

CHAPTER 2.1.19.

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 from foot and mouth disease (FMD), vesicular exanthema of swine (VES), or swine vesicular disease (SVD) when horses are not involved. Sheep, goats and many other wild species can be infected. Humans are also susceptible. The disease is limited to the Americas; however, it was previously described in France and in South Africa.

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. There is also some evidence that it could be a plant virus and that animals are the end of the epidemiological chain. The pathogenesis of the disease is unclear, and it has been observed that the humoral-specific antibodies do not always prevent infection with VS serogroup viruses.

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 or VES when only pigs are affected.

Identification of the agent: *Virus can be readily isolated by the inoculation of several tissue culture systems, unweaned mice or embryonated chicken eggs. Viral RNA can be detected from epithelial tissue and vesicular fluid by conventional and real-time reverse transcriptase polymerase chain reaction (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 (CF) test is also a good alternative. The virus neutralisation (VN) test may be used, but it is elaborate and time-consuming.*

Serological tests: *Convalescent animals develop serotype-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 VN. Other described tests are CF, agar gel immunodiffusion and counter immunoelectrophoresis.*

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 limited to the Americas; however, it was described in France (1915 and 1917) and in South Africa (1886 and 1897) (Hanson, 1952).

Influenza-like signs, normally without vesicles, have been observed in humans who are in contact with animals with VS or who handle infective virus. All manipulations involving virus, including infective materials from animals, should be undertaken with using proper biosafety procedures.

There are two distinct immunological classes of vesicular stomatitis virus (VSV) that have been recognised: New Jersey (NJ) and Indiana (IND). Both viruses are members of the genus *Vesiculovirus*, family Rhabdoviridae and have been extensively studied at the molecular level. Several other closely related rhabdoviruses have been isolated from sick animals over the past decades. There are three subtypes of the IND serogroup based on serological relationships: IND-1 IND-2 and IND-3; they are also known as classical IND virus (VSIV), coccal virus (COCV), and alagoas virus (VSAV), respectively (Federer *et al.*, 1967). Strains of the serotype NJ and subtype IND-1 are endemic in livestock in areas of southern Mexico, Central America, Venezuela, Colombia, Ecuador and Peru, with VSV NJ causing the vast majority (>80%) of the clinical cases. Sporadic activity of NJ and IND-1 VSV has been reported in northern Mexico and western United States. IND-2 has only been isolated in Argentina and Brazil and only from horses (Salto-Argentina/63, Maipú-Argentina/86, Rancharia-Brazil/66, Riberao-Brazil/79) (Alonso *et al.*, 1991; Alonso Fernandez & Sondahl, 1985). Cattle living together with the affected horses did not develop antibodies against VSV (Alonso *et al.*, 1991). The IND-3 subtype, (Alagoas-Brazil/64), has been identified, sporadically only in Brazil and only in horses until 1977. However, in 1977 the IND-3 serotype (Espinosa-Brazil/77 strain) was first isolated from cattle in Brazil; it has been observed that this serotype affects cattle to a lesser degree than horses (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 NJ and IND serotypes in horses, and subsequently in cattle and pigs; this same predilection has been observed in other VS outbreaks.

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 VS NJ has been demonstrated to occur from black flies (*Simulium vittatum*) to domestic swine and cattle (Mead *et al.*, 2004; 2009). There are also hypotheses that the VS virus is a plant virus present in pasture (Mason, 1978) and that animals are the end of the epidemiological chain and, in special circumstances, the virus could undergo an adaptation process to infect animals, followed by direct transmission between susceptible animals. During the 1982 epizootic in western USA, there were a number of cases where there was direct transmission from animal to animal (Sellers & Maarouf, 1990). While VS is not diagnosed in livestock every year in the USA, it is considered to be endemic in feral pigs on Ossabaw Island, Georgia (Boring & Smith, 1962).

