Annex 7. Chapter 3.1.8. Foot and Mouth Disease

MEETING OF THE BIOLOGICAL STANDARDS COMMISSION

Paris, 2–6 February 2026

Table 1. Test methods available for the diagnosis of FMD and their purpose

Method	Purpose					
	Population freedom from infection	**Individual animal freedom from infection prior to movement**	**Contribute to eradication policies**	**Confirmation of clinical cases**	**Prevalence of infection – surveillance**	**Immune status in individual animals or populations post-vaccination**
Detection and identification of the agent[a]						
Virus isolation	☐☐☐	☑☐☐	☑☑☑	☑☑☑	☐☐☐	☐☐☐
Antigen detection ELISA	☐☐☐	☐☐☐	☑☑☑	☑☑☑	☐☐☐	☐☐☐
LFD	☐☐☐	☐☐☐	☑☑☑	☑☑☑	☐☐☐	☐☐☐
Real-time RT-PCR	☑☐☐	☑☐☐	☑☑☑	☑☑☑	☑☐☐	☐☐☐
Conventional RT-PCR	☑☐☐	☑☐☐	☑☑☑	☑☑☑	☑☐☐	☐☐☐
Detection of immune response						
NSP Ab ELISA	☑☑☑	☑☑☐	☑☑☑	☑☑☑	☑☑☑	☐☐☐
SP Ab ELISA[b]	☑☑☐	☑☑☐	☑☑☑	☑☑☑	☑☑☐	☑☑☑
VNT[b]	☑☑☐	☑☑☐	☑☑☑	☑☑☑	☑☑☐	☑☑☑

Key: ☑☑☑ = recommended for this purpose; ☑☑☐ recommended but has limitations;
☑☐☐ = suitable in very limited circumstances; ☐☐☐ = not appropriate for this purpose.
ELISA = enzyme linked immunosorbent assay; LFD: lateral flow device; RT-PCR = reverse-transcriptase polymerase chain reaction; NSP Ab ELISA = ELISA for antibodies against nonstructural proteins; SP Ab ELISA = ELISA for antibodies against structural proteins; VNT = Virus neutralisation test.
[a]It is essential to confirm the presence of FMDV following virus isolation by an antigen or nucleic acid detection test.
[b]The tests do not distinguish infected from vaccinated animals.

Appendix 1
Foot and mouth disease
Intended purpose of test: population freedom from infection

Test with score and species	Sample type and target analytes	Accuracy*	Test population	Validation report	Advantages	Disadvantages	References
Real-time RT-PCR ☑☐☐	[1] Testing often focuses on oral swabs, nasal swabs and probang samples to identify sub-clinical cases and carrier ruminants (other sample types such as lymph nodes collected at abattoirs and bulk milk are also used for this purpose)	[1] High (approaching 100%), where there are animals that have been recently infected, but lower where infection has occurred historically	[1] Test purpose validated using samples from experimental studies	[1] See references and Appendix 4: Confirmation of clinical cases	[1] Real-time RT-PCR can be automated allowing large number of samples to be tested [2] Oral swabs and milk are simple to collect, time efficient, require less restraint and are relatively non-invasive	[1] Screening large numbers of samples need to carefully consider the scope for cross-contamination (false positives) [2] Ability to recognise FMDV-infected animals is dependent on when samples are taken as well as technique used [3] Collection of probang samples requires specific training and is semi-invasive [4] More expensive than ELISA methods	Armson *et al.* (2020) Callahan *et al.* (2002) Jamal *et al.* (2012) King *et al.* (2006) Moniwa *et al.* (2007) Reid *et al.* (2003) Shaw *et al.* (2004) Shaw *et al.* (2007) Stenfeldt *et al.* (2013)
RT-PCR ☑☐☐	[1] Testing often focuses on oral swabs, nasal swabs and probang samples to identify sub-clinical cases and carrier ruminants	[1] Usually lower sensitivity compared with real-time RT-PCR	[1] See above descriptions for real-time RT-PCR as there is limited information on the use of RT-PCR for this purpose	[1] See reference and Appendix 4: Confirmation of clinical cases	[1] Oral swabs are simple to take, time efficient, require less restraint and are relatively non-invasive	[1] Screening large numbers of samples need to carefully consider the scope for cross-contamination (false positives) [2] Collection of probang samples requires specific training and is semi-invasive	~~Parida *et al.* (2005)~~ Reid *et al.* (2000) Vangrysperre & De Clercq (1996)
NSP Ab ELISA ☑☑☑	[1] Sera	[1] DSe between 95% and 100% [2] DSp between 98% and 100% [3] various NSP ELISA kits exist, and these will have different DSe and DSp	[1] Samples from FMDV susceptible species collected as part of FMDV control programmes [2] This purpose is also supported by sera collected from experimentally vaccinated and/or infected animals	[1] See references	[1] High diagnostic sensitivity in non-vaccinated populations [2] A single test covers all FMDV serotypes [3] Tests are usually appropriate for all susceptible species [4] Easy to perform, suitable for large-scale application or automation [5] Simple-to-use commercial kits are available [6] Ideal for surveillance, control, and eradication	[1] This test is ideal to screen populations, but specificity is <100% therefore, confirmatory testing is often required [2] Lower test sensitivity should be expected if animals have been previously vaccinated	Bergmann *et al.* (2003) Brocchi *et al.* (2006) Browning *et al.* (2020) Bruderer *et al.* (2004) Campos *et al.* (2008) Lu *et al.* (2007) Malirat *et al.* (1998) Paton *et al.* (2006) Sharma *et al.* (2014) Sorensen *et al.* (2005)

