

Swine Disease Matrix Supplement

*Prepared for the Swine Health Information Center*

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List of Acronyms

AGID Agar gel immunodiffusion

BSL Biosafety level

CDC Centers for Disease Control and Prevention

cELISA Competitive enzyme linked immunosorbent assay

CF Complement fixation

EIA Enzyme immunoassay

ELISA Enzyme linked immunosorbent assay

EM Electron microscopy

FA Fluorescent antibody

FADDL-Plum Island Foreign Animal Disease Diagnostic Laboratory-Plum Island

HI Hemagglutination inhibition

IFA Immunofluorescence assay

IHC Immunohistochemistry

ISU VDL Iowa State University Veterinary Diagnostic Laboratory

NVSL-Ames National Veterinary Services Laboratories—Ames

Ohio ADDL Ohio Animal Disease Diagnostic Laboratory

OIE World Organization for Animal Health

PIADC Plum Island Animal Disease Center (now known as FADDL-Plum Island)

PCR Polymerase chain reaction

qRT-PCR Quantitative reverse transcriptase polymerase chain reaction

RT-LAMP Reverse transcription loop-mediated isothermal amplification

RT-PCR Reverse transcriptase polymerase chain reaction

UMN VDL University of Minnesota Veterinary Diagnostic Laboratory

USDA ARS United States Department of Agriculture-Agricultural Research Service

VI Virus isolation

VLP Virus-like particle

VN/NT Virus neutralization/virus neutralizing test

WHO World Health Organization

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**Note: Information about availability of assays is not guaranteed. For more information, contact the cited laboratory or reference.**

1. Encephalomyocarditis virus

# 1.1 Antigen Testing

**VI**—Virus can be grown in BHK-21 cells, Vero cells, HeLa cells, and chicken embryos.

Available at some diagnostic laboratories (e.g., ISU VDL) and at NVSL-Ames. 7–14 day turnaround at ISU; 15 day turnaround at NVSL-Ames.

**RT-PCR**—Primers and methods have been described in the literature; high sensitivity and moderate specificity.1,2 Contamination during testing can result in false-positives.

**qRT-PCR**— Primers and methods have been described in the literature; higher sensitivity and specificity compared to conventional RT-PCR.3 Qualitative real-time RT-PCR offered by Zoologix; sensitivities and specificities unknown.4

**FA**—Test has been described in the literature. Available at some veterinary diagnostic labs within the United States (e.g., ISU VDL, Ohio ADDL).

**IHC**—Test is sometimes used in research. Can be beneficial during diagnosis, but not commonly/commercially offered.

**OTHER**—RT-LAMP assay recently described by Yuan et al. 2014.5

# 1.2 Antibody Testing

**ELISA**—No commercial test available. Commonly described in the literature; a recent publication designed and implemented a double-antigen sandwich ELISA for detection of antibodies in both human and swine sera, but was not validated for sensitivity and specificity.6

**VN**— Available at some commercial testing laboratories (e.g., UMN VDL, Texas A&M VDL, Ohio ADDL, ISU VDL) and at NVSL-Ames. Currently the reference standard for diagnosis, recommended specimen of choice is fetal fluids or serum; detection of antibodies in adult animals is not diagnostic, as they can be seropositive due to subclinical/previous infections.7 If antibody titers are greater than 1:16 they may be significant, indicating active or recent infection.8

**IFA**, **AGID, and latex agglutination** have been used to identify antibodies, but are not commonly chosen for detection.8

2. Filoviruses: African and Reston species

# 2.1 Antigen Testing

**VI**—Available at the CDC; acceptable confirmatory test for clinical cases. Species-independent test. Requires BSL-4 laboratory.9 Maintaining optimal conditions while shipping is difficult and may lead to reduced sensitivity and specificity of the test.

**RT-PCR**— Available at the CDC; acceptable confirmatory test for clinical cases. Species-independent test. More sensitive than Ag-capture ELISA; allows for differentiation between ebolaviruses.9

**qRT-PCR**— Available at the CDC; acceptable confirmatory test for clinical cases. Species-independent test. More sensitive than Ag-capture ELISA, faster than RT-PCR; allows for differentiation between ebolaviruses.9

**FA**— No reliable testing methods available commercially for Ebola virus in swine.

**IHC**— Available at the CDC; acceptable confirmatory test for clinical cases. Species-independent test.9

**OTHER**—Ag-capture ELISA using monoclonal antibodies described in Saijo et al. 200610 was successfully modified for Reston virus in swine.11

# 2.2 Antibody Testing

No commercial antibody test available for Reston virus in pigs. Lateral flow immunodiagnostic (LFI) assays have recently been developed for use in human and non-human primates as a sensitive, point-of-contact diagnostic test that may be possible to use or adapt to use in swine.12

**ELISA**— No commercial test available. Methods for antibody-capture ELISA on swine sera described in Sayama et al. 2012.11

**VN**— No commercial test available. Methods for VN (called NT/neutralizing test in the text) on swine sera described in Sayama et al. 2012.11

**IFA/CF/HI**—No commercial test or reliable testing method is available for swine. Methods for IFA on swine sera described in Sayama et al. 2012, but efficacy was not described.11

3. Getah virus

# 3.1 Antigen Testing

**VI**—Gold standard method; slow, requires approximately one week for results.13 Can be grown in numerous cell lines. A good description of the method is available in Bannai et al. 2014.14

**RT-PCR**— Numerous primer sequences have been published in the literature; Wekeza et al. 2001 describes primers capable of replicating viral RNA from multiple species including horse, pig and mosquito.15 Nemoto et al. 2014 reports using a one-step RT-PCR primer kit from Qiagen to identify Getah virus in racehorses in Japan.16 The Jiangsu Entry-Exit Inspection and Quarantine Bureau of the People’s Republic of China appears to have submitted for and received a patent for a swine Getah virus RT-PCR detection kit in 2013 (patent CN 102337356B); commercial availability and validity of the process unknown.17

**qRT-PCR/FA/IHC** —Utilization of real-time RT-PCR for Getah virus detection in swine has not been published. FA and IHC not commercially available or commonly used in research for Getah virus detection in swine.

# 3.2 Antibody Testing

**ELISA**—No commercial test is available for swine serum, but Ab-capture ELISA (IgG and IgM) is commonly used in equines and has been used in swine. A swine-adopted ELISA method described in Hohdatsu et al. 1990 was more sensitive for Getah virus than HI, with the similarly high specificity.18

**VN/CF/HI**—Virus/serum neutralization has been reported to be more specific than CF and HI in differentiating Getah virus from other alphaviruses, but only CF is able to differentiate between Getah and the closely-related Saginawa virus (not a known pathogen in pigs).15,19

**IFA**—No reliable test available for detection of Getah virus antibodies in swine.

4. Hepatitis E virus

# 4.1 Antigen Testing

**VI**—Not available; virus is difficult to grow in cell cultures.

**RT-PCR**/**qRT-PCR**—Available at some veterinary diagnostic laboratories (UMN VDL); CDC offers hepatitis E virus RNA testing by real-time qRT-PCR on human sera, as well as genotyping.

**FA**/**IHC**—Not available for swine hepatitis E virus. IHC has been described in research, but is not applicable on a mass-testing regimen as it requires greater and more expensive technical capacity and expertise.20

# 4.2 Antibody Testing

**ELISA/EIA**—Standard methods of testing for swine hepatitis E globally. Commercially offered by several labs within the United States (i.e., swine hepatitis E virus ELISA at ISU VDL; human IgM and IgG hepatitis E EIA at CDC). Strong evidence shows that derived hepatitis E antigen of human or swine origin work equally well when detecting antibodies in either human or swine sera; commercial test kits prepared for human diagnostics are capable of detecting antibodies to hepatitis E virus in swine antibodies.21,22

Of commercial kits available, the EIA anti-HEV IgM test (no. E-152) from Diagnostics Systems in Italy23 had the highest sensitivity (98%; 95%CI: 88—99.9%) and specificity (95.2%; 95%CI: 91.3—97.4%) when compared to in-house and commercial test kits.24

**VN/IFA/CF/HI**—Not available and/or applicable to hepatitis E virus diagnosis. An abstract has recently been published on a potential virus neutralization method25, but efficacy data is not yet available.

5. Influenza C virus

# 5.1 Antigen Testing

**VI**—Gold standard and currently the primary method of definitive diagnosis. Influenza C viruses can be grown using the same methods and cell lines as influenza A and B viruses. Influenza isolation is available at many/most veterinary diagnostic labs. Identification of the virus is typically by HI.

**RT-PCR**—Primers are described for influenza C virus detection in swine.26

**qRT-PCR**—Primers have been described for detection of influenza C virus in humans27; efficacy for swine samples is unknown.

**FA/IHC**—Not typically used for influenza C diagnostics, except in research; virus isolation and RT-PCR are preferred over FA and IHC.

# 5.2 Antibody Testing

**HI**—Standard method of influenza identification; method for detecting human influenza C virus in swine and has been documented several times in the literature, shown to be sensitive and specific to influenza C.28,29

**ELISA/CF/VN/Western blot**—These tests have been used and described in literature for detection of human influenza C antibodies in swine, horses, and dogs 30,31; typically used to reinforce or elaborate on results from HI test. Methods utilized are similar to those used for detection of influenza C antibodies in humans; minimal efficacy information for swine is available. Several studies showed discordant results between HI and ELISA, with ELISA showing greater sensitivity.29,30 This may be due to the manufactured antibodies of the ELISA detecting both IgM and IgG within swine sera, whereas HI appears to only detect anti-influenza-C IgG antibodies.29

**IFA**—IFA has been described for testing human sera for influenza C antibodies, but no reliable information for testing swine sera was located.

