washing, 50 µl of bioreactor fluid is added to each well and plates are incubated at 37°C for 30 minutes.

iii) *Detector:* The plates are washed three times, and 50 µl of goat anti-mouse horseradish-peroxidase conjugate diluted in 1% nonfat dry milk with 10% normal goat serum is added to each well. The plates are incubated at 37°C for 30 minutes, washed three times, and 50 µl of tetramethyl-benzidine (TMB) substrate solution is added to each well. The plates are incubated at 25°C for 5–10 minutes and then 50 µl of 0.05 M sulphuric acid is added to each well. The plates are read at 450 nm and the optical density of the diluent control wells must be > 1.0.

iv) *Interpretation of the results:* A sample is positive if the absorbance is ≤50% of the absorbance of the diluent control. Note that horses naturally infected with VSNJV virus have been known to test positive by this assay for at least 8 years following infection.

2.3. Virus neutralisation test

Virus and cells: VSIV, COCV, VSAV and VSNJV are propagated in BHK, IB-RS-2 or Vero cell monolayers and stored in liquid nitrogen or frozen at –70°C (Allende *et al.,* 1992).

2.3.1. Test procedure

i) The test sera are heat-inactivated, including control standard sera, for 30 minutes at 56°C.

ii) Starting from 1/8 dilution, sera are diluted twofold or fourfold in cell culture medium, and the dilution series is continued in a cell-culture grade flat-bottomed 96-well microtitre plate using at least two rows of wells, preferably four rows (depending on the degree of precision required) and a volume of 50 µl per well. An extra well with 1/8 dilution test serum is used for toxicity control of sera. Dilutions of control sera with known titres (positive, weak positive and negative) are also included in the test.

iii) Add 50 µl per well of the VSV strain (VSIV, COCV, VSAV and VSNJV) stock at a dilution in culture medium calculated to provide 100 $TCID_{50}$ (50% tissue culture infective dose) per well. In the toxicity control wells, add 50 µl of culture medium in place of virus. Add 100 µl of

culture medium to one row of empty wells as cell controls. A back titration of virus stock is also undertaken, at least four wells per dilution, to check the potency of the virus (dose acceptance limits 32–320 $TCID_{50}$ per well). An alternative protocol can be a viral dose of 1000 $TCID_{50}$ per well (tolerance range between 750 and 1270 $TCID_{50}$) to increase the specificity of the test.

iv) Incubate the plates for 60 minutes at 37°C in a 3–5% CO_2 atmosphere to allow viral neutralisation.

v) Add 100 µl per well of the BHK, IB-RS-2 or Vero cell suspension at 3×10^5 ml, containing 10% FBS for cell growth.

vi) Incubate the plates for 48–72 hours at 37°C, either in a 3–5% CO_2 atmosphere or seed the plate with pressure-sensitive tape and incubate.

vii) Check the cell cultures for the onset of CPE and read the results: positive monolayer wells – where the virus has been neutralised, have no CPE and the cells remain intact (blue-stained cells sheets); negative wells – where virus has not been neutralised, have a CPE (empty cavity – no staining). If staining is used fix the cells with 10% formol/saline for 30 minutes. For staining, the plates are immersed in 0.1% crystal violet in 1 % ethanol and 5 % buffered formalin for 30 minutes.

vii) Validate the test by checking the back titration of the virus (which should give a value of 100 $TCID_{50}$ per well with a permissible range of 32–320 $TCID_{50}$), the control sera and the cell control wells. The positive control serum should give a titre of twofold dilution (±0.3 log_{10} units) from its target value. The weak positive serum should be positive. The negative serum should give no neutralisation. In the cell control wells, the monolayers should be intact.

iv) *Interpretation of the results:* Virus neutralising titres of serum antibody responses (titres are expressed as the final dilution of serum present in the serum/virus mixture where 50% of wells are protected). This can be calculated by the Spearman–Kärber or Reed Muench methods. The 50% neutralisation titre of each serum is expressed as log 10. When only two repetitions per dilution are performed, the highest inverse of the dilution that neutralised 100% of the cavities can be considered. In general, a titre of ≥32 (1.5) or more of the final serum dilution in the serum/virus mixture is regarded as positive for VSV antibodies. Laboratories are encouraged to verify this cut-off internally, with reagents provided by an OIE Reference Laboratory, where available. If cytotoxicity is observed in the control wells, the sample is reported to be toxic (no result) unless neutralisation of the virus without cytotoxicity is observed at higher dilutions and a titre can be read without ambiguity (Allende *et al.,* 1992).

