NDSU VETERINARY DIAGNOSTIC LABORATORY

General Submission Guidelines

This guide details general guidelines for proper specimen collection, storage, and submission. For more information regarding specific test requirements please see www.vdl.ndsu.edu or **contact the laboratory at 701-231-8307 or 701-231-7527. Properly collected, stored and submitted samples give the best results.**

Provide complete 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 (i.e. why you are submitting the sample). For continuity it is important to include any previously submitted cases; if the accession number is not available, just indicate that prior cases exist. History is critical as it dictates not only case set up but also interpretation of test results.

- Note: Send the submission form and any relevant paperwork with the sample in a second sealable bag to protect the paperwork in case of any sample container leaks. Glass should be well-packed as it can break in transport.

Knowledge of pathogenesis of suspected disease can aid in sample choice; the website provides acceptable samples for each test but the preferred specimen may vary depending on the stage of disease, manifestations, and species affected. Multiple samples or multiple samples collected over 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) and if samples from more than one animal are submitted, include the corresponding number ID from the submission form.

General Guidelines:

- Fresh samples yield better results. Many samples begin to degrade rapidly after collection; therefore samples should be received by the laboratory within 24 hours whenever possible.
- Send tissue or fluids in sterile, leak-proof containers. Gastrointestinal samples must be separate from other tissue.
- **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.**
- **Send feces in a clean, leak-proof container. No fecal material should be present on the outside of the container.**
- **Use refrigeration temperatures for storing and sending unfixed samples**
    - **Exceptions: Fungal, CSF culture, blood culture, anaerobic culture**
    - **Be aware of transit times and weather conditions that 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 significantly affect results. Ratio of tissue to 10% formalin should be approximately 1:10 to avoid under-fixation which allows tissues to continue deteriorating.
- Send fluids for cytology testing in EDTA tubes. Dried, unstained slides made at time of collection are also recommended. Note: If culture is also desired, a second set of samples collected without additives is required. (i.e. red-topped tube).
- In cold weather, add 1 part ethanol to 9 parts 10% buffered formalin to prevent freezing during transit.
- Use a pencil or solvent resistant marker (i.e. no “permanent” marker) when submitting unstained 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 interpreting of titers. For most diseases paired serum samples should, on average, be collected 2-4 weeks apart. The presence of maternal antibody in animals <6 months of age and a history of vaccination can complicate interpretation.

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

Proper processing of red top tubes for serology testing:

- After blood collection, stand tube upright and let sit at room temperature for 30 to 60 minutes.
    - Note: Times less than 30 minutes and greater than 60 minutes can increase the amount of cellular products and degree of hemolysis which affect test results.
- Centrifuge at 1000-1300 x g for 10 minutes.
- Remove serum into sterile tube.
- Maintain at 4ºC until transport and during transport. Avoid freeze-thaw cycles.
- > 1mL sera is ideal to ensure adequate sample for all testing requested and any repeat or follow-up testing 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.

Virology:

- **Multiple types of tests are available for virus detection. High quality, fresh samples are necessary to preserve either viable virus (viral culture) or to maintain viral morphology for direct fluorescent antibody detection or electron microscopy.** Swabs should always be sent in viral transport media (preferred), universal transport media, or in sterile red top tube with sufficient sterile saline to maintain moisture.
- Send tissues and fluids in sterile, leak-proof containers.
- Refrigerate samples after collection. Ship samples on cold packs and submit to the laboratory within 24 hours.

Molecular Diagnostics (PCR):

PCR is extremely sensitive and specific and it will detect both viable and non-viable pathogens. **Contamination is an important consideration, especially when multiple animals are sampled at the same time.**

- Tissues and fluids should be sent in sterile, leak-proof containers.
- Feces should be sent in clean, leak-proof container. **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 in sterile red top tube with sufficient sterile saline to maintain moisture. Dry swabs are acceptable but not ideal. Samples sent in serum separator tubes containing gel will be rejected.
- Refrigerate samples after collection. Ship samples on cold packs and submit to the laboratory within 24 hours.

