IOWA STATE UNIVERSITY

Veterinary Diagnostic Laboratory

Pathology Submission Guide

PORCINE ABORTION

Specimens to submit: Entire fetuses with placenta, minimally contaminated, fresh/chilled are preferred specimens. Do NOT freeze. 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)
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

SAMPLING TECHNIQUES

1. Do NOT freeze tissue.
2. Submit placenta whenever possible
3. Thorough investigation of abortion should include serology. Submit dam's sera. Retain 1/2 of sample frozen.

AGENTS DETECTED BY ROUTINE EXAMINATION

Bacteria	*Arcanobacterium pyogenes, Bacillus spp., Brucella spp., E. coli, Salmonella spp., Erysipelothrix, Streptococcus spp., etc*
Viruses	PRRSV, PCV2, PRV, parvovirus (see comments)

AGENTS REQUIRING SPECIAL TESTS (BY REQUEST)

Leptospira	If leptospirosis is suspected, extra effort should be made to deliver freshly aborted, chilled fetuses directly to the lab for PCR or FA tests which are on kidney or IHC. Serology on sow sera is probably the most reliable method for diagnosing leptospirosis.
PRRS virus	PRRSV virus is not present in all aborted fetuses. Virus isolation is not routinely conducted but PCR on pooled tissues or thoracic fluids is routine. Histopath may occasionally demonstrate lesions suggestive of PRRS virus in umbilical cords, lungs, heart or other tissues. Preferred samples are lung or serum from weakborn littermates or from pigs that develop pneumonia shortly after birth. PCR on serum from sick sows is often valuable. 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 parvovirus, PCV2 or PRRSV. Lungs and hearts from mummified fetuses are useful for detection of these viruses by PCR. Fetal serology 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 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

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. 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	*Streptococcus suis, Haemophilus parasuis, Arcanobacterium pyogenes, E. coli (intestine, edema disease)*
Viruses	*Pseudorabies virus, PRRS virus, PCV2*
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 Teschovirus, paramyxovirus, herpesviruses detected by PCR or VI on fresh/chilled brain and spinal cord)

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, 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.

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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, formalin-fixed
Ileum	10-15 cm segments, fresh/chilled Three 1 cm pieces, formalin-fixed
Jejunum	10-15 cm segments, fresh/chilled Three 1 cm pieces, formalin-fixed
Lesions	10-15 cm segments, fresh/chilled Several 1 cm pieces, 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 a 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. **Do not send whole, dead pigs** (intestines autolyze quickly).

AGENTS DETECTED BY ROUTINE EXAMINATION

Bacteria	*Clostridium difficile, Clostridium perfringens, E. coli, Enterococcus durans, Salmonella* spp.
Parasites	Isospora (coccidia), Cryptosporidia
Viruses	Rotavirus, PED virus, TGE virus, 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 TGEV or PEDV 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 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 hyodysenteriae* can occasionally be isolated from feces (swabs are even less reliable).
- Wet mounts of intestinal impression smears or fecal flotation may be of value for quick in-house detection of Isospora
- In cases where mesocolonic edema is prominent, *Clostridium difficile* is a differential and the entire colon or colon contents should be submitted for a *C. difficile* toxin ELISA.
- In cases of necrotic enteritis, submit both necrotic and adjacent non-necrotic segments, fresh and 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	10-15 cm segment, fresh/chilled Three 1 cm pieces, formalin-fixed
Jejunum	10-15 cm segment, fresh/chilled Three 1 cm pieces, formalin-fixed
Lesions	10-15 cm segment, fresh/chilled Seceral 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 a 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. **Do not send whole, dead pigs** (intestines autolyze quickly).