Note: Seroconversion is considered when there is a four-fold increase in antibody titre between paired serum samples collected with a minimum interval of 7–14 days between the first (acute phase of the disease) and the second blood collection (convalescent phase).

2.4. Complement fixation test

A detailed description of this test is given in Section B.1.5. This is modified as follows. The CFT may be used for quantification of early antibodies, mostly IgM. For this purpose, twofold serum dilutions are mixed with 2 CFU_{50} of known antigen and with 5% normal bovine or calf sera included in 4 CHU_{50} of complement. The mixture is incubated for 3 hours at 37°C or overnight at 4°C. Subsequently, the haemolytic system is added followed by incubation for 30 minutes at 37°C. The serum titre is the highest dilution in which no haemolysis is observed. Titres of 1/5 or greater are positive. This CFT has low sensitivity and is frequently affected by anticomplementary or nonspecific factors.

C. REQUIREMENTS FOR VACCINES

1. Background

1.1. Rationale and intended use of the product

VSV infections can have significant impacts on the health and production aspects of animals, resulting in considerable economic losses for producers. Reduced feed intake caused by oral lesions can result in weight loss and delays to market. Lesions on the feet can cause temporary locomotor problems affecting the ability of an animal to obtain food and water, and permanent foot problems that result in the animal being culled. Lesions of the mammary gland can impact the ability of the dam to nurse her offspring and for harvesting milk for sale. Animals may be culled if mammary or teat lesions are severe.

Although vaccination is not widely practised, vaccine is used to reduce the severity of clinical signs and the economic impacts of the disease.

Attenuated virus vaccines have been tested in the field in the USA, Panama, Guatemala, Peru and Venezuela (Lauerman *et al*., 1962; Mason, 1978) with unknown efficacy. Killed vaccines for VSIV and VSNJV are manufactured in Colombia and Venezuela (2002 OIE vaccine survey). Although a commercial vaccine combining VS and FMD antigens in a single emulsion for Andean countries has been tested in vaccination–challenge experimentation and published (House *et al*., 2003), the vaccine is not produced/applied routinely.

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.

2. Outline of production and minimum requirements for conventional vaccines

2.1. Characteristics of the seed

2.1.1. Biological characteristics

Identity of the seed and the source of the serum used in growth and passage of the virus should be well documented, including the source and passage history of the organism.

2.1.2. Quality criteria (sterility, purity, freedom from extraneous agents)

The purity of the seed and cells to be used for vaccine production must be demonstrated. The master seed virus (MSV) should be free from adventitious agents, bacteria, or *Mycoplasma*, using tests known to be sensitive for detection of these microorganisms. The test aliquot should be representative of a titre adequate for vaccine production, but not such a high titre that hyperimmune antisera are unable to neutralise seed virus during purity testing. Seed virus is neutralised with monospecific antiserum or monoclonal antibody against the seed virus and the virus/antibody mixture is cultured on several types of cell line monolayers. A cell line highly permissive for bovine viral diarrhoea virus, types 1 and 2, is recommended as one of the cell lines chosen for evaluation of the MSV. Bovine viral diarrhoea virus is a potential contaminant introduced using FBS in cell culture systems. Cultures are subpassaged at 7-day intervals for a total of at least 14 days, then tested for adventitious viruses that may have infected the cells or seed during previous passages.

2.2. Method of manufacture

2.2.1. Procedure

Once the vaccine is shown to be efficacious, and the proposed conditions for production are acceptable to regulatory authorities, approval may be granted to manufacture vaccine. Virus seed can be grown in cell culture. Selection of a cell type for culture is dependent on the degree of virus adaptation, growth in medium, and viral yield in the specific culture system. Vaccine products should be limited to the number of passages from the MSV that can be demonstrated to be effective. Generally, large-scale monolayer or suspension cell systems are operated under strict temperature-controlled, aseptic conditions and defined production methods, to assure lot-to-lot consistency. Dose of virus used to inoculate cell culture should be kept to a minimum to reduce the potential for viral defective interfering particles. When the virus has reached its appropriate titre, as determined by CPE, fluorescent antibody assay, or other approved technique, the virus is clarified, filtered, and inactivated (for killed vaccines).

