CHAPTER 2.8.8.

SWINE VESICULAR DISEASE

SUMMARY

***Description and importance of the disease**: Swine vesicular disease (SVD) is a contagious disease of pigs, caused by an enterovirus and characterised by vesicles on the coronary bands, heels of the feet and occasionally on the lips, tongue, snout and teats. Strains of SVD virus (SVDV) vary in virulence, and the disease may be subclinical, mild or severe, the latter usually only being seen when pigs are housed on abrasive floors in damp conditions. The main importance of SVD is that it can be clinically indistinguishable from foot and mouth disease (FMD), and any outbreaks of vesicular disease in pigs must be assumed to be FMD until investigated by laboratory tests and proven otherwise. However, subclinical infection has been the most frequent condition observed during recent years.*

***Identification of the agent:** Where a vesicular condition is seen in pigs, the demonstration by enzyme-linked immunosorbent assay (ELISA) of SVD viral antigen in a sample of lesion material or vesicular fluid is sufficient for a positive diagnosis. If the quantity of lesion material submitted is not sufficient (less than 0.5 g), or if the test results are negative or inconclusive, a more sensitive test, such as the reverse transcriptase polymerase chain reaction (RT-PCR) or isolation of virus (VI) in porcine cell cultures, may be used. If any inoculated cultures subsequently develop a cytopathic effect, the demonstration of SVD viral antigen by ELISA or viral RNA by RT-PCR will suffice to make a positive diagnosis. Subclinical infection may be detected by random sampling of pen-floor faeces tested for identification of SVD virus using RT-PCR or VI tests.*

***Serological tests:** Serological tests can be used to help confirm clinical cases as well as to identify subclinical infections. Specific antibody to SVDV can be identified using ELISA for screening and the microneutralisation test for confirmation. A small proportion (up to 0.1%) of normal, uninfected pigs will react positively in serological tests for SVD. The reactivity of these singleton reactors is transient, so that they can be differentiated from infected pigs by resampling of the positive animal and its cohorts.*

Diagnostic and standard reagents are available from reference laboratories.

***Requirements for vaccines:** There are currently no commercial vaccines available against SVD.*

A. INTRODUCTION

Swine vesicular disease (SVD) can be a subclinical, mild or severe vesicular condition depending on the strain of virus involved, the route and dose of infection, and the husbandry conditions under which the pigs are kept. When it occurs clinically, SVD can be indistinguishable from foot and mouth disease (FMD) and therefore a differential diagnosis and laboratory investigation is an urgent requirement. However, during the present century outbreaks of SVD have been characterised by less severe or no clinical signs and infection has been detected when samples are tested for serosurveillance programmes or for export certification.

After its first detection in 1966, the disease occurred with epidemics in eastern and western Europe (during the 1970s and 1980s), and was also detected in East Asia. Since then, SVD has only been sporadically reported, mainly from Italy where its circulation is investigated and controlled through a virological and serological surveillance plan.

The incubation period for SVD is between 2 and 7 days, after which a transient fever of up to 41°C may occur. Vesicles then develop on the coronary band, typically at the junction with the heel. These may affect the whole coronary band resulting in loss of the hoof. More rarely, vesicles may also appear on the snout, particularly on the dorsal surface, on the lips, tongue and teats, and shallow erosions may be seen on the knees. Affected pigs may be

lame and off their feed for a few days. Abortion is not a typical feature of SVD. Recovery is usually complete in 2–3 weeks (Loxam & Hedger, 1983). Affected pigs may excrete virus from the nose and mouth and in the faeces up to 48 hours before the onset of clinical signs. Most virus is produced in the first 7 days after infection, and virus excretion from the nose and mouth normally stops within 2 weeks. Virus may continue to be shed for up to 3 months in the faeces, though under usual circumstances virus is detectable in faeces only up to 1 month. The SVD virus (SVDV) is extremely resistant to inactivation in the environment, and is stable in the pH range 2.5–12.0 (Mann, 1981). This is in contrast to the FMD virus, which is very labile outside the pH range 6.0–8.0.

