

[^{X1}ANNEX I

UNGULATES

Editorial Information

- X1** Substituted by [Corrigendum to Commission Regulation \(EU\) No 206/2010 of 12 March 2010 laying down lists of third countries, territories or parts thereof authorised for the introduction into the European Union of certain animals and fresh meat and the veterinary certification requirements \(Official Journal of the European Union L 73 of 20 March 2010\)](#).

PART 6

Protocols for the standardisation of materials and testing procedures**(referred to in Article 5)**

Tuberculosis (TBL)

The single intradermal tuberculin test using bovine tuberculin shall be carried out [^{F1}in a manner equivalent to the standards in] Annex B to Directive 64/432/EEC. In the case of Suidae animals, the single intradermal tuberculin test using avian tuberculin shall be carried out [^{F1}in a manner equivalent to the standards in] Annex B to 64/432/EEC, except that the site of injection shall be the loose skin at the base of the ear.

Textual Amendments

- F1** Words in [Annex 1 Pt. 6](#) substituted (31.12.2020) by [The Import of, and Trade in, Animals and Animal Products \(Miscellaneous Amendments\) \(EU Exit\) Regulations 2020 \(S.I. 2020/1462\)](#), regs. 1(3), **56(28)(d)** (with regs. 69-71)

[^{F2}Brucellosis (*Brucella abortus*) (BRL)

The serum agglutination test, complement fixation test, buffered brucella antigen test, enzyme-linked immunosorbent assays (ELISA) and fluorescence polarisation assay (FPA) shall be carried out [^{F1}in a manner equivalent to the standards in] Annex C to Directive 64/432/EEC.] Brucellosis (*Brucella melitensis*) (BRL)

Tests shall be carried out [^{F1}in a manner equivalent to the standards in] Annex C to Directive 91/68/EEC.

Enzootic Bovine Leukosis (EBL)

The agar gel immuno-diffusion test and the enzyme linked immuno-absorbent assay test (ELISA) shall be carried out [^{F1}in a manner equivalent to the standards in] paragraphs A and C of Chapter II of Annex D to Directive 64/432/EEC.

Bluetongue (BTG)

- A. The blocking or competitive ELISA test shall be carried out according to the following protocol:

The competitive ELISA using monoclonal antibody 3-17-A3 is capable of identifying antibodies to all known serotypes of bluetongue virus (BTV).

Changes to legislation: There are currently no known outstanding effects for the Commission Regulation (EU) No 206/2010, PART 6. (See end of Document for details)

E	C+	C+										
F	C+	C+										
G	Cm	Cm										40
H	Cm	Cm										40

APPENDIX 2:

Serum titration format (10 sera/plate)

	Controls		Test Sera									
	1	2	3	4	5	6	7	8	9	10	11	12
A	Cc	C-	1:5									1:5
B	Cc	C-	1:10									1:10
C	C++	C++	1:20									1:20
D	C++	C++	1:40									1:40
E	C+	C+	1:80									1:80
F	C+	C+	1:160									1:160
G	Cm	Cm	1:320									1:320
H	Cm	Cm	1:640									1:640

Test protocol:

Conjugate control : Wells 1A and 1B are a blank control consisting of BTV antigen and conjugate. This may be used to blank the ELISA reader.

Mab control (Cm) : Columns 1 and 2, rows G and H are the monoclonal antibody control and contain BTV antigen, monoclonal antibody and conjugate. These wells represent maximum colour. The mean of the optical density readings from this control represents the 0 % inhibition value.

Positive control (C++, C+) : Columns 1 and 2, rows C-D-E-F. These wells contain BTV antigen, BTV strong and weak positive antiserum respectively, Mab and conjugate.

Negative control (C-) : Wells 2A and 2B are the negative controls, which contain BTV antigen, BTV negative antiserum, Mab and conjugate.

Test sera : For large-scale serological surveys and rapid screening, sera may be tested at a single dilution of 1:5 (Appendix 1). Alternatively, 10 sera may be tested over a dilution range from 1:5 to 1:640 (Appendix 2). This will give some indication of the titre of antibody in the test sera.

Procedure:

1. Dilute BTV antigen to pre-titrated concentration in PBS, sonicate briefly to disperse aggregated virus (if sonicator is not available, pipette vigorously) and add 50 µl to all wells of the ELISA plate. Tap sides of plate to disperse antigen.
2. Incubate at 37 °C for 60 minutes on an orbital shaker. Wash plates three times by flooding and emptying the wells with non-sterile PBS and blot dry on absorbent paper.
3. Control wells: Add 100 µl of blocking buffer to Cc wells. Add 50 µl of positive and negative control sera, at a dilution of 1:5 (10 µl sera + 40 µl blocking buffer), to respective wells C-, C+ and C++. Add 50µl blocking buffer to Mab control wells.