2.2.2. Requirements for substrates and media

Cell cultures should be demonstrated free of adventitious viruses. All animal origin products used in the production and maintenance of cells (i.e. trypsin, FBS) and growth of virus should be free of adventitious agents, with special attention paid to the presence of bovine viral diarrhoea virus.

2.2.3. In-process controls

Cell cultures should be checked macroscopically for abnormalities or signs of contamination and discarded if unsatisfactory. Virus concentration can be assessed using antigenic mass or infectivity assays.

An inactivation kinetics study should be conducted using the approved inactivating agent (β-propiolactone or 1 ml for 100 ml of viral suspension of 0.1 M binary ethyleneimine [BEI during 24 hours]) on each viral lot with a titre greater than the maximum production titre and grown using the approved production method. This study should demonstrate that the inactivation method is adequate to assure complete inactivation of virus. Samples taken at regular timed intervals during inactivation, then inoculated on to a susceptible cell line, should indicate a linear and complete loss of titre by the end of the inactivation process.

During production, antigen content is measured to establish that minimum bulk titres have been achieved. Antigen content is generally measured before inactivation (if killed vaccine) and prior to further processing.

2.2.4. Final product batch tests

Vaccine candidates should be shown to be pure, safe, potent, and efficacious.

i) Sterility and purity

During production, tests for bacteria, *Mycoplasma*, and fungal contamination should be conducted on both inactivated and live vaccine harvest lots and confirmed on the completed product (see Chapter 1.1.9 *Tests for sterility and freedom from contamination of biological materials intended for veterinary use*).

ii) Safety

The use of target animal batch release safety tests or laboratory animal batch release safety tests should be avoided wherever possible.

iii) Batch potency

Potency is examined on the final formulated product. Mirroring what is done for the potency test in FMD vaccines, a vaccination–challenge test has being proposed for testing VSV vaccines (House *et al.,* 2003). The gaps in knowledge regarding the pathogenesis of VSV infection and the immune mechanism that affords protection against viral infection are limitations for development and implementation of a validated protocol for a challenge test. However, for batch release, indirect tests can also be used for practicability and animal welfare considerations, as long as correlation has been validated to protection in the target animal during efficacy tests. Frequently indirect potency tests include antibody titration after vaccination of target species. Ideally, indirect tests are carried out for each strain for one species and each formulation of vaccine to establish correlation between the indirect test results and the vaccine efficacy test results.

Relative potency could be used to determine antigen content in final product. It is necessary to confirm the sensitivity, specificity, reproducibility, and ruggedness of such assays.

2.3. Requirements for regulatory approval

2.3.1. Safety requirements

i) Target and non-target animal safety

Final product may be evaluated in the host animal using two animals of the minimum age recommended for use, according to the instructions given on the label; the animals are observed for 21 days. Field safety studies conducted on vaccinates, in at least three divergent geographical areas, with at least 300 animals per area, are also recommended.

For killed and modified live virus (MLV) vaccines product safety will be based on an absence of adverse reactions such as shock, abscesses at site of inoculation, etc. In the specific case of MLV vaccines, it would not be expected to see clinical signs. If clinical signs of vesicular stomatitis virus are observed, use of the vaccine should be reconsidered. Residual virus should be evaluated for prior to mixing the antigen with adjuvant. Initial safety is evaluated in a few animals for 21 days under close observation to assess for gross safety issues. If the vaccine passes this first safety test, the vaccine is used in the field in a larger number of animals to evaluate if subtle safety issues are present: adverse reactions/swelling, abscesses, shock, etc.

ii) Reversion-to-virulence for attenuated/live vaccines

Reversion to virulence for live viral vaccines is often demonstrated by back passage through susceptible species. Virus is isolated from the vaccinated animal and the isolated virus is then used to inoculate additional animals. Sequential passage through animals should show that animals remain clinically healthy with no demonstration of typical vesicular stomatitis lesions.

iii) Environmental consideration

Inactivated vesicular stomatitis vaccines probably present no special danger to the user, although accidental inoculation may result in an adverse reaction caused by the adjuvant and secondary components of the vaccine. MLV vaccines may pose a hazard to the user depending on the level of inactivation of the virus.

Preservatives should be avoided if possible, and where not possible, should be limited to the lowest concentration possible. Vaccine bottles, syringes, and needles may pose an environmental hazard for vaccines using adjuvants or preservatives and for MLV vaccines. Instructions for disposal should be included within the vaccine packaging information and based on current environmental regulations in the country of use.

