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Introduction

Diagnostic Services has been an integral component of the Atlantic Veterinary College at the University of Prince Edward Island since the College opened in 1986. Over these last 30 years, we have fulfilled the diagnostic and research needs of clients in the Atlantic Provinces, across Canada and abroad and we are excited to continue with these endeavors. Our professional, technical and administrative staff has vast experience and take pride in offering outstanding customer service while meeting the needs of the aquatic, agricultural, companion, avian and wildlife communities. We also support the regulatory needs of government agencies. Please contact us at avcdiagnostics@upei.ca for any requests and read our Diagnostic Update for laboratory news regarding testing, staff and interesting clinical cases (posted to your clinic or available on-line) and are archived on our web-site.

We are proud to offer the Veterinary Laboratory Association Quality Assurance Program® (VLA-QAP) here at the Atlantic Veterinary College - an external proficiency program offering a specially designed proficiency test kit for veterinary laboratories. It provides a confidential means of comparing your laboratory's internal test results to those of your peers in the animal health field. It also provides a way to verify the successful execution of diagnostic methods, sample handling, and data collection within the laboratory. Please visit www.vlaqap.org for more information.

Quality Policy

It is Diagnostic Services' policy and mission to provide and maintain accurate and reliable results to its clients by ensuring the highest standard of analytical and diagnostic testing services in accordance with CAN-P-4E (ISO/IEC 17025:2005) and all other applicable client and regulatory requirements. Diagnostic Services' also strives to provide clients results for the requested tests within the expected turnaround times.

Diagnostic Services personnel and Management are committed to compliance with this Quality Manual, CAN-P-4E (ISO/IEC 17025:2005) and all other applicable quality documents, regulations and contract agreements through effective training. Diagnostic Services personnel and Management are also committed to continually improve the quality of laboratory operations and the effectiveness of the Quality Management System to meet the needs of its clients.

Quality Objectives

1. All personnel are adequately trained, possess proficient skills to perform their assigned tasks, and are familiar and comply with all Quality Management System policies and procedures.
2. The principles, methodologies, equipment, reagents, supplies, interpretations and other relevant information pertaining to diagnostic and analytical procedures conducted in Diagnostic Services are documented and made available to all laboratory personnel.
3. All testing equipment is properly maintained and monitored to ensure meeting the applicable required standards and specifications.
4. All chemicals, reagents and supplies are of suitable quality and meet the specifications required for use in diagnostic and analytical testing procedures.
5. The facilities are appropriately designed for performing efficient and safe testing activities, and where applicable, for adequately separating areas designated for "clean" procedures, such as tissue culture, or other incompatible activities from areas where specimens are received, processed and tested.
6. Sample handling and management practices incorporate adequate procedures for ensuring the security, receipt, identification, storage and disposal of all samples.
7. The data management system incorporates adequate procedures for ensuring the security, accurate recording, validation, authorization, reporting, storage, retrieval and disposal of testing data, results and associated records.
8. All laboratory work is performed in a safe manner for the benefit of the staff and the general public.
9. Workload management procedures are adequate to ensure that acceptable testing turnaround times and provision of quality service are consistently achieved.
10. An adequate mechanism for problem solving and corrective action is established, documented and implemented.

Diagnostic Services Information

Mailing Address

Diagnostic Services
Atlantic Veterinary College
University of Prince Edward Island
550 University Avenue
Charlottetown, PE C1A 4P3

Hours of Operation

Monday-Friday 8:00AM – 5:00PM
Saturday 8:00AM – 4:00PM (limited testing available)

For Results and General Inquires

Laboratories:	Phone:
General	902-566-0863
Bacteriology	902-566-0821
Biochemistry	902-566-0860
Electron Microscopy	902-566-0849
Endocrinology	902-566-0860
Hematology	902-566-0859
Histopathology	902-566-0864
Parasitology	902-566-0822
Post Mortem/Biopsy	902-566-0864
Toxicology	902-566-0833
Virology	902-566-0877
Quality Assurance Program	902-566-0990
	Fax:
Diagnostic Laboratories	902-566-0723
Post Mortem	902-566-0871

Enquiry Email avcdiagnostics@upei.ca

Website http://www.upei.ca/diagserv

Staff Information

(Current as of September 2019)

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VLA Quality Assurance Program	Richard Green, BSc	rwgreen@upei.ca	902-566-0990
	Ashley Patriquen	apatriquen@upei.ca	

Specimen Submission and Reporting

We only receive diagnostic specimens from licensed veterinarians and research samples from universities, industry and government researchers.

Submission Forms

The appropriate diagnostic submissions form(s), must accompany each case. The form(s) must include the names of the referring veterinarian and the veterinary clinic, as well as the name of the owner. It must also include the patient's data – including species, breed, age and sex, a concise clinical history and where possible a tentative diagnosis. Please type, print, or write clearly. Forms can be found on this website or directly from our laboratory.

We cannot process samples that have incomplete or inconsistent submission information and we are mandated to seek clarification from the submitter which will delay processing.

Reporting Results

Preliminary and Final reports in the form of a pdf file are automatically emailed as soon as the test is complete or faxed at 10:00am,12:00pm, 3:00 pm, 5:00pm and 7:00pm. Original copies of these reports will be kept on file in Diagnostic Services.
The veterinarian named on the submission form will receive the report. The laboratory will only forward results to another person/clinic upon the receipt of authorization in the form of a signed document from the submitting veterinarian. This form can be found by contacting the laboratory at (902)566-0860.

Electronic Access

Clients of Diagnostic Services also have access to viewing their results from their submissions through our web-based Diagnostic Results Viewer. Clinics will be assigned a unique Username by our administration to admit you this secure, password protected site.

Specimen Handling

i. Specimens submitted to the University of Prince Edward Island become the property of the University of Prince Edward Island and will not be returned to the client unless pre-approval has been obtained.
ii. Proper handling of specimens from the time of collection until their reception in the laboratory is essential for optimal results. Please follow the instructions in the appropriate sections of this reference guide. Improperly packaged samples can result in damage during shipping creating hazards and may not be processed.

iii. Use the proper containers, supplies, etc. as indicated in the appropriate sections of the reference guide. Follow appropriate biosafety guidelines when packaging and shipping samples to the laboratory.

iv. Each specimen must be clearly labelled as to source (urine, serum, nasal swab, fecal, etc.) and individual animal identification.

v. Please call the laboratory ahead of time if it is necessary to submit a large number of specimens and/or if a special test is required.