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Spot titration method: Add a 1:5 dilution of each test serum in blocking buffer to duplicate wells of columns 3 to 12 (10 µl sera + 40 µl blocking buffer),

or

Serum titration method: Prepare a two-fold dilution series of each test sample (1:5 to 1:640) in blocking buffer across eight wells of single columns 3 to 12.

4. Immediately after the addition of the test sera, dilute Mab 1:100 in blocking buffer and add 50 µl to all wells of the plate except for the blank control.
5. Incubate at 37 °C for 60 minutes on an orbital shaker. Wash three times with PBS and blot dry.
6. Dilute rabbit anti-mouse concentrate to 1/ 5 000 in blocking buffer and add 50 µl to all wells of the plate.
7. Incubate at 37 °C for 60 minutes on an orbital shaker. Wash three times with PBS and blot dry.
8. Thaw the O-Phenylenediamine dihydrochloride (OPD) and immediately before use add 5 µl of 30 % hydrogen peroxide to each 10 ml of OPD. Add 50 µl to all wells of the plate. Allow colour to develop for approximately 10 minutes and stop the reaction with 1 Molar sulphuric acid (50 µl per well). Colour should develop in the Mab control wells and in those wells containing sera with no antibody to BTv.
9. Examine and record the plates either visually or using a spectrophotometric reader.

Analysis of results:

Using the software package print out the optical density (OD) values, and the percentage inhibition (PI) for test and control sera based on the mean value recorded in the antigen control wells. The data expressed as OD and PI values are used to determine whether the test has performed within acceptable limits. The upper control limits (UCL) and lower control limits (LCL) for the Mab control (antigen plus Mab in the absence of test sera) are between OD values 0,4 and 1.4. Any plate that fails to conform to the above criteria must be rejected.

If a computer software package is not available print out the OD values using the ELISA printer. Calculate the mean OD value for the antigen control wells, which is equivalent to the 100 % value. Determine the 50 % OD value and manually calculate the positivity and negativity of each sample.

Percentage inhibition (PI) value = $100 - (\text{OD of each test control} / \text{Mean OD of } C_m) \times 100$.

The duplicate negative control serum wells and the duplicate blank wells must record PI values between + 25 % and – 25 %, and between + 95 % and + 105 %, respectively. Failure to be within these limits does not invalidate the plate but does suggest that background colour is developing. The strong and weak positive control sera must record PI values between + 81 % and + 100 %, and between + 51 % and + 80 %, respectively.

The diagnostic threshold for test sera is 50 % (PI 50 % or OD 50 %). Samples recording PI values >50 % are recorded negative. Samples that record PI values above and below the threshold for the duplicate wells are considered doubtful; such samples may be re-tested in the spot test and/or titration. Positive samples may also be titrated to provide an indication of the degree of positivity.

Visual reading: Positive and negative samples are easily discernible by eye; weakly positive or strong negative samples may be more difficult to interpret by eye.

Preparation of BTV ELISA antigen:

1. Wash 40-60 roux of confluent BHK-21 cells three times with serum-free Eagle's medium and infect with bluetongue virus serotype 1 in serum-free Eagle's medium.
2. Incubate at 37 °C and examine daily for cytopathic effect (CPE).
3. When CPE are complete in 90 % to 100 % of the cell sheet of each roux, harvest the virus by shaking any still-attached cells from the glass.
4. Centrifuge at 2 000 to 3 000 rpm to pellet the cells.
5. Discard the supernatant and re-suspend the cells in approximately 30 ml of PBS containing 1 % ' Sarkosyl ' and 2 ml phenylmethylsulphonyl fluoride (lysis buffer). This may cause the cells to form a gel and more lysis buffer may be added to reduce this effect. (NB: phenylmethylsulphonyl fluoride is harmful - handle with extreme caution.)
6. Disrupt the cells for 60 seconds using an ultrasonic probe at an amplitude of 30 microns.
7. Centrifuge at 10 000 rpm for 10 minutes.
8. Store the supernatant at + 4 °C and re-suspend the remaining cell pellet in 10 to 20 ml of lysis buffer.
9. Sonicate and clarify, storing the supernatant at each stage, a total of three times.
10. Pool the supernatants and centrifuge at 24 000 rpm (100,000 g) for 120 minutes at + 4 °C over a 5 ml cushion of 40 % sucrose (w/v in PBS) using 30 ml Beckmann centrifuge tubes and an SW 28 rotor.
11. Discard the supernatant, drain the tubes thoroughly and re-suspend the pellet in PBS by sonication. Store the antigen in aliquots at – 20 °C.

