

IOWA STATE UNIVERSITY

Veterinary Diagnostic Laboratory

Pathology Submission Guide

INTRODUCTION:

These “Quick Guides” are the basic guidelines for sample submission for common diseases.

For unique, urgent, and unusual clinical conditions or gross lesions, please call the laboratory for addition submission suggestions.

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PORCINE ABORTION/PREGNANCY WASTAGE

Specimens to submit: Entire fetuses with placenta, minimally contaminated, fresh/chilled are preferred specimens. Do not freeze samples intended for histopathology. Send 4-6 representative fresh fetuses and all mummified fetuses. Alternatively, remove the following tissues from 3 fetuses per litter:

Thoracic fluid	0.25 - 1 ml per aborted pig, may pool within litter for PRRS virus, PCV2
Brain	1/2 brain, fresh/chilled and formalin-fixed
Heart	1/2 of organ fresh/chilled, 1/2 cm slice formalin-fixed
Kidney	Fresh/chilled, formalin-fixed (1/2 cm slices)
Liver	Fresh/chilled (1/3 of organ), formalin-fixed (1/2 cm slice)
Lung	Fresh/chilled (1 entire lung), plus formalin-fixed (1/2 cm slice)
Spleen	Fresh/chilled (1/2 of organ), formalin-fixed ((1/2 cm slice)
Stomach contents	1-3 ml in sterile syringe or tube, fresh/chilled
Placenta	Fresh/chilled and several pieces formalin-fixed
Umbilicus	Formalin-fixed, several 1/2 cm slices
Sow serum	Optional, see notes on abortion serology. 1-3 ml from affected sows
Sow nasal swab(s)	Optional in cases where influenza is suspected

SAMPLING TECHNIQUES

1. Do not freeze tissue intended for histopathology.
2. Submit placenta whenever possible
3. Thorough investigation of abortion should include serology. Submit dam's sera. Retain 1/2 of sample frozen.
4. In populations thought naïve for PRRSV or IAV, oral fluids may be a useful sample type.

AGENTS DETECTED BY ROUTINE EXAMINATION

Bacteria	<i>Trueperella pyogenes</i> , <i>Bacillus</i> spp., <i>Brucella</i> spp., <i>E. coli</i> , <i>Salmonella</i> spp., <i>Erysipelothrix</i> , <i>Streptococcus</i> spp., etc
Viruses	PRRSV, PCV2, PRV, parvovirus (see comments)

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PORCINE ABORTION/PREGNANCY WASTAGE

AGENTS REQUIRING SPECIAL TESTS (BY REQUEST)

Leptospira	If leptospirosis is suspected, PCR tests on pooled kidneys are preferred sample type. Serology on sow sera is a useful method to implicate a role for leptospirosis.
PRRS virus	PRRSV virus is not present in all aborted fetuses. PRRSV may be detected in serum from sick sows and/or pools of fetal tissues from aborted litters and/or from weak-born littermates or from pigs that develop pneumonia shortly after birth. A serologic survey of the sow herd may be useful, but may be difficult to interpret in PRRS-endemic or vaccinated herds.
Toxicosis	Carbon monoxide (heart blood in EDTA; clotted heart blood or thoracic fluid as second choice).

COMMENTS

- Parvovirus and PCV2 usually do not cause abortion but may be present in mummified fetuses.
 - Mummified fetuses may harbor porcine parvovirus, PCV2, PCV3 or PRRSV as well as other viruses. Lungs and hearts from mummified fetuses are useful for detection of these viruses by PCR.
 - Fetal serology from fresh stillborns may aid in the diagnosis of porcine parvovirus, PCV2, *Leptospira* and PRRSV.
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PORCINE CENTRAL NERVOUS SYSTEM DISORDERS

Specimens to submit: One or more acutely affected live pigs. Alternatively, tissues from field necropsy should include:

Brain (including brain stem)	Swab of brain stem and base of cerebellum (for bacterial culture) 1/2 brain divided longitudinally, fresh/chilled 1/2 brain, formalin-fixed
Intestine	Optional, edema disease. One 10-15 cm slice of ileum and jejunum, fresh/chilled Several 1/2 cm slices of jejunum and ileum, formalin-fixed
Spinal cord	Optional, locomotor problems. Entire carcass or vertebral column, fresh/chilled Dissected cord, fresh/chilled Cross-sections (1/2 cm slices) of cord from 4-5 levels, formalin-fixed
Spleen	Fresh/chilled and formalin-fixed
Tonsil	Fresh/chilled and formalin-fixed