6. Influenza D virus

# 6.1 Antigen Testing

**VI**—Isolation available at some veterinary diagnostic labs and commercial laboratories. Previous successful documented isolations were conducted at Newport Laboratories.32,33

**RT-PCR**—Primers have been described for conservative RT-PCR detection of influenza D virus; sensitivity and specificity were not evaluated.34

**qRT-PCR**—Real-time RT-PCR primers specific and sensitive to influenza D virus have been described.32,35,36

**FA/IHC**—No reliable method has been described for influenza D antigen testing with FA or IHC.

# 6.2 Antibody Testing

**ELISA/VN/IFA/CF**—No reliable method has been described for influenza D virus antibody testing with ELISA, VN, IFA, or CF. However, all methods have been used successfully in influenza A, B, and C viruses, and should be readily adaptable for influenza D virus.

**HI**—Method has been described for swine and bovine sera32,33,35; shown to be highly sensitive and moderately to highly specific. Cross-reaction with influenza C virus antibodies only occurred when sera had very high levels; false positives may be seen if active or previously active influenza C infection is present.35

**OTHER**— An AGID has been described; specificity to influenza D virus antibodies was high.32 Historically, AGID sensitivity to influenza viruses has been documented as lower than other serological assays37 such as ELISA and HI, which may preclude AGID as a viable field test in the face of an outbreak.

7. Japanese encephalitis virus

# 7.1 Antigen Testing

**VI**— Gold standard for diagnosis, but time-consuming and may not be suitable in the event of a foreign animal disease emergency. Virus can be replicated in multiple cell lines including chicken embryo, African green monkey kidney (Vero) cells, baby hamster kidney (BHK) cells, and the C6/36 mosquito cell line. Indirect fluorescent antibody testing via monoclonal antibodies and/or hemagglutination inhibition testing with antiserum can be used to identify the virus/differentiate from similar viruses.38 A validated virus isolation method has been published by the OIE.38 CDC Arbovirus Diagnostic Laboratory offers virus isolation.39

**RT-PCR**—Available, but has lesser sensitivity than real time RT-PCR.40

**qRT-PCR**—Multiple commercial qRT-PCR primer kits are available: Japanese Encephalitis Virus Real Time RT-PCR Kit (Liferiver Biotech, United States)41; Japanese Encephalitis Virus Non structural protein 5 (ns5) Standard Kit (Genesig, United Kingdom).42

**FA**—Not recommended by the OIE or WHO.38

**IHC**—Method is described in Yamada et al. 2004.43 Time-consuming and not suitable for large-scale testing or surveillance.

**Other**—Mei 2012 describes an antigen-capture ELISA method using polyclonal antibodies, resulting in greater sensitivity than previous ELISA methods, using human, swine or mosquito samples. This method has moderate to high sensitivity and specificity; limitations to use include the short duration of viremia in affected animals (reduced sensitivity) and cross-reaction with antigenically similar West Nile virus. However, in circumstances of herd-wide or greater outbreak, this test would be an acceptable alternative to other viral detection testing methods that require more expensive equipment, less stable reagents, or more knowledgeable staff.44

The RT-LAMP-LFD method is fast, sensitive, specific, and requires less expensive equipment that traditional RT-PCR methods. Method and primers have been described.45 A different study comparing traditional RT-PCR and qRT-PCR to the RT-LAMP method showed RT-LAMP to have similar sensitivity and specificity to qRT-PCR and higher sensitivity than traditional RT-PCR.40

# 7.2 Antibody Testing

**ELISA**—IgM ELISA kits are commercially available for human specimens; efficacy with swine samples is unknown. However, Conlan 2012 successfully modified the human-specific AFRIMS MAC ELISA to react successfully with pig sera.46

**VN**—The most specific serological technique for identifying antibodies to Japanese encephalitis virus is the plaque reduction virus neutralization test, as described in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2015. This test is both specific and sensitive, making it ideal for immunological survey.38

**CF**—Requires paired sera, with a four-fold increase or decrease in titer to be diagnostic. Method is described in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2015.38

**HI**—Requires paired sera, with a four-fold increase or decrease in titer to be diagnostic. Method is described in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2015.38

Commercially available in Korea; designed by the Animal and Plant Quarantine Agency, Anyang, Gyeongii, Rep. of Korea, produced by a contracted company, and distributed to regional diagnostic laboratories. Sensitivity and specificity were not declared.47

**Other**—Immunochromatographic testing has been developed in Korea as a quick, portable (test kit fits in the palm of a hand) alternative to HI and IFA, and has been successfully deployed in regional surveillance programs in Korea. Although sensitivity and specificity has not been determined compared to the gold standard—plaque reduction neutralization test—the benefits of the test include portability, lack of expensive equipment needed, and a turnaround time of 20 minutes.48

8. Menangle virus

# 8.1 Antigen Testing

**VI**—Virus can be grown in multiple cell lines49-52; however, several passages are needed (time-consuming process), and virus has only been successfully isolated from a few swine tissue samples (false-negatives common). Newer methods have been described for virus isolation from bat urine, but applicability to large-scale diagnostic investigations is unknown and unlikely.50,53 The test has not yet been validated.

**qRT-PCR**—Primers and methods have been described in literature.49,50

**IHC**—Standard procedures using rabbit sera containing anti-Menangle virus antibodies has been described50; sensitivity and specificity are unknown.

# 8.2 Antibody Testing

Virus neutralization is currently the only available method of detecting Menangle antibodies in porcine sera. The method has been described in literature49,50,52, and is available at only a few veterinary diagnostic laboratories in Australia (Elizabeth Macarthur Agriculture Institute, Woodbridge Rd, Menangle, NSW, Australia). This test has not been validated.

9. Nipah virus

# 9.1 Antigen Testing

**VI**—Recommended by the OIE for confirmation of clinical cases; method and cell lines are described in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial animal 2015.38

BSL 4 pathogen; additional caution must be taken when isolating this agent.

**RT-PCR**—Recommended by the OIE for confirmation of clinical cases; primers and methods described in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2015.38

**qRT-PCR**—Recommended by the OIE for confirmation of clinical cases; primers and methods described in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2015.38

Highly sensitive and specific; faster than traditional RT-PCR 38.

**FA**—Listed by OIE as a “suitable” testing method for confirmation of clinical cases; method described in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2015.38

Sensitive, but has some cross-reaction with Hendra and Nipah viruses.

**IHC**—Listed by OIE as a “suitable” testing method for confirmation of clinical cases; method described in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2015.38

May have cross-reaction with Hendra virus.

# 9.2 Antibody Testing

**ELISA**—OIE-recommended method of surveillance; currently being used as a screening tool. A standardized method is provided in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2015. Indirect ELISA specificity is just over 98%; OIE recommends testing all ELISA-positive sera with virus neutralization for confirmation.38

**VN**—Considered the reference standard by the OIE; a method is outlined in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2015.38 Cross-reaction/neutralization occurs between Hendra and Nipah viruses, with greater neutralization between homologous virus and antibody than between cross-reactive virus and antibody.

**IFA/CF/HI—**Not applicable for testing of Nipah virus per the OIE.

**OTHER**—A microsphere immuno-assay has been described and validated for detecting Hendra virus in equine sera; a lower level of reactivity to Nipah virus in pig sera may indicate that this test is suitable for adaptation to Nipah virus detection in swine. Reagents used are recombinant, reducing the level of biosafety required when handling specimens compared to traditional ELISA.54

10. Porcine adenovirus

# 10.1 Antigen Testing

**VI**— Virus can be readily grown in cell culture (porcine kidney, thyroid, and testicular cells commonly used).55-57

**PCR**—No validated method available.

**qPCR**—Not commonly used for adenovirus detection. Primers have been described in the literature; no validation available for swine specimens.58-60

**FA/IHC**—No reliable testing method for swine adenovirus is available or has been validated.

# 10.2 Antibody Testing

No serological method has been validated for the detection of porcine adenovirus antibodies.

**ELISA**—Methods have been described in other species61,62, but no methods are available for swine sera.

**VN**—The most commonly used method of serological testing; methods have been described55,56,63, but efficacy data is not available. Antibodies do not appear to be cross-reactive between serotypes57,63, indicating that sensitivity may be low.

**CF**—Methods have been described55,57, but no sensitivity/specificity data is available.

11. Porcine astrovirus

# 11.1 Antigen Testing

No commercial antigen testing is available.

**VI**—Method has been described using cell lines ESK and PK-15.64,65

**RT-PCR**—Primers and methods have been described in literature.66-70 Specificity and sensitivity are reported as high, although actual data showing efficacy is not available. Although not publicly offered at veterinary diagnostic laboratories, the UMN VDL has previously performed RT-PCR for porcine astrovirus.71

**qRT-PCR**—No reliable testing method is available.

**FA**—The method for immunofluorescence detection has been described65; no validation data is available.

**IHC**—No reliable testing method is available.

**OTHER**—Electron microscopy has been used to detect astroviruses; under EM, these viruses exhibit a distinctive five- to six-pointed-star appearance65,72 EM is time-consuming and labor-intensive, making it impractical for large-scale diagnostics.