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 the NJ virus 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). Both NJ and IND-1 serotypes in the 1995, 1997 and 1998 US outbreaks primarily caused clinical disease in horses. Although some clinical signs were observed in cattle, the primary finding in cattle was seroconversion.

B. DIAGNOSTIC TECHNIQUES

VS cannot be reliably clinically differentiated from the other vesicular diseases, such as foot and mouth disease (FMD), vesicular exanthema of swine (VES), and swine vesicular disease (SVD) when horses are not involved. 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, VES and SVD, in order to facilitate the differential diagnosis of these vesicular diseases. Note: VS serogroup viruses can be human pathogens and appropriate precautions should be taken when working with potentially infected tissues or virus (see Chapter 1.1.3 *Biosafety and biosecurity in the veterinary microbiology laboratory and animal facilities*).

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 that animals should be sedated before samples are collected to avoid injury to helpers and for reasons of animal welfare. Samples from all species should be placed in containers of Tris-buffered tryptose broth with phenol red, pH 7.6. If complement fixation (CF) is to be carried out for antigen detection, samples from all species can be collected in glycerol/phosphate buffer, pH 7.2–7.6. (Note: glycerol is toxic to virus and decreases the sensitivity of virus isolation; it is therefore only recommended for collection of samples for CF test.) Samples should be kept refrigerated and if they can arrive at the laboratory within 48 hours after collection, they should be sent refrigerated. If samples are sent frozen with dry ice, precautions should be taken to protect the sample from contact with any CO₂. There are special packaging requirements for shipping samples with dry ice (see Chapter 1.1.1 *Collection and shipment of diagnostic specimens*, for further information on shipping of diagnostic samples).

When epithelial tissue is not available from cattle, samples of oesophageal–pharyngeal (OP) fluid can be collected by means of a probang (sputum) cup. In pigs, throat swabs can be taken for submission to a laboratory for virus isolation. This material should be sent to the laboratory refrigerated in Tris-buffered tryptose broth. If the samples will be in shipment for more than 48 hours after collection, they should be sent frozen with dry ice as described previously. Probang samples for isolation of virus should not be treated with solvents such as chloroform. Virus can be isolated from oral and nasal specimens up to 7 days post-infection.

When it is not possible to collect samples for identification of the agent, serum samples from recovered animals can be used for detecting and quantifying specific antibodies. Paired sera from the same animals, collected 1–2 weeks apart, are preferred for checking the change in antibody titre.

Specific reagents for VS diagnosis are not commercially available and each laboratory must produce its own or obtain them from a Reference Laboratory. The two OIE Reference Laboratories for vesicular stomatitis (see Table given in Part 4 of this *Terrestrial Manual*), and the Institute for Animal Health¹, produce and distribute diagnostic reagents on request.

1. Identification of the agent

For identification of VS serogroup viruses and the differential diagnosis of vesicular diseases, clarified suspensions of field samples suspected to contain virus should be submitted for immunological testing. For virus isolation, the same 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 serogroup 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.

Virus replicates and can be isolated in 8–10-day-old chicken embryos by inoculation into the allantoic sac, in 2- to 7-day-old unweaned mice by inoculation using any route, or in 3-week-old mice by intracerebral inoculation. In all three cases, virus causes death in between 2 and 5 days after inoculation.

The most susceptible route for horses and cattle is intradermal administration. Pigs are inoculated in the coronary band or on the snout. Vesicular lesions may be observed in the epithelial tissues of the mouth, teats and feet, 2–4 days after inoculation. The presence of secondary vesicles after inoculation of cattle and horses depends mainly on the VS virus isolate used. The snout is normally affected in pigs.

If a CPE develops in the cultures, the suspension fluids can be used for identification of the agent by different immunological tests and the cell culture can be stained with VS-specific fluorescent antibody conjugate and viral antigen detected by enzyme-linked immunosorbent assay (ELISA), complement fixation (CF) test or polymerase chain reaction (PCR). Similar tests can be performed on homogenate suspensions of the dissected musculo-skeletal tissues of dead mice and chicken embryos and with suspensions of epithelial samples. The brain tissue from mice is an excellent source of virus.

