Microbiology
Veterinary Diagnostic Services

The Microbiology Section of the Veterinary Diagnostic Services (VDS) encompasses diagnostic bacteriology, mycology and parasitology. Culture, microscopy and immunologic methods are used for the detection of pathogenic bacteria, fungi, protozoa, helminths and arthropods. See the Virology and Molecular Diagnostics Section for information on polymerase chain reaction (PCR) assays done for certain bacteria and protozoa.

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Contact Us

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

Turnaround Times

- The VDS Fee Schedule outlines the estimated turnaround times for all tests.

- The time for completion of bacterial culture is variable, as it depends on the nature of the specimen and the type of bacteria involved.
    - When specimens are collected from a normally sterile site and contain no bacteria or a single rapidly-growing species, final reports are usually sent within two working days.
    - When specimens contain slow growing bacteria (e.g., anaerobes, actinomycetes) or multiple bacterial species (e.g., chronic otitis cases, contaminated samples), it takes longer to report the final result. Preliminary reports are sent as appropriate.

Specimen Selection and Collection

- Directly sample the site of suspected infection as indicated by clinical signs, gross lesions or medical imaging.

- When collecting from normally sterile sites, avoid contaminating the specimen with environmental microorganisms or the commensal bacteria of skin (the animal's or the collector's), feces or mucosal surfaces.

- Place antemortem specimens in appropriate sterile containers.

- Do not use anticoagulant tubes or serum separator tubes.

- Use swabs with dacron, rayon or nylon tips and plastic handles. Cotton and wood materials can inhibit the growth of fastidious bacteria. Submitting swabs in transport media (e.g., Amies or Stuart's media) is preferable in most situations. If the swab is intended for culture, do not use transport media intended for virus isolation or PCR testing. Dry swabs are only acceptable if they will be processed at VDS within a few hours of collection.

- Specimens should be refrigerated and submitted to VDS as soon as possible after collection. If more than two days will elapse between specimen collection and processing at VDS, samples of tissue or exudate should be frozen.

Exceptions

- Maintain *Tritrichomonas* pouches and blood culture bottles at room temperature – never refrigerate or freeze these specimens.

- Specimen containers must be labeled with the specimen type and two unique identifiers (animal identification, owner name).

- Whenever possible, collect specimens for bacterial culture before starting antimicrobial therapy.
  - Drugs that fail to control an infection may still inhibit *in vitro* growth. For follow up testing, it is ideal to cease treatment for 48 to 72 hours before specimen collection. Specimens collected during antimicrobial therapy are still acceptable and may yield significant results; however, negative cultures obtained from these specimens will be questionable.

The following section describes information on sampling and testing for:

- superficial skin and ear canal
- wounds, cellulitis, draining tracts and abscess
- histotoxic clostridial infections
- conjunctivitis
- urine
- milk
- respiratory track
- blood
- joint, pleural and peritoneal fluids
- feces
- postmortem specimens
- antimicrobial sensitivity testing

Superficial Skin and Ear Canal

Otitis Externa and Pyoderma	Dermatophytosis	Acariasis	Macroparasites
• Gram staining is done on swabs submitted for bacterial culture. • Yeast such as *Malassezia* and *Candida* will be seen on the Gram stain and may grow on bacterial media, if concurrent bacterial infection is absent. • Requesting fungal culture is generally not warranted. If fungal culture is desired, submit a separate swab. • Swab ears separately. • The Microbiology lab will not pool multiple swabs for a single culture.	• Collect at least 2 cubic centimetres of hair and scab material from the periphery of lesions and submit for fungal culture. • If enough sample is received, it will be examined by the KOH-calcofluor white method which has the potential to detect fungal elements rapidly. • Dermatophyte growth usually takes at least 5 days; negative culture will be reported after 2 weeks.	• If mange is suspected, submit skin scrapings for a parasitology direct examination. • Mites such as *Sarcoptes* and *Demodex* are often difficult to detect. Multiple skin scrapings are recommended.	• Submit lice, fleas and ticks for parasite identification. • These can be submitted in 70% ethanol.

Wounds, Cellulitis, Draining Tracts and Abscesses

- Gram stain, aerobic culture and anaerobic culture are conducted on specimens from lesions of this type. Swabs are acceptable, but samples of exudate (1 ml or more) or fresh tissue biopsies are better, especially for anaerobic culture. Sample from the periphery of abscesses, as the central exudate may not contain viable bacteria.