# 11.2 Antibody Testing

No reliable antibody testing methods exist. Very few descriptions of serological analysis of porcine astrovirus exist in the literature, with none of the described methods offering any validation data. The neutralization test was utilized and described during early accounts of astrovirus diagnosis in pigs, but the method was not validated. A method utilizing a Luciferase Immunoprecipitation System assay has been described to characterize human antibody response to human-astrovirus73, and may be modifiable to porcine samples and porcine astrovirus; however, antigen prevalence studies indicate 80% or more of healthy finishing pigs have porcine astrovirus in their stool samples67,71,74 and seroprevalence may be high enough in the general population to make serological diagnosis impractical.

12. Porcine cytomegalovirus

# 12.1 Antigen Testing

**VI**—Not available commercially; has been used in research for virus detection. Methods described in literature require long turnaround time, usually greater than one week. Porcine cytomegalovirus grows in porcine lung macrophage and porcine fallopian tube cell lines.75,76

**RT-PCR**—Primers described in the literature; highly sensitive and specific with fast-turnaround time.77

**qRT-PCR**—Zoologix offers commercial qRT-PCR testing; reported as highly sensitive and specific, but supportive data is not reported on their website.78

**FA**—Not commonly used in research; not commercially available.

**IHC**—Methods have been described in the literature; low sensitivity (70%) and extensive preparation time and materials needed preclude this as a viable option for large-scale diagnostics.79

**OTHER**—Electron microscopy has been utilized to identify the virus75; however, this method is time-consuming and not applicable on a large-scale.

# 12.2 Antibody Testing

Due to high seroprevalence worldwide80-82, antibody testing may not be applicable in the event of suspected disease, except if herd was known-negative previously or paired sera show significant change in antibody levels over time indicating active infection.

**ELISA**—Method has been described.80,81,83 Most sensitive of the antibody-detecting testing methods.

**VN**—Not as sensitive as ELISA or IFA.80

**IFA**—Method has been described; not as sensitive as ELISA.80,81

**CF/HI**—Not applicable.

13. Porcine kobuvirus

# 13.1 Antigen Testing

**VI**—Has been reported for human Aichi virus but required at least 3 weeks for diagnosis.84

**RT-PCR**—Most commonly utilized method of antigen detection; highly sensitive and specific; primers have been published numerous times.

**qRT-PCR**—Not commonly used; a method has been described85 but no validation has been conducted on the method. Has been used for human kobuvirus detection with increased sensitivity over traditional RT-PCR methods.86

**FA/IHC**—Not developed and/or not used for porcine kobuvirus detection.

# 13.2 Antibody Testing

No reports of antibody testing for porcine kobuvirus exist.

14. Porcine rubulavirus (“blue eye”)

# 14.1 Antigen Testing

**VI**—Historically used for virus identification. Methods have been described in various publications; cytopathic effects can be seen within 72 hours.87,88 Modern qRT-PCR techniques appear to have higher sensitivity. Not available at commercial laboratories within the United States; available through NVSL-Ames.

**RT-PCR**—A nested RT-PCR has been described for research purposes, but no efficacy data is available.89

**qRT-PCR**—A recently described method has been shown to have higher sensitivity and specificity than virus isolation,90 and may be suitable for large-scale detection in the face of an outbreak.

**FA**/**IHC**—Techniques for avidin/biotin peroxidase immunohistochemistry and direct immunofluorescence assay have been described with reportedly high correlation to clinical and histological signs noted; however, no efficacy data was established.91,92 Furthermore, these tests are post-mortem only, and were tested on piglets—diagnostic application to adult animals is unknown.

# 14.2 Antibody Testing

**ELISA**—Indirect-ELISA has low sensitivity and specificity and is not useful in identifying the disease if prevalence is low; may be useful for screening in the face of an outbreak if it can be modified into a field-test kit. There is one description of a blocking-ELISA with sensitivity of 99% and specificity of 97%, making it a viable option for quick, inexpensive testing of large numbers of field samples; however, no further work or comparisons were identified, and testing was only performed on one strain of rubulavirus (LPMV), so actual field application and efficacy with various strains is unknown.93

**VN**—Has been described numerous times in the literature; more time-consuming and labor-intensive than HI, but has higher sensitivity (95.65%) and specificity (100%) than HI (70.83%, 100%) or ELISA (73.91%, 82.35%).94

**IFA/CF—**Not commonly used or available for porcine rubulavirus antibody testing.

**HI—**Lower sensitivity than VN, but more easily used in mass-quantity field testing and epidemiological surveys; requires antigen for testing—in a rubulavirus-free zone, an inactivated antigen with full hemagglutinating and antigenic characteristics such as described in Giron et al. 200695 can be used. Not available at commercial laboratories within the United States; available through NVSL-Ames.

15. Porcine sapelovirus

# 15.1 Antigen Testing

**VI**—Virus is readily grown in porcine kidney-15 (PK-15) cells, and has a unique cytopathic effect relative to other related picornaviruses. Methods have been described often in literature 96-98, but are time-consuming and expensive; molecular detection methods appear to be more useful when testing large numbers of specimens or when time is of essence.

**RT-PCR**—Methods have been described in literature99,100; sensitivity is very high (down to 0.36 TCID/100μL), but specificity was only measured against primary swine pathogens that are not closely related to porcine sapelovirus, and so are less likely to cross-react than porcine teschoviruses or other porcine enteroviruses. More testing to determine accurate specificity is recommended before utilizing this test in field conditions. Primers used in the nested-RT-PCR method described in Zell et al. 2000 appear to be specific when tested on related virus species, and may be a viable alternative, but no sensitivity and specificity comparison data was provided.96

**qRT-PCR**—A method has been documented for the real-time RT-PCR detection of porcine sapeloviruses, with primers described. The primers for porcine sapelovirus were sensitive and specific, not reacting with porcine teschoviruses or porcine enteroviruses.

**FA**/**IHC**—Not commonly used for detection of porcine sapelovirus.

**OTHER**—An RT-LAMP procedure was recently published, with primers; sensitivity was high (able to detect down to 10 copies/μL RNA) and highly specific (100%) with no cross-reaction with other viruses within the same family (*Picornaviridae*). This testing method is more rapid and less expensive than traditional and quantitative RT-PCR techniques.101

# 15.2 Antibody Testing

No reliable tests to detect porcine sapelovirus antibodies exist or are described in literature. Some literature alludes to antibody testing in the past, when porcine sapelovirus was considered a serotype of porcine enterovirus, but no research specific to sapelovirus seroprevalence and serodetection were identified during literature searches.

16. Porcine sapovirus

# 16.1 Antigen Testing

**VI**—Not available; does not readily grow in cell culture.

**RT-PCR**—Moderately sensitive and specific for porcine sapovirus; primers have been described in the literature for a capsid region102,103 from porcine sapovirus—Cowden strain. Cross-reactivity to non-Cowden sapoviruses is unknown (such as an unknown, genetically different porcine sapovirus), but unlikely due to the genetic variation within caliciviruses.

**qRT-PCR**—Approximately 10-fold more sensitive than traditional RT-PCR; specificity is moderate, and field trials showed some detection of porcine norovirus GGII.11B in addition to porcine sapovirus.104 This test may be applicable in field conditions due to sensitivity and fast processing time.

**FA/IHC**—No verified or applicable testing method available; immunocytochemistry was briefly described in literature for verification of growth of a vector plasmid containing antigen proteins in culture,105 but is not applicable for diagnostic field testing.

# 16.2 Antibody Testing

Due to the difficulty in cultivating caliciviruses (including porcine sapoviruses), serological assays utilizing whole, live virus are not available.

**ELISA**—Two antibody-detecting ELISAs have been developed for porcine sapovirus: a fixed-cell ELISA and a VLP (virus-like particle) ELISA. Both showed high sensitivity, detecting all known positive samples during the trial; however, efficacy for the ELISAs was tested using recombinant proteins, and although the ELISAs were tested on sow sera, cross-reactivity with other strains of porcine sapovirus is unknown.103,105

17. Porcine teschovirus

# 17.1 Antigen Testing

**VI**—A preferred test by OIE standards. Method is described in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2015.38 Available through NVSL-Ames and FADDL-Plum Island. Labor-intensive and time-consuming method; estimated turnaround time is 25 days and 14 days for Ames and Plum Island, respectively.

**RT-PCR/qRT-PCR**—Not yet accepted by OIE for diagnosis. Methods and primers have been described in literature for both traditional RT-PCR96,99 and quantitative RT-PCR.106 RT-PCR available at some veterinary diagnostic laboratories as well as at NVSL-Ames and FADDL-Plum Island, 3 day turnaround for each.

**IFA—**A preferred test by OIE standards. Method is described in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2015.38 Available through NVSL-Ames, 5–7 day turnaround.

**IHC**—Technique is described in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2015; high levels of false-negatives make this technique unsuitable for large-scale diagnostic testing.38

# 17.2 Antibody Testing

Due to high seroprevalence worldwide, the OIE does not recommend serology as a method of diagnosis, unless with paired sera showing a four-fold or greater increase/decrease in conjunction with clinical symptoms.38 Antigen testing is preferred for definitive diagnosis.