Test with score and species	Sample type and target analytes	Accuracy*	Test population	Validation report	Advantages	Disadvantages	References
SP Ab ELISA ☑☑☐	[1] Sera	[1] DSe between 93% and 100%	[1] Samples from FMDV susceptible species collected as part of FMDV control programmes [2] This purpose is also supported by testing of sera collected from experimentally infected animals	[1] See references and Appendix 3: Contribute to eradication policies	[1] Simple-to-use commercial kits are available [2] SP-abs appear early after infection or vaccination so SP-ELISAs have higher diagnostic sensitivity compared with NSP ELISAs [3] Tests are usually appropriate for all susceptible species [4] Easy to perform, suitable for large-scale application or automation	[1] Cannot differentiate between antibodies generated after vaccination or infection. Therefore, SP Ab ELISAs cannot be used if vaccines are used [2] Tests have high sensitivity for FMDV-specific antibodies but often have poor inter-serotype specificity. Therefore, serotype confirmation using another method may be required [3] The solid-phase competitive ELISA is more specific but as sensitive as the liquid-phase blocking ELISA	Hamblin *et al.* (1986) Hamblin *et al.* (1987) Ludi *et al.* (2022) Mackay *et al.* (2001) Paiba *et al.* (2004)
VNT ☑☑☐	[1] Sera	[1] DSp≈100% [2] DSe≈100% [3] Test performance is influenced by the specific antigen used [4] Test conditions (cells and condition of cell culture) influence test performance	[1] Samples from FMDV susceptible species collected as part of FMDV control programmes [2] This purpose is also supported by sera collected from experimentally infected animals	[1] See references and Appendix 3: Contribute to eradication policies	[1] Test that is often used for confirmatory purposes with high sensitivity and specificity	[1] Test requires 1–3 days for execution [2] Maintenance and use of live virus and tissue culture cells, thus biocontainment restriction procedures are required	Willems *et al.* (2020) Hamblin *et al.* (1986)

*NB: Sensitivity and specificity estimates presented in these tables are generally sourced from single studies and should be interpreted as indicative rather than definitive measures of test performance. Published evaluations often rely on non-random or restricted sample sets; therefore, the reported values may not be generalisable to all species, populations, epidemiological contexts, or assay formats. Variability is further influenced by the range of test designs available and their respective capacities to detect different FMDV serotypes and lineages. It is recommended that individual laboratories conduct verification exercises, following WOAH-prescribed procedures, to establish accuracy parameters appropriate to their specific testing conditions and to ensure that the assays used are fit-for-purpose.

Appendix 2
Foot and mouth disease
Intended purpose of test: individual animal freedom from infection prior to movement

Test with score and species	Sample type and target analytes	Accuracy*	Test population	Validation report	Advantages	Disadvantages	References
Virus Isolation ☑☐☐	[1] Testing of samples from FMDV antibody-negative animals (blood and swabs) to rule out pre-clinical/sub-clinical infection [2] Testing of probang samples can be used to confirm FMDV-antibody results	[1] Considered to be the gold standard method [2] Test performance is dependent upon the cell line and culture conditions that are used [3] For appropriate samples collected from acutely infected animals, DSe and DSp can approach 100% using highly sensitive cell lines (example LFBKαVβ6)	[1] This test purpose is supported by data generated from experimental studies in cattle and other species	[1] See references and Appendix 4: Confirmation of clinical cases	[1] Potentially useful for subsequent studies for virus characterisation including vaccine selection	[1] Needs confirmatory testing to confirm the agent causing CPE is FMDV (e.g. Ag-ELISA or sequencing) [2] Material submitted for testing must be in good condition requiring that a cold chain is maintained from sample collection to the laboratory [3] Testing can be impacted by gross bacterial/fungal contamination, despite the use of antibiotics in the cell culture media [4] Time-consuming and specialist facilities are needed (with appropriate biocontainment)	Ferris *et al.* (2006) Fukai *et al.* (2015) Gray *et al.* (2020) LaRocco *et al.* (2015)
Real-time RT-PCR ☑☐☐	[1] Testing of samples from FMDV antibody-negative animals (blood and swabs) to rule out pre-clinical/sub-clinical infection [2] Testing of probang samples can be used to confirm FMDV-antibody results	[1] WOAH *Terrestrial Manual* describes two methods 3D and 5'UTR and these have different sensitivity and specificity [2] DSp ~100% DSe ~100%	[1] This test purpose is supported by data generated from experimental studies in cattle and other species [2] Further validation data including the use of RT-PCR to detect pre-clinically infected animals arises from FMD outbreaks	[1] See references and Appendix 4: Confirmation of clinical cases	[1] Rapid [2] High analytical and diagnostic sensitivity and specificity [3] Test can be used for all sample types [4] Can adapt testing to high throughput	[1] Test performance dependent upon primers selected [2] requires specific equipment [3] Cross-contamination during sample preparation can lead to false positive results [4] Sequence variability can generate false negative results (particularly for the 5'UTR test [5] Panserotypic real time RT-PCRs in the WOAH *Terrestrial Manual* do not give details of serotype	Callahan *et al.* (2002) King *et al.* (2006) Moniwa *et al.* (2007) Reid *et al.* (2003) Shaw *et al.* (2004) Shaw *et al.* (2007)
RT-PCR ☑☐☐	[1] Testing of samples from FMDV antibody-negative animals (blood and	[1] DSe 97% [2] DSp ~100% [3] Analytical sensitivity reported	Limited validation data is available for this purpose	[1] See references and Appendix 4: Confirmation of clinical cases	[1] Alternative method when there is no real time RT PCR equipment available	[1] Test performance dependent upon primers selected [2] Running a gel increases the risk of cross-contamination	Reid *et al.* (2000) Reid *et al.* (2003) Vangrysperre & De Clercq (1996)

