Anatomic Pathology

Veterinary Diagnostic Services



The Anatomic Pathology Section of the Veterinary Diagnostic Services (VDS) provides necropsy and histopathology services.

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Contact Us

- Call us at 204-945-8220 in Winnipeg
- Email us at <u>vetlab@gov.mb.ca</u>
- Go to manitoba.ca/agriculture/vds

Necropsy

General Information for Necropsy Submissions

Animals must be submitted through a licensed veterinarian or other authorized agency, with a complete submission form.

VDS does not have the facilities to euthanize animals, other than live piglets (under 15 kg) for diarrhea workup and live poultry. Submit bodies that are freshly dead, representative of the problem, untreated, and early in the disease course:

- Keep small carcasses refrigerated. Put them in the freezer if the interval between death and delivery will be more than two days.
- Large carcasses should be kept cool and delivered as soon as possible.
- Avoid head trauma when euthanizing animals with nervous system signs.

Recommended submission size:

- companion animal: one
- poultry from large operation: 10 or fewer
- poultry from small operation: five or fewer
- aborted piglets: three per litter + placenta
- swine: three or fewer
- aborted ruminants with placenta: one or two
- ruminants: one or two

Submission Form and History

Visit our website for instructions on required information on forms. The history should include:

- questions you would like answered
- clinical signs, production, and management practices
 - o lameness, coughing, a decrease in feed or water consumption
 - o an increase in mortality, a decrease in egg production, a decrease in growth
 - o new medications, temperature fluctuation in the barn

Provide as much information as possible, as this improves diagnostic testing. History related to clinical signs, production, and management practices also help pathologist interpret gross examination findings.

Necropsy Billing and Tests Included

All necropsies include gross examination and a pathology report. Carcass disposal is included for food animals. Companion animals are released directly to Precious Pet Cremation. Precious Pet Cremation will bill directly.

Necropsy on Abortion Cases

Certain tests are included with the fee for necropsy on fetuses. The pathologist may order these tests at no additional charge. If additional testing is required, please specify this on the submission form. Additional charges will apply.

Equine abortion necropsy includes:

- gross examination
- histopathology
- bacterial culture (up to four samples)
- one polymerase chain reaction (PCR) test for Equid herpesvirus,
- one PCR test for Equine arteritis virus

This applies to each fetus submitted.

Bovine abortion necropsy includes:

- gross examination
- histopathology
- bacterial culture (up to four samples)
- fungal culture (up to two samples)
- one PCR test for Bovine herpesvirus 1
- one PCR test for Bovine viral diarrhea (BVD) virus
- one PCR test for Neospora caninum
- one PCR test for *Ureaplasma diversum*

This applies to each fetus submitted.

Sheep and goat abortion necropsy includes:

- gross examination
- histopathology
- bacterial culture (up to four samples)
- fungal culture (up to two samples)
- one PCR test for Coxiella burnetii
- one PCR test for Chlamydophila abortus
- one PCR test for Toxoplasma gondii

This applies to each fetus submitted.

Porcine abortion necropsy examination fee includes:

- gross examination
- histopathology
- bacterial culture (up to four samples)

- one PCR test for Porcine circovirus 2
- one PCR test for Porcine parvovirus
- one PCR test for Porcine reproductive and respiratory syndrome (PRRS) virus

The pathologists will examine only three fetuses per litter.

Necropsy on Poultry Cases

The necropsy fee for commercial poultry (facilities with more than 1,000 birds) includes gross examination of up to 10 birds. The birds must be from the same barn and exhibiting the same clinical signs. Submission of more birds is subject to an additional charge. Please note that histopathology and other tests are not included in the necropsy fee. Additional tests can be ordered on the submission form.

Testing for small flock (less than 1,000 birds) is subsidized for surveillance purposes. See Useful Links on the VDS homepage (Poultry Flock Avian Influenza Program).

Histopathology

General Information for Histopathology Submissions

Label containers of formalin or alcohol according to Transport of Dangerous Goods regulations. We also recommend the following:

- Use wide-mouthed, leak proof, unbreakable containers.
 - Fixed tissues become rigid and can be difficult to remove from narrow-mouthed containers, such as plastic pill bottles.
 - Regular tape around the rim of containers does not prevent leakage.
- Place the formalin container in a bag with absorbent material.
- Place the submission form in a separate bag to prevent contamination.

Tissue selection, size, preservative used and freezing can all impact histological examination.

Tissue Selection

- Tissue selection depends on history, clinical signs, and gross postmortem findings. Discussion with a pathologist may aid in sample selected for testing. If in doubt, cover differential diagnoses by taking representative sections of all major organs for histopathology.
- Organs with focal or multifocal lesions should have multiple sites sampled for histology, including affected and unaffected areas.
- Remember to take at least one section at the edge of a lesion to include some normal, as well as diseased tissue.