We cannot process samples that have incomplete or inconsistent submission information and we are mandated to seek clarification from the submitter which will delay processing.

Packaging & Shipping Laboratory Submissions – General Guidelines

1. Collect the right specimens: The Fee Guide may provide adequate information but if you are in doubt please call the laboratory,

2. DO:
    - Refer to specific discipline sections of this manual for detailed information regarding your diagnostic requirements.
    - Submit separate samples for each test (bacteriology, virology, etc.) in individually labelled containers. Ensure there is at least 1 identifier present on the sample container which matches that on the sample submission form (patient name, reference ID, etc.).
    - Submit tissues in separate, labelled, properly sealed non-leak new or clean containers. Choose non-leak containers and ensure that they are not cross threaded and leaking any fluids.
    - Separate serum from clot after clotting is complete and place in a labelled non-additive tube for shipment. Whole blood is required for some testing – please see the referral guide for specific test requirements.
    - We recommend that tubes are contained in Styrofoam tube boxes or in other suitable material to prevent breakage during shipping.
    - Bubble wrap is also recommended to provide additional cushioning.
    - Place sample submission form(s) in a separate plastic bag.
    - Place ice packs in a separate plastic bag or wrap in paper towel or newspaper.

3. DO NOT:
    - Do not submit sample in rectal gloves.
    - Do not submit samples in syringes with needles.
    - Do not submit samples in glass containers.
    - Do not submit large volumes of formalin. (see page 11)
    - Do not ship perishable samples to arrive on holidays.

4. Complete the sample submission form:
    - Information on species, breed, age, sex, morbidity, mortality, history and treatment is essential for interpretation.
    - Supply full name and address of clinic and veterinarian and owner or producer.
    - Please indicate if zoonotic disease,(i.e. rabies, leptospirosis,) is a consideration.

5. Identify each specimen:
    - Give source (urine, serum, aspirate etc.)
    - Ensure there is at least 1 identifier present on the sample container which matches that on the sample submission form (patient name, reference ID, etc.).
    - Specify test requested on each container (urinalysis, culture, chemistry profile, etc.)

6. Prepare for shipping:
    - Place samples in proper containers. Package carefully to prevent leakage. Contain the requisition sheet in a plastic bag to prevent moisture damage.
    - Use mailing containers that meet standards for Transportation of Dangerous Goods, Type IB packaging, https://www.tc.gc.ca/eng/tdg/safety-menu.htm, which consists of watertight inner packaging surrounded by absorbent material, watertight secondary inner packaging and sturdy outer packaging (corrugated cardboard).
    - Frozen specimens: pack with gel-type ice packs. Wrap in 5-6 layers of absorbent paper and place inside an insulated container.
    - All biohazardous samples must be packaged in a new or disinfected suitable primary container which has been disinfected on the outside and then placed in a suitable secondary container with enough absorbent material, and the outside disinfected.

7. Unsuitable specimens:
    - A specimen may not be accepted and/or processed if contamination of the packaging poses a risk to personnel or if the specimen is spoiled, contaminated, or inappropriate.
    - The consignor will be notified and fees will reflect administration and disposal of the specimen.
    - Formalin – see ensuing notes

8. Multiple sample testing – Mammalian/Aquatic - Research or Diagnostic
   (Number of animals greater than 20)
    - Please make prior arrangements with laboratory for multiple sample submissions.

9. Follow-up samples – Convalescent, re-submissions
    - When submitting additional samples, please reference the original Diagnostic Services Laboratory Number on a new sample submission form.

Delivery to Diagnostic Services

Local Veterinary practices

Local veterinary practices may deliver samples in person to the reception window of Diagnostic Services or to the Pathology and Post-Mortem Admitting office (located just inside Door 14 which is in the courtyard off of the perimeter road). Do not drop off samples at reception of the Veterinary Teaching Hospital.

Outside Charlottetown

On-Island clinics (those that were grand-fathered into an existing agreement with McInnis Express) have specimens delivered morning or afternoon Monday to Friday.

The University of PEI Shipping and Receiving department receives specimens from most couriers from off-Island packages by mid-morning Monday to Friday. On Saturday packages from clinics in the Atlantic Provinces are delivered by Midland Courier only.

Midland Courier

Diagnostic Services negotiates special rates and arrangements with Midland Courier to deliver packages to our laboratory overnight from most locations in the Atlantic Provinces. The packages are typically delivered to the Atlantic Veterinary College by 8:30am, Monday through Saturday.

Please contact our laboratory to request Midland Service. You will then be directed to contact Midland Courier to be supplied with pre-printed labels for your clinic to include Clinic name, address and phone number and our delivery address. When preparing a shipment, complete the waybill by indicating how many packages are being sent, ground or air, and indicate Recipient billing. If you are shipping for Saturday delivery – please indicate this on your waybill as well. Diagnostics will invoice each collect waybill with your monthly bill. Courier prices for packages less than 10 pounds can be found in the Fee Schedule. Shipments that weigh more than 10 pounds will have an additional fee calculated.

Formalin notes and Specimen Container Recommendations

The health and safety of people involved in the transport and handling of samples received by Diagnostic Services is very important. Samples for histopathology are received daily by our laboratory, most of which are shipped in 10% formalin. There are many people who handle these samples from the time it leaves your clinic to when it arrives at Diagnostic Services. These include courier drivers, people at the courier sorting facility, the shipping/receiving clerks at the Atlantic Veterinary College and sample reception clerks and technologists in Diagnostic Services. Occasional samples in formalin solutions have been shipped in glass jars which break, plastic bags which split, loose topped urine collection bottles which crack, or containers with poor fitting or cross threading lids which leak. When sample containers of these types are used, leaks occur and expose all of the above to formalin.