Test with score and species	Sample type and target analytes	Accuracy*	Test population	Validation report	Advantages	Disadvantages	References
	swabs) to rule out pre-clinical/sub-clinical infection [2] Testing of probang samples can be used to confirm FMDV-antibody results	to be less than 1 pfu					
NSP Ab ELISA ☑☑☐	[1] Sera collected to rule out historical exposure to FMDV	[1] DSe between 95% and 100% [2] DSp between 98% and 100% [3] Various NSP ELISA kits exist, and these will have different DSe and DSp	[1] Test validated for cattle, sheep, goats and pigs [2] Samples from vaccinated and/or infected animals	[1] See references and Appendix 1: Population freedom from infection	[1] High diagnostic sensitivity in non-vaccinated populations [2] A single test covers all FMDV serotypes [3] Tests are usually appropriate for all susceptible species [4] Easy to perform, suitable for large-scale application or automation [5] Simple-to-use commercial kits are available [6] Ideal for surveillance, control, and eradication	[1] Specificity is < 100% therefore, confirmatory testing may be required [2] Lower specificity is expected when animals have previously been vaccinated [3] Test will only generate a positive result for historical exposure to FMDV >7–10 days after infection. If more recent infection cannot be ruled out, other (molecular or culture) test methods may be required.	Bergmann *et al.* (2003) Brocchi *et al.* (2006) Browning *et al.* (2020) Bruderer *et al.* (2004) Campos *et al.* (2008) Lu *et al.* (2007) Malirat *et al.* (1998) Paton *et al.* (2006) Sharma *et al.* (2014) Sorensen *et al.* (2005)
SP Ab ELISA ☑☑☐	[1] Sera collected to rule out historical exposure to FMDV	[1] DSe between 93% and 100%	[1] Test validated for cattle, sheep, goats and pigs	[1] See references and Appendix 3: Contribute to eradication policies	[1] Simple-to-use commercial kits are available [2] SP-abs appear early after infection or vaccination so SP-ELISAs have higher diagnostic sensitivity compared with NSP ELISAs [3] Tests are usually appropriate for all susceptible species [4] Easy to perform, suitable for large-scale application or automation	[1] SP Ab ELISAs cannot be used if vaccines are used as the test cannot differentiate between antibodies generated after vaccination or infection [2] Test will only generate a positive result for historical exposure to FMDV >7–10 days after infection. If more recent infection cannot be ruled out, other (molecular or culture) test methods may be required. [3] Often cross-reactivity across serotype [4] The solid-phase competitive ELISA is more specific but as sensitive as the liquid-phase blocking ELISA	Hamblin *et al.* (1986) Hamblin *et al.* (1987) Ludi *et al.* (2022) Mackay *et al.* (2001) Paiba *et al.* (2004)

Member	Comment
USA	**Category**: Editorial **Proposed amended text**: The solid-phase competitive ELISA is **as sensitive as** but more specific **than** ~~**but as sensitive as**~~ the liquid-phase blocking ELISA **Rationale**: Grammatical correction to improve readability **Supporting Evidence**: not relevant

Test with score and species	**Sample type and target analytes**	**Accuracy***	**Test population**	**Validation report**	**Advantages**	**Disadvantages**	**References**
VNT ☑☑☐	Sera collected to rule out historical exposure to FMDV	[1] DSp≈100% [2] DSe≈100% [3] Test performance is influenced by the specific antigen used [4] Test conditions (cells and condition of cell culture) influence test performance	[1] Test validated for cattle, sheep, goats and pigs	[1] See references and Appendix 3: Contribute to eradication policies	[1] High sensitivity and specificity	[1] VNT test cannot be used if vaccines are used as the test cannot differentiate between antibodies generated after vaccination or infection [2] Test will only generate a positive result for historical exposure to FMDV >7–10 days after infection. If more recent infection cannot be ruled out, other (molecular or culture) test methods may be required. [3] It requires 1–3 days for execution [4] Maintenance and use of live virus and tissue culture cells, thus biocontainment restriction procedures are required	Willems *et al.* (2020) Hamblin *et al.* (1986)

NOTE: If a suspect non-negative antibody ELISA is received, probang with PCR testing could be used a complementary test to demonstrate freedom.