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PORCINE CENTRAL NERVOUS SYSTEM DISORDERS

SAMPLING TECHNIQUES

1. Entire head can be submitted. Chill before shipment if possible.
2. Do NOT freeze fresh brain or head.
3. Fresh half of brain should be packed carefully to avoid crushing.
4. Fixed half of brain should be incised transversely (not longitudinally) into the ventricle to aid in fixation if brain is large.
5. Cerebrospinal fluid (CSF) can be collected prior to removing the skull. When a bacterial meningitis is suspected, CSF is an excellent sample as there is less opportunity for contamination compared to most methods of opening the skull.

AGENTS DETECTED BY ROUTINE EXAMINATION

Bacteria	<i>Streptococcus suis</i> , <i>Haemophilus parasuis</i> , <i>Trueperella pyogenes</i> , <i>E. coli</i> (small intestine needed for edema disease)
Viruses	Pseudorabies virus, PRRS virus, PCV2, Teschovirus, Sapelovirus, Astrovirus, Enteroviruses
Non-infectious	Water deprivation/sodium toxicity

AGENTS REQUIRING SPECIAL TESTS (BY REQUEST)

Toxicosis	Selenium (liver and spinal cord - lumbar intumescence, fresh/chilled) Organophosphate (whole blood in EDTA, brain, stomach contents, fresh/chilled)
Viruses	Rabies (FA on brain); other viruses (e.g. HEV, porcine paramyxovirus, herpesviruses, enteroviruses, etc. detected by PCR or VI on fresh/chilled brain and spinal cord)
Bacteria	<i>Clostridium tetani</i> (tetanus)

COMMENTS

- Cerebellum and brain stem are affected by most infectious causes of CNS disease and should always be included in submitted samples.
 - Many toxic causes of CNS disease do not induce lesions in the brain and must be diagnosed by analysis of other tissues. For most toxicoses, submission of stomach contents, liver, kidney, feed, water, serum and whole blood (in EDTA), as well as brain, would include the tissues necessary for diagnosis.
 - Spinal cord is essential for diagnosis of causes of posterior paresis or paralysis.
 - Next Generation Sequencing (NGS) is available to complement investigations where no infectious agent is detected, but is suspected based on lesions.
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PORCINE ENTERITIS – NURSING PIGS

Specimens to submit: The best specimens are acutely-ill (<24 hours) live untreated pig(s). Alternatively, necropsy of euthanized pig(s) with intestines collected in formalin within 10 minutes of death.

Colon/cecum contents	2-10 ml fresh/chilled
Colon and cecum	Entire organ, fresh/chilled Several 1 cm pieces or section across multiple loops, formalin-fixed
Ileum	10 cm segments, fresh/chilled Three 1 cm pieces, formalin-fixed
Jejunum	10 cm segments, fresh/chilled Three 1 cm pieces, formalin-fixed
Lesions (e.g. liver, other)	2 cm cubes, fresh/chilled Several 1 cm slices, formalin-fixed

Samples removed at necropsy in the field are better than a whole dead pig submitted to the lab.

SAMPLING TECHNIQUES

1. Samples must be taken as soon after death as possible (within minutes).
2. Intestines do not need to be tied off at the ends.
3. Flush intestinal segments for histopathologic examination with formalin and drop in fixative. Or, gently open ends of 1/2" segments with scissors or forceps to expose mucosa as immersed.
4. Pool all formalin-fixed tissues from each pig in one bag; individual pigs can be pooled or kept separate as desired. Package fresh intestines separately from other tissues and each pig in a separate bag. Chill fresh tissues before mailing. Do NOT freeze.
5. Pooled serum from pigs from several litters can be used to rule out acute systemic viral infections.
6. **Do not send whole, dead pigs** (intestines autolyze quickly).