Due to the different morphological characteristics of the rhabdovirus (VS serogroup viruses), picornavirus (FMD virus and SVD virus), 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.

The preferred immunological methods for the identification of the viral antigens in the laboratory are the ELISA (Alonso *et al.*, 1991; Ferris & Donaldson, 1988), the CF test (Alonso *et al.*, 1991; Jenny *et al.*, 1958) and fluorescent antibody staining. The virus neutralisation (VN) test, with known positive antisera against the VS virus NJ and IND serotypes, may be used in tissue cultures, unweaned mice or embryonated eggs, but it is more time-consuming.

a) Virus isolation

- i) Inoculate cell culture in Leighton tubes and 25 cm² flasks with the clarified suspension of tissues or vesicular fluid.
- ii) Incubate inoculated cell cultures at 37°C for 1 hour.
- iii) 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).

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- iv) Incubate Leighton tube cell cultures at 33–35°C and observe for CPE.
- v) After 18–24 hours of incubation, the cover-slip from one Leighton tube culture per specimen inoculated is stained with New Jersey and Indiana VS virus-specific fluorescent antibody (FA) conjugate.
- vi) Remaining Leighton tube cultures and 25 cm² flask cultures are incubated at 35–37°C for 6 more days and observed daily for CPE.
- vii) At 7 days post-inoculation, the remaining Leighton tube cover-slips are stained with FA conjugate.
- viii) If CPE is observed and the FA staining is negative, a second passage as is made, as described above, using the cells from the 25 cm² flask. Note: First passage cultures with significant CPE may yield false-negative immunofluorescence results. Serial tenfold dilutions may be prepared and inoculated to provide distinct plaques of fluorescing cells.
- ix) *Interpretation of the results:* If no fluorescence is observed and no CPE evident in the flask culture, the sample is negative for virus isolation. If specific fluorescence is observed, the sample is positive for virus isolation.
- x) Alternatively cell culture in flasks can be inoculated with field samples, incubated at 35–37°C for 48 hours and observed daily for CPE. If no CPE is observed after 48 hours, the flask cultures are frozen and thawed and a sample of the supernatant is inoculated into fresh cell culture. Up to three passages are made, of 48 hours each. To detect the presence of VSV antigen, clarified supernatants of each passage are tested by ELSA or CF test.

b) Enzyme-linked immunosorbent assay

The indirect sandwich ELISA (IS-ELISA) (Alonso *et al.*, 1991; Ferris & Donaldson, 1988) is currently the diagnostic method of choice for identification of viral serotypes of VS and other vesicular diseases. Specifically, the ELISA procedure with a set of polyvalent rabbit/guinea-pig antisera, prepared against virions of the representative strains of the three subtypes of the IND serotype, identifies all strains of the VS virus IND serotype (Alonso *et al.*, 1991). For detection of VS virus NJ strains, a monovalent set of rabbit/guinea-pig antisera is suitable (Alonso *et al.*, 1991; Ferris & Donaldson, 1988).

• 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 Allende *et al.*, 1992), and optimally diluted in carbonate/bicarbonate buffer, pH 9.6. Subsequently, the plates are washed once with phosphate buffered saline (PBS) and blocked for 1 hour at room temperature with 1% ovalbumin in PBS. The plates are used immediately or are washed three times and stored at –20°C for future use.
- ii) *Test samples:* Antigen suspensions of test samples (10–20% epithelial tissue suspension, musculo-skeletal tissue of chicken embryo or mice in PBS 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 and polyvalent guinea-pig antisera to VS virus NJ and IND serotypes, respectively, that are homologous to coated rabbit serum and that have been diluted appropriately in PBS containing 0.05% Tween 20, 1% ovalbumin, 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:* H₂O₂-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 five times between each stage with PBS containing 0.05% Tween 20. Controls for the reagents used are included.