*NB: Sensitivity and specificity estimates presented in these tables are generally sourced from single studies and should be interpreted as indicative rather than definitive measures of test performance. Published evaluations often rely on non-random or restricted sample sets; therefore, the reported values may not be generalisable to all species, populations, epidemiological contexts, or assay formats. Variability is further influenced by the range of test designs available and their respective capacities to detect different FMDV serotypes and lineages. It is recommended that individual laboratories conduct verification exercises, following WOAH-prescribed procedures, to establish accuracy parameters appropriate to their specific testing conditions and to ensure that the assays used are fit-for-purpose.

Appendix 3
Foot and mouth disease
Intended purpose of test: contribute to eradication policies

Test with score and species	Sample type and target analytes	Accuracy*	Test population	Validation report	Advantages	Disadvantages	References
Virus isolation ☑☑☑	[1] Vesicular liquid, epithelia, swabs, milk, probangs, sera*, blood* *FMD viraemia is transient (~7 days), therefore use of blood sampling should consider the likely pathogenic cycle of infection	[1] Considered to be the gold standard method [2] Test performance is dependent upon the cell line and culture conditions that are used [3] For appropriate samples collected from acutely infected animals, DSe and DSp can approach 100% using highly sensitive cell lines (example LFBKαVβ6)	[1] Samples collected from suspected/confirmed cases and after experimental infection	[1] See references	[1] Generates isolates that are useful for subsequent studies for virus characterisation including vaccine selection	[1] Needs a confirmatory testing to confirm the agent causing CPE is FMDV (e.g., Ag-ELISA or sequencing) [2] Material submitted for testing must be in good condition requiring that a cold chain is maintained from sample collection to the laboratory [3] Testing can be impacted by gross bacterial/fungal contamination, despite the use of antibiotics in the cell culture media [4] Time-consuming and specialist facilities are needed (with appropriate biocontainment)	Ferris *et al.* (2006) Fukai *et al.* (2015) Gray *et al.* (2020) LaRocco *et al.* (2015)
Antigen detection ELISA ☑☑☑	[1] Field samples can be directly tested without VI if suitable (i.e. epithelia, vesicular fluid) [2] Other sample types may require virus isolation first	[1] DSe 83% virus-positive epithelia where viral loads are high in samples collected from acute cases, but lower where sampling focusses on convalescent animals [2] ELISA formats can differ, using polyclonal, monoclonal and/or recombinant integrin. The selection of these reagents impacts on the DSe and DSp of the test	[1] Samples collected from suspected/confirmed cases and after experimental infection	[1] See references	[1] Possibility of virus typing when the laboratory lacks equipment for PCR and sequencing [2] Can be used to serotype isolates generated by culture methods [3] Commercial kit version of this test are available	[1] Lower analytical sensitivity in relation to other methods available (e.g. RT-PCR) [2] DSe is impacted by the ability of the selected antibody reagents to detect diverse FMDV lineages [3] Direct testing of clinical samples is only applicable to epithelial suspensions and fluids	Ferris *et al.* 2011 Grazioli *et al.* 2020 Roeder & Le Blanc Smith (1987)

Test with score and species	Sample type and target analytes	Accuracy*	Test population	Validation report	Advantages	Disadvantages	References
LFD ☑☑☑	[1] Saliva, tissue, vesicular fluid and cultivated virus [2] Other sample types such as blood and serum may also be appropriate	[1] DSe 87–99% [2] DSp 99–100%	[1] Most validation data focusses on samples from cattle or pigs	[1] See references [2] Further validation studies for other sample-types are currently underway	[1] Rapid and simple to perform [2] No need for laboratory equipment [3] FMDV positive LFDs can be submitted for molecular analyses	[1] As per antigen detection ELISA	Ferris *et al.* (2009) Ferris *et al.* (2012) Foglia *et al.* (2025) Oem *et al.* (2009) Yang *et al.* (2022)
Real-time RT-PCR ☑☑☑	[1] All samples collected from acutely infected animals (vesicular liquid, epithelia, sera, blood, oral and nasal swabs, milk) [2] Environmental samples (including air) [3] Probang samples from convalescent animals	[1] WOAH *Terrestrial Manual* describes two methods 3D and 5'UTR and these have different sensitivity and specificity [2] DSp ~100% DSe ~100%	[1] Samples collected from suspected/confirmed cases and after experimental infection	[1] See references	[1] Rapid [2] High analytical and diagnostic sensitivity and specificity [3] Test can be used for all sample types [4] Can adapt testing to automated platforms to achieve high throughput	[1] Test performance dependent upon primers selected [2] Requires specific equipment [3] Cross-contamination during sample preparation can lead to false positive results [4] Sequence variability can generate false negative results (particularly for the 5'UTR test [5] Panserotypic real time RT-PCRs in the WOAH *Terrestrial Manual* do not give details of serotype	Callahan *et al.* (2002) King *et al.* (2006) Moniwa *et al.* (2007) Reid *et al.* (2000) Reid *et al.* (2009) Shaw *et al.* (2004) Shaw *et al.* (2007)
RT-PCR ☑☑☑	[1] All samples collected from acutely infected animals (vesicular liquid, epithelia, sera, blood, oral and nasal swabs, milk)	[1] DSe 97% [2] DSp ~100% [3] Analytical sensitivity reported to be less than 1 pfu	[1] Samples collected from suspected/confirmed cases and after experimental infection		[1] Alternative when there is no real time RT PCR equipment available	[1] Test performance dependent upon primers selected [2] Running a gel increases the risk of cross-contamination	~~Reid *et al.* (2000)~~ Reid *et al.* (2003) Vangrysperre & De Clercq (1996)
NSP Ab ELISA ☑☑☑	[1] Sera	[1] DSe between 95% and 100% [2] DSp between 98% and 100% [3] various NSP ELISA kits exist and these will	[1] Test validated for cattle, sheep, goats and pigs [2] Samples collected from suspected/confirmed cases and after experimental infection	[1] See references	[1] High diagnostic sensitivity in non-vaccinated populations [2] A single test covers all FMDV serotypes [3] Tests are usually appropriate for all susceptible species [4] Easy to perform, suitable for large-scale application or	[1] Specificity is < 100% therefore, confirmatory testing often required [2] Lower sensitivity should be expected if animals have been previously vaccinated	Bergmann *et al.* (2003) Brocchi *et al.* (2006) Browning *et al.* (2020) Bruderer *et al.* (2004) Campos *et al.* (2008) Lu *et al.* (2007) Malirat *et al.* (1998) Paton *et al.* (2006)