AGENTS DETECTED BY ROUTINE EXAMINATION

Bacteria	<i>Clostridium difficile</i> , <i>Clostridium perfringens</i> , <i>E. coli</i> , <i>Enterococcus durans</i> , <i>Salmonella</i> spp.
Parasites	<i>Cystoisospora</i> (coccidia), <i>Cryptosporidia</i> and protozoans
Viruses	Rotaviruses, PEDV, PDCoV, TGEV, PRRSV

COMMENTS

- Accurate diagnosis of diarrhea in suckling piglets usually requires submission of tissues.
- Feces from acutely affected pigs are useful for detection of epidemic agents such as PEDV, PDCoV, or TGEV by PCR. Results of tests on feces only (both positive and negative) may not be completely definitive and must be evaluated with consideration of clinical signs. Samples (10-20 ml) should be taken from acutely affected, nonmedicated piglets on the first day of diarrhea.
- Accurate diagnosis of endemic agents requires both the detection of the offending agent(s) as well as the presence of compatible histologic lesions.
- *Brachyspira* spp. can be isolated from feces or fecal swabs in positive herds.
- Wet mounts of intestinal impression smears or fecal flotation may be of value for quick in-house detection of *Cystoisospora*
- In cases where mesocolonic edema is prominent, *Clostridium difficile* is a differential. Entire colon can be submitted; colon contents for *C. difficile* toxin ELISA and culture, with multiple colon sections in formalin for histopathology.
- In cases of necrotic enteritis, submit both necrotic and adjacent non-necrotic segments, fresh and formalin-fixed.

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PORCINE ENTERITIS - WEANED PIGS

Specimens to submit: The best specimen is an acutely-ill (< 24 hours) live untreated pig(s). Alternatively, tissues may be removed from euthanized pigs.

Colon and cecum	Several 10 cm sections, fresh/chilled Several 1 cm pieces, formalin-fixed
Feces/colon content	2-10 ml fluid contents, fresh/chilled
Ileum	10 cm segment, fresh/chilled Three 1 cm pieces, formalin-fixed
Jejunum	10 cm segment, fresh/chilled Three 1 cm pieces, formalin-fixed
Mesenteric lymph nodes	Fresh / chilled Several formalin-fixed
All suspected lesions	10 cm segment, fresh/chilled Several 1 cm pieces, formalin-fixed

Samples removed at necropsy in the field are often better than a whole, dead pig submitted to the lab.

SAMPLING TECHNIQUES

1. Samples must be taken as soon after death as possible (within minutes).
2. Intestines do not need to be tied off at the ends.
3. Flush intestinal segments for histopathologic examination with formalin and drop in fixative. Or, gently open ends of 1/2" segments with scissors or forceps to expose mucosa as immersed.
4. Pool all formalin-fixed tissues from each pig in one bag; individual pigs can be pooled or kept separate as desired. Package fresh intestines separately from other tissues and that from each pig in a separate bag. Chill fresh tissues before mailing. Do NOT freeze.
5. Do not send whole, dead pigs (intestines autolyze quickly).

AGENTS DETECTED BY ROUTINE EXAMINATION

Bacteria	<i>E. coli</i> , <i>Salmonella</i> spp., <i>Clostridium perfringens</i> , <i>Enterococcus durans</i> , <i>Brachyspira</i> spp., <i>Lawsonia intracellularis</i>
Parasites	<i>Coccidia</i> , roundworms, whipworms
Viruses	Rotavirus, TGEV, PEDV, PDCoV, PCV2

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PORCINE ENTERITIS - WEANED PIGS

COMMENTS

- Detection of endemic agents from feces does not provide a definitive diagnosis.
 - Detection of *Lawsonia* or rotaviruses detected by PCR or *Salmonella* or hemolytic *E. coli* detected by culture or parasite ova/oocysts detected by fecal flotation should be interpreted in context of clinical signs and lesions. Histopathology on tissues for compatible histologic lesions is usually required for definitive diagnosis.
 - Feces or OF used to detect TGEV, PDCoV and PEDV by PCR is diagnostic when expected negative.
 - Fecal samples (10-20 ml) should be collected from acutely affected, nonmedicated pigs on the first day of diarrhea.
 - Colitis associated with *Brachyspira* spp. should be confirmed by culture and histopathology for a definitive diagnosis. *Brachyspira* and compatible lesions are only found in large intestine.
 - Porcine proliferative enteritis associated with *Lawsonia intracellularis* can be confirmed by IHC or PCR.
 - Ill-defined conditions such as dietary hypersensitivity or nonspecific colitis may be implied but cannot be confirmed by routine diagnostic investigations.
 - In cases of necrotic enteritis submit both necrotic and adjacent non-necrotic segments, fresh and fixed.
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- Call the laboratory to discuss the value of feces for diagnosis or monitoring for specific pathogens of interest.