2.3.2. Efficacy requirements

The gaps in knowledge regarding the pathogenesis of VSV infection and the immune mechanism that affords protection against viral infection are limitations for the development and implementation of a validated protocol for an efficacy test. Ideally vaccine efficacy should be estimated in vaccinated animals directly by evaluating their resistance to live virus challenge. Vaccine efficacy should be established for every strain to be authorised for use in the vaccine.

Live reference VSV viruses corresponding to the virus strains circulating in the region are stored at ultralow temperatures. Each challenge virus is prepared as follows. Tongue tissue infected by VSV should be obtained from original field case of VS and received at the Reference Laboratory in glycerol buffer as described in Section B. *Diagnostic Techniques*.

The preparation of cattle challenge virus follows the process described in Chapter 3.1.8 *Foot and mouth disease*, Section B.1.1 *Virus isolation*, with the view of obtaining a sterile 10% suspension in Eagles minimal essential medium with 10% sterile FBS.

The preparation of the stock of challenge virus to be aliquoted is prepared starting from lesions collected in two cattle over 6 months of age, previously recognised to be free of VSV antibodies. These animals are tranquillised, for example using xylazine 100 mg/ml (follow instructions for use), then inoculated intradermally (i.d.) in the tongue with the suspension in about 20 sites, 0.1 ml each. The vesiculated tongue tissue is harvested at the peak of the lesions, approximately 2 days later.

A 2% suspension is prepared as above and filtered through a 0.2 µm filter, aliquoted and frozen in the gas phase of liquid nitrogen, and constitutes the stock of challenge virus. The infective titres of this stock are determined both in cell culture ($TCID_{50}$) and in two cattle (BID_{50}: 50% bovine infective dose). These two cattle that have been tranquilised using xylazine, are injected intradermally in the tongue with tenfold dilutions (1/10 through 1/10,000), using four sites per dilution (Henderson, 1949). The cattle titrations are read 2 days later. Most frequently, titres are above 10^6 $TCID_{50}$ for 0.1 ml and above 10^5 BID_{50} for 0.1 ml calculated using the Spearmann–Kärber method. The dilution for use in cattle challenge test is 10 000 DIB_{50} in a total volume of 4× 0.1 ml by intralingual injection for both the 50% protective dose (PD_{50}) test and the PGP (protection against generalised foot infection) test (House *et al.,* 2003).

i) Vaccination–challenge method

For this experimental method, a group of 12 VSV sero-negative cattle of at least 6 months of age are vaccinated with a bovine dose by the route and in the volume recommended by the manufacturer at day 0 and day 40. These animals and a control group of two non-vaccinated animals are challenged 2 weeks or more after the second vaccination. The challenge strain is a suspension of bovine virus that is fully virulent and appropriate to the virus types in the vaccine under test by inoculating a total of 10,000 BID_{50} intradermally into four sites (0.1 ml per site) on the upper surface of the tongue. Animals are observed at 7–8 days after challenge.

It was proposed that vaccinated animals showing no lesion on the tongue should be considered fully protected. Vaccinated animals showing lesions at one, two, or three inoculation sites should be considered partially protected, and animals showing lesions at four sites are considered not protected (House *et al.,* 2003). Control animals must develop lesions at four sites. Vaccine should fully protect at least nine animals out of 12 vaccinated (75% protection), the remaining animals being partially or not protected. This test gives a certain measure of the protection following the injection of two commercial bovine doses of vaccine in a limited cattle population.

Although the vaccination-challenge method has been described and published (House *et al.*, 2003) data on the validation under field conditions for the efficacy of released vaccine are not available.

ii) Efficacy in other species

Efficacy tests in other target species, such as horses, are not yet described or standardised. In general, a successful test in cattle should be considered to be sufficient evidence of the quality of a VS vaccine to endorse its use in other species.

2.3.3. Duration of immunity

The duration of immunity (D.O.I) of a VS vaccine will depend on the efficacy (formulation and antigen payload). As part of the approval procedure the manufacturer should be required to demonstrate the D.O.I. of a given vaccine by either challenge or the use of a validated alternative test, such as serology at the end of the claimed period of protection.

2.3.4. Stability

The stability of all vaccines including oil emulsion vaccines should be demonstrated as part of the shelf-life determination studies for approval. Vaccines should never be frozen or stored above the target temperature.

