Manitoba
Agriculture, Food
and Rural Development

VETERINARY DIAGNOSTIC SERVICES
ANATOMICAL PATHOLOGY
Updated: October 1, 2014

The Anatomical Pathology Section of Veterinary Diagnostic Services provides necropsy and histopathology services.

Procedure	Specimen
Necropsy	1. Must be submitted through a licensed veterinarian or other authorized agency with a completed submission form. 2. All species, freshly dead (when possible), symptomatic, untreated, early in disease course. EXCEPTION: Live is preferable for diarrhea in young production animals and birds - Carcass should be held at refrigeration temperature until delivered - Do not freeze unless interval between death and delivery >3 days. This is subject to type of animal and ambient temperatures. - Do not euthanize neurological animals by brain trauma. 3. Suggested submission size: - Companion animal: 1 - Poultry from large operation: 5-10 - Poultry from small operation: 1-5 - Aborted piglets with placenta 3/litter including freshest; plus all mummified - Neonatal pigs: 3 - Weaner pigs: 2-3 - Feeder pigs: 1-3 - Aborted ruminants with placenta: 1-2 - Neonatal ruminants: 1-2 - Older ruminants: 1-2
Field Post Mortem	1. See General Principles of Sample Collection. 2. The history, clinical signs and gross lesions should determine which tissues are collected. 3. If unclear, fix tissue from major organs including brain; take fresh tissue for testing of differentials 4. See Microbiology and Virology on how to submit fresh tissue
Biopsy	1. If entire specimen **<3 mm** in any dimension, submit in histologic cassette with sponges to avoid loss. 2. For multiple specimens submit in individually labelled containers to indicate site. 3. If specimen is large, slice **partially through** to allow fixation. 4. Use dye or suture tags to orient specimen or identify margins, if pertinent. 5. Amputations: small specimens, e.g. digits, may be submitted whole; larger specimens, e.g. limbs, should be refrigerated and delivered within 24 hours.

ANATOMICAL PATHOLOGY: GENERAL PRINCIPLES OF SAMPLE COLLECTION

- Proper selection and preservation of samples is essential to make the most efficient and economical use of the laboratory.
- Digital photographs of lesions are always helpful.
- Use sharp and clean instruments for specimen collection. Use forceps to handle tissue by margins or mesenteric attachments. Rough handling and crushing exacerbates tissue artifacts.
- Fixation of tissues should take place at the time of necropsy. DO NOT send fresh tissues for histopathology as tissue autolysis is rapid.
- Use good quality 10% neutral buffer formalin with 10:1 formalin to tissue ratio. Excess formalin can be decanted after 1-2 days fixation
- Use a wide mouth leak proof unbreakable container as fixed specimens do not bend easily. Zip lock bags ARE NOT acceptable for formalin.
- Freezing seriously impairs histological examination of specimens. If tissue is partially frozen when taken, let section unthaw before placing into formalin.
- To prevent freezing, place samples in 70% ethyl alcohol but only after the samples were fixed in formalin as otherwise tissue will become rigid making tissue trimming difficult. The containers must be labeled with the chemical it contains as per Transport of Dangerous Goods regulations.
- Tissues for histology should be no thicker than 0.5-1.0 cm (pencil width) to ensure fixation all the way through. Thin (potato chip) pieces will curl and are difficult to orientate and trim. Thin tissues can be placed flat on a piece of cardboard.
- Tissues to collect will depend on history, clinical signs and gross post mortem. If in doubt take representative sections of all major organs for histology and collect fresh tissue to cover differentials. Not all needs to be sent to the laboratory initially but will be available if additional evaluation is required. Discussion with laboratory pathologist can help you decide what approach to take.
- Organs with focal or multifocal lesions should have multiple areas sampled for histology, including affected and unaffected; remember to take at least one section at the edge of a lesion to include some normal as well as diseased tissue.
- Tips by organ:

    - **Lung**: Palpation can often detect affected areas best. Each affected area that is different should be sampled. Cranial ventral lobes are most often affected but representative sections of caudal should also be taken. In small animals can fix whole lobe if < 1 cm in one dimension.

    - **Heart**: As a minimum take samples of right and left ventricle and interventricular septum including large papillary muscles; can be T shaped section in smaller animals. If heart disease is suspected in small animals open chambers and fix entire heart (if fresh not required). In larger

animals fix representative sections and consider submitting reminder of heart fresh for pathologist to examine.

- **Liver:** Multiple slices are necessary to detect deeper lesions. Each affected area that is different should be sampled. Can submit whole lobe if < 1 cm in one dimension.

- **Intestine**: Segments should be ~2-3cm in length; gently swish in 10% neutral buffered formalin by one edge to hasten flow into lumen. Segments do not need to be opened longitudinally. NEVER tie at ends as this greatly delays formalin reaching mucosa. For optimal preservation sections should be fixed < 15 minutes after death. For intestinal diseases multiple duodenum, jejunum, ileum, spiral colon, and colon should be taken.

- **Kidneys:** Section longitudinally to ensure poles are examined which are often sites of infarction. In small animals 1/2 of kidney can be fixed if < 1 cm in one dimension. Otherwise take representative 1 cm sections that include cortex and medulla to pelvic epithelium – do not miss medulla when taking sections from pyramidal kidneys.

