

Best Practice Sample Submission

Tissue/Necropsy

Sample collection

- Whenever possible, submission of the entire animal is preferred.
- Under ideal circumstances, the animal should be submitted as soon as possible after death.
- If a period of time must elapse prior to submission, the animal should be kept cold, but not frozen.
 - Freezing produces artifacts that make interpretation difficult. Please include a complete clinical history with all submissions. It is most helpful when the attending veterinarian writes or contributes to the history.
- Submit sections of lesions and samples of major organs: lung, heart, liver, kidney, spleen, brain, ileum, small intestine, endocrine tissues, colon and others as may be dictated by the clinical syndrome. Contact the laboratory if you have questions.
- Ship fresh unfixed brain by itself in a hard container such as a Tupperware or similar plastic container. If you don't have a hard container, ship the brain in a whirl pac or ziplock bags by itself.

Fixation

Ten percent (10% of a 37-40% concentrated stock solution) neutral buffered formalin is the fixative of choice.

- Place in the fixative as soon as possible.
- Fix tissue slices 24-48 hours. Tissue volume should be less than 1/10th that of the formalin solution. Slices should be no thicker than 1 cm. If formalin is not available, the tissues should be refrigerated and shipped cold (frozen gel packs or equivalent). Do not freeze.
- Brain submitted for Rabies testing needs to be wholly intact in order to get the correct samples and cannot be fixed.
- Brains from food animal cases not intended for rabies testing require one half of the brain (right or left side) should be fixed without slicing into smaller pieces.

- Use wide mouthed, leak proof containers. A tissue that is fresh and pliable can easily be put into a narrow mouthed jar, but it becomes hard when fixed and cannot be readily removed.
- Alternatively, tissues can be fixed in formalin solution for 24 - 48 hours, removed from the solution, wrapped in a formalin-soaked gauze sponge, placed in a plastic bag, and sealed for shipment. This technique decreases the probability of formalin leakage during shipment.
- Special histopathology requests - Some samples, such as eyes, may require different fixation or handling procedures. Please contact the laboratory prior to taking these samples.

Fixation Recipe

- Commercial Formaldehyde (37-40%) - 100 ml
- Distilled water - 900 ml
- Sodium phosphate monobasic - 4.0 g
- Sodium phosphate dibasic (anhydrous) - 6.5 g (pH should be 7.2 ± 0.5)

Packaging and shipping

- Protect formalin - fixed specimens from freezing
 - Freezing produces artifacts in tissues that generally result in the sections being unsuitable for interpretation. Frozen tissues should be formalin fixed after thawing.
- Protect formalized samples from direct contact with frozen packs OR frozen specimens as this may result in freezing of the fixed tissue.
- During cold weather it may be necessary to use an insulated container for shipping formalin-fixed specimens.

For surgical pathology samples

Sample collection

Attention to certain details will help assure that a sample will provide the diagnostic information needed.

- Provide pertinent clinical history, physical examination findings (distribution and duration of the lesions) and clinical laboratory findings. This will help the pathologist interpret the

findings in the context of the clinical case and helps to ensure that useful information is obtained. This information is of most critical importance for skin biopsies.

- Provide a precise description of the lesion that was biopsied as well as the site of the biopsy. These features are often critical for proper interpretation of the lesion.
- If the lesion was previously biopsied, the case accession number (if previously sent to the VDL) or previous biopsy report, is often useful in evaluating whether the lesion is the same disease process as in the previous biopsy and whether progression or regression has occurred.
- For excision biopsies in which the completeness of excision margins is evaluated, it is very helpful if the surgical margins are stained by the surgeon with india ink (other inks will dissolve in the solvents used in tissue processing).

For herd samples (Cattle, Sheep, Pigs and Goats)

Sample collection

- In cases involving a herd problem, the best specimens for submission are live, acutely affected, untreated animals.
- When submission of a live animal is not possible, the second best submission would be samples obtained by the submitting veterinarian or trained personnel at the time of field necropsy.
- Prior to euthanasia, collect blood in red top and purple top tubes.
- Perform a complete postmortem examination and submit appropriate specimens and a complete history.

It is important that the veterinarian or the person most familiar with the clinical syndrome select which animals to submit when there is a herd problem.

For farmed cervidae samples

Sample collection

- Collect a section of brain stem that includes obex and one medial retropharyngeal lymph node. Place both tissues in 10% buffered formalin (ensure formalin does not freeze). If buffered formalin is not available, tissues can be packed in a hard container in ice and submitted fresh. It is also acceptable to submit frozen tissues when submission is delayed, although this method is less desirable.

