

General Submission Guidelines

This document is intended to provide guidance for proper collection, storage and submission of samples to the NDSU-Veterinary Diagnostic Laboratory. For more information regarding specific test requirements, please visit vdl.ndsu.edu or call 701-231-8307. **Properly collected, stored and submitted samples will give the best results.**

Provide complete case information on the submission form. Relevant information includes how the sample was collected (include source and collection date), veterinarian information, signalment, clinical signs, lab abnormalities, contributing disease, treatment, differential diagnosis and, in particular, specific questions you have (that is, why you are submitting the sample).

For continuity, include any previously submitted case numbers. If the accession number is not available, just indicate that prior cases exist.

History is critical because it dictates not only case setup but also interpretation of test results.

- Protect the submission form and any relevant paperwork from moisture or leaking samples by placing them in a sealable plastic bag in the sample box.

Knowledge of pathogenesis of suspected diseases can aid in sample choice. The website provides acceptable sample information for each test, but the preferred sample may vary depending on the stage of disease, manifestations and species affected. Multiple samples from different sites or multiple samples collected during a period of time may be needed for a diagnosis. Typically, the best samples are collected during the acute stage of disease prior to treatment.

Clearly label all samples. Include any animal identifiers (name, breed, sex, ID number). If multiple samples are submitted, label them in numerical and consecutive order (1, 2, 3, etc.), corresponding with the numbers on the Sample Identification Form found at vdl.ndsu.edu/forms.

General Guidelines

- **Fresh samples yield the best results.** Many samples begin to degrade rapidly after collection; therefore, samples should be received by the laboratory within 24 hours of collection whenever possible.
- Tissues, fluids, aspirates or fecal material are always preferable to swabs. Submit swabs only if no other sample options are available.
- Never send a syringe with the needle attached. Use a syringe cap to secure material in the syringe if transfer of the fluid to another tube is not possible.
- Send tissues and fluids in sterile, leak-proof containers. Gastrointestinal samples must be separate from other tissues.
- Send feces in a clean, leak-proof container. No fecal material should be present on the outside of the container.
- If testing for bacterial culture and polymerase chain reaction (PCR) testing are requested, please submit two sets of swabs. Submit one swab in transport media (Amies, Stuart's, etc.) for culture and another with about 1 milliliter (ml) of sterile saline in a sterile tube to maintain moisture for PCR.
- Use refrigeration temperatures for storing and sending unfixed samples.
 - Exceptions: Fungal, cerebrospinal fluid (CSF), blood or anaerobic cultures
 - Be aware that transit times and weather conditions may impact sample quality. For example, extra ice may be necessary in July, while freezing may be a concern in January.

Histopathology

- Send fixed and fresh tissues. Any delay in fixation can affect results significantly. The ratio of tissue to 10% formalin should be approximately 1:10 to avoid underfixation, which allows tissues to continue deteriorating. Be mindful of the container size. Fresh tissues may fit in the container initially, but after fixation with formalin, the tissues expand and may be difficult to remove from the container.
- Send fluids for cytology testing in EDTA tubes. Dried, unstained slides made at the time of collection also are recommended. Note: If culture also is desired, a second set of samples collected without additives is needed (that is, red-top tube).
- In cold weather, add one part ethanol to nine parts 10% buffered formalin to prevent freezing during transit.
- Use a pencil or solvent resistant marker (no "permanent" marker) to label slides.

► Serology

The detection of a specific antibody response can provide indirect support for infection/disease. Submitting acute and convalescent (paired) serum samples are often critical for meaningful interpretation of titers. For most diseases, paired serum samples should, on average, be collected two to four weeks apart. The presence of maternal antibody in animals less than 6 months of age and a history of vaccination can complicate interpretation.

- False negative results can occur when testing early in the infection.
- Contaminated or hemolyzed sera can affect results significantly, leading to false positive or false negative results.
 - Samples with moderate to severe levels of hemolysis will be rejected.
- Samples collected prior to colostrum intake are critical for diagnosis of in utero infections.