- vi) *Interpretation of the results:* An antiserum giving an absorbance more than 20% greater than the other antisera, negative serum and controls is considered to be positive for the corresponding virus subtype.

c) Complement fixation test

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

- **Test procedure**

- i) *Antisera*: Guinea-pig monovalent anti-NJ VS virus and polyvalent anti-IND VS virus, diluted in veronal buffer (VB) at a dilution containing 2.5 CFU₅₀ (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 CHU₅₀ (50% complement haemolytic units) are added to the serum and antigen. (An alternative is to use 7.5, 10 and 20 CHU₅₀ with the goal of reaching 4 CHU₅₀ in the test.) The mixture of antisera, test samples and complement is incubated at 37°C for 30 minutes.
- iv) *Haemolytic system*: A suspension of sheep red blood cells (SRBC) in VB, sensitised with 10 HU₅₀ (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 eight times greater than those indicated for the CF 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 are 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 on the ELISA or CF test should be inoculated into cell culture or unweaned mice. If there is no evidence of viral infection after three passages, the specimen is considered to be negative for virus.

d) Nucleic acid recognition methods

The PCR can be used to amplify small genomic areas of the VS virus (Hofner *et al.*, 1994; 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. In general, PCR techniques have not been routinely used for screening diagnostic cases for viruses causing VS.

2. Serological tests

For the identification and quantification of specific antibodies in serum, the ELISA and the VN test are preferable. The CF test 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 CF and for extended periods for the VN and ELISA (Katz *et al.*, 1997).

a) Enzyme-linked immunosorbent assay (a prescribed test for international trade)

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 VN (Allende *et al.*, 1992).

- **Test procedure**

- i) *Solid phase*: As described above in Section B.1.a for the IS-ELISA.
- ii) *Liquid phase*: Duplicate, twofold dilution series of each test serum, starting at 1/4, are prepared in U-bottomed microtitre plates. An equal volume of VS virus NJ or IND glycoprotein, in a dilution providing 70% reaction, 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 reagents and methods are used as those indicated for the IS-ELISA.

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

- **Competitive enzyme-linked immunosorbent assay (a prescribed test for international trade)**

A competitive 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 NJ and IND-1 recombinant antigens as described by Katz *et al.* (1995).

- **Test procedure**

- Solid phase:* Antigens are diluted in carbonate/bicarbonate buffer, pH 9.6, and 75 μ l is added to each well of a 96-well ELISA plate. The plates are incubated overnight at 4°C; coated plates can be frozen at –70°C for up to 30 days. The plates are thawed, antigen is decanted, and 100 μ l 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.
- Liquid phase:* 50 μ l 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 serotype should be included on each ELISA plate. The plates are incubated at 37°C for 30 minutes. Without washing, 50 μ l of polyclonal ascites fluid is added to each well and plates are incubated at 37°C for 30 minutes.
- Detector:* The plates are washed three times, and 50 μ l 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 μ l 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 μ l 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 .
- Interpretation of the results:* A sample is positive if the absorbance is $\leq 50\%$ of the absorbance of the diluent control. Note that horses naturally infected with New Jersey virus have been known to test positive by this assay for at least 5 years following infection.

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

The VN test is carried out in tissue culture microtitre plates with flat-bottomed wells using inactivated serum as test sample, 1000 TCID₅₀ (50% tissue culture infective dose) of VS NJ or IND virus, and Vero M cells, or preformed monolayer (Allende *et al.*, 1992) or a suspension IB-RS-2 cells to test for the presence of unneutralised virus.