Titration of BTV ELISA antigen:

Bluetongue ELISA antigen is titrated by the indirect ELISA. Twofold dilutions of antigen are titrated against a constant dilution (1/100) monoclonal antibody 3-17-A3. The protocol is as follows:

1. Titrate a 1:20 dilution of BTV antigen in PBS across the microtitre plate in a twofold dilution series (50 µl/well) using a multichannel pipette.
2. Incubate for one hour at 37 °C on an orbital shaker.
3. Wash plates three times with PBS.
4. Add 50 µl of monoclonal antibody 3-17-A3 (diluted 1/100) to each well of the microtitre plate.
5. Incubate for one hour at 37 °C on an orbital shaker.
6. Wash plates three times with PBS.
7. Add 50 µl of rabbit anti-mouse globulin conjugated to horseradish peroxidase, diluted to a pre-titrated optimal concentration, to each well of the microtitre plate.
8. Incubate for one hour at 37 °C on an orbital shaker.

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9. Add substrate and chromogen as described previously. Stop the reaction after 10 minutes by the addition of 1 Molar sulphuric acid (50 µl/well).

In the competitive assay, the monoclonal antibody must be in excess, therefore a dilution of antigen is chosen which falls on the titration curve (not on the plateau region) which gives approximately 0,8 OD after 10 minutes.

- B. The agar gel immuno-diffusion test shall be carried out according to the following protocol:

Antigen:

Precipitating antigen is prepared in any cell culture system that supports the rapid multiplication of a reference strain of bluetongue virus. BHK or Vero cells are recommended. Antigen is present in the supernatant fluid at the end of virus growth but requires 50 to 100-fold concentration to be effective. This may be achieved by any standard protein concentration procedure; virus in the antigen may be inactivated by the addition of 0,3 % (v/v) beta-propiolactone.

Known positive control serum:

Using the international reference serum and antigen a national standard serum is produced, standardised for optimal proportion against the international reference serum, freeze-dried and used as the known control serum in each test.

Test serum

Procedure : 1 % agarose prepared in borate or sodium barbitol buffer, pH 8,5 to 9,0, is poured into a petri dish to a minimum depth of 3,0 mm. A test pattern of seven moisture-free wells, each 5,0 mm in diameter, is cut in the agar. The pattern consists of one centre well and six wells arranged round it in a circle of radius 3 cm. The central well is filled with the standard antigen. Peripheral wells 2, 4 and 6 are filled with known positive serum, wells 1, 3 and 5 are filled with test sera. The system is incubated for up to 72 hours at room temperature in a closed humid chamber.

Interpretation : A test serum is positive if it forms a specific precipitin line with the antigen and forms a complete line of identity with the control serum. A test serum is negative if it does not form a specific line with the antigen and it does not bend the line of the control serum. Petri dishes must be examined against a dark background and using indirect illumination.

Epizootic haemorrhagic disease (EHD)

The agar gel immuno-diffusion test shall be carried out according to the following protocol:

Antigen:

Precipitating antigen is prepared in any cell culture system that supports the rapid multiplication of the appropriate serotype(s) of epizootic haemorrhagic disease virus. BHK or Vero cells are recommended. Antigen is present in the supernatant fluid at the end of virus growth but requires 50 to 100-fold concentration to be effective. This may be achieved by any standard protein concentration procedure; virus in the antigen may be inactivated by the addition of 0,3 % (v/v) beta-propiolactone.

Known positive control serum:

Using the international reference serum and antigen a national standard serum is produced, standardised for optimal proportion against the international reference serum, freeze-dried and used as the known control serum in each test.

Test serum

Procedure : 1 % agarose prepared in borate or sodium barbitol buffer, pH 8,5 to 9,0, is poured into a petri dish to a minimum depth of 3,0 mm. A test pattern of seven moisture-free wells, each 5,0 mm in diameter, is cut in the agar. The pattern consists of one centre well and six wells arranged round it in a circle of radius 3 cm. The central well is filled with the standard antigen. Peripheral wells 2, 4 and 6 are filled with known positive serum, wells 1, 3 and 5 are filled with test sera. The system is incubated for up to 72 hours at room temperature in a closed humid chamber.