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PORCINE SYSTEMIC/MULTISYSTEMIC DISEASE INVESTIGATIONS

This section pertains to those cases where systemic disease or polymicrobial disease is suspected, including pigs found dead (with or without gross lesions).

Specimens to submit: Acutely affected live untreated pig(s). Alternatively, tissues from euthanized clinically-affected, nonmedicated pigs. Tissues from pigs found dead are also useful if not autolyzed.

Tissues / sample to submit should include:

Brain (including brain stem)	1/2 Fresh and 1/2 formalin-fixed
Turbinate	Turbinate swab (chilled) and turbinate in formalin Entire organ, fresh chilled
Heart	Fresh/chilled and formalin-fixed/swabs of fibrin if present
Kidney	Fresh/chilled and formalin-fixed
Liver	Fresh/chilled and formalin-fixed
Lung	Entire or 6 cm cube of lung with lesions, fresh/chilled 4-6 slices (1 cm) of affected and adjacent unaffected lung, formalin-fixed
Joint swabs/synovium	Swabs chilled; synovium fresh/chilled and in formalin
Lymph nodes, tonsil	Fresh/chilled and formalin-fixed, preferably those that are enlarged
Spleen	6 cm fresh / chilled; 1 cm slice formalin-fixed
Intestine	Two 10-15 cm slices of ileum and two jejunum, fresh/chilled Several (6-10) 1/2 cm slices ileum and jejunum, formalin fixed
Colon and cecum	Several 1 cm sections fixed / loop fresh-chilled
Feces	30 grams, fresh chilled
Spinal cord	Entire carcass or vertebral column, fresh/chilled, or Dissected cord, fresh/chilled Cross-sections (1/2 cm slices) of cord from 4-5 levels, formalin-fixed
Skeletal muscle	1 cm slices, formalin-fixed
Whole blood / serum	Chilled; useful for clinical pathology, PCR, serology, chemistry
Feed / water	Chilled and available should analysis be indicated

SAMPLING TECHNIQUES

1. Fresh tissues should be chilled before shipping. Do NOT freeze.
2. Pool all formalin-fixed tissues from each pig in one bag; individual pigs can be pooled or kept separate as desired. Package fresh intestines separately from other tissues and keep each pig in a separate bag.

AGENTS DETECTED BY ROUTINE EXAMINATION

Bacteria	<i>Pasteurella multocida</i> , <i>Streptococcus suis</i> , <i>Actinobacillus pleuropneumoniae</i> , <i>Actinobacillus suis</i> , <i>Trueperella pyogenes</i> , <i>Bordetella bronchiseptica</i> , <i>Haemophilus parasuis</i> <i>Erysipelothrix</i> , <i>Salmonella</i> spp., <i>E. coli</i> , <i>Clostridium</i> <i>perfringens</i> type A and C, <i>Streptococcus suis</i> , <i>Lawsonia intracellularis</i> , <i>Brachyspira</i> spp.
Parasites	Round worms, whipworms

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PORCINE SYSTEMIC/MULTISYSTEMIC DISEASE INVESTIGATIONS

Viruses	PRRS virus, PCV2/3, IAV, cytomegalovirus/inclusion body rhinitis (only if turbinates are submitted), PRV, PEDV, rotavirus, TGEV, Teschovirus, other viruses
Mycoplasma	<i>Mycoplasma hyopneumoniae/hyorhinitis/hyosynoviae</i> by PCR
Non-infectious	Water deprivation, toxicities, deficiencies

COMMENTS

- Oral fluids (OF) are useful for monitoring presence of agents in a population. Detection of and agent by PCR in OF usually does not confirm disease status unless expected negative.
 - Virus isolation is often challenging from OF. If VI is desired, tissues/lesions are often better samples.
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PORCINE PNEUMONIA/RHINITIS