There are many well documented adverse effects to formalin exposure in humans, including irritation to the eyes, respiratory tract and skin. Skin exposure can lead to local redness, tingling and skin sensitization with the possibility of an allergic reaction with subsequent exposure. Symptoms involving the respiratory tract can include coughing, wheezing, burning pain of the nose and throat, shortness of breath and possibly pulmonary edema. Formalin is documented as a cancer causing agent, which is a concern with chronic exposure.

Samples from our courier arrive in a large bag. When there is a formalin leak, the formalin therefore spills over the other specimens shipped by many clinics. This can lead to damaged or unreadable submission forms and exposure to formalin fumes can adversely affect slide staining which can impact evaluation of blood smears and cytology specimens. How can you help? We are asking our clients to please use leak proof containers when shipping histology samples fixed in formalin. An example of an ideal container already used by some of our clients is LeakBuster™ Specimen Containers (StarplexR). These containers come in a variety of sizes and can be purchased from VWR or Fisher Canada.

Most of the samples submitted to our laboratory are packaged appropriately and we sincerely appreciate your diligence. However, to further minimize the risk of exposure to formalin by all those involved in the handling and shipping of samples, please evaluate the type of sample containers used by your clinic and ensure that proper packaging is used for shipping these samples.

If you have any questions concerning the packaging and shipping of samples, please do not hesitate to contact the staff at Diagnostic Services. We look forward to helping you.

Bacteriology & Mycology

General specimen collection guidelines:
Please complete a laboratory submission form for each submission. As a minimum, we require the following information:

- Brief but relevant history (please note if the animal was recently travelling outside of Canada)
- Animal species and age
- Site of specimen
- Symptom and treatment history
- Previous AVC laboratory number (if this is a resubmission)

Note: If submitting samples via courier please ensure the requisitions arrive clean and dry by enclosing them in a plastic zip-lock bag, separate from the samples and ice packs.

AVC Diagnostic Bacteriology Laboratory is a Risk Group 2 (Level 2) lab. Therefore, the following Level 3 samples will NOT BE ACCEPTED:

- Brain Tissue (swabs from brain tissue are acceptable)
- Tissue or swabs from primates
- Suspect cases of blastomycosis, histoplasmosis, psittacosis. Q fever, rabies, tuberculosis or tularaemia, regardless of serology testing result. Organisms may still be present in samples when serology testing is negative. Please contact the Bacteriology Lab to determine where to send the sample.
- For Aquatic Species suspect cases of pathogens identified as Exotic to Canada by the Canadian Food Inspection Agency (CFIA)

General Information:

- Culture swabs with transport media are the preferred specimen container for most specimens. Transport media is a non-nutrient medium that sustains the viability of organisms, without allowing significant growth. Dry swabs usually yield no bacterial growth and are not recommended.
- Label swabs/containers with the owner's name, animal ID as well as the site of the sample
- Collect sample before antimicrobial treatment is started. If this is not possible, indicate on the submission form which antibiotic was used and when treatment was started.
- Collect specimens in sterile manner (to avoid contamination with normal bacterial flora) using a sterile swab with transport media (NO dry swabs) and /or by placing it in a sterile specimen container. Take care to ensure that the exterior of swabs/containers are kept clean and uncontaminated to not endanger handlers. It is important to recognize that all samples have the potential of zoonotic transmission, and that many people handle the samples between collection and arrival at the laboratory.

- If collecting sample with a syringe, transfer contents of the syringe into a sterile container or onto a sterile swab with transport media. Do not use syringes to transport samples because they are a danger to handlers and may leak.
- Store the sample in the refrigerator until shipment and ship with freezer packs to the laboratory.
- When submitting *anaerobic culture*, Do not refrigerate sample or ship with freezer packs. (See Anaerobic Culture submitting guidelines below).

Sample Type	**Recommended Sampling and Guidelines**	**Shipping (if different than general guidelines**	**Comments**
Abscesses & Wounds	Submit swab of internal wall or abscess, excised abscess or fluid contents		Contact lab if anaerobic infection is suspected.
Anaerobic Culture	Refer to notes on anaerobic cultures	Anaerobes are cold sensitive. Samples must be stores at room temp.	Please phone the lab prior to submitting anaerobic cultures for suggestions on best specimen to submit.
Blood	Perform a surgical preparation and withdraw appox. 10 ml of blood and inoculate directly into a specialized "blood culture media" bottle (e.g. Oxoid Signal System).	It is not recommended to ship blood culture systems. Please contact lab for further instructions.	DO NOT refrigerate. Blood samples in red vacutainer tubes or blood samples in anticoagulant tubes are not suitable for culture.
Campylobacter Culture	Submit fresh fecal sample (at least 5 grams); morning sample preferred. See Feces for more details. DO NOT send swabs.	Store in refrigerator immediately following collection. Send on freezer packs.	Contact lab for further instructions and fees.
Canine Brucellosis Testing	Submit at least 2 mL of serum. Keep Refrigerated.		Samples are sent to reference laboratory for Brucella canis antibodies using rapid slide agglutination testing (RSAT) and agar gel immunodiffusion (AGID) assays. STAT testing cannot be accommodated. Testing requires 2 business days from the time of receipt by the reference laboratory.