Bacteriology:

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

- Tissues, fluids, aspirates, or fecal material are the best samples. 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 >24 hours to lab increases likelihood of commensal bacterial overgrowth/loss of pathogen.
- It is critical that sample for bacterial culture maintain moisture.
- Inappropriate temperatures may allow for overgrowth in samples with normal flora or may cause the loss of a pathogen.
    - Note: CSF, blood, and samples collected for anaerobic culture should be maintained at room temperature and submitted to the laboratory within 24 hours.
    - All other samples should be refrigerated after collection.
- If anaerobic pathogens are suspected submit at least 1 ml fluid or more than 1cm^3 tissue. Alternatively an anaerobic transport system can be used to maintain the appropriate atmospheric condition.
- Certain bacteria require special transport media (*Mycoplasma* sp., *Campylobacter* sp.) for optimal recovery. *See Bacterial Transport Systems Guide.*

Please note some bacteria require special growth media and conditions (*Mycoplasma* sp., *Campylobacter* sp., anaerobes, *Yersinia* sp. (not *pestis), Salmonella* sp., *Streptococcus equi)* and if suspected must be noted on the submission form so they are set up on the appropriate media.

Collection Guidelines by Sample Source

Source	Ideal Collection Method	Container	Temperature Requirements	Shipping
Blood	-Surgical prep with percutaneous blood collection -2-3 samples over 24 hours -Sample when febrile	-Blood culture bottle or Yellow-top Isolator tube	Room temperature	Room temperature and to the lab within 18 hours for Isolator Tube, within 24 hours for blood culture bottle.
Dermatophyte	-Clean lesion with 70% alcohol; pluck hair from edge of lesion -Toothbrush over coat	Sealed envelope or bag	Room temperature	Room temperature and to the laboratory within 24 hours.
Eye	-Corneal scrapings, swab of conjunctiva or edge of corneal ulcer. -Remove crusts/contaminants before sampling. -Topical dyes and anesthetics can interfere. Rinse eye prior to sampling. -Sample from unaffected eye can help interpret growth in affected eye if unilateral	-Aerobic transport system -Universal or viral transport media for *mycoplasma, chlamydia,* and viruses if suspected.	Refrigeration temperature after collection	With cold packs and to the lab within 24 hours.
Feces	Per rectum or immediately collected from the ground	-Send 5-10 grams in sealed, leak-proof container or bag. -Cary-Blair Transport Medium acceptable for Bacteriology ONLY	Refrigeration temperature after collection	With cold packs to the lab within 24 hours.
Joint	-Surgical prep with percutaneous fluid collection -Synovium (surgical sampling)	-Sterile Container, Port-A-Cult fluid transport, blood culture bottle	Room temperature	Room temperature and to the lab within 24 hours.
Lower Respiratory	-Transtracheal wash, bronchioalveolar lavage, tissue, aspirate	-Sterile container or Port-A-Cul fluid transport container -Collect fluid into EDTA for cytology or send slides; do NOT send fluid for culture in EDTA	Refrigeration temperature after collection	With cold packs and to the lab within 24 hours.
Outer Ear	Clear debris from canal with saline, then swab ear canal. Two swabs, one for culture and one for Gram stain (Same tube)	-Aerobic bacterial transport system. Note: Gel systems are not ideal for direct Gram stains	Refrigeration temperature after collection	With cold packs and to the lab within 24 hours.
Skin	-Surgical prep with biopsy or active inflammation or aspiration of pustules. -Cleanse surface of skin with saline; swab the affected area, do not touch unaffected areas.	-Aerobic bacterial transport system. -Submit two swabs, can be in same tube Note: Gel systems are not ideal for direct Gram stain.	Refrigeration temperature after collection	With cold packs and to the lab within 24 hours.
Upper Respiratory	-Remove crusts and/or debris -Swab nasal cavity or nasopharynx Note: Do not culture nasal passage to determine pathogen of lower respiratory tract	-Aerobic transport system -Universal or viral transport media for mycoplasma, chlamydia, and viruses if suspected	Refrigeration temperature after collection	With cold packs and to the lab within 24 hours.
Urine	-Sterile prep of skin with cystocentesis -Mid-stream free catch after cleansing of genital region -Samples from indwelling catheter not recommended	-Send 3-5 mL in sterile urine cup, red top tube, urine transport system (grey top)	-Refrigeration temperature within 1 hour of collection (sterile tube) -Room temperature for urine transport system	-Sterile cup or tube, with cold packs to the lab within 24 hours -Urine transport system, room temperature and to the lab within 48 hours.
Uterine	Clean external genitalia and sample uterus with double guarded swab. Biopsy with histopathology is recommended procedure for breeding soundness exams and to aid in culture interpretation.	-Aerobic transport system for swab or tissue - 10% Formalin for biopsy	Refrigeration temperature after collection	With cold packs to the lab within 24 hours.