Vaccines should be stored at 4–8°C, with minimal exposure to light. The shelf life should be determined by use of the approved potency test (Section C.2.2.4.iii) over the proposed period of viability.

i) For animal production

Virus(es) used in vaccine production should be antigenically relevant to virus(es) circulating in the field. A vaccination/challenge study in the species for which the vaccine will be used will indicate the degree of protection afforded by the vaccine. Species used in vaccination/challenge studies should be free of antibodies against vesicular stomatitis. Vaccination/challenge studies should be conducted using virus produced by the intended production method, at the maximum viral passage permitted, and using an experimental animal model. It is necessary to confirm the sensitivity, specificity, reproducibility, statistical significance and confidence level of such experimental model.

Antibody levels after vaccination measured *in vitro* could be used to assess vaccine efficacy provided a statistically significant correlation study has been made. For vaccines containing more than one virus (for example, VSNJV and VSIV), the efficacy of the different components of these vaccines must each be established independently and then as a combination in case interference between different viruses exists.

The duration of immunity and recommended frequency of vaccination of a vaccine should be determined before a product is approved. Initially, such information is acquired directly using host animal vaccination/challenge studies. The period of demonstrated protection, as measured by the ability of vaccinates to withstand challenge in a valid test, can be incorporated into claims found on the vaccine label.

If the vaccine is to be used in horses, swine, cattle, or other ruminants destined for market and intended for human consumption, a withdrawal time consistent with the adjuvant used (generally 21 days) should be established by such means as histopathological examination submitted to the appropriate food safety regulatory authorities.

ii) For control

The same principles apply as for animal production usage. In addition, it should be noted that antibody responses in vaccinated animals may not be differentiated from animals exposed to field virus. Therefore, vaccinated animals will need to be clearly identified if serological methods will be used in conjunction with compatible clinical signs to assess field virus exposure.

REFERENCES

AFSHAR A., SHAKARCHI N.H. & DULAC G.C. (1993). Development of a competitive enzyme linked immunosorbent assay for detection of bovine, equine, ovine and porcine antibodies to vesicular stomatitis virus. *J. Clin. Microbiol*., **31**, 1860–1865.

ALONSO A., MARTINS M., GOMES M.P.D., ALLENDE R. & SONDAHL M.S. (1991). Development and evaluation of an enzyme-linked immunosorbent assay for detection, typing and subtyping of vesicular stomatitis virus. *J. Vet. Diagn. Invest.,* **3**, 287–292.

ALONSO FERNANDEZ A. & SONDAHL M.S. (1985). Antigenic and immunogenic characterisation of various strains of the Indiana serotype of vesicular stomatitis isolated in Brazil. *Bol. Cen. Panam. Fiebre Aftosa,* **51**, 25–30.

ALLENDE R., SEPULVEDA L., MENDES DA SILVA A., MARTINS M., SONDAHL M.S. & ALONSO FERNANDEZ A. (1992). An enzyme-linked immunosorbent assay for the detection of vesicular stomatitis virus antibodies. *Prev. Vet. Med.,* **14**, 293–301.

BORING W. & SMITH D. (1962). Vesicular Stomatitis Virus: A Survey and Analysis of the Literature. Technical Study No. 43, US Army Biological Laboratories, Fort Detrick, USA.

CARGNELUTTI J.F., OLINDA R. G., MAIA L.A., AGUIAR G.M.N., NETO E.G.M., SIMÕES S.V.D., LIMA T.G., DANTAS A.F.M., WEIBLEN R., FLORES E.F. & RIET-CORREA F. (2014). Outbreaks of vesicular stomatitis Alagoas virus in horses and cattle in northeastern Brazil. *J. Vet. Diagn. Invest*., **26**, 788–794.

COMER S.A., CORN J.L., STALLKNECHT D.E., LANDGRAF J.G. & NETTLES V.F. (1992). Titers of vesicular stomatitis virus New Jersey serotype in naturally infected male and female *Lutzomyia shannoni* (Diptera: Psychodidae) in Georgia. *J. Med. Entomol.,* **29**, 368–370.

COTTON W.E. (1927). Vesicular stomatitis. *Vet. Med.,* **22**, 169–175.

DE OLIVEIRA A.M., FONSECA A.A. JR, CAMARGOS M.F., ORZIL L.M., LAGUARDIA-NASCIMENTO M., OLIVEIRA A.G.G., RODRIGUES J.G., SALES M.L., DE OLIVEIRA T.F.P. & DE MELO C.B. (2018). Development and validation of RT-qPCR for vesicular stomatitis virus detection (*Alagoas vesiculovirus*). *J. Virol. Methods*, **257**, 7–11.