Test with score and species	Sample type and target analytes	Accuracy*	Test population	Validation report	Advantages	Disadvantages	References
		have different DSe and DSp			automation [5] Simple-to-use commercial kits are available [6] Ideal for surveillance, control, and eradication		Sharma *et al.* (2014) Sorensen *et al.* (2005)
SP Ab ELISA ☑☑☑	[1] Sera	[1] DSe between 93% and 100% [2] DSp between 98% and 100% [2] A range of different test formats are available including in-house and commercial assays. The performance of these tests vary.	[1] Test validated for cattle, sheep, goats and pigs [2] Samples collected from suspected/confirmed cases and after experimental infection	[1] See references	[1] Simple-to-use commercial kits are available [2] SP-abs appear early after infection or vaccination so SP-ELISAs have higher diagnostic sensitivity compared with NSP ELISAs [3] Tests are usually appropriate for all susceptible species [4] Easy to perform, suitable for large-scale application or automation	[1] Cannot differentiate between antibodies generated after vaccination or infection [2] Often cross-reactivity across serotype [3] The solid-phase competitive ELISA is more specific but as sensitive as the liquid-phase blocking ELISA	~~Browning *et al.* (2020)~~ Hamblin *et al.* (1986) Hamblin *et al.* (1987) Ludi *et al.* (2022) Mackay *et al.* (2001) Paiba *et al.* (2004)
VNT ☑☑☑	Sera	[1] DSp≈100% [2] DSe≈100% [3] Test performance is influenced by the specific antigen used [4] Test conditions (cells and condition of cell culture) influence test performance	[1] Test validated for cattle, sheep, goats and pigs [2] Samples collected from suspected/confirmed cases and after experimental infection	[1] See references	[1] High sensitivity and specificity	[1] It requires 1–3 days for execution [2] Maintenance and use of live virus and tissue culture cells, thus biocontainment restriction procedures are required	Willems *et al.* (2020) Hamblin *et al.* (1986)

*NB: Sensitivity and specificity estimates presented in these tables are generally sourced from single studies and should be interpreted as indicative rather than definitive measures of test performance. Published evaluations often rely on non-random or restricted sample sets; therefore, the reported values may not be generalisable to all species, populations, epidemiological contexts, or assay formats. Variability is further influenced by the range of test designs available and their respective capacities to detect different FMDV serotypes and lineages. It is recommended that individual laboratories conduct verification exercises, following WOAH-prescribed procedures, to establish accuracy parameters appropriate to their specific testing conditions and to ensure that the assays used are fit-for-purpose.

Appendix 4
Foot and mouth disease
Intended purpose of test: confirmation of clinical cases