Because SVD may be mild or subclinical, it is essential when submitting samples from suspect clinical cases that serum samples from both the suspect pigs and other apparently unaffected animals in the group be included. It is possible for SVD to circulate unnoticed until it affects a particularly susceptible group. Therefore, in order to ascertain how long infection has been present, it is necessary to look for seroconversion to SVDV in apparently healthy animals. Also the identification of the isotype of the immunoglobulins (M or G) to SVDV may help to ascertain the time of exposure to infection.

SVDV has been classified as a porcine variant of a human coxsackievirus B5 (*Enterovirus B*), in the family *Picornaviridae*. All isolates are classified in a single serotype, with four distinguishable antigenic/genomic variants (Brocchi *et al*., 1997), which evolved sequentially in different time-periods, except for the third and fourth variants that were co-circulating in Italy during 1992–1993. All SVDVs occurring since then diverge from a common origin and cluster in a unique antigenic/genomic lineage corresponding to the fourth and most recent group; however, two genomic sub-lineages are distinguishable within it (Knowles *et al*., 2007). Antigenically and genetically, SVDV is closely related to the human virus coxsackievirus B5 and it has been suggested that it arose through recombination with another human enterovirus, coxsackievirus A9 (Bruhn *et al.,* 2015). A second adaptation of a human enterovirus to cause vesicular disease in pigs was reported in Russia in 1975 involving coxsackievirus B4, which is serologically distinct from SVDV/CV-B5 (Lomakina *et al.,* 2016).

There are reports of seroconversion to SVDV in laboratory workers handling the agent. However, clinical disease in humans is reported to be mild, with the exception of a single case of meningitis associated with SVDV infection, and there have been no reported cases of seroconversion or disease in farmers or veterinarians working with infected pigs. Under experimental conditions, it has not been possible to show transmission of coxsackievirus B5 between pigs. Laboratory manipulations should be carried out at an appropriate biosafety and containment level determined by biorisk analysis (see Chapter 1.1.4 *Biosafety and biosecurity: Standard for managing biological risk in the veterinary laboratory and animal facilities*).

B. DIAGNOSTIC TECHNIQUES

Table 1. *Test methods available for the diagnosis of swine vesicular disease 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
Agent identification						
Virus isolation	–	+	+	+++	–	n/a
RT-PCR	–	+++	+++	+++	–	n/a
ELISA for antigen detection	–	–	–	+++	–	n/a
Detection of immune response						
Virus neutralisation	+	+++	+	+	+	n/a
Competitive ELISA for Ab screening	+++	+++	+++	+	+++	n/a

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
ELISA for IgG and IgM identification	+	+++	+++	+	+	n/a

Key: +++ = recommended method, validated for the purpose shown; ++ = suitable method but may need further validation;
+ = may be used in some situations, but cost, reliability, or other factors severely limits its application;
– = not appropriate for this purpose; n/a = purpose not applicable.
RT-PCR = reverse-transcriptase polymerase chain reaction; ELISA = enzyme-linked immunosorbent assay.
NOTE: Selection of assays suited for different purposes should take into account the different kinetics of the diagnostic targets (agent and antibodies) during infection.

1. Identification of the agent

Any vesicular condition in pigs could represent an FMD infection and differential diagnosis between FMD and other vesicular conditions, including SVD, is necessary. The diagnosis of SVD requires the facilities of a specialised laboratory. Countries that lack such a facility should send samples for investigation to an OIE Reference Laboratory for SVD (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/).

For clinical cases, the detection of antigens or genome of SVDV by means of enzyme-linked immunosorbent assay (ELISA) and reverse-transcriptase polymerase chain reaction (RT-PCR) has the same diagnostic value as virus isolation. Due to their speed, ELISA and RT-PCR make suitable screening tests. However, virus isolation is the reference method and should be used if a positive ELISA or RT-PCR result is not associated with the detection of clinical signs of disease, the detection of seropositive pigs, or a direct epidemiological connection with a confirmed outbreak.