FEDERER K.E., BURROWS R. & BROOKSBY J.B. (1967). Vesicular stomatitis virus – the relation between some strains of the Indiana serotype. *Res. Vet. Sci.*, **8**, 103–117.

FERRIS N.P. & DONALDSON A.I. (1988). An enzyme-linked immunosorbent assay for the detection of VSV antigen. *Vet. Microbiol.*, **18**, 243–258.

FOWLER V.L., KING D.J., HOWSON E.L.A., MADI M., PAUSZEK S.J., RODRIGUEZ L.L, KNOWLES N.J., MIOULET V. & KING D.P. (2016). Genome sequences of nine vesicular stomatitis virus isolates from South America. *Genome Announc.,* **4**, e00249-16. doi: 10.1128/genomeA.00249-16.

FRANCY D.B., MOORE C.G., SMITH G.C., TAYLOR S.A. & CALISER C.H. (1988). Epizootic vesicular stomatitis in Colorado, 1982: isolation of virus from insects collected along the northern Colorado Rocky Mountain Front Range. *J. Med. Entomol.,* **25**, 343–347.

HENDERSON W.M. (1949). The quantitative study of foot and mouth disease virus. Agricultural Research Council Report Series No. 8, HMSO, London, UK, page 5.

HOFNER M.C., CARPENTER W.C., FERRIS N.P., KITCHING R.P. & BOTERO F.A. (1994). A hemi-nested PCR assay for the detection and identification of vesicular stomatitis virus nucleic acid. *J. Virol. Methods*, **50**, 11–20.

HOLE K., VELAZQUES-SALINAS L. & CLAVIJO A. (2010). Improvement and optimization of a multiplex real time RT-PCR assay for the detection and typing of vesicular stomatitis virus. *J. Vet. Diagn. Invest*., **22**, 428–433.

HOUSE J.A, HOUSE C., DUBOURGET P. & LOMBARD M. (2003). Protective immunity in cattle vaccinated with a commercial scale inactivated, bivalent vesicular stomatitis vaccine. *Vaccine,* **21**, 1932–1937.

ICTV (International Committee on Taxonomy of Viruses) (2019). Virus Taxonomy: 2019 EC 51 Release. Available at: https://talk.ictvonline.org/taxonomy/

JENNY E.W., MOTT L.O. & TRAUB E. (1958). Serological studies with the virus of vesicular stomatitis. I. Typing of vesicular stomatitis by complement fixation. *Am. J. Vet. Res.*, **19**, 993–998.

KATZ J.B., EERNISSE K.A., LANDGRAF J.G. & SCHMITT B.J. (1997). Comparative performance of four serodiagnostic procedures for detecting bovine and equine vesicular stomatitis virus antibodies. *J. Vet. Diagn. Invest.,* **9**, 329–331.

KATZ J.B., SHAFER A.L. & EERNISSE K.A. (1995). Construction and insect larval expression of recombinant vesicular stomatitis nucleocapsid protein and its use in competitive ELISA. *J. Virol. Methods,* **54**, 145–157.

KILLMASTER L.F., STALKNECHT D.E., HOWERTH E.W., MOULTON J.K., SMITH P.F. & MEAD D.G. (2011). Apparent disappearance of vesicular stomatitis New Jersey virus from Ossabaw Island, Georgia. *Vector Borne Zoonotic Dis.*, **11**, 559–565.

KITCHING R.P. & DONALDSON A.I. (1987). Collection and transportation of specimens for vesicular virus investigation. *Rev. Sci. Tech*., **6**, 251–283. doi: 10.20506/rst.6.1.291.

LAUERMAN L.H., KUNS M.L. & HANSON R.S. (1962). Field trial of live virus vaccination procedure for prevention of vesicular stomatitis in dairy cattle. I: Preliminary immune response. Proceedings of the 66th Annual Meeting of the United States Animal Health Association, 365–369.

MASON J. (1978). The epidemiology of vesicular stomatitis. *Bol. Cen. Panam. Fiebre Aftosa,* **29**–**30**, 35–53.

MEAD D.G., LOVETT K.R., MURPHY M.D., PAUSZEK S.J., SMOLIGA G., GRAY E.W., NOBLET R., OVERMYER J. & RODRIGUEZ L.L. (2009). Experimental transmisión of vesicular stomatitis New Jersey virus from *Simulium vittatum* to cattle: clinical outcome is influenced by sie of insect feeding. *J. Med. Entomol.,* **46**, 866–872.