- **Spleen:** If have infarcted or hemorrhagic lesions remember to include sections at edge of lesions as centre is often non-diagnostic.

- **Brain**: The entire brain should be removed in neurological cases. Sagittally section brain and submit one-half in formalin and one-half chilled. Be sure to include cerebellum and brainstem in addition to cerebrum. Brain should always be submitted from animals found dead without gross lesions in the thoracic or abdominal viscera. Do not slice brain into small strips, especially longitudinally, since it is nearly impossible to cut good sections from these brains. If the brain cannot be removed submit entire chilled head.

- **Spinal cord:** Chilled vertebral column (entire or portion) can be submitted if unable to remove spinal cord. Column can be cut into segments to fit container. If remove spinal cord, gently open meninges to allow proper fixation.

- **Eye:** Remove and fix as soon as possible after death. Extraocular tissue should be removed and if necessary submitted separately. At least 5mm of optic nerve should be present. Inject globe with 0.1-0.3cc of buffered 10% formalin (until globe is turgid) and then immerse entire globe in formalin.

Abortion

Species	Fixed	Fresh (chilled)	Included Tests
All	tongue, lymph node, lung, heart, liver, spleen, kidney, diaphragm, brain, skeletal muscle, ileum, colon, placenta	lung, stomach fluid, placenta	Culture
Bovine	Plus eyelid, salivary gland, thyroid, adrenal	Plus thymus, liver, spleen, brain	Plus PCR BVD, IBR, Neospora, Ureaplasma. Trace Mineral
Goats Sheep	Plus salivary gland, thyroid, adrenal	Plus Liver	Plus PCR Chlamydophilia. Coxiella, Toxoplasma. Trace Mineral
Horses	Plus salivary gland, thyroid, adrenal	Plus liver, lung, spleen	Plus PCR for EVR, EVA. Trace Mineral
Pigs		Include mummified lungs separately for Parvovirus	Plus PCR PRRS, PCV2, PPV

Central Nervous Disorders

Species	Fixed	Fresh (chilled)	Optional Tests
All	Brain, spinal cord	Brain, spinal cord	Culture
Bovine	Brain, spinal cord	Brain, spinal cord, meningeal swab	Culture, UV-light
Goats Sheep	Brain, spinal cord	Brain, spinal cord, meningeal swab	Culture, UV-light
Horses	Brain, spinal cord	Brain, spinal cord, CSF	PCR EVR, WNV
Pigs	Brain, spinal cord	Brain, spinal cord, meningeal swab	Culture

Enteritis

Species	Fixed	Fresh (chilled)	Optional Tests
All	Small intestine, large intestine, stomach, mesenteric lymph node	Small intestine, large intestine, mesenteric lymph node, feces, GI parasites, liver	Culture aerobic & anaerobic, direct smear, fecal flotation, parasite ID, PCR, FAT
Bovine	Small intestine, large intestine, rumen, abomasum, mesenteric lymph node	Abomasum, small intestine, large intestine, mesenteric lymph node, liver	Plus PCR BVDV, Mycobacterium avium paratuberculosis, , Bovine Coronavirus, Rotavirus, Cryptosporidium parvum, FAT Crypto and Giardia panel
Goats Sheep	Small intestine, large intestine, rumen, abomasum, mesenteric lymph node	Abomasum, small intestine, large intestine, mesenteric lymph node, liver	Plus PCR Mycobacterium paratuberculosis, BVDV *Check abomasum for Ostertagia & Haemonchus
Horses	Stomach, small intestine, large intestine, mesenteric lymph node	Stomach, small intestine, large intestine, mesenteric lymph node, feces, GI parasites, liver	Plus ELISA C. difficile, PCR Lawsonia
Pigs	Stomach, small intestine, large intestine, mesenteric lymph node, lung, brain	Stomach, small intestine, large intestine, mesenteric lymph node, feces, GI parasites, liver	Plus PCR Rotavirus, TGEV, PEDV, Delta coronavirus, Lawsonia intracellularis, Brachyspira hyodysenteriae, B. pilosicoli, ELISA C. difficile, Typing: Clostridium perfringens, E.coli,

Pneumonia

Species	Fixed	Fresh (chilled)	Optional Tests
All	Lung, bronchial lymph nodes	Lung, bronchial lymph nodes	Culture
Bovine	Lung, bronchial lymph nodes	Lung, bronchial lymph nodes	Plus PCR BVDV, IBR, BRSV, BCV, Mycobacterium bovis
Goats Sheep	Lung, bronchial lymph nodes	Lung, bronchial lymph nodes	Plus MCF
Horses	Lung, bronchial lymph nodes		Plus EIV, EVA, EVR
Pigs	Lung, tonsil, bronchial lymph nodes	Lung, tonsil, bronchial lymph nodes	Plus PCR Mycoplasma hyopneumoniae, PCV-2, PRRSV, Suid Herpesvirus 2 (PCMV), SIV (matrix) and SIV H1N1, H3N2 and pandemic 2009 H1N1, Typing Pasteurella multocida