Interpretation : A test serum is positive if it forms a specific precipitin line with the antigen and forms a complete line of identity with the control serum. A test serum is negative if it does not form a specific line with the antigen and it does not bend the line of the control serum. Petri dishes must be examined against a dark background and using indirect illumination.

Infectious bovine rhinotracheitis (IBR) / infectious pustular vulvo-vaginitis (IPV)

A. The serum neutralisation test shall be carried out according to the following protocol:

Serum : All sera are heat-inactivated at 56 °C for 30 minutes before use.

Procedure : The constant virus-varying serum neutralisation test on microtitre plates employs MDBK or other susceptible cells. The Colorado, Oxford or any other reference strain of the virus is used at 100 TCID₅₀ per 0,025 ml; inactivated undiluted serum samples are mixed with an equal volume (0,025 ml) of virus suspension. The virus/serum mixtures are incubated for 24 hours at 37 °C in the microtitre plates before the MDBK cells are added. Cells are used at a concentration which forms a complete monolayer after 24 hours.

Controls : (i) virus infectivity assay, (ii) serum toxicity controls, (iii) uninoculated cell culture controls, (iv) reference antisera.

Interpretation : The results of the neutralisation test and the titre of the virus used in the test are recorded after three to six days incubation at 37 °C. Serum titres are considered negative if there is no neutralisation at a dilution of 1/2 (undiluted serum).

B. Any other test recognised in the framework of Decision 2004/558/EC ⁽¹⁾ .
Foot-and-mouth disease (FMD)

A. Collecting oesophageal/pharyngeal samples and testing shall be carried out according to the following protocol:

Reagents : Prior to sampling, transport medium is prepared. Two ml volumes are dispensed in as many containers as there are animals to be sampled. The containers used must withstand freezing over solid CO₂ or liquid nitrogen. Samples are obtained by the use of a specially-designed sputum collector or 'probang'. To obtain a sample the probang cup is passed through the mouth, over the dorsum of the tongue and down into the upper part of the oesophagus. Attempts are made to scrape the surface epithelium of the upper oesophagus and pharynx by movements directed laterally and dorsally. The probang is then withdrawn, if possible after the animal has swallowed. The cup must be full and contain a mixture of mucus, saliva, oesophageal fluid and cellular debris. Care must be taken to ensure that each specimen contains some visible cellular material. Very rough handling which causes bleeding must be avoided. Samples from some animals may be heavily contaminated with ruminal contents. Such samples must be

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- discarded and the mouth of the animal flushed with water, or preferably physiological saline, before repeat sampling.
- Treatment of samples:** : Each sample collected in the probang cup is examined for quality and 2 ml added to an equal volume of transport medium in a container which can withstand freezing. The containers are tightly closed, sealed, disinfected and labelled. The samples are kept cool (+ 4 °C) and examined within three to four hours or placed over dry ice (- 69 °C) or liquid nitrogen and kept frozen until examined. Between animals the probang is disinfected and washed in three changes of clean water.
- Testing for FMD virus:** : Samples are inoculated into cultures of primary bovine thyroid cell cultures using at least three tubes per sample. Other susceptible cells such as primary bovine or porcine kidney cells can be used but it must be kept in mind that for some strains of FMD virus they are less sensitive. The tubes are incubated at 37 °C on a roller apparatus and examined daily for 48 hours for the presence of a cytopathic effect (CPE). If negative, cultures are blind passaged onto new cultures and re-examined for 48 hours. The specificity of any CPE must be confirmed.
- Recommended transport media:**
1. 0,08M phosphate buffer pH 7,2 containing 0,01 % bovine serum albumin, 0,002 % phenol red and antibiotics.
 2. Tissue culture medium (such as Eagle's MEM) containing 0,04 M HEPES buffer, 0,01 % bovine serum albumin and antibiotics, pH 7,2.
 3. Antibiotics (per ml final) must be added to the transport medium such as penicillin 1 000 IU, neomycin sulphate 100 IU, polymyxin B sulphate 50 IU, mycostatin 100 IU.
- B.** The virus neutralisation test shall be carried out according to the following protocol:
- Reagents** : Stock FMDV antigen is prepared in cell cultures or on cattle tongues and stored at - 70 °C or less or at - 20 °C after the addition of 50 % glycerol. This is the stock antigen. FMDV is stable under these conditions and titres vary little over a period of months.
- Procedure** : The test is carried out in flat-bottomed tissue culture grade microtitre plates using susceptible cells such as IB-RS-2, BHK-21 or calf kidney cells. Sera for the test are diluted 1/4 in serum-free cell culture medium with the addition of 100 IU/ml neomycin or other suitable antibiotics. Sera are inactivated at 56 °C for 30 minutes and 0.05 ml amounts are used to prepare a twofold series on microtitre plates using 0,05 ml diluting loops. Pre-titrated virus also diluted in serum-free culture medium and containing 100 TCID₅₀/0.05 ml is then added to each well. Following incubation at 37 °C for one hour to allow neutralisation to take place, 0,05 ml of suspension cells containing 0,5 to 1.0 × 10⁶ cells per 1 ml in cell culture medium containing serum free of FMD antibody is added to each well and the plates are sealed. Plates are incubated at 37 °C. Monolayers are normally confluent within 24 hours. CPE is usually sufficiently advanced at 48 hours for a microscopic reading of the test. At this time a final microscopic reading may be made or the plates may be fixed and stained for macroscopic reading, for instance using 10 % formal-saline and 0,05 % methylene blue.
- Controls** : Controls in each test include homologous antiserum of known titre, a cell control, a serum toxicity control, a medium control, and a virus titration from which the actual amount of virus in the test is calculated.