**ELISA/VN**—If screening of serology is necessary, ELISA and/or VN in microtiter plates is the preferred method of the OIE; methods are described in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2015.38 VN is available through FADDL-Plum Island.

18. Porcine torovirus

# 18.1 Antigen Testing

**VI**—No available; porcine torovirus does not grow in cell culture.

**RT-PCR**—Primers for both a porcine torovirus-specific fragment of the N gene and for full coding sequences have been described107-109 but sensitivity/specificity data was not given.

**qRT-PCR**—Primers and a method for SYBR-Green detection and melting temperature (Tm) analysis is described in the literature; sensitivity and specificity are both declared as high, although the author does note that the reaction will also detect and amplify bovine torovirus, due to the similarity of the N gene.110 This is likely to occur with other real-time and quantitative RT-PCR tests utilizing primers for the N gene region.

**FA**—A method has been described in literature; however, no sensitivity/specificity data is available.111

**IHC**—Not commonly used in research; no efficacy data has been published. Unlikely to be of use in large-scale diagnostic testing due to the increased labor and time needed over real-time RT-PCR.

**OTHER**—In 2014, Newport Laboratories sequenced the first United States isolate of porcine torovirus112; they may provide other diagnostics, such as RT-PCR, in a time of need.

# 18.2 Antibody Testing

High seroprevalence/endemicity of porcine torovirus has been documented in several countries109,113,114; because of this, serological testing may not be useful for diagnosis of cases or outbreaks. No testing appears to be available at commercial laboratories within the United States.

**ELISA**—Methods for ELISA detection of antibodies has been published.107,111 ELISA is sensitive and specific, and is less-costly than other methods for testing of large numbers of samples

**VN**—Plaque neutralization methods have been published, utilizing bovine torovirus107, and a heterologous neutralization assay utilizing equine torovirus has also been described.109 Equine and bovine torovirus cross-react with porcine torovirus antibodies; as such, specificity to porcine torovirus in particular is not possible with these tests. Sensitivity of either method is unknown.

**IFA/CF**—Not commonly used for torovirus detection; no efficacy data available.

**HI**—A method utilizing DFP-inactivated HE52.7 and HE52.11 cell lysates has been described; this method avoids artifacts due to the esterase activity hemagglutinin-esterase molecule. HI testing utilizing these antigens appeared to be specific to the particular strain that the modified antigen was derived from111; sensitivity across strains may be too low to be useful as a large-scale diagnostic method.

**OTHER**— Western blot assay has been documented as a method to confirm ELISA positives.107

19. Pseudorabies virus

# 19.1 Antigen Testing

**VI**—Gold standard method of antigen detection; method published in OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals.38

**qRT-PCR**—A commercially-available, qualitative real-time PCR kit (ADIAVET PRV REALTIME) has been validated by an OIE reference laboratory for use in surveillance and/or routine diagnosis by local veterinary laboratories; the kit is non-species-specific.115 The USDA ARS has also validated a real-time dual PCR method utilizing two genes that allows for differentiation between wildtype and commercial marker vaccine; sensitivity was high (>94%) but specificity was low (79% at best).116

**FA/IHC**—Not recommended for pseudorabies virus detection. Methods have been published in the literature, but not validated for detection in large-scale testing situations.117,118

# 19.2 Antibody Testing

**ELISA**—OIE prescribed test for international trade. Commercial ELISAs are available both within the United States as well as worldwide. Licensed kits include: PRV antibody test kit (Calbiotech Veterinary Diagnostics); PRV gB antibody test kit (IDEXX Laboratories Inc.); and PRV gpI antibody test kit (IDEXX Laboratories Inc.).119 Sensitivity of ELISA is generally higher than 1-hour virus neutralization.38

**VN**—OIE prescribed test for international trade; method published in OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals.38 Cannot be used to differentiate between wildtype and commercial vaccine virus.

**IFA/CF/HI**—Not recommended by the OIE for detection of antibody. Methods have been described in literature, but not validated for detection of pseudorabies antibody in outbreak situations.118

**Other**—Latex agglutination has been utilized in research and field settings for detection of pseudorabies antibodies120 with similar level of sensitivity to other serological tests including ELISA.121,122

20. Sendai virus

# 20.1 Antigen Testing

No antigen testing methods for swine specimens have been validated for Sendai virus detection; however, virus isolation and molecular diagnostics are commonly used in laboratory rodent specimens and would likely be applicable to swine specimens.

**VI**—In rodents, Sendai virus is readily isolated from multiple cell cultures. A method for isolating a paramyxovirus (an unidentified and two variants of bovine parainfluenzavirus 3) from swine specimens has been described using fetal porcine kidney (FPK) cells123,124, and may be applicable for culturing Sendai virus from swine specimens.

**qRT-PCR**—Commercial primer kits for Sendai virus real-time RT-PCR are available (Thermo-Fischer).125

# 20.2 Antibody Testing

Diagnostic tests for Sendai virus antibodies in swine are not available. Historically, HI, VN and CF have been used; however, specific methods utilized are not available and there appears to be poor agreement between testing methods.126 In mice, ELISA and IFA are preferred over HI and CF, due to the lower sensitivity and specificity of the latter set of tests.127

**ELISA**—Commercial ELISA test kits are available for laboratory rodents (e.g., mouse, hamster, etc.); no data on efficacy of these kits in pigs is available.

21. Seneca Valley virus

# 21.1 Antigen Testing

**VI**—Methods have been described.128 Sensitivity and specificity are unknown.

**RT-PCR**—Available at some veterinary diagnostic laboratories (e.g., ISU VDL; UMN VDL, unvalidated; Kansas State University; South Dakota State University; etc.). Although not listed in the FADDL-Plum Island diagnostic catalog, veterinarians have sent samples for RT-PCR testing for Seneca Valley virus to Plum Island. Primers have been published.129,130

**qRT-PCR**—Not currently available.

**IHC**—Method has been described128, but is not yet validated.

# 21.2 Antibody Testing

No test detecting Seneca Valley virus antibody is commercially available.

**ELISA**—A method has been published in the literature.128 The ISU VDL and UMN VDL are reportedly in the process of creating and validating ELISA methods.

**VN**—Method has been described128,129; no validation tests were conducted—however, a comparison with the cELISA developed showed similar levels of detection.128

**IFA/CF/HI**—No reliable testing methods are available.

22. Swine papillomavirus

# 22.1 Antigen Testing

**VI**—Method has been described; cell lines of porcine origin appear to be most effective for culture of swine papillomavirus.131 However, virus isolation is not commonly used due to the unreliability of the method for this virus, and would not likely be a suitable choice for large-scale diagnostic testing.

**RT-PCR**—Currently the most common method used for papillomavirus detection in literature; primers have been described.132 Sensitivity is reported as high; specificity within the *Papillomaviridae* is designed as low, so that the test will detect novel papillomaviruses.

**qRT-PCR**/**FA**—No reliable testing method available.

**IHC**—A method has been described133; sensitivity appears to be quite low, however, and modifications to the method would be recommended before using as a diagnostic tool.

# 22.2 Antibody Testing

Detection methods for swine papillomavirus antibody have not been published; no reliable method exists. One description of a serum neutralization test was published131, but validation has not been conducted, and no repeated applications of the method appear to have been conducted since the publication in 1972.

23. Swine pox virus

# 23.1 Antigen Testing

**VI**—Virus isolation methods have been described134-136, but are not commonly used for diagnosis due to the increased speed and sensitivity of PCR assays.

**PCR**—A rapid duplex PCR assay has recently been developed for simultaneous and independent detection of swinepox virus and vaccinia virus; no cross-reaction with 9 other related pox-viruses was evident (high specificity) and the PCR assay was able to detect down to 0.1ng/reaction of viral DNA (high sensitivity).137 Primers were also published in another recent research paper138, but no sensitivity/specificity data was published.

**qPCR**—There is no record of quantitative PCR being used for swinepox diagnosis.

**FA/IHC**—Methods have been described, but are not commonly used for swinepox diagnosis, and no sensitivity/specificity data exists.135 Monoclonal antibodies specific for swinepox virus have been described, and would be useful for antigen detection.139

**OTHER**—Electron microscopy on formalin-fixed skin, skin homogenate, or vesicular fluid has been used for diagnosis through the demonstration of poxvirus-like particles in conjunction with clinical signs136,140; however, other pox-viruses (such as vaccinia virus) can cause similar symptoms in swine, and may result in misdiagnosis using EM alone due to morphological similarity.135

# 23.2 Antibody Testing

No reliable or commercially available testing method for swinepox antibodies has been described. Some literature has described attempts at characterizing the immune response to swinepox, but methods such as virus neutralization, ELISA, or immunofluorescent assay have either failed to detect swinepox antibodies or were not expanded upon in research.141,142 One description of an indirect-ELISA used for swinepox antibody detection in sows was located, but details were unavailable.143

24. Vesicular exanthema of swine virus

# 24.1 Antigen Testing

For diseases causing vesicular lesions, the OIE considers the following tests to be acceptable: antigen-binding ELISA, RT-PCR or qRT-PCR, or virus isolation.38

**VI**—Reference method for all vesicular diseases. Standardized methods for virus isolation of vesicular diseases has been described by the OIE 38.