Blastomycosis	Granulomas
• If *Blastomycosis* is strongly suspected by the clinician, smears should be sent to the Clinical Pathology Section for cytology.	• If lesions are suspected to be granulomas submit fresh specimens for bacterial culture, fungal culture and acid-fast staining; samples may be sent to a referral laboratory for mycobacterial detection. • Fixed samples for histopathology are also recommended.

Histotoxic Clostridial Infections

- In cases of suspected blackleg or malignant edema/gas gangrene in ruminants, submit samples of skeletal muscle and/or subcutaneous tissue for the clostridial fluorescent antibody test (FAT) panel. The panel includes *Clostridium chauvoei, Clostridium septicum, Clostridium novyi* and *Clostridium sordellii.* A positive result for *C. chauvoei* almost always indicates blackleg. The other species rapidly colonize tissues after death; if autolysis is advanced, consider testing for *C. chauvoei* only.

- Heart and other internal organs can be appropriate specimens for the *Clostridium chauvoei* FAT test if necropsy reveals myocardial necrosis or serofibrinous exudate in the pericardial sac, thoracic cavity or abdominal cavity. The full clostridial panel is not appropriate for internal organs.

- If bacillary hemoglobinuria or black disease (infectious necrotic hepatitis) are suspected, submit a portion of necrotic liver for the *Clostridium novyi* FAT. This test will detect both *C. novyi* type B (the agent of black disease) and *C. haemolyticum* (the agent of bacillary hemoglubinuria, formerly *C. novyi* type D).

Conjunctivitis

- Moisten the swab with sterile saline and avoid contact with the eyelid skin when swabbing.
- If corneal ulceration is present, particularly in horses, collect a separate swab for fungal culture.
- In cattle, submit a separate swab for *Bovine herpesvirus* 1 PCR testing. Use a viral transport media swab or a synthetic swab in a red-top tube with saline (see Virology and Molecular Diagnostics Section).

Urine

- Specify the urine collection method on the submission form. This information is needed to interpret qualitative culture results.
- Urine collected by cystocentesis is the ideal specimen for quantitative bacterial culture. Free catch (midstream voided) samples and catheterization samples are acceptable for cats and male dogs, as well as large animals. Swabs are not recommended for urine because quantitative culture requires a liquid sample.
- During cystotomy, urine or visibly inflamed bladder wall can be collected for culture. While urolithiasis in dogs is commonly associated with urease-producing bacteria, bladder stones are not useful for culture.
- When requesting both urine culture and urinalysis, submit urine in two separate containers for the Microbiology and Clinical Pathology Sections.

Milk

Bovine Mastitis

- Individual quarter samples are ideal and should be collected in clinical mastitis cases. Composite samples (milk from 2-4 quarters) can be used to screen herds for major mastitis pathogens.

- The California Mastitis Test (CMT) will be done on quarter samples that have not been frozen. If the CMT indicates mastitis, but no pathogens are detected by routine culture, consider ordering the PCR test for *Mycoplasma bovis*.

- Mention udder abscesses, other lesions or any signs of systemic illness in the history section of the submission form.

- Milk samples are often collected by dairy producers. Veterinarians are responsible for instructing their clients in proper collection techniques that will avoid contamination. Many of the minor mastitis pathogens (e.g., coagulase-negative staphylococci) are normal residents on skin and therefore are common contaminants. Environmental pathogens and *Staphylococcus aureus* can be present in feces and reside transiently on skin, so severely contaminated milk samples can give misleading culture results. When contamination is severe (3 or more species isolated), only major mastitis pathogens will be reported.

Other Species

- Collect milk specimens from individual mammary glands. Do not pool milk samples from multiple glands.

- Describe milk abnormalities and any lesions such as abscesses in the history section of the submission form.

- Consider aspirating exudate directly from abscesses.

Respiratory Tract

Rhinitis

- Nasal swabs or nasal flushes can be submitted for bacterial culture.

- Submit separate swabs for fungal culture.

- If PCR testing is also required, submit a separate swab in viral transport media or a synthetic swab in a red-top tube with saline.

Tracheobronchitis and Pneumonia

- Transtracheal aspirates and bronchoalveolar lavages are the ideal samples. Nasal swabs are acceptable but will often be contaminated by nasopharyngeal flora. Note that many of the bacteria involved in causing pneumonia can also be nonpathogenic residents of the upper respiratory tract.

- Take lung samples collected at necropsy from areas with visible pneumonia or pleuritis. If sampling a lung that lacks definite lesions, collect from the cranial and middle lung lobes.