- **Test procedure**

- Virus:* VS NJ or IND virus is grown in Vero cell monolayers and stored in liquid nitrogen or frozen at –70°C.
- Test samples:* Sera are inactivated at 56°C for 30 minutes before testing. Positive and negative control standard sera are included in the test.
- Virus neutralisation:* Sera are diluted in a twofold or four-fold dilution series across the plates, starting from 1/4 dilution. Two rows of wells are used per serum. The same volume of NJ or IND VS virus suspension containing about 1000 TCID₅₀/25 μ l is added and incubated at 37°C for 60 minutes to allow neutralisation to take place. Subsequently, 50 μ l of the mixtures is deposited on preformed cell monolayers in microtitre plates or 150 μ l of 300,000/ml IB-RS-2 or Vero cell suspension is added to each well with the serum/virus mixtures. The plates are covered with loosely fitting lids and incubated for 48–72 hours at 37°C in an atmosphere of 5% CO₂ or sealed with pressure-sensitive tape and incubated in a normal atmosphere. (It has been determined that a virus titre of 1000 TCID₅₀ will decrease the nonspecific reactions and maintain a high test sensitivity.)
- Interpretation of the results:* Wells without CPE are considered to be positive. End-point titres of test serum titres are determined by the Spearman–Kärber method when the virus titres are between 750 and 1330 TCID₅₀ and when titres of positive and negative standard sera are within twofold of their mean values as estimated from previous titration. The 100% neutralisation titres of each serum are expressed at $\log 10$. Sera with values of 1/32 or greater are considered to be positive for antibodies against VSV. Note that horses naturally infected with New Jersey virus have been known to test positive by this test method for at least 5 years following infection. In an alternative protocol, the end-point titre of the test serum is determined when the virus doses are between $10^{2\pm 0.5}$ /100 μ l and when titres of positive and negative standard sera are within twofold of their mean values as estimated from

the previous titration. The 50% neutralisation titre of each serum is expressed as log₁₀. Sera with values of 1.3 (1/20) or greater are considered to be positive for VS antibodies (Allende *et al.*, 1992).

c) Complement fixation (a prescribed test for international trade)

A detailed description of this test is given in Section B.1.b. This is modified as follows. The CF test may be used for quantification of early antibodies, mostly IgM. For this purpose, twofold serum dilutions are mixed with 2 CFU₅₀ of known antigen and with 5% normal bovine or calf sera included in 4 CHU₅₀ 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 considered to be positive. This CF has low sensitivity and is frequently affected by anticomplementary or nonspecific factors.

C. REQUIREMENTS FOR VACCINES

1. Background

a) Rationale and intended use of the product

Vesicular stomatitis virus 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. Where vaccination is 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 the Indiana and New Jersey serotypes are manufactured in Colombia and Venezuela (2002 OIE vaccine survey).

Guidelines for the production of veterinary vaccines are given in Chapter 1.1.6 *Principles of veterinary vaccine production*. The guidelines given here and in chapter 1.1.6 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

a) Characteristics of the seed

i) *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.

ii) *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 through the use of fetal bovine serum 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.

b) Method of manufacture

i) *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).

ii) *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, fetal bovine sera) and growth of virus should be free of adventitious agents, with special attention paid to the presence of bovine viral diarrhoea virus.

iii) *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 on a 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.

iv) *Final product batch tests*

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

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 1.1.7 *Tests for sterility and freedom from contamination of biological materials*).

Safety

Final container samples of completed product from inactivated vaccines should be tested.

Batch potency

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

c) Requirements for authorisation

i) *Safety requirements*

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.

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.

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. Modified live virus 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 modified live virus vaccines. Instructions for disposal should be included within the vaccine packaging information and based on current environmental regulations in the country of use.

ii) *Efficacy requirements*

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, New Jersey and Indiana-1), 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.

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.

iii) *Stability*

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.5.b) over the proposed period of viability.

3. Vaccines based on biotechnology

a) **Vaccines available and their advantages**

None

b) **Special requirements for biotechnological vaccines, if any**

None

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NB: There are OIE Reference Laboratories for Vesicular stomatitis
(see Table in Part 4 of this *Terrestrial Manual* or consult the OIE Web site for the most up-to-date list:
<http://www.oie.int/en/our-scientific-expertise/reference-laboratories/list-of-laboratories/>).

Please contact the OIE Reference Laboratories for any further information on
diagnostic tests, reagents and vaccines for vesicular stomatitis