**RT-PCR**—A multiplex RT-PCR and microarray assay has been developed and primers published for simultaneous detection and differentiation between foot-and-mouth disease (FMDV), vesicular stomatitis virus (VSV), swine vesicular disease virus (SVDV), and vesicular exanthema of swine virus (VESV). Specificity was listed as 100%; sensitivity is slightly lower than real-time RT-PCR. Due to small sample size, further validation was recommended before adoption as a diagnostic test144; upon further validation, sensitivity of 93.9% and specificity of 98.1% were determined.145 Traditional RT-PCR is significantly less sensitive than virus isolation in IB-RS-2 cells.146

**rRT-PCR**—Primers for real-time RT-PCR detection of marine caliciviruses have been published; sensitivity was high with a detection limit similar to VI, and specificity was high—no cross-reaction was noted with any other vesicular disease-causing virus.147

**FA/IHC**—Not OIE-preferred tests for diagnosis of vesicular diseases. Methods have been described in literature; sensitivity/specificity unknown.148,149

**OTHER**—An Ag-capture ELISA to detect vesicular exanthema of swine virus, San Miguel sea lion virus, and other caliciviruses has been described.150 San Miguel sea lion viruses and other marine calic Miguel sea lion viruses and other marine caliciviruses is described.

# 24.2 Antibody Testing

Anti-sera has historically been acquired from Plum Island-FADDL (called PIADC at that time).151

**ELISA**—No commercial test kit is available, but testing procedures have been described in the literature.150 Sensitivity and specificity data are unclear; all comparisons were made with guinea-pig antisera from a single inoculation, and authors noted differences on cross-reactions between serotypes upon repeat of the procedure.

**VN**—Has been described in literature.151 Serotype specific; may not be useful for testing for unknown virus. Time-consuming method; can require several days.

**IFA--** A method for IFA antibody detection has been documented152; sensitivity was low (82%) but higher than VN (12%) when compared using the same sera. Specificity was high—no cross-reaction was noted to other major swine pathogens showing similar clinical symptoms. The specificity of this assay was not tested using non-porcine caliciviruses.

**CF**—Applicable in large-scale diagnostic situations due to rapid speed and high specificity151; has been used extensively for diagnosis of vesicular diseases. Serotype specific; may not be useful for testing for unknown virus.

**HI**—No reliable, validated testing methods available.

**OTHER**— AGID has been attempted previously; however, due to cross-reaction between all tested serotypes of vesicular exanthema of swine virus, the assay was not applicable for serotype differentiation.151 It may be useful for as a screening method, but would likely be less useful than ELISA and other testing methods due to time and labor intensity.

25. Vesicular stomatitis virus

# 25.1 Antigen Testing

OIE recommends only using agent identification tests on animals with clinical symptoms; negative results do not rule out disease, but could indicate that the animal is no longer actively shedding 38.

**VI**— Available at NVSL (Ames and Plum Island). Six day turnaround, Plum Island; seven day turnaround, Ames. Recommended method of clinical case confirmation by OIE; method described in OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2015.

**RT-PCR/qRT-PCR** — Acceptable method of clinical case confirmation by OIE.38 A multiplex qRT-PCR and microarray for VSV, FMDV, VESV, and SVDV has been described; although time to results is slightly longer than real time RT-PCR, sensitivities and specificities were similar, and the test was able to accurately differentiate between 49 strains of the vesicular viruses.144 Available at NVSL (Ames and Plum Island). One day turnaround.

**FA**—Not recommended by the OIE for testing for vesicular stomatitis.38

**IHC**— Described in literature, not typically used in vesicular stomatitis virus diagnosis except in research. Not recommended by the OIE for testing for vesicular stomatitis.38

**OTHER**—Ag-capture ELISA available at NVSL (Plum Island). One day turnaround. Recommended method of clinical case confirmation by OIE; method described in OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2015.38

# 25.2 Antibody Testing

OIE states that serological testing does not differentiate between current vs. previous exposure; use combination testing or paired sera showing four-fold titer increase or decrease to determine current status.38

**ELISA**—Ab-capture ELISA offered by NVSL (Plum Island). One day turnaround.

c-ELISA available at NVSL (Ames). Two day turnaround. Not recommended by OIE for confirmation of clinical cases.38

**VN**—Elaborate and time-consuming.38 Available at some veterinary diagnostic laboratories (UMN VDL, ISU VDL, Washington ADDL-cattle only) and at NVSL (Ames and Plum Island). Three day turnaround, Ames and Plum Island. Low serum concentrations may lead to nonspecific neutralization and hence false positive results.153

**IFA**— Described in literature, not typically used in vesicular stomatitis diagnosis except in research. Not recommended by the OIE for testing for vesicular stomatitis.38

**CF**— A prescribed test for international trade; can be used to quantify early antibody levels.38 Available at NVSL (Ames and Plum Island). One day turnaround, Plum Island; two day turnaround, Ames.

**HI**—Not applicable for vesicular stomatitis.

References

1. Duke GM, Hoffman MA, Palmenberg AC. Sequence and structural elements that contribute to efficient encephalomyocarditis virus RNA translation. *J Virol.* 1992;66(3):1602-1609.

2. Vanderhallen H, Koenen F. Rapid diagnosis of encephalomyocarditis virus infections in pigs using a reverse transcription-polymerase chain reaction. *J Virol Methods.* 1997;66(1):83-89.

3. Yuan W, Zheng Y, Sun M, Zhang X, Qi Y, Sun J. Development of a TaqMan-based real-time reverse transcription polymerase chain reaction assay for the detection of encephalomyocarditis virus. *J Virol Methods.* 2014;207:60-65.

4. Zoologix. Encephalomyocarditis. <http://www.zoologix.com/primate/Datasheets/Encephalomyocarditis.htm>. Accessed February 2, 2016.

5. Yuan W, Wang J, Sun M, et al. Rapid detection of encephalomyocarditis virus by one-step reverse transcription loop-mediated isothermal amplification method. *Virus Res.* 2014;189:75-78.

6. Feng R, Wei J, Zhang H, et al. National serosurvey of encephalomyocarditis virus in healthy people and pigs in China. *Arch Virol.* 2015.

7. ISU VDPAM. EMC. *Swine Disease Manual* 2015. Accessed September 23, 2015.

8. Koenen F. Encephalomyocarditis virus. 9th ed. Diseases of Swine: Blackwell Publishing; 2006:331-336.

9. Centers for Disease Prevention and Control (CDC). Questions and Answers about Ebola-Reston virus in pigs, Phillipines. 2013. Accessed September 23, 2015.

10. Saijo M, Niikura M, Ikegami T, Kurane I, Kurata T, Morikawa S. Laboratory diagnostic systems for Ebola and Marburg hemorrhagic fevers developed with recombinant proteins. *Clin Vaccine Immunol.* 2006;13(4):444-451.

11. Sayama Y, Demetria C, Saito M, et al. A seroepidemiologic study of Reston ebolavirus in swine in the Philippines. *BMC Vet Res.* 2012;8:82.

12. Boisen ML, Oottamasathien D, Jones AB, et al. Development of prototype filovirus recombinant antigen immunoassays. *J Infect Dis.* 2015;212 Suppl 2:S359-367.

13. Dong D, Fu SH, Wang LH, Lv Z, Li TY, Liang GD. Simultaneous detection of three arboviruses using a triplex RT-PCR: enzyme hybridization assay. *Virol Sin.* 2012;27(3):179-186.

14. Bannai H, Nemoto M, Ochi A, et al. Epizootiological investigation of Getah virus infection among racehorses in Japan in 2014. *J Clin Microbiol.* 2015;53(7):2286-2291.

15. Wekesa SN, Inoshima Y, Murakami K, Sentsui H. Genomic analysis of some Japanese isolates of Getah virus. *Vet Microbiol.* 2001;83(2):137-146.

16. Nemoto M, Bannai H, Tsujimura K, et al. Getah Virus infection among racehorses, Japan, 2014. *Emerg Infect Dis.* 2015;21(5):883-885.

17. Hou Y, Tang TS, Jiang Y, et al., Inventors. Swine getah virus reverse transcription-polymerase chain reaction (RT-PCR) detection kit and application thereof. 2011.

18. Hohdatsu T, Ide S, Yamagishi H, et al. Enzyme-linked immunosorbent assay for the serological survey of Getah virus in pigs. *Nihon Juigaku Zasshi.* 1990;52(4):835-837.

19. Fukunaga Y, Kumanomido T, Kamada M. Getah virus as an equine pathogen. *Vet Clin North Am Equine Pract.* 2000;16(3):605-617.

20. Ha SK, Chae C. Immunohistochemistry for the detection of swine hepatitis E virus in the liver. *J Viral Hepat.* 2004;11(3):263-267.

21. Engle RE, Yu C, Emerson SU, Meng XJ, Purcell RH. Hepatitis E virus (HEV) capsid antigens derived from viruses of human and swine origin are equally efficient for detecting anti-HEV by enzyme immunoassay. *J Clin Microbiol.* 2002;40(12):4576-4580.

22. Zhang H, Mohn U, Prickett JR, et al. Differences in capabilities of different enzyme immunoassays to detect anti-hepatitis E virus immunoglobulin G in pigs infected experimentally with hepatitis E virus genotype 3 or 4 and in pigs with unknown exposure. *J Virol Methods.* 2011;175(2):156-162.

23. DSI S.R.L. Hepatitis E. 2015; <http://dsitaly.com/data/pages/products/re_files/HEV.pdf>. Accessed September 28, 2015.