If there are clinical signs, investigation should start with the examination of a 10% suspension of lesion material in phosphate buffered saline (PBS) or tissue culture medium and antibiotics. Faecal samples are the specimen of choice for the detection of virus where subclinical SVD is suspected. Faecal samples can be collected from individual pigs or from the floor of premises suspected to contain, or to have contained, pigs infected with SVD. The level of virus in faeces is usually insufficient for detection by ELISA and the use of RT-PCR and/or virus isolation is required. A significant proportion of faecal samples inoculated into cell cultures will give rise to the growth of other enteroviruses. These can be differentiated from SVDV by ELISA or RT-PCR, but they may also outgrow SVDV that is present, and give rise to false negative results. Therefore, RT-PCR is more sensitive than virus isolation when applied to faecal samples.

1.1. Preparation of samples

1.1.1. Lesion material

Samples should be transported in phosphate buffered saline (PBS) with antibiotics, mixed with glycerol (1/1), pH 7.2–7.6. A suspension is prepared by grinding the sample in sterile sand in a sterile pestle and mortar with a small volume of PBS or tissue culture medium and antibiotics. Further medium should be added to obtain approximately a 10% suspension. This is clarified by centrifugation at 2000 ***g*** for 20–30 minutes and the supernatant is harvested.

1.1.2. Faecal samples

Faecal material (approximately 20 g) is resuspended in a minimal amount of tissue culture medium or phosphate buffer (0.04 M phosphate buffer or PBS). The suspension is homogenised by vortexing and clarified by centrifugation at 2000 ***g*** for 20–30 minutes; the supernatant is harvested and filtered through 0.45 µm filter.

1.2. Virus isolation

A portion of the clarified epithelial or faecal suspension is inoculated on to monolayers of IB-RS-2 cells or other susceptible porcine cell line, grown in appropriate containers (25 cm^2 flasks, rolling tubes, 24-, 12-, 6-well plates). For differential diagnosis (e.g. FMD) in case of clinical lesions bovine cell culture systems should also be employed in parallel. Generally SVDV will grow in cells of porcine origin only.

Tissue culture medium is supplemented with 10% bovine serum for cell growth, with 1-3% bovine serum for maintenance, and with antibiotics.

Cultures are examined daily. If a cytopathic effect (CPE) is observed, the supernatant fluid is harvested and virus identification is performed by ELISA (or other appropriate test, e.g. RT-PCR). Negative cultures are blind-passaged after 48 or 72 hours, and observed for a further 2–3 days. If no CPE is evident after the second passage, the sample is recorded "NVD" (no virus detected). When isolating virus from faeces in which the amount of virus present may be low, a third tissue culture passage may be required.

1.3. Immunological methods

1.3.1. Enzyme-linked immunosorbent assay

The detection of SVD viral antigen by an indirect sandwich ELISA has replaced the complement fixation test as the method of choice. The test has the same format as that used for FMD diagnosis. Wells of ELISA plates are coated with rabbit antiserum to SVDV. This is the capture serum. Test sample suspensions are added and incubated. Appropriate controls are also included. Guinea-pig anti-SVD detection serum is added at the next stage followed by rabbit anti-guinea-pig serum conjugated to horseradish peroxidase. Extensive washing is carried out between each stage to remove unbound reagents. A positive reaction is indicated if there is a colour reaction on the addition of chromogen (for example orthophenylenediamine) and substrate (H_2O_2). With strong positive reactions this will be evident to the naked eye, but results can also be read spectrophotometrically at the appropriate wavelength, in which case an absorbance reading ≥0.1 above background indicates a positive reaction. As an alternative to guinea-pig and rabbit antisera, suitable monoclonal antibodies (MAbs) can be used, coated to the ELISA plate as the capture antibody and peroxidase conjugated as detector antibody. For example, a simple sandwich ELISA performed with MAb 5B7 as both catching and conjugated/detector antibody, that represents also the reference method for the serological competitive ELISA, is suited for the detection of SVD viral antigen.

A MAb-based ELISA can also be used to study antigenic variation among strains of SVDV. Tissue-culture grown viral strains are trapped by a rabbit hyperimmune antiserum to SVDV adsorbed to the solid phase. Appropriate panels of MAbs are then reacted and the binding of MAbs to field strains is compared with the binding of MAbs to the parental strains. Strong binding indicates the presence of epitopes shared between the parental and the field strains (Brocchi *et al*., 1997).