Cerebral Spinal Fluid	Submit ~1 mL of CSF in sterile container		Contact lab if anaerobic infection is suspected.
Cervical Swab	Submit swabs; guarded swabs preferred		
Clostridial Infections	For Clostridial FAT: collect affected tissue (e.g. muscle, heart or diaphragm) immediately after death. DO NOT submit liver For enterotoxemia; submit fresh intestinal contents or ligated small intestine. DO NOT send swabs.		Clostridial Fluorescent Antibody Test (FAT): *C. chauvoei, C. novyi, C. septicum, C. sordellii*
Clostridium difficile culture	Submit fresh fecal sample (at least 5 grams); morning sample preferred. See Feces for more details. DO NOT send swabs.		Sent to referral lab at AVC.
Dermatophilosis	Collect scabs and send in a sterile container for culture and Giemsa stains.		
Ear	If submitting swabs for both ears, please ensure they are labeled accordingly.		
Eye	Submit swab from infected area/lesion or from secretions.		
Feces	Submit in sterile wide-mouth leak-proof container, no more than half-full. Morning sample preferred. Submit at least 5 grams. DO NOT send samples in rectal gloves. Very small samples (e.g. from budgies) may dry out in a large container – use a swab with transport media.	Store in refrigerator immediately following collection. Send on freezer packs.	**Caution**: Feces contain many bacteria. In a confined space, gasses build up and my cause the top to blow off the container, or cause the contents to "spray" once the lid is loosened.
Johne's Disease (M.avium subsp Paratuberculosis)	Submit fecal sample (at least 5 grams) for culture or 1 ml of serum for serology testing.	Store feces in the refrigerator immediately following collection. Send on freezer packs.	Serology is not as sensitive as culture. Turn-around time for serology:5-7 days; for culture: 8-9 weeks. Sent to referral lab at AVC.
Joint Fluid	Submit sample on a swab with transport media. We do not recommend the submission of fluid samples.		Joint fluid submitted in a vacutainer without anticoagulant will clot, enmeshing the bacteria; vacutainers with anticoagulant will inhibit bacterial growth.
Leptospires	Submit 1 ml of serum or 1 ml of fetal fluid.		Sent to referral lab. Contact lab for information on fees.

Lower respiratory tract	Transtracheal wash, tracheal swab, pleural fluid.		
Methicillin-Resistant *Staph.* screening	Submit nasal and/or rectal swabs. Use swabs with transport media.		Screening for carrier status.
Milk	Refer to milk collection guidelines.		For herd health testing, please notify lab ahead of time.
Mycology	Refer to mycotic sampling guidelines.		
Mycoplasmas	Collect tissues or swabs.	Freeze the sample until shipment and ship with freezer packs.	Sent for referral lab. Contact lab for information on fees and detection methods (culture, PCR)
Nasal	Submit nasal swab, nasal biopsies, nasal flush		
Oral Cavity	Submit swabs of infected area, tissues of biopsies.		Contact lab if anaerobic infection is suspected.
Salmonella screening	Submit at least 5 grams of feces. Refer to Feces for more details. DO NOT – send swabs	Store feces in the refrigerator immediately following collection. Send on freezer packs.	
Skin	Swab on infected area/pustules or collect scabs in sterile container (see Dermatophilosis)		
Tissues	Place individual tissues in plastic "Whirl-Pak" bags or leak-proof plastic containers. For intestines, make sure the ends are ligated. DO NOT use the same instruments to open the GI tract and other organs.	Store the sample in the refrigerator until shipment and ship with freezer packs. Pack in second outer plastic bag within shipping container. If not transporting to lab for more than 2 days, freeze the tissues.	
Urine	The preferred method of collection is cystocentesis. Samples collected by free-catch and catheter are more likely to be contaminated.	Store the sample in the refrigerator immediately following collection until shipment, and ship with freezer packs. DO NOT freeze.	For urine samples that will not arrive at the lab within 24 hours, submit urine on a swab with transport media. One charge will apply for fluid and swab specimens from the same case.
Vaginal, small animal pre-breeding	Vaginal swabs generally yield profuse growth of mixed flora. Please include a history including any signs of discharge and/or inflammation. Pre-breeding checks rely more on cytology. We cannot culture *Brucella.* Please see "Canine		

	Brucellosis Screening" for details.		

Milk Culture Collection Procedures

For the best possible results from the laboratory, take samples directly from the cow using sterile technique as outlined below. Do not take samples via the milking equipment or weight meter.

Please Note: Laboratory fees will be applied regardless of whether clean or contaminated samples are submitted.

- Label bottles prior to sampling with a waterproof marker with the vial identification numbers, and indicate cow name and date. If quarter samples are collected, designate each quarter sampled as RF, RH, LF, or LH.
- Collect the samples immediately before milking. Wear gloves for sampling.
- Clean and dry the udder using standard good udder preparation, as would be done for milking. Use individual paper towels to dry the teats prior to sampling.
- Scrub each teat-end opening thoroughly in three directions with a cotton ball soaked in 70% alcohol. A separate cotton ball should be used for each teat.
- Discard one or two streams of milk.
- Remove the vial cap carefully and fill the bottle about 2/3 full. Do not touch the rim with the teat or your fingers. Begin sample collection from the closest teat then to the teats on the far side of the udder. The tube or vial should be at a 45-degree angle when collecting the sample.
- To collect cow composite samples, collect an approximately equal volume of milk from each quarter in one sample bottle. There is a greater risk of contamination of composite sampling.
- Collect milk samples from quarters with clinical mastitis before treatment.
- Cool samples on cold packs in a portable cooler as they are collected. Refrigerate samples immediately. Wrap and package samples thoroughly to insulate them and keep them cool during shipment to the laboratory. Samples much arrive within 24 hours of collection. Samples to be cultured at a later date (after 24-48 hours) should be frozen.
- Ship samples to diagnostic services at the Atlantic Veterinary College.
- Please call the Bacteriology Laboratory at 902-566-0821 for more information or assistance if required.

Mycology Submissions

With a skin lesion, the area of infection should be washed with a mild disinfectant and the hair should be trimmed to a length of 0.5 cm. The sample should be collected from the active borders of several suspect sites with hemostats by grasping the hair shafts close to the skin and rolling the hairs from the follicles. It is important to ensure that:

- The root hairs just beneath the skin surface are obtained, and
- There is sufficient sample for microscopic examination and 2 cultures.

This sample should be placed in a sterile container and submitted to the laboratory.

Do not use oil to collect the sample. DO NOT use a swab for mycotic culturing. If including a scalpel blade, place the sample in a rigid container.

The recommended specimen container should have a wide mouth and be sterile (e.g. a sterile urine container or a pill bottle). The specimen should always be contained completely within the container – for example, there should be no hairs sticking out to eliminate the chance of contaminating anyone/thing it may contact during transit to the laboratory. Unacceptable containers are Whirl-pak bags, plastic bags, vacutainers, or paper envelopes.