24. Drobeniuc J, Meng J, Reuter G, et al. Serologic assays specific to immunoglobulin M antibodies against hepatitis E virus: pangenotypic evaluation of performances. *Clin Infect Dis.* 2010;51(3):e24-27.

25. Yang F, Tang Z, Wang S, et al. [The establishment of high-throughput neutralization titer evaluation model for hepatitis E virus (HEV)]. *Bing Du Xue Bao.* 2015;31(1):1-6.

26. Kimura H, Abiko C, Peng G, et al. Interspecies transmission of influenza C virus between humans and pigs. *Virus Res.* 1997;48(1):71-79.

27. Pabbaraju K, Wong S, Wong A, May-Hadford J, Tellier R, Fonseca K. Detection of influenza C virus by a real-time RT-PCR assay. *Influenza Other Respir Viruses.* 2013;7(6):954-960.

28. Brown IH, Harris PA, Alexander DJ. Serological studies of influenza viruses in pigs in Great Britain 1991-2. *Epidemiol Infect.* 1995;114(3):511-520.

29. Yamaoka M, Hotta H, Itoh M, Homma M. Prevalence of antibody to influenza C virus among pigs in Hyogo Prefecture, Japan. *J Gen Virol.* 1991;72 ( Pt 3):711-714.

30. Manuguerra JC, Hannoun C. Natural infection of dogs by influenza C virus. *Res Virol.* 1992;143(3):199-204.

31. Kawano J, Onta T, Kida H, Yanagawa R. Distribution of antibodies in animals against influenza B and C viruses. *Jpn J Vet Res.* 1978;26(3-4):74-80.

32. Hause BM, Collin EA, Liu R, et al. Characterization of a novel influenza virus in cattle and Swine: Proposal for a new genus in the *Orthomyxoviridae* family. *MBio.* 2014;5(2):e00031-00014.

33. Collin EA, Sheng Z, Lang Y, Ma W, Hause BM, Li F. Cocirculation of two distinct genetic and antigenic lineages of proposed influenza D virus in cattle. *J Virol.* 2015;89(2):1036-1042.

34. Jiang WM, Wang SC, Peng C, et al. Identification of a potential novel type of influenza virus in bovine in China. *Virus Genes.* 2014;49(3):493-496.

35. Hause BM, Ducatez M, Collin EA, et al. Isolation of a novel swine influenza virus from Oklahoma in 2011 which is distantly related to human influenza C viruses. *PLoS Pathog.* 2013;9(2):e1003176.

36. Ducatez MF, Pelletier C, Meyer G. Influenza D virus in cattle, France, 2011-2014. *Emerg Infect Dis.* 2015;21(2):368-371.

37. Peng D, Hu S, Hua Y, et al. Comparison of a new gold-immunochromatographic assay for the detection of antibodies against avian influenza virus with hemagglutination inhibition and agar gel immunodiffusion assays. *Vet Immunol Immunopathol.* 2007;117(1-2):17-25.

38. World Organization for Animal Health (OIE). OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Paris, France: Office International des Epizooties; 2015.

39. Centers for Disease Control and Prevention (CDC). Instructions for Submitting Diagnostic Specimens to the DVBD Arbovirus Diagnostic Laboratory. 2014. Accessed September 23, 2015.

40. Dhanze H, Bhilegaonkar KN, Ravi Kumar GV, et al. Comparative evaluation of nucleic acid-based assays for detection of Japanese encephalitis virus in swine blood samples. *Arch Virol.* 2015;160(5):1259-1266.

41. LifeRiver Biotech. JEV Real-Time RT-PCR Kit. <http://www.liferiverbiotech.com/Pages/Product/ProductDetail.aspx?productId=411>. Accessed February 2, 2016.

42. Genesig. Japanese encephalitis virus. <http://www.genesig.com/products/9321>. Accessed February 2, 2016.

43. Yamada M, Nakamura K, Yoshii M, Kaku Y. Nonsuppurative encephalitis in piglets after experimental inoculation of Japanese encephalitis flavivirus isolated from pigs. *Vet Pathol.* 2004;41(1):62-67.

44. Mei L, Wu P, Ye J, et al. Development and application of an antigen capture ELISA assay for diagnosis of Japanese encephalitis virus in swine, human and mosquito. *Virol J.* 2012;9:4.

45. Deng J, Pei J, Gou H, Ye Z, Liu C, Chen J. Rapid and simple detection of Japanese encephalitis virus by reverse transcription loop-mediated isothermal amplification combined with a lateral flow dipstick. *J Virol Methods.* 2015;213:98-105.

46. Conlan JV, Vongxay K, Jarman RG, et al. Serologic study of pig-associated viral zoonoses in Laos. *Am J Trop Med Hyg.* 2012;86(6):1077-1084.

47. Japanese encephalitis. Serologic tests. *OIE Reference Laboratory*.

48. Cha GW, Lee EJ, Lim EJ, et al. A novel immunochromatographic test applied to a serological survey of Japanese encephalitis virus on pig farms in Korea. *PLoS One.* 2015;10(5):e0127313.

49. Bowden TR, Bingham J, Harper JA, Boyle DB. Menangle virus, a pteropid bat paramyxovirus infectious for pigs and humans, exhibits tropism for secondary lymphoid organs and intestinal epithelium in weaned pigs. *J Gen Virol.* 2012;93(Pt 5):1007-1016.

50. Barr JA, Smith C, Marsh GA, Field H, Wang LF. Evidence of bat origin for Menangle virus, a zoonotic paramyxovirus first isolated from diseased pigs. *J Gen Virol.* 2012;93(Pt 12):2590-2594.

51. Philbey AW, Ross AD, Kirkland PD, Love RJ. Skeletal and neurological malformations in pigs congenitally infected with Menangle virus. *Aust Vet J.* 2007;85(4):134-140.

52. Kirkland PD, Love RJ, Philbey AW, Ross AD, Davis RJ, Hart KG. Epidemiology and control of Menangle virus in pigs. *Aust Vet J.* 2001;79(3):199-206.

53. Barr J, Smith C, Smith I, et al. Isolation of multiple novel paramyxoviruses from pteropid bat urine. *J Gen Virol.* 2015;96(Pt 1):24-29.

54. McNabb L, Barr J, Crameri G, et al. Henipavirus microsphere immuno-assays for detection of antibodies against Hendra virus. *J Virol Methods.* 2014;200:22-28.

55. Haig DA, Clarke MC, Pereira MS. Isolation of an adenovirus from a pig. *J Comp Pathol.* 1964;74:81-84.

56. Hirahara T, Yasuhara H, Matsui O, et al. Growth activity of porcine adenoviruses in primary porcine testicular cell cultures. *Nihon Juigaku Zasshi.* 1990;52(5):1089-1091.

57. Hirahara T, Yasuhara H, Matsui O, et al. Isolation of porcine adenovirus from the respiratory tract of pigs in Japan. *Nihon Juigaku Zasshi.* 1990;52(2):407-409.

58. Bofill-Mas S, Rusiñol M, Fernandez-Cassi X, Carratalà A, Hundesa A, Girones R. Quantification of human and animal viruses to differentiate the origin of the fecal contamination present in environmental samples. *Biomed Res Int.* 2013;2013:192089.

59. Hundesa A, Maluquer de Motes C, Albinana-Gimenez N, et al. Development of a qPCR assay for the quantification of porcine adenoviruses as an MST tool for swine fecal contamination in the environment. *J Virol Methods.* 2009;158(1-2):130-135.

60. Wolf S, Hewitt J, Greening GE. Viral multiplex quantitative PCR assays for tracking sources of fecal contamination. *Appl Environ Microbiol.* 2010;76(5):1388-1394.

61. Anderson LJ, Godfrey E, McIntosh K, Hierholzer JC. Comparison of a monoclonal antibody with a polyclonal serum in an enzyme-linked immunosorbent assay for detecting adenovirus. *J Clin Microbiol.* 1983;18(3):463-468.

62. Harmon MW, Drake S, Kasel JA. Detection of adenovirus by enzyme-linked immunosorbent assay. *J Clin Microbiol.* 1979;9(3):342-346.

63. Hammond JM, Johnson MA. Porcine adenovirus as a delivery system for swine vaccines and immunotherapeutics. *Vet J.* 2005;169(1):17-27.

64. Indik S, Valícek L, Smíd B, Dvoráková H, Rodák L. Isolation and partial characterization of a novel porcine astrovirus. *Vet Microbiol.* 2006;117(2-4):276-283.

65. Shimizu M, Shirai J, Narita M, Yamane T. Cytopathic astrovirus isolated from porcine acute gastroenteritis in an established cell line derived from porcine embryonic kidney. *J Clin Microbiol.* 1990;28(2):201-206.

66. Monini M, Di Bartolo I, Ianiro G, et al. Detection and molecular characterization of zoonotic viruses in swine fecal samples in Italian pig herds. *Arch Virol.* 2015;160(10):2547-2556.

67. Luo Z, Roi S, Dastor M, Gallice E, Laurin MA, L'homme Y. Multiple novel and prevalent astroviruses in pigs. *Vet Microbiol.* 2011;149(3-4):316-323.

68. Blomström AL, Ley C, Jacobson M. Astrovirus as a possible cause of congenital tremor type AII in piglets? *Acta Vet Scand.* 2014;56(1):82.