Interpretation : Wells with evidence of CPE are considered to be infected and neutralisation titres are expressed as the reciprocal of the final dilution of serum present in the serum/virus mixtures at the 50 % end point estimated according to the Spearman-Kärber method. (Karber, G., 1931, Archiv fuer Experimentelle Pathologie und Pharmakologie, 162, 480.). Tests are considered to be valid when the actual amount of virus used per well in the test is between 101,5 and 102,5 TCID₅₀ and when the titre of the reference serum is within twofold of its expected titre, estimated from the mode of previous titrations. When the controls are outside these limits the tests are repeated. An end point titre of 1/11 or less is taken as negative.

C. The detection and quantification of antibody by ELISA shall be carried out according to the following protocol:

Reagents : Rabbit antisera to 146S antigen of seven types of foot-and-mouth disease virus (FMDV) used at a predetermined optimum concentration in carbonate/bicarbonate buffer, pH 9,6. Antigens are prepared from selected strains of virus grown on monolayers of BHK-21 cells. The unpurified supernatants are used and pretitrated according to the protocol but without serum, to give a dilution which after the addition of an equal volume of PBST (phosphate buffered saline containing 0,05 % Tween-20 and phenol red indicator) would give an optical density reading of between 1,2 and 1,5. The viruses can be used inactivated. PBST is used as a diluent. Guinea-pig antisera are prepared by inoculating guinea pigs with 146S antigen of each serotype. A predetermined optimum concentration is prepared in PBST containing 10 % normal bovine serum and 5 % normal rabbit serum. Rabbit anti-guinea-pig immunoglobulin conjugated to horseradish peroxidase is used at a predetermined optimum concentration in PBST containing 10 % normal bovine serum and 5 % normal rabbit serum. Test sera are diluted in PBST.

Procedure:

1. ELISA plates are coated with 50 µl of rabbit antiviral sera overnight in a humidity chamber at room temperature.
2. Fifty microlitres of a duplicate, twofold series of each test serum starting at 1/4 are prepared in U-bottomed multiwell plates (carrier plates). Fifty microlitres of a constant dose of antigen are added to each well and the mixtures are left overnight at 4 °C. The addition of the antigen reduces the starting serum dilution to 1/8.
3. The ELISA plates are washed five times with PBST.
4. Fifty microlitres of serum/antigen mixtures are then transferred from the carrier plates to the rabbit-serum-coated ELISA plates and incubated at 37 °C for one hour on a rotary shaker.
5. After washing, 50 µl of guinea-pig antiserum to the antigen used in point 4 is added to each well. The plates are incubated at 37 °C for one hour on a rotary shaker.
6. The plates are washed and 50 µl of rabbit anti-guinea-pig immunoglobulin conjugated to horseradish peroxidase is added to each well. The plates are incubated at 37 °C for one hour on a rotary shaker.

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7. The plates are washed and 50 µl of orthophenylene diamine containing 0,05 % H₂O₂ (30 %) w/v is added to each well.

8. The reaction is stopped after 15 minutes with 1,25M H₂SO₄.

The plates are read spectrophotometrically at 492 nm on an ELISA reader linked to a microcomputer.

Controls : For each antigen used 40 wells contain no serum but contain antigen diluted in PBST. A duplicated twofold dilution series of homologous bovine reference antiserum. A duplicate twofold dilution series of negative bovine serum.