Specimens to submit: Live acutely affected pig(s). Alternatively, tissues should include:

Lung	Entire lung (one side) or generous portion of lesion and adjacent unaffected lung, fresh/chilled 4 to 6 thin slices (1 cm) through affected and adjacent unaffected lung, formalin-fixed. At least 3-4 cross sections through anteroventral lung are recommended
Tracheobronchial lymph node	Fresh/chilled; formalin fixed enlarged lymph nodes
Airway swabs, BAL	Swab/lavage of large airways (chilled, appropriate transport media)
Nasal swab	Dacron-tipped, slightly moistened, for bacterial and viral detection
Snout or turbinate	Turbinate scroll from one side removed at junction with midline septum, formalin-fixed
Tonsil	1/2 fresh, and 1/2 formalin-fixed

SAMPLING TECHNIQUES

1. Fresh tissue should be chilled before shipping. Do NOT freeze.
 2. Samples are best for diagnosis or primary agents if taken at the onset of respiratory signs.
 3. Nasal swab preservation: swabs must be kept moist and cool before and during shipment.
 4. Fixed turbinate must be submitted to confirm the presence of porcine cytomegalovirus (inclusion body rhinitis)
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AGENTS DETECTED BY ROUTINE EXAMINATION

Bacteria	<i>Pasteurella multocida</i> , <i>Streptococcus suis</i> , <i>Salmonella choleraesuis</i> , <i>Actinobacillus pleuropneumoniae</i> , <i>Actinobacillus suis</i> , <i>Trueperella pyogenes</i> , <i>Bordetella bronchiseptica</i> , <i>Haemophilus parasuis</i>
Viruses	PRRS virus, PCV2, SIV, PRV, PRCV, PPIV-1; Cytomegalovirus/inclusion body rhinitis (if turbinates are submitted for histopath)
Mycoplasma	<i>Mycoplasma hyopneumoniae</i>

AGENTS REQUIRING SPECIAL TESTS (BY REQUEST)

Viruses	Isolation, sequencing
Bacteria	Genotyping, serotyping

COMMENTS

- Isolation attempts for *Mycoplasma hyopneumoniae* can be done on fresh/chilled lung but are not routine because of the difficulty of recovering these fragile organisms in the presence of heavy contamination or concurrent bacterial or other mycoplasmal infections.
 - In populations thought naïve for PRRSV or IAV, oral fluids may be a useful sample type.
 - PCR is used on nasal swabs, oral fluids or lung tissues for detection of swine influenza virus with subtyping to determine hemagglutinin (H) and neuraminidase (N) subtypes routine. Sequencing has higher success rate from specimens with lower cycle threshold values.
 - PRRSV is often best isolated from lung lavage samples or serum. The lung can be lavaged (with cell culture growth media or Lactated Ringers Solution) and the fluid submitted. Lung lavages can be done at the lab if at least one half of the lung is submitted without holes or slices.
 - PRRSV sequencing has higher success rate from specimens with lower cycle threshold values.
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PORCINE LOCOMOTOR SYSTEM DISORDERS

Specimens to submit: Live acutely affected pig(s) or freshly dead pigs.

Alternatively, tissues should include:

Affected whole limb	Fresh/chilled
Affected joint(s)	Fresh/chilled
Bones (affected and 2 nd rib)	Fresh/chilled
Costochondral junction	Formalin-fixed
Muscle	2 cm x 2 cm pieces of different muscle groups formalin-fixed (heart, diaphragm, ham, loin)
Brain (including brainstem)	1/2 fresh/chilled and 1/2 formalin-fixed
Spinal cord	Entire carcass or vertebral column, fresh/chilled Dissected cord, fresh/chilled Cross-sections (1/2 cm slices) of cord from 4-5 levels, formalin-fixed
Synovial fluid	Fresh/chilled
Synovium	Fresh/chilled, and formalin-fixed
Liver	Fresh/chilled, and formalin-fixed
Spleen	Fresh/chilled, and formalin-fixed
Kidney	Fresh/chilled, and formalin-fixed
Serum and whole blood	Fresh/chilled
Urine	Fresh/chilled
Feed / water samples	Fresh/chilled

SAMPLING TECHNIQUES

1. Fresh tissue should be chilled before shipping. Do NOT freeze tissues intended for histopathology.
 2. Feed
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AGENTS DETECTED BY ROUTINE EXAMINATION