69. Laurin MA, Dastor M, L'homme Y. Detection and genetic characterization of a novel pig astrovirus: relationship to other astroviruses. *Arch Virol.* 2011;156(11):2095-2099.

70. Lee S, Jang G, Lee C. Complete genome sequence of a porcine astrovirus from South Korea. *Arch Virol.* 2015;160(7):1819-1821.

71. Mor SK, Chander Y, Marthaler D, Patnayak DP, Goyal SM. Detection and molecular characterization of Porcine astrovirus strains associated with swine diarrhea. *J Vet Diagn Invest.* 2012;24(6):1064-1067.

72. Shirai J, Shimizu M, Fukusho A. Coronavirus-, calicivirus-, and astrovirus-like particles associated with acute porcine gastroenteritis. *Nihon Juigaku Zasshi.* 1985;47(6):1023-1026.

73. Burbelo PD, Ching KH, Esper F, et al. Serological studies confirm the novel astrovirus HMOAstV-C as a highly prevalent human infectious agent. *PLoS One.* 2011;6(8):e22576.

74. Amimo JO, Okoth E, Junga JO, et al. Molecular detection and genetic characterization of kobuviruses and astroviruses in asymptomatic local pigs in East Africa. *Arch Virol.* 2014;159(6):1313-1319.

75. Shirai J, Narita M, Iijima Y, Kawamura H. A cytomegalovirus isolated from swine testicle cell culture. *Japan J Vet Sci.* 2015;47(5):697-703.

76. Kawamura H, Tajima T, Hironao T, Kajikawa T, Kotani T. Replication of porcine cytomegalovirus in the 19-PFT cell line. *J Vet Med Sci.* 1992;54(6):1209-1211.

77. Hamel AL, Lin L, Sachvie C, Grudeski E, Nayar GPS. PCR Assay for detecting porcine cytomegalovirus. *J Clin Microbiol.* 1999;37(11):3767-3768.

78. Zoologix. Porcine cytomegalovirus. <http://www.zoologix.com/avian/Datasheets/PorcineCytomegalovirus.html>. Accessed February 2, 2016.

79. Sekiguchi M, Shibahara T, Miyazaki A, et al. In situ hybridization and immunohistochemistry for the detection of porcine cytomegalovirus. *J Virol Methods.* 2012;179(1):272-275.

80. Tajima T, Hironao T, Kajikawa T, Kawamura H. Application of enzyme-linked immunosorbent assay for the seroepizootiological survey of antibodies against porcine cytomegalovirus. *J Vet Med Sci.* 1993;55(3):421-424.

81. Assaf R, Bouillant AM, Di Franco E. Enzyme linked immunosorbent assay (ELISA) for the detection of antibodies to porcine cytomegalovirus. *Can J Comp Med.* 1982;46(2):183-185.

82. Plowright W, Edington N, Watt RG. The behaviour of porcine cytomegalovirus in commercial pig herds on JSTOR. *J Hygiene.* 1975;76(1):125-135.

83. Liu X, Zhu L, Shi X, et al. Indirect-blocking ELISA for detecting antibodies against glycoprotein B (gB) of porcine cytomegalovirus (PCMV). *J Virol Methods.* 2012;186(1-2):30-35.

84. Yamashita T, Kobayashi S, Sakae K, et al. Isolation of Cytopathic Small Round Viruses with BS-C-1 Cells from Patients with Gastroenteritis on JSTOR. *J Infect Dis.* 1991;164(5):954-957.

85. Xiu J-s, Chen X-q, Wang B, Li T. Establishment of a real-time RT-PCR method based on SYBR Green for diagnosis of porcine kobuvirus. *Chinese J Zoonoses.* 2012;9:015.

86. Houde A, Poitras E, Leblanc D, et al. Aichi virus in Alberta, Canada: A one year examination of human diarrheic stool samples by RT-PCR. *Appl Microbiol Microb Biotechnol.* 2010:767-772.

87. Stephan HA, Gay GM, Ramírez TC. Encephalomyelitis, reproductive failure and corneal opacity (blue eye) in pigs, associated with a paramyxovirus infection. *Vet Rec.* 1988;122(1):6-10.

88. Reyes-Leyva J, Espinosa B, Santos G, et al. Purification and characterization of the Hemagglutinin-Neuraminidase o. *Glycoconjugate J.* 1999;16(9):517-522.

89. Cuevas JS, Rodriguez-Ropon A, Kennedy S, Moreno-Lopez J, Berg M, Hernandez-Jauregui P. Investigation of T-cell responses and viral mRNA persistence in lymph nodes of pigs infected with porcine rubulavirus. *Vet Immunol Immunopath.* 2009;127:148-152.

90. Rivera-Benitez JF, Garcia-Contreras AC, Reyes-Leyva J, Hernandez J, Sanchez-Betancourt JI, Ramirez-Mendoza H. Efficacy of real-time RT-PCR for quantification of the N gene from different PorPV isolates. Paper presented at: 23rd IPVS Congress2014; Mexico.

91. Mendoza GR, Pradal-Roa P, Doporto DJM, Constatino CF, Mercado GC. The role of immunohistochemical techniques on Blue Eye Disease diagnosis in pigs. Paper presented at: 16th IPVS Congress2000; Melbourne, Australia.

92. Ramirez-Mendoza H, hernandez-Jauregui P, Reyes-Leyva J, Zenteno E, Moreno-Lopez J, Kennedy S. Lesions in the reproductive tract of boars experimentally infected with porcine rubulavirus. *J Comp Pathol.* 1997;117:237-252.

93. Nordengrahn A, Svenda M, Moreno-Lopez J, et al. Development of a blocking ELISA for screening antibodies to porcine rubulavirus, La Piedad Michoacan Virus. *J Vet Diagn Invest.* 1999;11:319-323.

94. Quezada-Monroy F, Echeveste-Garcia de Alba R, Verde-Rojo E, et al. Comparison between virus neutralization (VN) test, ELISA test and hemmaglutination inhibition (HI) test for Blue Eye Disease (BED) diagnosis. Paper presented at: 21st IPVS Congress2010; Vancouver, Canada.

95. Giron PC, Martinez-Lara A, Santacruz JP, Lopez DC, Ayala MAC. Evaluation of an inactivated antigen useful for the HI serodiagnosis in free areas of Blue Eye disease. Paper presented at: 19th IPVS Congress2006; Copenhagen, Denmark.

96. Zell R, Krumbholz A, Henke A, et al. Detection of porcine enteroviruses by nRT-PCR: differentiation of CPE groups I-III with specific primer sets. *J Virol Methods.* 2000;88(2):205-218.

97. Lan D, Ji W, Yang S, et al. Isolation and characterization of the first Chinese porcine sapelovirus strain. *Arch Virol.* 2011;156(9):1567-1574.

98. Cano-Gómez C, García-Casado MA, Soriguer R, Palero F, Jiménez-Clavero MA. Teschoviruses and sapeloviruses in faecal samples from wild boar in Spain. *Vet Microbiol.* 2013;165(1-2):115-122.

99. Palmquist JM, Munir S, Taku A, Kapur V, Goyal SM. Detection of porcine teschovirus and enterovirus type II by reverse transcription-polymerase chain reaction. *J Vet Diagn Invest.* 2002;14(6):476-480.

100. Schock A, Gurrala R, Fuller H, et al. Investigation into an outbreak of encephalomyelitis caused by a neuroinvasive porcine sapelovirus in the United Kingdom. *Vet Microbiol.* 2014;172(3-4):381-389.

101. Wang C, Yu D, Hua X, Yuan C, Sun H, Liu Y. Rapid and real-time detection of Porcine Sapelovirus by reverse transcription loop-mediated isothermal amplification assay. *Journal of Virological Methods.* 2014;203:5-8.

102. Chao DY, Wei JY, Chang WF, Wang J, Wang LC. Detection of multiple genotypes of calicivirus infection in asymptomatic swine in Taiwan. *Zoonoses Public Health.* 2012;59:434-444.

103. Guo M, Qian Y, Chang KO, Saif LJ. Expression and self-assembly in baculovirus of porcine enteric calicivirus capsids into virus-like particles and their use in an enzyme-linked immunosorbent assay for antibody detection in swine. *J Clin Microbiol.* 2001;39(4):1487-1493.

104. Mauroy A, Van der Poel WH, der Honing RH, Thys C, Thiry E. Development and application of a SYBR green RT-PCR for first line screening and quantification of porcine sapovirus infection. *BMC Vet Res.* 2012;8:193.

105. Alcalá AC, Rodríguez-Díaz J, de Rolo M, et al. Seroepidemiology of porcine enteric sapovirus in pig farms in Venezuela. *Vet Immunol Immunopathol.* 2010;137(3-4):269-274.

106. Krumbholz A, Wurm R, Scheck O, et al. Detection of porcine teschoviruses and enteroviruses by LightCycler real-time PCR. *J Virol Methods.* 2003;113(1):51-63.

107. Pignatelli J, Jimenez M, Luque J, Rejas MT, Lavazza A, Rodriguez D. Molecular characterization of a new PToV strain. Evolutionary implications. *Virus Res.* 2009;143(1):33-43.

108. Shin DJ, Park SI, Jeong YJ, et al. Detection and molecular characterization of porcine toroviruses in Korea. *Arch Virol.* 2010;155(3):417-422.