- Please use the CWD submission form and provide the owner's name, address, animal identification, species, sex and age.
- Form should be within the shipping box but separated from the ice packs and/or sample.

An entire frozen head can also be submitted for an additional charge. (A 10% out of state surcharge applies for out-of-state submissions).

Please Note: It is critical for test accuracy that the proper lymph node be submitted. Improper samples will be reported as unsuitable for testing. If you would like training on CWD sample submission procedures, please contact the [Minnesota Board of Animal Health](#) at 651-296-2942 or toll free at 800-627-3529.

For wild cervids samples

Sample collection

- Collect one or both medial retropharyngeal lymph nodes. Large animal veterinarians and a video on the DNR website can offer assistance, if needed.
- Place tissues in 10% buffered formalin (ensure formalin does not freeze). If buffered formalin is not available, fresh tissues can be packed in a hard container (so they do not get crushed) and submitted on ice. It is also acceptable to submit frozen tissues in a hard container when submission is delayed, although this method is less desirable.
- On the Necropsy Sample Submission Form - Ruminant request CWD testing for hunter-harvested deer.
- Please print the owner's name, address, email address (for results report) species and animal sex. Form should be within the shipping box, but separated from the ice packs and/or sample.

An entire frozen head can also be submitted for an additional charge. (A 10% out of state surcharge applies for out-of-state submissions)

Please Note: It is critical for test accuracy that the proper lymph node be submitted. Improper samples will be reported as unsuitable for testing. If you would like training on CWD sample submission procedures, please contact the [Minnesota DNR](#).

Testing is done by immunohistochemistry (IHC) and results are typically available in two weeks.

For fish samples

Sample collection

- It is critical that the fish are collected properly. Live fish showing clinical signs of the disease will greatly increase the chances of an accurate diagnosis.
 - To increase the probability of catching sick fish, first try finding them swimming slowly near the edge of the pond/lake near the surface. When none are seen, catching fish randomly (snagging/netting) is best.
 - Feeding fish is not recommended, since sick fish rarely take feed.
- Fish that are freshly dead (clear eyes, red gills, and slimy skin) are the second choice, however even at this stage several diseases cannot be diagnosed due to invasion of environmental bacteria, decomposition of tissue, and loss of external parasites.
- Fish that are beginning to decompose are as difficult as live-healthy fish with no clinical signs of disease.

Packaging and shipping

- Water samples should accompany unhealthy fish sent to the Veterinary Diagnostic Laboratory or the water data from the time the fish are collected.
- At least 8oz of water should be collected 1 foot below the surface of the water as far from the shoreline as you can reach.
- The water should be in a clean bottle that can seal tight. Water in the bag or bucket of fish is not suitable for many water quality tests.
- In addition, if plants or algae are sent, they should be in their own bag or bottle.

Personal delivery (Recommended):

- Delivering the fish personally to the Diagnostic Laboratory will greatly reduce the shipping stresses and possibility of death in transit.
- At least one pathologist is on staff from 7:45 am to 4:30 pm Monday thru Friday to assist you with your fish upon arrival. However, calling the Diagnostic Laboratory prior to your arrival will help ensure immediate attention.
- If the fish are expected to die en route, putting them in a clear plastic bag without water on frozen cold-packs is the best option, as dead fish in water are rarely suitable for diagnosis.

- Wet ice may be used (personal delivery only) if kept separate from the fish and not allowed to leak from the container.

Shipping by mail:

- If a trip to the Diagnostic Laboratory is not an option, shipping the fish overnight express is an alternative.
- Live fish must be in a bag of water with oxygen to sustain them for the trip.
- Fish too sick to survive or freshly dead fish should be placed in a clear plastic bag without water and buried among frozen ice packs.
- Wet ice should not be used because it commonly leaks from shipping containers.
- Fish may also be sent frozen, but dry ice must be used.
- Another alternative is to place small fish or pieces of large fish in a formaldehyde solution.
 - If formaldehyde is to be used, it should be clearly marked in a sealable container, then triple bagged to prevent leaks and contact with the skin of carriers or Laboratory personnel.
 - Formaldehyde and containers are provided by the Diagnostic Laboratory (pick-up only).
- It is always recommended that you notify the Diagnostic Laboratory prior to shipping fish so proper attention can be given upon their arrival.

For rabies

Sample collection

- It is recommended to submit whole body or head samples instead of brain tissue only for this testing as it has the potential to expose multiple people to the disease.