If collecting a sample from an asymptomatic animal, it is suggested that you use the "brush method." A sterilized toothbrush or a surgical scrub brush is recommended for this technique. Brush the animal's coat thoroughly and extensively, and send only the brush to the lab fully contained in a sealed plastic bag with the submission form in a separate bag.

* Please call the Bacteriology Laboratory at 902-566-0821 for further information regarding the collection of fungal specimens other than ringworm.

Reference: Greene. Infectious Disease of the Dog and Cat. 2nd Edition. Philadelphia: W.B. Saunders Company, 1990.

Systemic Mycoses

The deep-seated mycoses (histoplasmosis, blastomycosis, and coccidioidomycosis) are caused by dimorphic fungi. These fungi are members of the Risk Group 3 category, based on the inherent risks of handling them. Samples suspected of containing dimorphic fungi cannot be processed at AVC's Diagnostic Bacteriology Laboratory. Please call the lab to determine an appropriate sample to collect, and for contact information of a referral laboratory.

Anaerobic Culture Submission Guidelines

In an effort to avoid workup on inappropriate samples (which are quite costly), Diagnostic Services recommends calling the laboratory for suggestions on the best specimen to submit. A sample from an inappropriate site, or an improperly collected specimen, may produce misleading lab results and may lead to the patient being treated with unnecessary antimicrobials or to false negative results.

General Guidelines:

Anaeobes are usually collected from a warm, moist environment that is low in oxygen. It is important to avoid "shocking" the anaerobes by exposing them to oxygen or allowing them to dry.

Anaerobic organisms are cold sensitive. Samples for anaerobic culture should not be refrigerated. They must be stored at room temperature.

Collection Using Needle & Syringe

Specimens collected by needle and syringe are better for anaerobic bacteriology than those collected by swabs (Swab fibres contain ambient air and introduce oxygen to the sample). After collecting the aspirated specimen, any air present in the needle and syringe should be expelled. Carefully place an alcohol-soaked gauze pad over the needle and cautiously expel the air.

Aspirated material may then be injected into one of the following for transportation to the lab:

a) An ANAEROBIC swab (see below for suggested specimens)
b) Thioglycollate enrichment broth
c) An oxygen-free transport tube/vial (see below for suggested specimens)

Transportation using Anaerobic Swabs (Submit ASAP)

Suggested specimens: Surgical sites, thick pus, and other specimens as listed above (only if the oxygen-free transport tube/vial is not available).

The specimen (that has been either expelled from a syringe onto the swab, or collected directly from the site by the swab) should be placed immediately into an anaerobic transport system. It is important to ensure that the system is for isolation of fastidious anaerobes. Manufacturer's instructions must be carefully followed.

Transportation using an Oxygen-Free Transport Tube/Vial (Submit ASAP)

Suggested specimens: aspirate, biopsy, bone, fluid and tissue

The sample should be placed into an oxygen-free transport system – preferably a PRAS transport system. This system contains oxygen-free gas and Cary-Blair transport media containing resazurin, an anaerobic indicator. This system consists of an anaerobic transport tube with a stopper that has a rubber diaphragm and a plastic screw cap. Aspirates are injected into the transport system through the rubber diaphragm after is has been decontaminated (with alcohol or other disinfectant). Swabs or small tissue samples can be inserted into the

transport media by removing the cap. The tube is then tightly recapped and sent ASAP to the laboratory.

Acceptable & Unacceptable Clinical Specimens for Anaerobic Culture

Category of Specimen	Specimen Type	Comments
Acceptable	Transtracheal aspirates	
	Centesis samples from surgically prepared sites and usually sterile body sites (urinary bladder, blood, thoracic/ pleural cavity, peritoneal cavity, pericardial cavity, cerebrospinal fluid, joints)	
	Fistulous tracts	Clean skin first; use syringe to obtain specimen.
	Abscesses	
	Deep wound and aspirates from other soft tissues	Use a guarded swab
	Endometrial swabs	
	Surgical specimens obtained from usually sterile sites	The deeper the better; debride wounds before swabbing
Unacceptable	Saliva or nasopharyngeal swabs	Except for tooth root abscesses
	Gingival swabs	
	Bronchoscopy cultures	
	Vaginal or cervical swabs	
	Skin or superficial wounds	
	Gastric washes	
	Urine (free catch or catheter)	
	Feces, intestinal tract	Except for clostridial cultures.

Biochemistry & Special Chemistry

The accuracy of the biochemistry tests and their interpretation is primarily dependent upon the quality of the submitted sample. Keep the following important factors in mind when submitting samples.

Fasting your patient before blood work is always desirable for all and essential for some blood tests. Please refer to the Fee Guide for test specifications.

Sampling prior to therapy is vital to the reliability of most types of samples.

1. Submit a complete clinical history including physical examination findings, current medication and time and date of obtaining the sample from the patient.

Selection of the proper volumes, type of samples and appropriate containers or vacutainer tubes. The Fee Schedule listed sample type, volume and storage requirements for each test(s).

2. Submission of volumes in excess of the requirement is ideal as it enables further investigation without resampling.

Turn Around Time

Routine Chemistry, Endocrinology, Hematology, Urinalysis and Serology will be reported by noon for samples arriving before 10:30 am and 5:00pm for samples arriving before 3:30 pm. Samples that arrive after 3:30 pm may be processed on the same day if the clinician deems the case to be urgent, otherwise testing will not be completed until the following day.

Emergency or "STAT" Requests

All requests for "STAT" service should be addressed to the laboratory. Consideration should be given to which individual tests are required "STAT" and which tests can be done at the standard work schedule. The Diagnostic Laboratory is always receptive to requests from submitters to send reports as soon as they are completed on critical cases.