Test with score and species	Sample type and target analytes	Accuracy*	Test population	Validation report	Advantages	Disadvantages	References
Virus isolation ☑☑☑	[1] Vesicular liquid, epithelia, swabs, probangs, sera*, blood* *FMD viraemia is transient (~7 days), therefore use of blood sampling should consider the likely pathogenic cycle of infection	[1] Considered to be the gold standard method [2] Test performance is dependent upon the cell line and culture conditions that are used [3] For appropriate samples collected from acutely infected animals, DSe and DSp can approach 100% using highly sensitive cell lines (example LFBKαVβ6)	[1] Samples collected from suspected/confirmed cases and after experimental infection	[2] See references.	[1] Generates isolates that are useful for subsequent studies for virus characterisation including vaccine selection	[1] Needs a confirmatory testing to confirm the agent causing CPE is FMDV (e.g. Ag-ELISA or sequencing) [2] Material submitted for testing must be in good condition requiring that a cold chain is maintained from sample collection to the laboratory [3] Testing can be impacted by gross bacterial/fungal contamination, despite the use of antibiotics in the cell culture media [4] Time-consuming and specialist facilities are needed (with appropriate biocontainment)	Ferris *et al.* (2006) Fukai *et al.* (2015) Gray *et al.* (2020) LaRocco *et al.* (2015)
Antigen detection ELISA ☑☑☑	[1] Field samples can be directly tested without VI if suitable (i.e. epithelia, vesicular fluid) [2] Other sample types may require virus isolation first	[1] DSe 83% virus-positive epithelia where viral loads are high in samples collected from acute cases, but lower where sampling focuses on convalescent animals [2] ELISA formats can differ, using polyclonal, monoclonal and recombinant integrin. The selection of these reagents impacts on the DSe and DSp of the test	[1] Samples collected from suspected/confirmed cases and after experimental infection	[1] See references and/or kit inserts	[1] Possibility of virus typing when the laboratory lacks equipment for PCR and sequencing [2] Can be used to serotype isolates generated by culture methods [3] Commercial kit version of this test are available	[1] Lower analytical sensitivity in relation to other methods available (e.g., RT-PCR) [2} DSe is impacted by the ability of the selected antibody reagents to detect diverse FMDV lineages [3] Direct testing of clinical samples is only applicable to epithelial suspensions and fluids	Ferris *et al.* 2011 Grazioli *et al.* 2020 Roeder & Le Blanc Smith (1987)
LFD ☑☑☑	[1] Saliva, tissue homogenates, vesicular fluid and cultivated virus	[1] Similar test performance to Ag-ELISA	[1] Samples collected from suspected/confirmed	[1] See references [2] Further validation	[1] Rapid and simple to perform [2] No need for laboratory equipment	As per antigen detection ELISA	Ferris *et al.* (2009) Ferris *et al.* (2012) Foglia *et al.* (2025)

Test with score and species	Sample type and target analytes	Accuracy*	Test population	Validation report	Advantages	Disadvantages	References
	[2] Other sample 3types such as blood and serum may also be appropriate	[2] DSe 87–99% [] DSp 99–100%	cases and after experimental infection	studies for other sample-types are currently underway	[3] FMDV positive LFDs can be submitted for molecular analyses		Oem *et al.* (2009) Yang *et al.* (2022)
Real-time RT-PCR ☑☑☑	[1] All samples collected from acutely infected animals (vesicular liquid, epithelia, sera, blood, milk, oral and nasal swabs) [2] Environmental samples (including air) [3] Probang samples from convalescent animals	[1] WOAH *Terrestrial Manual* describes two methods 3D and 5'UTR and these have different sensitivity and specificity [2] DSp ~100% DSe ~100%	[1] Samples collected from suspected/confirmed cases and after experimental infection	[1] See references	[1] Rapid [2] High analytical and diagnostic sensitivity and specificity [3] Test can be used for all sample types [4] Can adapt testing to automated platforms to achieve high throughput	[1] Test performance dependent upon primers selected [2] Requires specific equipment [3] Cross-contamination during sample preparation can lead to false positive results [4] Sequence variability can generate false negative results (particularly for the 5'UTR test [5] Panserotypic real time RT-PCRs in the WOAH *Terrestrial Manual* do not give details of serotype	Callahan *et al.* (2002) King *et al.* (2006) Moniwa *et al.* (2007) Reid *et al.* (2003) Shaw *et al.* (2004) Shaw *et al.* (2007)
RT-PCR ☑☑☑	[1] All samples collected from acutely infected animals (vesicular liquid, epithelia, sera, blood, milk, oral and nasal swabs)	[1] DSe 97% [2] DSp ~100% [3] Analytical sensitivity reported to be less than 1 pfu	[1] Samples collected from suspected/confirmed cases and after experimental infection		[1] Alternative when there is no real time RT PCR equipment available	[1] Test performance dependent upon primers selected [2] Running a gel increases the risk of cross-contamination	Reid *et al.* (2000) ~~Reid *et al.* (2003)~~ Vangrysperre & De Clercq (1996)
NSP Ab ELISA ☑☑☑	[1] Sera	[1] DSe between 95% and 100% [2] DSp between 98% and 100% [3] various NSP ELISA kits exist and these will have different DSe and DSp	[1] Samples from all susceptible species that have been exposed to FMDV (field and experimental studies) [2] Validation of this purpose includes sera collected from vaccinated and/or infected animals	[1] See references	[1] High diagnostic sensitivity in non-vaccinated populations [2] A single test covers all FMDV serotypes [3] Tests are usually appropriate for all susceptible species [4] Easy to perform, suitable for large-scale application or automation [5] Simple-to-use commercial kits are available	[1] FMDV-specific antibodies arise after infection. Therefore, these tests are only appropriate if infection >7–10 days is suspected. [2] Specificity is < 100% therefore, confirmatory testing is required [3] Lower sensitivity should be expected if animals have been previously vaccinated	Bergmann *et al.* (2003) Brocchi *et al.* (2006) Browning *et al.* (2020) Bruderer *et al.* (2004) Campos *et al.* (2008) Lu *et al.* (2007) Malirat *et al.* (1998) Paton *et al.* (2006) Sharma *et al.* (2014) Sorensen *et al.* (2005)