AGENTS DETECTED BY ROUTINE EXAMINATION	
Bacteria	*E. coli, Salmonella spp., Clostridium perfringens, Enterococcus durans, Brachyspira spp. Lawsonia intracellularis*
Parasites	Coccidia, roundworms, whipworms
Viruses	Rotavirus, TGE virus, PED virus, PRRSV, PCV2

COMMENTS

- Feces can be used to detect TGEV and PEDV by PCR and is diagnostic when expected negative.
- 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. Histopathology on tissues for compatible pathologic lesions is required for definitive diagnosis. Fecal samples (10-20 ml) should be taken on the first day of diarrhea. Call the laboratory to discuss the value of feces for diagnosis or monitoring for specific pathogens of interest.
- Colitis associated with *Brachyspira hyodysemeteriae* and *Brachyspira pilosicoli* should be confirmed by culture and histopathology for a definitive diagnosis.
- 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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PORCINE MULTISYSTEMIC DISEASE INVESTIGATIONS	
Specimens to submit: The best specimen is a live untreated pig(s). Alternatively, tissues should include:	
Brain	1/2 fresh and 1/2 formalin-fixed
Turbinate	Turbinate swab (chilled) and turbinate in formalin Entire organ, fresh chilled
Colon and Cecum	Several 1 cm pieces, formalin fixed
Heart	Fresh/chilled and formalin-fixed/swabs of fibrin if present
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
Kidney	Fresh/chilled and formalin-fixed
Liver	Fresh/chilled and formalin-fixed
Lung	Entire lung (one side) or generous portion of lung containing the lesions and adjacent unaffected lung, fresh/chilled 4-6 thin slices (1 cm) through affected and adjacent unaffected lung, formalin-fixed
Joint swabs/synovium	Swabs chilled; synovium fresh/chilled and in formalin Spleen, tonsil, and lymph nodes
Lymphoid tissues	Fresh/chilled and formalin-fixed
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
Whole blood/serum	Chilled; useful for clin-path, PCR, serology, chemistry
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	*Pasteurella multocida, Streptococcus suis, Actinobacillus pleuropneumoniae, Actinobacillus suis, Arcanobacterium pyogenes, Bordetella bronchiseptica, Haemophilus parasuis Erysipelothrix, Salmonella spp., E. coli, Clostridium perfingens type A and C, Enterococcus durans, Lawsonia intracellularis, Brachyspira spp.*
Parasites	*Coccidia, Cryptosporidia*, round worms, whip worms
Viruses	PRRS virus, PCV2, SIV, cytomegalovirus/inclusion body rhinitis (only if turbinates are submitted), PRV, PEDV, rotavirus, TGE virus, Teschovirus, and more.
Mycoplasma	*Mycoplasma hyopneumoniae/hyorhinis/hyosynoviae* by PCR
Non-infectious	Water deprivation, toxicities, deficiencies
COMMENTS	
• PRRS virus is often best isolated from lung lavage fluids. The lung can be lavaged (with cell culture growth media or Lactated Ringers Solution) and the fluid submitted or the lavage can be done at the lab if at least one half of the lung is submitted without holes or slices in it.	

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PORCINE PNEUMONIA / RHINITIS

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

Brain	1/2 fresh, and 1/2 formalin-fixed
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
Nasal swab	Swab of deep airway, chilled (Dacron-tipped, slightly moistened, for bacterial and viral cultures)
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. *Do not submit fresh samples in glycerin (glycerin has not proven to be of value and prevents freezing of sections for FA tests).*
3. Samples for virus detection need to be taken at the onset of respiratory signs.
4. Nasal swab preservation: swabs must be kept moist and cool before and during shipment.
5. Fixed turbinate must be submitted to confirm the presence of porcine cytomegalovirus (inclusion body rhinitis)

AGENTS DETECTED BY ROUTINE EXAMINATION	
Bacteria	*Pasteurella multocida, Streptococcus suis, Salmonella choleraesuis, Actinobacillus pleuropneumoniae, Actinobacillus suis, Arcanobacterium pyogenes, Bordetella bronchiseptica, Haemophilus parasuis*
Viruses	PRRS virus, PCV2, SIV, PRCV, cytomegalovirus/inclusion body rhinitis (if turbinates are submitted for histopath)
Mycoplasma	*Mycoplasma hyopneumoniae*
AGENTS REQUIRING SPECIAL TESTS (BY REQUEST)	
Viruses	Isolation, sequencing

COMMENTS

- Cultures 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 infection.
- Porcine respiratory coronavirus is more readily detected from nasal swabs than lung tissues. High serum antibody titers to TGE virus in herds with no evidence of TGE diarrhea also may suggest the presence of PRCV. A differential ELISA serology test is available through the VDL.
- 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 of slices.
- PRRSV sequencing has higher success rate from specimens with lower cycle threshold values.

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