Multiple Sample Submission

Diagnostic or Research Submissions

Projects involving many samples (20 or more) must to be discussed with the laboratory prior to sampling so that we can discuss expectations regarding Projects may require the purchase of additional reagent or supplies and scheduling of laboratory staff. Pricing for larger projects is provided upon request and we strive to meet your

Biochemistry

The most frequent causes of inaccurate biochemical tests are:

a) Aged sample

b) Hemolysis, icterus and/or lipemia

c) Insufficient sample quantity

d) Drug interference from post therapy sampling or contamination

Test requirements for routine chemistry panels are specified on the submission form.
Test requirements for special chemistry are briefly outlined on the submission form and additional details are listed in the Fee Schedule. The following points apply to general specimen handling for clinical chemistry:

a) THE SUBMISSION OF A WHOLE CLOTTED UNSEPARATED SAMPLE IS GENERALLY UNSATISFACTORY FOR MOST BIOCHEMICAL TESTS.

Following clot retraction of the whole blood (allow 20-30 minutes at room temperature), rim the tube with a wooden applicator stick, centrifuge, aspirate the serum by pipette or syringe and place into a second red-stoppered tube or plastic screw capped shipping vial. Ensure that all red blood cells are removed to prevent hemolysis during shipping. Usually about 0.3 ml of serum can be obtained from each l ml of clotted blood. Additional centrifugation of the clot could yield additional serum if required. Label the separated sample as serum and submit fresh if delivery within 48 hours is possible or frozen if longer transit time is likely. Serum can ALWAYS be frozen and stored before shipment.

b) When <u>plasma</u> samples are submitted they must be collected in proper vacutainers, such as heparin or sodium fluoride. Plasma is immediately separated by centrifugation, pipetted off and submitted in a red-stoppered tube or a plastic screw cap shipping vial.

IT IS IMPORTANT WHEN SEPARATING SERUM OR PLASMA TO LABEL THE SAMPLE ACCORDINGLY.
<u>STATE THE TYPE OF ANTICOAGULANT USED</u>.

c) Any test requiring fresh frozen serum or plasma must be marked frozen on outside of the container and arrive in a frozen state.

Cytology

Body Fluids

Samples should arrive at the laboratory before 3:30 pm to allow sufficient time for processing. Results from samples submitted after 3:30 pm will usually be available the next day, but occasionally may be reported the same day.

For cytological evaluation of peritoneal, thoracic and synovial fluid submit:

a) Fluid in EDTA (lavender – topped tube) for cell counts, protein, etc. (If bacterial culture is a possibility, please also submit a fluid in a plain tube or a swab.)
b) Freshly made air-dried, unstained smears.
c) Freshly made unstained smears from centrifuged sediment if the fluid appears to be of low cellularity.

Smears should be labelled with the animal's and owner's names and the site.

Cerebral Spinal Fluid (CSF)

Samples require special cytocentrifugation and cell counting techniques before preparing the appropriate smears for cytologic evaluation. Cells do not preserve well in CSF and therefore the specimens must be analyzed immediately upon submission. Collect the CSF into an EDTA purple topped tube. Please notify the laboratory before drawing the CSF.

If submitting CSF via overnight courier, please call (902) 566-0859 for handling recommendations.

Solid Tissues

Impression smears and smears of fine needle aspirates of tissue are best submitted air-dried, unfixed and non-refrigerated.
Air-Dried – 2 or 3 slides prepared as for routine blood smear examination.
Smears should be labelled with the animal's and owner's names and the site.
Please carefully label all submissions as to whether centrifugation has been used. If in doubt about the best samples to submit, call the laboratory before collecting samples.

Bone Marrow

Submit 10 -15 air- dried smears. Aspirate into a syringe containing EDTA. Smear marrow spicules by slide over slide crush technique.
If the marrow clots, fix in formalin and send to histopathology.

Urine

Urines should be split into a red-topped tube and a conical tube. Store the red-topped tube in the refrigerator. Centrifuge the remaining urine in a conical tube. Dispose of the supernatant and prepare smears from the sediment. Allow the smears to air-dry and maintain them at room temperature. Submit both the intact urine and the air-dried, unstained smears for cytologic evaluation.

Coagulation

TEST	SAMPLE REQUIREMENTS	COMMENTS
Direct Coombs Test	Whole blood (EDTA) Lavender vial – fresh 3.0 ml	Canine - done daily. (Feline & Equine – referred out)
Compatibility Studies (Crossmatch)	Whole blood EDTA and serum Lavender vial – fresh 3.0 ml Serum (red-topped) vial – 3.0 ml from recipient	Done daily. Please notify the laboratory in advance.
Coagulation Profile a) Prothrombin Time (PT) b) Activated Partial Thromboplastin Time (APTT) c) Fibrin-Degradation Products (FDP) d) Thrombin Clotting Time (TCT) e) Fibrinogin Titer (FT)	Whole blood (Sodium citrate) Blue tube (3.2% citrate 1:9 dilution with whole blood) (withdraw plasma & freeze immediately) d) & e) are referred out	PT, APTT, FDP – done daily

Endocrinology

Sample Arrival Time
Endocrine test samples must arrive at Diagnostic Services BEFORE 10 AM on the day the assay is to be run.

Assay Schedule

Cortisol	Monday - Saturday
Progesterone	Monday - Saturday
T4	Monday - Saturday
TSH	Monday – Saturday
Estrone Sulfate – Quantitative	Referred
Testosterone, Estradiol	Referred

Assay Reference Values
A list of endocrine assay reference intervals or guidelines is included. Reference values will continue to be updated from time to time and made available to all users of the Endocrinology Diagnostic Service.

Specimen Handling and Submission Requirements

Collect blood by venipuncture or capillary stick into red-top vacutainers. CENTRIFUGE AND SEPARATE SERUM FROM CELLS. Red blood cells present in Bovine samples submitted for ***progesterone*** analysis are a source of inaccurate results due to continued enzymatic activity. Most endocrine tests can use only serum. If you are uncertain what to collect, please check with the laboratory.

All biological fluids sent for endocrine assay should be sent frozen if there is more than a 24-hour delay in delivery of the sample to Diagnostic Services.

Label separated samples clearly with complete collection and identification information.
0.5 ml of serum is adequate for most endocrine assays.