1.4. Nucleic acid recognition methods

1.4.1. Reverse-transcriptase-polymerase chain reaction

Reverse-transcriptase followed by the PCR (RT-PCR) is a useful method to detect SVD viral genome in a variety of samples from clinical and subclinical cases. Several methods have been described (Benedetti *et al*., 2010; Callens & De Clercq, 1999; Fallacara *et al*., 2000; Hakhverdyan *et al*., 2006; Lin *et al*., 1997; McMenamy *et al*., 2011; Nunez *et al*., 1998; Reid *et al*., 2004a; 2004b; Vangrysperre & De Clercq, 1996), employing different techniques for RNA extraction, targeting different parts of the SVDV genome and using different approaches to detect the DNA products of amplification. However, in a comparative study on positive faecal samples from many different SVD outbreaks, the one step RT-PCR (Benedetti *et al.,* 2010) had the best diagnostic performance, with the capability to reveal all the circulating genomic sub-lineages, compared with two real-time RT-PCR assays targeting the 5'-untranslated region (Reid *et al.,* 2004a; 2004b*)*, and an RT-loop-mediated isothermal amplification (LAMP) assay (Blomström *et al*., 2008).

The method reported below describes an RNA immune-extraction protocol and a one-step RT-PCR protocol targeting SVDV *3D* region, which codes for the RNA-polymerase.

To isolate RNA the immunocapture technique using a SVDV-specific MAb has been shown to be particularly effective in the case of faecal samples (Fallacara *et al*., 2000): it enables enrichment and purification of the SVDV, usually present in low concentration in faeces, with efficient removal of potential reaction inhibitors.

This method is suitable for laboratories without equipment for real-time detection of DNA amplification products, but where such facilities are available an approach such as that

described by Reid *et al.* (2004a; 2004b) offers advantages in terms of ease of use and reduced risk of laboratory contamination by PCR products.

1.4.2. RNA immune-extraction

i) RNA Immune-extraction: Coat wells of an ELISA plate with a saturating solution of MAb 5B7 (200 µl/well, diluted in carbonate-bicarbonate buffer) by overnight incubation at 4°C. Wash plates three times with PBS. Use plates immediately or store at –20°C for up to 2–3 weeks, or more if stabilised.

ii) Distribute each sample (faeces suspension) into three wells of the 5B7-coated plate (200 µl/well, 600 µl of sample in total).

iii) After incubation for 1 hour at 37°C with very slow shaking, wash wells three times with PBS. Washing is performed manually, in order to avoid cross-contamination between wells.

iv) RNA is extracted from each sample by adding approximately 100 µl/well of lysis buffer (4 M guanidine thiocyanate, 25 mM sodium citrate, pH 7, 0.5% Sarkosyl). Incubate wells for 3–5 minutes and recover the sample from the three wells (300–350 µl total), and transfer into a single tube.

v) RNA is then precipitated by adding a mixture of 750 µl of absolute ethanol and 35 µl of 3 M sodium acetate (pH 5.2); vials are vortexed and incubated at –20°C for a minimum of 1 hour (prolonged overnight precipitation at –20°C may also be suitable).

vi) Centrifuge the sample at 15,500–16,000 ***g*** for 30 minutes at 4°C, after which a pellet should be visible which should be washed with 500 µl of 70% cold ethanol (centrifuged at 15,500–16,000 ***g*** for 10 minutes at 4°C) and dried.

vii) Resuspend the RNA pellet in 20 µl of DEPC water, or commercially available RNase-free water.

NOTE: As an alternative to immunocapture, RNA extraction can be performed using suitable commercially available kits based on chaotropic salt lysis and silica RNA affinity.