Blood

- When bacteremia is suspected, blood collected in a red-top or anticoagulant tube is not a suitable specimen for culture.

- Special blood culture bottles are required. These bottles can be obtained from VDS at no charge.

Joint, Pleural and Peritoneal Fluids

- A fluid sample (at least 0.5 ml) is preferable to a swab.

- Fluid submitted in an anticoagulant tube (EDTA) for fluid analysis cannot be used for bacterial culture (refer to Clinical Pathology Section). Submit a separate sample in a red-top tube.

Feces

- Submit fecal samples in rigid, sealable plastic containers. Tied-off disposable gloves are not a suitable specimen container.

Fecal Bacteriology

- Culture on feces and fecal swabs will routinely include *Salmonella* culture and, in susceptible species, *Campylobacter* culture.

- *Escherichia coli* is almost universally present in feces – it will only be reported if there is a reason to suspect *E. coli* diarrhea. *E. coli* isolated from calves with onset of diarrhea during the first 3 days of life, will be reported and tested for the F5 (K99) fimbrial antigen – F5 positive isolates should be considered significant. Hemolytic *E. coli* isolates from swine in susceptible age groups will be reported and tested for the F4 (K88) antigen. *E. coli* may be reported in other scenarios if there is histologic evidence of infection. PCR testing for *E. coli* virulence factor genes is also available (refer to the Virology and Molecular Diagnostics Section).

- *Clostridium perfringens* is part of the large intestinal flora, so isolating it from a fecal sample is a poor indicator of infection. In dogs and cats, the ELISA test for the *C. perfringens* enterotoxin is used to test for clostridial diarrhea. For diagnosis of *C. perfringens* type A diarrhea in neonatal piglets, an anaerobic culture should be done on small intestinal samples collected shortly after euthanasia. Isolates will be sent for PCR typing and to detect the beta-2 toxin gene that is associated with piglet diarrhea. When *C. perfringens* type D enterotoxemia (lambs) or *C. perfringens* type C necrotizing enteritis (multiple species) is suspected, anaerobic culture should be done on small intestine as soon as possible after death, and any *C. perfringens* isolates should be sent for PCR typing. For necrotic enteritis of poultry, affected segments of intestine should be submitted for aerobic and anaerobic culture.

- A *Clostridium difficile* toxin ELISA is available for piglet fecal or colon samples and canine fecal samples.

Fecal Parasitology

- A direct smear examination can be done when only a small volume is available. Fecal flotation is generally preferred for detecting cestodes, nematodes and coccidia. Flotation requires about 10 ml of liquid feces or a “walnut-sized” portion of solid feces.

- To determine the quantity of strongyle eggs in equine and ruminant feces, order a fecal egg count.

- A fluorescent antibody test panel is done to detect *Cryptosporidium parvum* and *Giardia* in fecal samples. This test requires about 3 grams of feces. If this amount is not available, PCR testing for *Cryptosporidium parvum* can be done in the Virology and Molecular Diagnostics Section.

- Macroscopic worms recently passed by the animal or found during necropsy can be submitted for parasite identification.

Postmortem Specimens

- After death, body sites that normally contain few or no microorganisms are rapidly colonized by endogenous bacteria originating mainly from the gastrointestinal tract and upper respiratory tract. Even mild autolysis can produce bacterial culture results that are unrewarding or difficult to interpret. Collect specimens as soon as possible after death.

- During necropsy, avoid touching the oral cavity and gastrointestinal tract until culture samples have been collected. Alternatively, change gloves before collecting bacteriology specimens. Place organ samples in separate labeled bags. Different organs from the same animal should not be pooled – mixing thoracic and abdominal organs is especially detrimental. It is also not acceptable to pool the same organ from different animals. When submitting multiple organ samples from multiple animals, label each bag with the animal identification and the specimen identification (e.g., Calf A lung, Calf B lung or Turkey 1 liver, Turkey 2 liver).

Antimicrobial Susceptibility Testing (AST)

- VDS uses the Kirby-Bauer disk diffusion method for AST. Zones of bacterial growth inhibition around antimicrobial drug-impregnated disks are measured and interpreted according to criteria set by the Clinical and Laboratory Standards Institute (CLSI).

- This method works well for fast-growing aerobic bacteria but cannot be done on anaerobes or certain fastidious bacteria. When disk diffusion is not appropriate, minimum inhibitory concentration determination by microbroth dilution may be available at a referral laboratory.