Adrenal Gland Function Testing Protocols

Please note: All samples should be labelled clearly with the patient and owner name as well as information as to the time of sampling (baseline, 2 hours post-ACTH, 8 hours post LDDS, etc). Please give the ACTH type, dose per kilogram, route of administration and the times of collection on the submission sheet. Providing this information will help to avoid mis-interpretations by the duty clinical pathologist.

ACTH Stimulation Test for Dog

a) Ideally fast the patient overnight but offer water.
b) Weigh the patient in kilograms.
c) Collect a blood sample in a red-stoppered tube for a baseline cortisol concentration. Allow the blood to clot, remove the serum and place it in a new red-stoppered tube. The assay requires 0.5 ml of serum. Refrigerate as soon as possible and freeze if shipment will be delayed more than 24 hours.
d) If using **ACTH gel**, inject **intramuscularly** at 2.2 U/kg body weight and collect a second blood sample at 2 hours post-ACTH.
e) If using **synthetic ACTH (Cortrosyn**), inject **intravenously** at a dose of 250 ug/dog (1 vial), regardless of body weight and collect a second blood sample at 1 hour post-ACTH.
f) If using **synthetic ACTH (Synacthen Depot**), inject **intramuscularly** at a dose of 0.25 mg/dog if the dog's weight is less than 15 kg and 0.5 mg/dog if the weight is greater than 15 kg. Collect a second blood sample at 2 hrs post –ACTH.

ACTH Stimulation Test for Cats

a) Ideally fast the patient overnight but offer water.
b) Weigh the patient in kilograms.
c) Collect a blood sample for a baseline cortisol concentration. Allow the blood to clot, remove the serum and place it in a new red-stoppered tube. The assay requires 0.5 ml of serum. Refrigerate as soon as possible and freeze if shipment will be delayed more than 24 hour.
d) If using **synthetic ACTH (Cortrosyn)**, inject **intravenously** at a dose of 125 ug/cat (½ vial), regardless of body weight. **Note: the synthetic ACTH gives more consistent results in cats, so is currently recommended over the gel for testing.** Collect a second specimen 1 hour post-ACTH. Note: additional samples may be collected at 30 and 90 minutes post-administration (individual preference), but the 1 hour post-administration sample is the most important.
e) If using ACTH gel, inject intramuscularly at 2.2 U/kg body weight. Collect blood samples for cortisol determination at both 1 and 2 hours post-ACTH in cats.
f) There is no established protocol for **Synthetic ACTH (Synacthen Depot)** in cats.

Patients on Trilostane to control hyperadrenocorticism should have an ACTH Stimulation test started 2-6 hours following oral administration of the drug. An ACTH Stimulation test should be performed 7-10 days after commencing therapy.

ACTH Stimulation Test for Horses

a) Weigh the horse in kilograms.
b) Collect a blood sample for a baseline cortisol concentration. Allow the blood to clot, remove the serum and place it in a red-stoppered tube. The assay requires 0.5 ml of serum. Refrigerate as soon as possible and freeze if shipment will be delayed more than 24 hours.
c) If using synthetic ACTH, inject IV at 1 unit/kg body weight (to a maximum of 100 units) and collect a blood sample at 2 hours post-ACTH administration.
d) If using ACTH gel, inject intramuscularly at a dose of 1 unit/kg body weight and collect a blood sample at 8 hours post-ACTH administration.

Low Dose Dexamethasone Suppression Test for Dogs

a) Fast the patient overnight but offer water.
b) Weigh the patient in kilogram.
c) Collect a blood sample for baseline cortisol concentration. Allow the blood to clot, remove the serum and place in a new red-stoppered tube. The assay requires 0.5 ml of serum. Refrigerate as soon as possible and freeze if shipment will be delayed more than 24 hours.
e) Inject dexamethasone intravenously at a dose of 0.01 mg/kg.
f) Collect blood samples for cortisol determination at 4 hours and 8 hours after dexamethasone injection.

High Dose Dexamethasone Suppression Test for Dogs

a) Fast the patient overnight but offer water.
b) Weigh the patient in kilograms.
c) Collect a blood sample for baseline cortisol concentration. Allow the blood to clot, remove the serum and place in a new red-stoppered tube. The assay requires 0.5 ml of serum. Refrigerate as soon as possible and freeze if shipment will be delayed more than 24 ours.
d) Inject dexamethasone intravenously at a dose of 0.1 mg/kg.
e) Collect a blood sample for cortisol determination at 8 hours following dexamethasone injection. A 4 hour post-dexamethasone sample can be collected as well but is optional.

Low Dose Dexamethasone Suppression Test for Cats

a) Ideally fast the patient overnight but offer water.
b) Weigh the patient in kilograms.
c) Collect a blood sample for baseline cortisol concentration. Allow the blood to clot, remove the serum and place it in a new red-stoppered tube. The assay requires 0.5 ml of serum. Refrigerate as soon as possible and freeze if shipment will be delayed more than 24 hours.
d) Inject dexamethasone intravenously at 0.1 mg/kg.
e) Collect post-dexamethasone blood samples at 4 and 8 hours after dexamethasone injection.

High Dose Dexamethasone Suppression Test for Cats

a) Ideally fast the patient overnight but offer water.
b) Weigh the patient in kilograms.
c) Collect a blood sample for baseline cortisol concentration. Allow the blood to clot, remove the serum and place it in a new red-stoppered tube. The assay requires 0.5 ml of serum. Refrigerate as soon as possible and freeze if shipment will be delayed more than 24 hours.
d) Inject dexamethasone intravenously at 1.0 mg/kg.
e) Collect a post-dexamethasone blood sample at 8 hours after dexamethasone injection. A 4 hour post-dexamethasone sample may be taken as well but is optional.

Overnight Dexamethasone Suppression Test for Horses

First day - 5 pm:

a) Weigh the patient in kilograms.
b) Collect a blood sample for baseline cortisol concentration. Allow the blood to clot before removing the serum and placed in a new red-stoppered tube. The assay requires 0.5 ml of serum. Refrigerate as soon as possible and freeze if shipment will be delayed more than 24 ours.
c) Inject dexamethasone IM at a dose of 0.04 mg/kg (equivalent to 40 ug/kg).