1.4.2.1. One step RT-PCR

The following protocol for the conventional RT-PCR may need to be adjusted to suit particular reagents used.

i) Assemble the reaction mix (20 µl is the final volume for each test sample)

Rnase free water	10.75 µl
5× RT-PCR buffer	5 µl
dNTP mix (10 mM each dNTP)	1 µl
pSVDV-SS4 Forward Primer 10 pmol/ul*	1 µl
pSVDV-SA2 Reverse Primer 10 pmol/ul*	1 µl
RNAse inhibitor	0.25 µl (equivalent to 5 U)
RT-PCR enzyme mix	1 µl

*Primers sequence: pSVDV-SS4 5'-TTC-AGA-ATG-ATT-GCA-TAT-GGG-G-3'
pSVDV-SA2 5'-TCA-CGT-TTG-TCC-AGG-TTA-CY-3'

ii) Add 5 µl of each template RNA to 20 µl reaction mix.

iii) Run the following program in a thermal cycler:

One cycle at 50°C for 30 minutes (reverse transcription step)

One cycle at 95°C for 15 minutes (initial activation step)

40 cycles of 94°C for 20 seconds (denaturation), 60°C for 20 seconds (annealing), 72°C for 45 seconds (extension)

One cycle at 72°C for 10 minutes (final extension).

iv) Mix a 20 µl aliquot of each sample with 4 µl of loading dye and load onto a 2% agarose gel containing an appropriate DNA intercalating dye. After electrophoresis, a positive result is indicated by the presence of a 154 bp fragment of the SVDV RNA polymerase (3D) encoding region in the gel. Alternatively, gel can be stained after

electrophoresis to reduce contamination of equipment by soaking in a staining solution.

1.4.2.2. Real-time RT-PCR

This test can also be adapted to the format of real-time RT-PCR with dedicated reagents/kits, in the presence of a suitable DNA stain, using the following adjusted programme in a real-time PCR cycler:

i) 1 cycle at 50°C for 30 minutes (reverse transcription step)

ii) 1 cycle at 95°C for 15 minutes (initial activation step)

iii) 40 cycles, each composed of 94°C for 15 seconds (denaturation), 58°C for 30 seconds (annealing), 72°C for 30 seconds (extension), 77°C for 15 seconds (detection).

For the melting cycle:

iv) 1 cycle at 72°C for 1 minute and increasing temperature from 72°C to 95°C, by incremental steps of 0.5°C for 5 seconds each. Specific amplification products for SVDV generate melting curves with a peak within the temperature range 79.5–82.5°C.

1.4.3. Sequence analyses

Comparative analysis of sequences of the viral genome is useful to establish relationships between isolates of SVDV. By sequencing the 1D region, which codes for the major structural protein VP1, or the 3D region, it has been possible to group strains of SVDV according to their sequence homology, and epidemiologically to relate strains causing disease in different locations or at different times (Brocchi *et al*., 1997). The databases of *1D* and *3D* gene sequences of SVDVs are held at the OIE Reference Laboratory, Pirbright, UK and the OIE Reference Laboratory, Brescia, Italy, respectively. Further sequences (including those for complete SVDV genomes) are available via the International Nucleotide Sequence Database Collaboration (including GenBank, ENA, and DDBJ).

2. Serological tests

Serological assays are used in the laboratory confirmation of outbreaks, for serological surveillance and for export certification of pigs. SVD is often diagnosed solely on the evidence of serological tests. Because of the subclinical or mild nature of the disease, it is often first suspected following routine serology for disease surveillance or export certification. The virus neutralisation (VN) test, the double immunodiffusion test, the radial immunodiffusion test, the counter immunoelectrophoresis test and the ELISA have all been described for the detection of antibodies to SVDV (Brocchi *et al*., 1995; Donaldson *et al*., 1983; Golding *et al*., 1976). However, the VN test and the ELISA are the only techniques commonly used. The VN test is the accepted confirmatory test, but has the disadvantage that it takes 2–3 days to complete and requires tissue culture facilities and handling of live virus in appropriate biosafety and containment facilities as determined by biorisk analysis (see chapter 1.1.4). The ELISA is quicker and simpler. A small proportion of sera from animals with no previous exposure to SVDV may react positively in serological tests for antibody to SVDV. The 5B7 MAb competitive ELISA (MAC-ELISA) is a reliable technique for detecting SVD antibody (Brocchi *et al*., 1995; Heckert *et al*., 1998) and similar results have been obtained with other ELISAs (Chenard *et al*., 1998; Ko *et al*., 2005). Results from a small proportion, 0.2%–0.4%, of sera from normal pigs are borderline or positive by the MAC-ELISA and should be retested by the VN test. Up to approximately 50% of these sera will also be positive by the VN test (i.e. 0.1–0.2% of the original population). Animals that test positive by ELISA but negative by VN test can be regarded as uninfected. Repeat samples should be collected from animals positive in both tests and from cohorts. A constant or declining titre in the positive animal and the absence of antibody to SVDV in cohorts confirms the status of the positive animal as a 'singleton reactor'. The factors responsible for 'singleton reactors' are unknown. Serological cross-reactivity with SVDV might arise due to infection with another, as yet unidentified, picornavirus or may be due to other non-specific factors present in the serum. Identification of the isotype of antibody present in positive sera (Brocchi *et al*., 1995) can be helpful as sera from 'singleton' reactors usually contain exclusively IgM and do not convert to IgG (De Clercq, 1998). IgM/IgG isotype-specific ELISAs are also helpful in assessing the time of infection in the pig or on the infected premises. The presence of IgM, alone or together with IgG, is evidence of recent infection and indicative of virus shedding, while detection of IgG alone suggests an older exposure to infection (Brocchi *et al*., 1995).