Test with score and species	Sample type and target analytes	Accuracy*	Test population	Validation report	Advantages	Disadvantages	References
SP Ab ELISA ☑☑☑	[1] Sera	[1] DSe between 93% and 100% [2] DSp between 98% and 100% [2] A range of different test formats are available including in-house and commercial assays. The performance of these tests vary	[1] Samples from all susceptible species that have been exposed to FMDV (field and experimental studies)	[1] See references	[1] Simple-to-use commercial kits are available [2] SP-abs appear early after infection or vaccination so SP-ELISAs have higher diagnostic sensitivity compared with NSP ELISAs [3] Tests are usually appropriate for all susceptible species [4] Easy to perform, suitable for large-scale application or automation	[1] FMDV-specific antibodies arise after infection. Therefore, these tests are only appropriate if infection >7–10 days is suspected. [2] Cannot differentiate between antibodies generated after vaccination or infection [3] Test exhibit cross-reactivity across FMDV serotypes requiring that further sampling (such as with a probang) may be required to verify the serotype present [4] The solid-phase competitive ELISA is more specific but as sensitive as the liquid-phase blocking ELISA	~~Browning *et al.* (2020)~~ Hamblin *et al.* (1986) Hamblin *et al.* (1987) Ludi *et al.* (2022) Mackay *et al.* (2001) Paiba *et al.* (2004)
VNT ☑☑☑	Sera	[1] DSp≈100% [2] DSe≈100% [3] Test performance is influenced by the specific antigen used [4] Test conditions (cells and condition of cell culture) influence test performance	[1] Samples from all susceptible species that have been exposed to FMDV (field and experimental studies)	[1] See references	[1] High sensitivity and specificity	[1] FMDV-specific antibodies arise after infection. Therefore, these tests are only appropriate if infection >7–10 days is suspected. [2] Test requires 1–3 days for execution and maintenance and use of live virus and tissue culture cells, thus biocontainment restriction procedures are required	Willems *et al.* (2020) Hamblin *et al.* (1986) Rweyemamu *et al.* (1984) Rweyemamu (1984)

*NB: Sensitivity and specificity estimates presented in these tables are generally sourced from single studies and should be interpreted as indicative rather than definitive measures of test performance. Published evaluations often rely on non-random or restricted sample sets; therefore, the reported values may not be generalisable to all species, populations, epidemiological contexts, or assay formats. Variability is further influenced by the range of test designs available and their respective capacities to detect different FMDV serotypes and lineages. It is recommended that individual laboratories conduct verification exercises, following WOAH-prescribed procedures, to establish accuracy parameters appropriate to their specific testing conditions and to ensure that the assays used are fit-for-purpose.

Appendix 5
Foot and mouth disease
Intended purpose of test: prevalence of infection – surveillance

Test with score and species	Sample type and target analytes	Accuracy*	Test population	Validation report	Advantages	Disadvantages	References
Real-time RT-PCR ☑☐☐	[1] All samples collected from acutely infected animals (vesicular liquid, epithelia, sera, blood, milk, oral and nasal swabs) [2] Environmental samples (including air) [3] Probang samples from convalescent animals	[1] WOAH *Terrestrial Manual* describes two methods 3D and 5'UTR and these have different sensitivity and specificity [2] DSp ~100% DSe ~100%	[1] Suspected infected animals	[1] See references and Appendix 4: Confirmation of clinical cases	[1] Rapid [2] High analytical and diagnostic sensitivity and specificity [3] Test can be used for all sample types [4] Can adapt testing to high throughput	[1] Test performance dependent upon primers selected [2] Requires specific equipment [3] Cross-contamination during sample preparation can lead to false positive results [4] Sequence variability can generate false negative results (particularly for the 5'UTR test [5] Panserotypic real time RT-PCRs in the WOAH *Terrestrial Manual* do not give details of serotype	Armson *et al.* (2020) Callahan *et al.* (2002) King *et al.* (2006) Moniwa *et al.* (2007) Reid *et al.* (2003) Shaw *et al.* (2004) Shaw *et al.* (2007)
RT-PCR ☑☐☐	[1] All samples collected from acutely infected animals (vesicular liquid, epithelia, sera, blood, milk, oral and nasal swabs)	[1] DSe 97% [2] DSp ~100% [3] Analytical sensitivity reported to be less than 1 pfu	[1] Samples from epithelia collection of different serotypes [2] 5 negative samples	[1] See Appendix 4: Confirmation of clinical cases	[1] Alternative when there is no real time RT PCR equipment availability	[1] Test performance dependent upon primers selected [2] Running a gel increases the risk of cross-contamination	Reid *et al.* (2000) ~~Reid *et al.* (2003)~~ Vangrysperre & De Clercq (1996)
NSP Ab ELISA ☑☑☑	[1] Sera	[1] DSe between 95% and 100% [2] DSp between 98% and 100% [3] various NSP ELISA kits exist and these will have different DSe and DSp	[1] Cattle, sheep, goats and pigs [2] samples collected as part of sero-surveillance studies [3] Samples from vaccinated and/or infected animals	[1] See references [2] Available from WOAH Ref. Lab.	[1] High diagnostic sensitivity in non-vaccinated populations [2] A single test covers all FMDV serotypes [3] Tests are usually appropriate for all susceptible species [4] Easy to perform, suitable for large-scale application or automation [5] Simple-to-use commercial kits are available [6] Ideal for surveillance, control, and eradication	[1] Specificity is < 100% therefore, confirmatory testing is required [2] Lower specificity should be expected if animals have been previously vaccinated	Bergmann *et al.* (2003) Brocchi *et al.* (2006) Browning *et al.* (2020) Bruderer *et al.* (2004) Campos *et al.* (2008) Lu *et al.* (2007) Malirat *et al.* (1998) Paton *et al.* (2006) Sharma *et al.* (2014) Sorensen *et al.* (2005)