109. Kroneman A, Cornelissen LA, Horzinek MC, de Groot RJ, Egberink HF. Identification and characterization of a porcine torovirus. *J Virol.* 1998;72(5):3507-3511.

110. Pignatelli J, Jiménez M, Grau-Roma L, Rodríguez D. Detection of porcine torovirus by real time RT-PCR in piglets from a Spanish farm. *J Virol Methods.* 2010;163(2):398-404.

111. Pignatelli J, Alonso-Padilla J, Rodríguez D. Lineage specific antigenic differences in porcine torovirus hemagglutinin-esterase (PToV-HE) protein. *Vet Res.* 2013;44:126.

112. Anbalagan S, Peterson J, Wassman B, Elston J, Schwartz K. Genome sequence of torovirus identified from a pig with porcine epidemic diarrhea virus from the United States. *Genome Announc.* 2014;2(6).

113. Alonso-Padilla J, Pignatelli J, Simon-Grifé M, Plazuelo S, Casal J, Rodríguez D. Seroprevalence of porcine torovirus (PToV) in Spanish farms. *BMC Res Notes.* 2012;5:675.

114. Pignatelli J, Grau-Roma L, Jiménez M, Segalés J, Rodríguez D. Longitudinal serological and virological study on porcine torovirus (PToV) in piglets from Spanish farms. *Vet Microbiol.* 2010;146(3-4):260-268.

115. Pol F, Deblanc C, Oger A, Le Dimna M, Simon G, Le Potier MF. Validation of a commercial real-time PCR kit for specific and sensitive detection of Pseudorabies. *J Virol Methods.* 2013;187(2):421-423.

116. Zanella EL, Miller LC, Lager KM, Bigelow TT. Evaluation of a real-time polymerase chain reaction assay for Pseudorabies virus surveillance purposes. *J Vet Diagn Invest.* 2012;24(4):739-745.

117. Grau-Roma L, Segalés J. Detection of porcine reproductive and respiratory syndrome virus, porcine circovirus type 2, swine influenza virus and Aujeszky's disease virus in cases of porcine proliferative and necrotizing pneumonia (PNP) in Spain. *Vet Microbiol.* 2007;119(2-4):144-151.

118. Song Y, Jin M, Zhang S, et al. Generation and immunogenicity of a recombinant pseudorabies virus expressing cap protein of porcine circovirus type 2. *Vet Microbiol.* 2007;119(2-4):97-104.

119. IDEXX Laboratories Inc. <https://www.idexx.com/livestock-poultry/swine/prv.html>. Accessed February 2, 2016.

120. Corn JL, Stallknecht DE, Mechlin NM, Luttrell MP, Fischer JR. Persistence of pseudorabies virus in feral swine populations. *J Wildl Dis.* 2004;40(2):307-310.

121. White AK, Ciacci-Zanella J, Galeota J, Ele S, Osorio FA. Comparison of the abilities of serologic tests to detect pseudorabies-infected pigs during the latent phase of infection. *Am J Vet Res.* 1996;57(5):608-611.

122. Yong T, Huan-chun C, Shao-bo X, Ya-li Q, Qi-gai H, Yu-qi R. Development of a latex agglutination test using the major epitope domain of glycoprotein E of pseudorabies virus expressed in E. coli to differentiate between immune responses in pigs naturally infected or vaccinated with pseudorabies virus. *Vet Res Commun.* 2005;29(6):487-497.

123. Janke BH, Paul PS, Landgraf JG, Halbur PG, Huinker CD. Paramyxovirus infection in pigs with interstitial pneumonia and encephalitis in the United States. *J Vet Diagn Invest.* 2001;13(5):428-433.

124. Qiao D, Janke BH, Elankumaran S. Complete genome sequence and pathogenicity of two swine parainfluenzavirus 3 isolates from pigs in the United States. *J Virol.* 2010;84(2):686-694.

125. Thermo Fisher Scientific. TaqMan® iPSC Sendai Detection Kit. <https://www.thermofisher.com/order/catalog/product/A13640>. Accessed February 2, 2016.

126. Ishida N, Homma M. Sendai virus. *Adv Virus Res.* 1978;23:349-383.

127. Faísca P, Desmecht D. Sendai virus, the mouse parainfluenza type 1: a longstanding pathogen that remains up-to-date. *Res Vet Sci.* 2007;82(1):115-125.

128. Yang M, van Bruggen R, Xu W. Generation and diagnostic application of monoclonal antibodies against Seneca Valley virus. *J Vet Diagn Invest.* 2012;24(1):42-50.

129. Knowles NJ, Hales LM, Jones BH, et al. Epidemiology of Seneca Valley virus: identification and characterization of isolates from pigs in the United States. Europic; 2006; Saariselka, Inari, Finland.

130. Willcocks MM, Locker N, Gomwalk Z, et al. Structural features of the Seneca Valley virus internal ribosome entry site (IRES) element: a picornavirus with a pestivirus-like IRES. *J Virol.* 2011;85(9):4452-4461.

131. Newman JT, Smith KO. Characteristics of a swine papovavirus. *Infect Immun.* 1972;5(6):961-967.

132. Stevens H, Rector A, Van Der Kroght K, Van Ranst M. Isolation and cloning of two variant papillomaviruses from domestic pigs: Sus scrofa papillomaviruses type 1 variants a and b. *J Gen Virol.* 2008;89(Pt 10):2475-2481.

133. Sironi G, Caniatti M, Scanziani E. Immunohistochemical detection of papillomavirus structural antigens in animal hyperplastic and neoplastic epithelial lesions. *Zentralbl Veterinarmed A.* 1990;37(10):760-770.

134. Kasza L, Bohl EH, Jones DO. Isolation and cultivation of swine pox virus in primary cell cultures of swine origin. *Amer J Vet Res.* 1960;21:269-273.

135. Cheville NF. Immunofluorescent and morphologic studies on swinepox. *Vet Pathol.* 1966;3:559-564.

136. De Boer GF. Swinepox. Virus isolation, experimental infections and the differentiation from Vaccinia virus infections. *Arch Virol.* 1975;49:141-150.

137. Medaglia MLG, Sa NMB, Correa IA, Costa LJ. One-step duplex polymerase chain reactionf for the detection of swinepox and vaccinia viruses in skin lesions of swine with poxvirus-related disease. *J Virol Methods.* 2015;219:10-13.

138. Jindal N, Barua S, Riyesh T, Lather A, Narang G. Molecular detection of swinepox virus in two piggery units in Haryana state. *Haryana Vet J.* 2015;54(1):72-74.

139. Ouchi M, Fujiwara M, Hatano Y, Yamada M, Nii S. Analysis of swinepox virus antigens using monoclonal antibodies. *J Vet Med Sci.* 1992;54(4):731-737.

140. Thibault S, Drolet R, Alain R, Dea S. Congenital swine pox: a sporadic skin disorder in nursing piglets. *Swine Health Prod.* 1998;6(6):276-278.

141. Shope RE. Swine pox. *Arch Virol.* 1940;1(4):457-467.

142. Massung RF, Moyer RW. A molecular biology of swinepox virus: II. The infectious cycle. *Virology.* 1990;180:355-364.

143. Shunzhou D, Xinhua J, Chuang L. Diagnosis and virus isolation of sow swine pox. *Jiangxi Nongye Daxue Xueboa.* 2012;32(5).

144. Lung O, Fisher M, Beeston A, et al. Multiplex RT-PCR detection and microarray typing of vesicular disease viruses. *J Virol Methods.* 2011;175(2):236-245.

145. Hindson BJ, Reid SM, Baker BR, et al. Diagnostic evaluation of multiplexed reverse transcription-PCR microsphere array assay for detection of foot-and-mouth and look-alike disease viruses. *J Clin Microbiol.* 2008;46(3):1081-1089.

146. Reid SM, Ansell DM, Ferris NP, Hutchings GH, Knowles NJ, Smith AW. Development of a reverse transcription polymerase chain reaction procedure for the detection of marine caliciviruses with potential application for nucleotide sequencing. *J Virol Methods.* 1999;82(1):99-107.

147. Reid SM, King DP, Shaw AE, et al. Development of a real-time reverse transcription polymerase chain reaction assay for detection of marine caliciviruses (genus *Vesivirus*). *J Virol Methods.* 2007;140(1-2):166-173.

148. Zee YC, Hackett AJ, Madin SH. A study of the cellular pathogenesis of vesicular exanthema of swine virus in pig kidney cells. *J Infect Dis.* 1967;117(3):229-236.

149. Gelberg HB, Lewis RM. The pathogenesis of vesicular exanthema of swine virus and San Miguel sea lion virus in swine. *Vet Pathol.* 1982;19(4):424-443.

150. Ferris NP, Oxtoby JM. An enzyme-linked immunosorbent assay for the detection of marine caliciviruses. *Vet Microbiol.* 1994;42(2-3):229-238.

151. Edwards JF, Yedloutschnig RJ, Dardiri AH, Callis JJ. Vesicular exanthema of swine virus: isolation and serotyping of field samples. *Can J Vet Res.* 1987;51(3):358-362.

152. Wilder FW. Detection of swine caliciviruses by indirect immunofluorescence. *Can J Comp Med.* 1980;44(1):87-92.

153. *Diseases of swine.* 10th ed. Ames, IA: Wiley-Blackwell; 2012.