Bacteria	<i>Streptococcus suis</i> , <i>Trueperella pyogenes</i> , <i>Haemophilus parasuis</i> , <i>E. coli</i> , <i>Actinobacillus suis</i> , <i>Erysipelothrix rhusiopathiae</i> , <i>streptococci</i> , <i>staphylococci</i>
Viruses	Classical swine fever virus
Mycoplasma	<i>Mycoplasma hyorhinis</i> , <i>Mycoplasma hyosynoviae</i>

AGENTS REQUIRING SPECIAL TESTS (BY REQUEST)

Viruses	Isolation, sequencing
Bacteria	Genotyping, serotyping

COMMENTS

- Bone profile may be performed in bone samples (2nd rib) to evaluate the possibility of metabolic bone disease.
 - Different interactions of Ca, P and Vitamin D may play a role in the development of rickets, osteomalacia, osteoporosis, fibrous osteodystrophy. Feed analysis may be warranted.
 - Vesicular diseases can cause lameness. If a vesicular disease is suspected contact the state veterinarian to initiate a Foreign Animal Disease (FAD) investigation. For a detailed description of samples to collect in case a vesicular disease is suspected consult the "Porcine Skin Conditions" guide.
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PORCINE SKIN CONDITIONS

Specimens to submit: Live or freshly euthanized acutely affected pig(s). Alternatively, tissues should include:

Affected skin (biopsies or sections with range of lesions)	Fresh/chilled, 2-4 formalin-fixed sections of 2 cm x 2 cm
Fluid or swabs from vesicles*	Fresh/chilled
Skin scraping	Fresh/chilled
Lung	Fresh/chilled; formalin-fixed
Liver	Fresh/chilled; formalin-fixed
Spleen	Fresh/chilled; formalin-fixed
Kidney	Fresh/chilled; formalin-fixed
Lymph nodes	Fresh/chilled; formalin-fixed (favor enlarged lymph nodes)

SAMPLING TECHNIQUES

1. Fresh tissue should be chilled before shipping. Do NOT freeze tissues intended for histopathology.
2. Skin scraping (mange, fungal). For mange skin scraping with a knife or scalpel to get superficial layers of skin (where mites burrow) as well as skin exudates is an appropriate sample to detect mange mites (i.e., *Sarcoptes*). In sows, deep inner ears or skin behind the ears are likely locations for mites. Mites in weaners are more dispersed.
3. For skin lesions suspected as manifestation of systemic disease, please use "SYSTEMIC" guidelines listed above (see systemic disease).
4. **If vesicles* are suspected**, collect vesicular fluid and/or swabs of acute lesions and submit fresh/chilled. Biopsy of site(s) can be collected with punch biopsy tool or surgically, with portions submitted fresh/chilled and portions formalin-fixed.
5. If a **vesicular disease*** is suspected contact the state veterinarian to initiate a Foreign Animal Disease (FAD) investigation.

AGENTS DETECTED BY ROUTINE EXAMINATION

Bacteria	<i>Staphylococcus hyicus</i> , <i>Streptococcus spp</i> , <i>Erysipelothrix rhusiopathiae</i> , <i>Actinobacillus suis</i> , <i>E. coli</i>
Viruses	Senecavirus A, Foot-and-mouth disease virus, vesicular stomatitis, swine vesicular disease virus, vesicular exanthema, Swinepox, PCV2, Classical swine fever virus
Parasites	<i>Sarcoptes scabiei</i> ; other mites
Fungi	<i>Microsporum spp.</i> , <i>Trichophyton spp.</i> , <i>Candida albicans</i>

AGENTS REQUIRING SPECIAL TESTS (BY REQUEST)

Viruses	Isolation, sequencing
Bacteria	Genotyping, serotyping

COMMENTS

- To diagnose toxicities and/or deficiencies that may affect skin (e.g., parakeratosis due to zinc deficiency) submit liver and feed in addition to the skin samples.
- For samples to diagnose systemic manifestations of specific agents see the "Porcine Systemic Disease Investigations" guide.

***If a vesicular disease is suspected, contact the state veterinarian to initiate a Foreign Animal Disease (FAD) investigation.**