2.1. Virus neutralisation

The quantitative VN microtest for antibody to SVDV is performed using IB-RS-2 cells (or suitable susceptible porcine cells) in flat-bottomed tissue-culture grade microtitre plates.

Virus is grown on IB-RS-2 cell monolayers and stored at –20°C after the addition of an equal volume of glycerol. SVDV has been found to be stable under these conditions for at least 1 year. The sera are inactivated at 56°C for 30 minutes before testing. A suitable medium is Eagle's complete medium/LYH with antibiotics.

The test is an equal volume test in 50 µl volumes:

i) Starting from a 1/4 dilution, sera are diluted in a twofold dilution series across the plate, two rows of wells per serum and a volume of 50 µl.

ii) Previously titrated virus is added; each 50 µl unit volume of virus suspension contains about 100 $TCID_{50}$ (50% tissue culture infective dose).

iii) Controls include at least a weak positive serum and a negative serum, a cell control, a medium control and a virus titration used to calculate the actual virus titre used in the test.

iv) Incubate at 37°C for 1 hour with the plates covered.

v) A cell suspension at 10^6 cells/ml is prepared in medium containing 10% bovine serum for cell growth. 50 µl of cell suspension is added to each well.

vi) Plates are sealed with pressure-sensitive tape and incubated at 37°C for 2–3 days. Alternatively, the plates may be covered with loosely fitting lids and incubated in an atmosphere of 5% carbon dioxide at 37°C for 2–3 days.

vii) Microscopic readings are feasible after 48–72 hours; the plates may be finally fixed and stained on the third day. Fixation is effected with 10% formalin/saline for 30 minutes; staining is done by immersion in 0.05% methylene blue in 10% formalin for 30 minutes. The plates are rinsed in tapwater. Positive readings are blue-stained cell sheets (where the virus has been neutralised and the cells remain intact), whilst empty wells (where virus has not been neutralised) are read as negative.

viii) Interpretation of the results

The test is considered to be valid when the amount of virus actually used per well is between $10^{1.5}$ and $10^{2.5}$ $TCID_{50}$, and the positive standard sera are within twofold of their expected titre. Titres are expressed as the final dilution of serum present in the serum/virus mixture where 50% of wells are protected. Laboratories should establish their own cut-off titres by reference to both results from a negative population and standard reagents available from the OIE Reference Laboratories, in particular the low-positive serum defining the lowest level of antibodies that Laboratories should consistently score positive.