Sample Processing:

- Collect blood in a red-top tube. Label the samples (animal ID and corresponding sample number from the submission form). Label acute or convalescent, if applicable.
- Allow the sample to clot at room temperature.
 - Note: Processing too quickly and delaying separation of the serum from the clot can increase the amounts of cellular products and degree of hemolysis, potentially affecting test results.
- Centrifuge the sample at 1,000 to 1,300 x g for 10 minutes.
- Remove the serum into a clean tube (no additives). Avoid transfer of any cellular elements.
- Maintain the sample at 4°C. For best results, submit the sample to be received by the laboratory within 72 hours of collection.
- Package the sample for shipping. Include a submission form with the samples. Record any pertinent sample or case history information to help with the interpretation of results.
- Transport sample on ice pack. Avoid freeze-thaw cycles.
- Greater than 1 ml of sera is ideal to ensure adequate sample for all testing requested and allows for repeat or follow-up testing if needed.

► Toxicology

Consultation with the laboratory prior to collecting and submitting samples for toxicology testing is recommended. Sample volume and conditions during transit are of particular importance and vary based on a variety of factors. Guides are available for water and nitrate feedstuffs at vdl.ndsu.edu/resources.

► Virology

- High-quality, fresh samples are necessary to maintain viral morphology for direct fluorescent antibody detection or electron microscopy. Rabies testing requires an intact whole fresh brain; refer to our rabies submission guide at vdl.ndsu.edu/resources for detailed information.
- Send tissues and fluids in sterile, leak-proof containers.
- Refrigerate samples after collection. Ship samples on ice packs and submit to the laboratory within 24 hours.

► Molecular Diagnostics

Polymerase chain reaction (PCR) is extremely sensitive and specific but will detect viable and nonviable pathogens. Contamination is an important consideration, especially when multiple animals are sampled at the same time.

- Tissues and fluids should be sent in sterile, rigid, leak-proof containers (no Whirl-paks).
- Feces should be sent in clean, well-labeled, leak-proof containers. No fecal material should be present on the outside of the container.
- Swabs are acceptable if no other samples are available or when sampling mucosal surfaces. Send in viral transport media, universal transport media or sterile red-top tubes with sufficient saline to maintain moisture (approximately 1 ml). Dry swabs are acceptable but are not ideal. Do not submit swabs in serum separator (gel) tubes or in gel transport media because it interferes with PCR. Submit two swabs if PCR and microbiology testing are requested.
- Refrigerate samples after collection. Ship samples on ice packs and submit to the laboratory within 24 hours of collection when possible.
- See our Molecular/Microbiology Collection Guide at vdl.ndsu.edu/resources for detailed collection guidelines.

► Microbiology

Sample quality is critical for successful detection of pathogens and interpretation of culture growth. Factors to consider when submitting samples for culture:

- Submission form: Include history and antimicrobial treatment (if any), and specify the sample source and method of collection – for example, urine collected via cystocentesis.
- Tissues, fluids, aspirates or fecal material are the best samples for testing. Send swabs only if no other sample options are available.
- When sampling, avoid contamination with normal flora or environmental bacteria. Collect as aseptically as possible.
- Delay in culture setup increases the likelihood of commensal bacteria overgrowth/loss of pathogen. Submit samples to the laboratory within 24 hours of collection whenever possible.
- Samples for bacterial culture must maintain moisture.
- Inappropriate temperatures may allow for overgrowth in samples with normal flora or may cause the loss of a pathogen.
 - Cerebrospinal fluid (CSF), blood, fungal and anaerobic cultures should be maintained at room temperature.
 - All other samples should be refrigerated after collection.
- If anaerobic pathogens are suspected, submit at least 1 ml of fluid or more than 1 centimeter³ of tissue. Alternatively, an anaerobic transport system can be used to maintain the appropriate atmospheric condition.
- Certain bacteria require special culture medium for testing (*Mycoplasma* sp., *Clostridium perfringens*, *Campylobacter* sp., anaerobes, *Yersinia* sp. (not *pestis*), *Salmonella* sp., *Streptococcus equi*). If these organisms are suspected, note it on the submission form to ensure cultures are setup on the appropriate media.
- See our Molecular/Microbiology Collection Guide at vdl.ndsu.edu/resources for detailed collection guidelines.