Specimen Transport Systems Guide				
Type	**Purpose/Function**	**Sample Type**	**Storage/Transport Temperature**	**Notes**
Amies	Improved recovery of fastidious organisms- charcoal enhances maintenance of fastidious organisms by scavenging bacterial waste products.	Ocular swab, respiratory swab, wound swab	Room temperature or refrigerate after collection	-Gel systems are not ideal for direct Gram stains. Use of a liquid media transport system is recommended if direct Gram stain is desired.
Blood culture bottle	Nutritive support of microorganisms; contains additive to neutralize bacteriocidal tendencies of blood and prevent coagulation.	Blood for culture only	Room temperature	-Top of blood culture bottle is not sterile, prep with 70% alcohol prior to inoculation. -Use is critical for a successful blood culture
EDTA Tube (purple top)	Contains anticoagulant to prevent clotting.	Whole blood, fluids for cytology	Refrigerate if not analyzed within 2 hours	-**Not acceptable for bacterial culture**
Isolator Tube	Designed for isolation and concentration of microorganisms; lyses leukocytes and erythrocytes in the blood and helps prevent coagulation.	Blood for culture	Room temperature or refrigerate after collection	-Top of tube is not sterile, prep with 70% alcohol prior to inoculation - Use is critical for a successful blood culture
Modified Cary-Blair Transport Media	Maintains enteric pathogens and helps prevent overgrowth of normal flora. Media is buffered to prevent pH changes. Only add fecal material to the fill line.	Fecals only	Refrigerate after collection, ship on cold pack	**Only acceptable for bacterial culture.** Send second specimen in clean container for fecal floats and molecular assays.
Port-A-Cul, Starswab, or other Anaerobic transport media	Maintains anaerobic environment and is suitable for both facultative anaerobic (most bacteria) and anaerobic bacteria.	Fluids, tissues, swabs	Room temperature	
Sterile Red Top Tube	Sterile tube without preservatives.	Fluids, ear notches, swabs (with 1 mL sterile saline added), blood, preputial washings, urine	Refrigerate after collection, ship on cold packs	-Acceptable transport container for most specimen types. -Do not use for submission of fecal samples. -Top of tube is not sterile, prep with 70% alcohol prior to inoculation -Plastic tubes are recommended
Stuart's Transport System	General bacteriostatic media designed to preserve bacterial viability and reduce overgrowth. May have gel, sponge, or liquid in tube.	Ocular swabs, orogpharyngeal swabs	Room temperature or refrigerate after collection	
Universal Transport Media	Maintains viruses, *Ureaplasma, Mycoplasma,* and *Chlamydia sp.* for up to 48 hours.	Ocular swabs, oropharyngeal swabs	Refrigerate after collection, ship on cold packs	
Urine Transport tube (grey top)	Contains buffered boric acid which enhances recovery of pathogens in urine and helps prevent overgrowth. Maintains samples up to 48 hours without refrigeration.	Urine	Room Temperature	Not acceptable for urinalysis or PCR testing.
Viral Transport Media	Buffered salt solution to maintain viruses at appropriate pH; may contain pH indicator, antibiotics, and cyropreservatives.	Ocular, respiratory, and mucosal swabs	Refrigerate after collection, ship on cold packs	-Fluid samples should be submitted in sterile containers without the addition of preservatives -Not for culture submissions
Weybridge Media	Critical for isolation of *Campylobacter sp.* from genitals	Genital tract	Ship on cold packs to the laboratory within 24 hours of collection, and protect from light.	-Preputial washings and cervical mucous in a sterile red top tube are acceptable for testing by PCR for *C. fetus* subsp. *venerealis.*