MEAD D.G., GRAY E.W., MURPHY M.D., HOWERTH E.W. & STALLKNECHT D.E. (2004). Biological transmission of vesicular stomatitis virus (New Jersey seroype) by *Simulium vittatum* (Diptera: Simuliidae) to domestic swine (*Sus scrofa*). *J. Med. Entomol*., **41**, 78–82.

OLTSKY P.K., TRAUM J. & SCHOENING H.W. (1926). Comparative studies on vesicular stomatitis and foot and mouth disease. *J. Am. Vet. Med. Assoc.,* **70**, 147–167.

PANAFTOSA-OPS/OMS (2019). Informe de Situación de los Programas de Erradicación de la Fiebre Aftosa. Sudamerica y Panamá en 2019. https://iris.paho.org/handle/10665.2/51789.

PAUSZEK S.J., ALLENDE R. & RODRIGUEZ L.L. (2008). Characterization of the full-length genomic sequences of vesicular stomatitis Cocal and Alagoas viruses. *Arch. Virol*., **153**, 1353–1357.

PAUSZEK S.J., BARRERA J. DEL C., GOLDBERT T., ALLENDE R. & RODRIGUEZ L.L. (2011). Genetic and antigenic relationships of vesicular stomatitis viruses from South America. *Arch. Virol*., **156**, 1961–1968.

RAINWATER-LOVETT K., PAUSZEK S.J., KELLEY W.N. & RODRIGUEZ L.L. (2007). Molecular epidemiology of vesicular stomatitis New Jersey virus from the 2004–2005 US outbreak indicates a common origin with Mexican strains. *J. Gen. Virol.*, **88**, 2042–2051. DOI 10.1099/vir.0.82644-0.

ROCHA C.S., OLIVEIRA I.V.P.M., MOURA G.H.F., BEZERRA J.A.B., RONDON F.C.M., VASCONCELOS D.C., ALMEIDA M.M., CORTEZ A.A., CLABUIG C. & ANTUNES J.M.A.P. (2020). Vesicular stomatitis due to Indiana III (Alagoas/VSIV-3) is endemic in Brazilian state of Ceará. *Ciênc. Rural,* **50**, 6, Santa Maria, e20190846. http://doi.org/10.1590/0103-8478cr20190846.

RODRIGUEZ L.L., LETCHWORTH G.J., SPIROPOULOU C.F. & NICHOL S.T. (1993). Rapid detection of vesicular stomatitis virus New Jersey serotype in clinical samples by using polymerase chain reaction. *J. Clin. Microbiol.*, **31**, 2016–2020.

SALES M.L., DALL'AGNOL M., DE OLIVEIRA A.M., CAMARGOS M.F., FONSECA A.A. JR & PIMENTA DOS REIS J.K.P. (2020). RT-qPCR for the diagnosis of the vesiculovirus Cocal virus. *Arch. Virol.,* **165**, 1843–1847.

SELLERS R.F. & MAAROUF A.R. (1990). Trajectory analysis of winds in vesicular stomatitis in North America. *Epidemiol. Infect.,* **104**, 313–328.

SEPULVEDA L. M., MALIRAT V., BERGMANN I.E., MANTILLA A. & NASCIMENTO E.R. (2007). Rapid diagnosis of vesicular stomatitis virus in Ecuador by the use of polymerase chain reaction. *Braz. J. Microbiol*., **38**, 500–506.

WILSON W.C., LETCHWORKTH G.J., JIMENEZ C., HERRERO M.V., NAVARRO R., PAZ P., CORNISH T.E., SMOLIGA G., PAUSZEK S.J., DORNAK C., GEORGE M. & RODRIGUEZ L.L. (2009). Field evaluation of a multiplex real-time reverse transcription polymerase chain reaction assay for detection of Vesicular stomatitis virus. *J. Vet. Diagn. Invest.*, **21**, 179–186.

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NB: There are OIE Reference Laboratories for vesicular stomatitis
(please consult the OIE Web site:
https://www.oie.int/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3).
Please contact the OIE Reference Laboratories for any further information on
diagnostic tests, reagents and vaccines for vesicular stomatitis

NB: FIRST ADOPTED IN 1990. MOST RECENT UPDATES ADOPTED IN 2021.