Test with score and species	Sample type and target analytes	Accuracy*	Test population	Validation report	Advantages	Disadvantages	References
SP Ab ELISA ☑☑☐	[1] Sera	[1] DSe between 93% and 100%	[1] See reference	[1] See Appendix 3: Contribute to eradication policies [2] Available from WOAH Ref. Lab.	[1] Simple-to-use commercial kits are available [2] SP-abs appear early after infection or vaccination so SP-ELISAs have higher diagnostic sensitivity compared with NSP ELISAs [3] Tests are usually appropriate for all susceptible species [4] Easy to perform, suitable for large-scale application or automation	[1] SP Ab ELISAs cannot be used if vaccines are used. Cannot differentiate between antibodies generated after vaccination or infection [2] ~~Less sensitive than RT-PCR~~ [2] Tests often suffer from cross-reactivity across serotype which limits their use for serotype-specific surveillance in countries/regions where multiple serotypes co-circulate ~~[4] SP Ab ELISAs cannot be used if vaccines are used as the test cannot differentiate between antibodies generated after vaccination or infection~~	Hamblin *et al.* (1986) Hamblin *et al.* (1987) Ludi *et al.* (2022) Mackay *et al.* (2001) Paiba *et al.* (2004)
VNT ☑☑☐	Sera	[1] DSp≈100% [2] DSe≈100% [3] Test performance is influenced by the specific antigen used [4] Test conditions (cells and condition of cell culture) influence test performance	[1] Susceptible animals	[1] See Appendix 3: Contribute to eradication policies [2] Recognised as the gold standard method and consequently initial test validation was not performed	[1] High sensitivity and specificity	[1] It requires 1–3 days for execution [2] Maintenance and use of live virus and tissue culture cells, thus biocontainment restriction procedures are required [3] VNT cannot be used if vaccines are used as the test cannot differentiate between antibodies generated after vaccination or infection	Willems *et al.* (2020) Hamblin *et al.* (1986)

*NB: Sensitivity and specificity estimates presented in these tables are generally sourced from single studies and should be interpreted as indicative rather than definitive measures of test performance. Published evaluations often rely on non-random or restricted sample sets; therefore, the reported values may not be generalisable to all species, populations, epidemiological contexts, or assay formats. Variability is further influenced by the range of test designs available and their respective capacities to detect different FMDV serotypes and lineages. It is recommended that individual laboratories conduct verification exercises, following WOAH-prescribed procedures, to establish accuracy parameters appropriate to their specific testing conditions and to ensure that the assays used are fit-for-purpose.

Appendix 6
Foot and mouth disease
Intended purpose of test: Immune status in individual animals or populations post-vaccination

Test with score and species	Sample type and target analytes	Accuracy*	Test population	Validation report	Advantages	Disadvantages	References
SP Ab ELISA ☑☑☑	[1] Sera from vaccinated animals [2] Sera collected for the purposes of vaccine control	[1] DSe ~100% DSp ~87%	[1] Validation data mostly available from cattle in controlled infection or vaccination studies	[1] See references and Appendix 3: Contribute to eradication policies	[1] Tests are simple-to use [2] Where extensive validation has been undertaken (such as in South America), SP-ELISA testing of vaccinated animals correlates with PGP results avoiding the requirement for challenge tests to assess FMD vaccine quality	[1] Tests suffer from poor inter-serotype specificity which can limit their use when multivalent FMD vaccines are used. [2] Estimating protective responses using SP-ELISA serology is not as precise as VNT	Barnett *et al.* (2003) Goris *et al.* (2008) ~~Gubbins *et al.* (2022)~~ Maradei *et al.* (2008) Periolo *et al.* (1993)
VNT ~~TEST~~ ☑☑☑	[1] Sera from vaccinated animals	[1] DSp≈100% [2] DSe≈100% [3] Test performance is influenced by the specific viral antigen used [4] Test conditions (cells and condition of cell culture) influence test performance	[1] Samples from vaccinated animals (including potency tests)	[1] See references	[1] High sensitivity and specificity compared with SP-ELISA [2] Test measures neutralising antibodies which are important correlates of protection	[1] Test requires 1–3 days for execution [2] Maintenance and use of live virus and tissue culture cells, thus biocontainment restriction procedures are required	Barnett *et al.* (2003) Goris *et al.* (2008) Gubbins *et al.*, (2022 Hamblin *et al.* (1986) Willems *et al.* (2020)

*NB: Sensitivity and specificity estimates presented in these tables are generally sourced from single studies and should be interpreted as indicative rather than definitive measures of test performance. Published evaluations often rely on non-random or restricted sample sets; therefore, the reported values may not be generalisable to all species, populations, epidemiological contexts, or assay formats. Variability is further influenced by the range of test designs available and their respective capacities to detect different FMDV serotypes and lineages. It is recommended that individual laboratories conduct verification exercises, following WOAH-prescribed procedures, to establish accuracy parameters appropriate to their specific testing conditions and to ensure that the assays used are fit-for-purpose.

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