2.2. Enzyme-linked immunosorbent assay

Commercial kits are available for antibody detection in pig specimens. In the ELISA developed by Brocchi *et al.* (1995), the SVD viral antigen is trapped to the solid phase using the MAb 5B7. The ability of test sera to inhibit the binding of peroxidase-conjugated MAb 5B7 to the trapped antigen is then evaluated. Finally, the amount of conjugated MAb bound is detected by the addition of substrate and chromogen.

i) ELISA plates are coated with 50 µl/well of MAb 5B7 at a saturating dilution in carbonate/bicarbonate buffer, pH 9.6, by overnight incubation at 4°C.

ii) The plates are washed three times with PBS containing 0.05% Tween 20, and 50 µl of SVD antigen (SVDV grown in IB-RS-2 cells, clarified, filtered and BEI-inactivated) at a predetermined optimal dilution, is added to each well. The optimal dilution of antigen is determined by checkerboard titrations of antigen and conjugated MAb that define the working dilution giving an absorbance on the upper part of the linear region of the antigen titration curve (between 1.5 and 2.0 optical density units). Plates are then incubated for 1 hour at 37°C.

iii) After three additional washes, 50 µl of diluted test sera (inactivation is irrelevant) and control sera are incubated with the trapped antigen for 1 hour at 37°C. Sera can be tested at a single dilution (1/7.5) or titrated. In the latter case, three-fold dilutions of sera are obtained directly in ELISA wells by adding 10 µl of serum to 65 µl of buffer (1/7.5 dilution) then transferring 25 µl to

sequential wells containing 50 µl of buffer, mixing, and finally discarding 25 µl. For spot-test, the screening dilution 1/7.5 is obtained by adding 7µl of each test serum (and control sera) to 45 µl of buffer previously distributed into wells.

iv) After incubation for 1 hour, 25 µl of an optimal dilution of peroxidase-conjugated MAb 5B7 (see step ii above) is added to each well and the plates are incubated at 37°C for a further 1 hour.

v) After a final series of washes, the colorimetric reaction is developed by distributing 50 µl per well of the substrate solution (for example 0.5 mg/ml orthophenylene-diamine in phosphate/citrate buffer, pH 5, containing 0.02% H_2O_2).

vi) The reaction is stopped after 10 minutes by adding 50 µl of 2N H_2SO_4. The absorbance is read at the appropriate wavelength using a microplate reader.

Antigen, sera and conjugate are diluted in PBS, pH 7.4, containing 0.05% Tween 20 and 1% yeast extract; the dilution buffer for sera contains, in addition, 1.0% mouse serum (or alternatively another source of murine immunoglobulins) to prevent nonspecific binding of pig serum to MAb 5B7 either coated to the plate or conjugated to peroxidase.

vii) *Controls:* Four wells on each plate containing all reactants except test serum confirm the maximum absorbance reading for the antigen; negative pig serum; a low positive standard pig serum; optionally, a strong positive pig serum at four dilutions, previously calibrated in order to give ≥50% inhibition (see step viii below) at the highest dilution.

viii) Interpretation of the results: Reactions are expressed as the percentage inhibition by each test serum of the MAb reaction with the SVD antigen. Sera are considered to be positive when producing an inhibition ≥80% at the 1/7.5 dilution; negative when producing an inhibition <70% at the 1/7.5 dilution; doubtful when producing an inhibition ≥70% and <80% at the 1/7.5 dilution. The second dilution (1/22.5) provides an indication of the level of antibodies: strongly positive sera show >80% inhibition at both 1/7.5 and 1/22.5 dilutions, while sera registering >80% inhibition at the 1/7.5 dilution but <50% inhibition at the 1/22.5 dilution are considered to be low positive or borderline. All positive, borderline and doubtful sera should be confirmed using the VN test.

STANDARD REFERENCE SERA FOR SVD SEROLOGY

The OIE Reference Laboratory, Pirbright, UK maintains a panel of reference sera that have been extensively validated by the National SVD Reference Laboratories of the Member States of the European Union. This panel includes the low-positive serum defining the lowest level of antibodies that should consistently provide a positive result by ELISA and Virus Neutralisation (RS01-04-94 or equivalent). Positive sera equivalent to these reference standards and Mab 5B7 are available at the OIE Reference Laboratory, Brescia, Italy.

C. REQUIREMENTS FOR VACCINES

No commercial SVD vaccines are currently available.

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NB: There are OIE Reference Laboratories for Swine vesicular disease
(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 and reagents for swine vesicular disease

NB: FIRST ADOPTED IN 1989; MOST RECENT UPDATES ADOPTED IN 2018.