Tissue Size

• A thickness of 0.5 to 1.0 cm ensures fixation through the tissue.

 Thinner pieces will curl and are difficult to orientate and trim. These tissues should be placed flat on cardboard and placed in fixative.

Tissue Preservation

- Use 10% neutral buffered formalin.
- Formalin to tissue ratio should be 10:1.
- Samples (necropsy or biopsy) should be placed in formalin immediately. Delayed preservation results in autolyzed tissues with reduced diagnostic value.

Tissue Freezing

- Tissue freezing impairs histological evaluation of specimens.
 - Frozen biopsies are difficult to interpret and may be non-diagnostic.
- To prevent issues related to freezing:
 - Fix first in 10% buffered formalin, then place in 70% ethyl alcohol for transport.
 - Note that fixing samples using only 70% ethyl alcohol prevents tissue from freezing, but tissues are firmer and may fragment during shipping or during trimming.

Submission Form and History

Visit our website for instructions on required information on forms. The history should include:

- age, breed, coat colour and sex (there are breed and sex-related skin diseases)
- duration of problem
- treatment, therapy (especially dose and duration of corticosteroid therapy)
- lesion/mass location and appearance, we encourage sending us photographs prior to removal
- for bone tumor and leg amputations we also recommend submitting <u>radiographs prior to</u> amputation
- questions you would like answered

Histopathology Billing and Tests Included

Histopathology includes fixation as needed, tissue trimming, embedding, cutting and staining, using hematoxylin and eosin staining. Additional slides with special stains may be required (see the fee schedule for special stain charges).

Histopathology for food animals is charged per animal.

There are two levels of charges for companion animal submissions:

- A single charge will apply if you are submitting a tissue smaller than 2.5 cm in diameter or up to five biopsies.
- A multiple charge will apply if you are submitting a tissue larger than 2.5 cm in diameter or more than five biopsies.

Field Necropsies

General Information for Collection, Packing and Shipping

Fresh organ samples should be placed in different bags, labelled with at least two identifiers (e.g., animal identification and organ type). Different organs, and organs from different individual animals, should not be mixed in one bag. Mixing organs severely impacts quality of testing, especially bacterial culture.

General Guide for Tissue Selection

The history, clinical signs and gross lesions should determine which organs are sampled. If in doubt, cover differential diagnoses by taking representative sections of all major organs for histopathology and fresh for ancillary testing (e.g., culture and sensitivity, PCR, toxicology, fungal culture).

Organs with focal or multifocal lesions should have multiple areas sampled for histopathology, 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. Cranioventral and middle lobes are most often affected, but representative sections of caudal lung should also be taken. In small animals, a whole lobe less than one cm in one dimension can be fixed.

Heart: As a minimum, take samples of right and left ventricle and interventricular septum, including large papillary muscles. One T-shaped section can be collected from smaller animals. If heart disease is suspected in small animals, open chambers and fix the entire heart (if a fresh sample is not required). In larger animals, fix representative sections and consider submitting the remainder of the heart fresh for the pathologist to examine.

Liver: Multiple slices are necessary to detect deeper lesions. Each affected area that is different should be sampled. Whole lobes less than one cm in one dimension can be submitted.

Intestine: Segments should be about two to three cm in length. Hold by one edge and gently swish in 10% neutral buffered formalin to hasten flow into lumen. Segments do not need to be opened longitudinally. NEVER tie off the ends, as this greatly delays formalin reaching mucosa. For optimal preservation, sections should be fixed as soon as possible after death, ideally within 20 minutes. For intestinal diseases, sample the duodenum, jejunum, ileum and colon.

Kidneys: Section longitudinally to ensure poles are examined, because infarcts often occur here. In small animals, the kidney can be fixed if less than one cm in one dimension. Otherwise, take representative one cm sections that include cortex and medulla to pelvic epithelium.

Spleen: If there are infarcts or hemorrhagic lesions, remember to include sections at the edge of lesions, as the centre may be non-diagnostic.

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

Spinal cord: The chilled vertebral column (entire or portion) can be submitted instead of removing the spinal cord. The column can be cut into segments to fit the container. If removing the spinal cord, gently open the dura mater to allow proper fixation. It is preferable to submit the entire body when spinal column lesions are suspected.

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

Photographs & Video

Photographs taken during necropsy can be a useful supplement to a description of gross lesions. Clients are encouraged to submit photographs, especially if unusual lesions are encountered during the field necropsy.

If an animal is exhibiting atypical clinical signs, it can be useful to take a video prior to euthanasia and submit this to the lab.