Interpretation : Antibody titres are expressed as the final dilution of tests serum giving 50 % of the mean OD value recorded in the virus control wells where test serum is absent. Titres in excess of 1/40 are considered positive.

References : Hamblin C, Barnett ITR and Hedger RS (1986) ' A new enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies against foot-and-mouth disease virus. I. Development and method of ELISA. ' Journal of Immunological Methods, 93, 115 to 121.11.

Aujeszky's disease (AJD)

A. The serum neutralisation test shall be carried out according to the following protocol:

Serum : All sera are heat-inactivated at 56 °C for 30 minutes before use.

Procedure : The constant virus-varying serum neutralisation test on microtitre plates employs Vero or other sensitive cell systems. Aujeszky's disease virus is used at 100 TCID₅₀ per 0,025 ml; inactivated undiluted serum samples are mixed with an equal volume (0,025 ml) of virus suspension. The virus/serum mixtures are incubated for two hours at 37 °C in the microtitre plates before the appropriate cells are added. Cells are used at a concentration which forms a complete monolayer after 24 hours.

Controls : (i) virus infectivity assay, (ii) serum toxicity controls, (iii) uninoculated cell culture controls, (iv) reference antisera.

Interpretation : The results of the neutralisation test and the titre of the virus used in the test are recorded after three to seven days incubation at 37 °C. Serum titres less than 1/2 (undiluted serum) are considered negative.

B. Any other test recognised in the framework of Decision 2008/185/EC ⁽²⁾.

Transmissible gastro-enteritis (TGE)

The serum neutralisation test shall be carried out according to the following protocol:

Serum : All sera are heat-inactivated at 56 °C for 30 minutes before use.

Procedure : The constant virus-varying serum neutralisation test on microtitre plates employs A72 (dog tumour) cells or other sensitive cell systems. TGE virus is used at 100 TCID₅₀ per 0,025 ml; inactivated undiluted serum samples are mixed with an equal volume (0,025 ml) of virus suspension. The virus/serum mixtures are incubated for 30 to 60 minutes at 37 °C in the microtitre plates before the appropriate cells are added. Cells are used at a concentration which forms a complete monolayer after 24 hours. Each cell receives 0,1 ml of cell suspension.

Controls : (i) virus infectivity assay, (ii) serum toxicity controls, (iii) uninoculated cell culture controls, (iv) reference antisera.

Interpretation : The results of the neutralisation test and the titre of the virus used in the test are recorded after three to five days incubation at 37 °C. Serum titres less than 1/2 (final dilution) are considered negative. If undiluted

serum samples are toxic to the tissue cultures, these sera may be diluted 1/2 before being used in the test. This is equivalent to 1/4 final dilution of serum. Serum titres of less than 1/4 (final dilution) are considered negative in these cases.

Swine vesicular disease (SVD)

Tests for swine vesicular disease (SVD) shall be carried out according to Decision 2000/428/EC ⁽³⁾.

Classical swine fever (CSF)

Tests for classical swine fever (CSF) shall be carried out according to Decision 2002/106/EC ⁽⁴⁾.

The performance of tests for CSF must follow the guidelines set out in the relevant chapter of the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals.

The evaluation of sensitivity and specificity of the serological test for CSF must be carried out in a national laboratory with a quality assurance scheme in place. Tests employed must be shown to recognise a range of weak and strong positive reference sera and allow detection of antibody in early phase and convalescence.

F³ Vesicular stomatitis (VS)

The virus neutralisation (VN) test shall be carried out in accordance with the testing protocols for vesicular stomatitis set out in Chapter 2.1.19 of the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals.

Sera that prevent cytopathic effect (CPE) at dilutions of 1 in 32 or greater shall be considered to contain antibodies to the vesicular stomatitis virus.]]

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- (1) [^{XI}OJ L 249, 23.7.2004, p. 20.]
- (2) [^{XI}OJ L 59, 4.3.2008, p. 19.]
- (3) [^{XI}OJ L 167, 7.7.2000, p. 22.]
- (4) [^{XI}OJ L 39, 9.2.2002, p. 71.]

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- X1** Substituted by [Corrigendum to Commission Regulation \(EU\) No 206/2010 of 12 March 2010 laying down lists of third countries, territories or parts thereof authorised for the introduction into the European Union of certain animals and fresh meat and the veterinary certification requirements \(Official Journal of the European Union L 73 of 20 March 2010\)](#).

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