Next day - 12 noon:

a) Collect a blood sample for 19 hour post-dexamethasone cortisol concentration.

Patients on Trilostane to control hyperadrenocorticism should have an ACTH Stimulation test started 2-6 hours following oral administration of the drug. An ACTH Stimulation test should be performed 7-10 days after commencing therapy.

ACTH Stimulation for Dogs

Drug	**Synthetic ACTH (Cortrosyn** (Cosyntropin for injection)**)**	**Synthetic ACTH (Synacthen Depot** (Cosyntropin-Zinc Hydroxide)**)**	**ACTH gel (Bexco** (Corticotropin injection, USP)**)**
Formulation	0.25 mg/vial reconstituted with 1 mL sterile saline, 0.25 mg = 250 ug = 25 U	1 mg/vial, 1 mL bottle	40 U/mL, 5 mL bottle
Dose	250 ug (1 vial)/dog regardless of body weight **or** (Cost-effective lower dose of 5 ug/kg to a maximum of 250 ug/dog)	body weight <15 kg - 0.25 mg/dog body weight >15 kg - 0.5 mg/dog	2.2 U/kg of body weight
Administration	intravenously	intramuscularly	intramuscularly
Collection	Pre and 1 hour post	Pre and 2 hour post	Pre and 2 hours post

ACTH Stimulation for Cats

Drug	**Synthetic ACTH (Cortrosyn** (Cosyntropin for injection)**)**	**Synthetic ACTH (Synacthen Depot** (Cosyntropin-Zinc Hydroxide)**)**	**ACTH gel (Bexco** (Corticotropin injection, USP)**)**
Formulation	0.25 mg/vial reconstituted with 1 mL sterile saline, 0.25 mg = 250 ug = 25 U	No established protocol	40 U/mL, 5 mL bottle
Dose	125 ug (½ vial)/cat regardless of body weight		2.2 U/kg of body weight
Administration	intravenously		intramuscularly
Collection	Pre, 30 min. and 1 hour post		Pre, 1 and 2 hours post

ACTH Stimulation for Horses			
Drug	Synthetic ACTH (Cortrosyn (Cosyntropin for injection))	Synthetic ACTH (Synacthen Depot (Cosyntropin-Zinc Hydroxide))	ACTH gel (Bexco (Corticotropin injection, USP))
Formulation	0.25 mg/vial reconstituted with 1 mL sterile saline, 0.25 mg = 250 ug = 25 U	No established protocol	40 U/mL, 5 mL bottle
Dose	1 U/kg body weight (to a maximum of 100 units per horse)		1 U/kg of body weight
Administration	Intravenously		intramuscularly
Collection	Pre and 2 hour post		Pre and 8 hours post

Dexamethasone Suppression for Dogs

	Low Dose Dexamethasone Suppression	High Dose Dexamethasone Suppression
Drug	Dexamethasone Sodium Phosphate	Dexamethasone Sodium Phosphate
Dose	0.01 mg/kg of body weight	0.1 mg/kg of body weight
Administration	Intravenously	intravenously
Collection	Pre, 4 hours and 8 hours Post	Pre and 8 hours post

Dexamethasone Suppression for Cats

	Low Dose Dexamethasone Suppression	High Dose Dexamethasone Suppression
Drug	Dexamethasone Sodium Phosphate	Dexamethasone Sodium Phosphate
Dose	0.1 mg/kg of body weight	1.0 mg/kg of body weight
Administration	Intravenously	intravenously
Collection	Pre, 4 hours and 8 hours Post	Pre and 8 hours post

Dexamethasone Suppression for Horses

	Overnight Dexamethasone Suppression
Drug	Dexamethasone Sodium Phosphate
Dose	0.04 mg/kg of body weight (equivalent to 40 ug/kg)
Administration	Intramuscularly
Collection	Pre(5pm on day 1) and 19 hours post (12 noon on day 2)

Thyroid Hormone Assays

Sample Collection and Handling

Assays for thyroid hormones routinely evaluated (total T4 and TSH) and those auto-antibodies to T4 and thyroglobulin **all utilize serum samples.**

- Blood should be collected 4-8 hours post pill from animals on thyroid replacement medication.
- Blood should be collected any time from cats on methimazole after they reach therapeutic levels.
    * Oral medication – 2 weeks
    * Ear gel – 3-4 weeks
    * Dietary control – 4-8 weeks
- Blood should be collected into a red-topped tube and allowed to clot fully before centrifugation so that fibrin strands are not present in the supernatant.
- Following centrifugation, the serum should be removed from the clot as soon as possible and, in most cases, placed into a new red- topped tube for submission. The exception to this is free T4; as concentrations can increase with storage in glass tubes, shipment in plastic vials is ideal.
- At least 0.5 ml is required for measurement of either T4.
- At least 1.0 ml is required for TSH or free T4 testing.
- At least 2.0 ml is required for auto-antibody determination.
- Refrigerate serum as soon as possible; do not store at room temperature.
- Ship to the AVC Diagnostic Services Laboratory in a cool state (using gel-packs). If shipment to the AVC is delayed more than 24 hours after collection, the serum should be frozen and shipped in a frozen state.
- The samples should be labelled clearly with the patient and owner name.
- If T4 concentrations are being evaluated for therapeutic monitoring, please give the drug type, dose per kilogram and the times of collection on the submission sheet. Blood should be collected 4-8 hours post pill from animals on thyroid replacement medication. Providing this information will help to avoid misinterpretations by the clinical pathologist.

Summary Chart of Tests for Diagnosis of Canine Hypothyroidism

Test	Advantages	Disadvantages
Total T4	Inexpensive Sensitive - usually low in hypothyroidism Can usually rule out hypothyroidism if in normal range - therefore good first step in evaluation	Easily decreased by non-thyroidal illnesses and many drugs - therefore cannot diagnose hypothyroidism if low
Total T3	If high values obtained in a patient with suspected hypothyroidism, can help identify a situation of auto-antibody interference	Easily decreased by non-thyroidal illnesses and many drugs - therefore cannot diagnose hypothyroidism if low Low in only 10 % of hypothyroid dogs - very insensitive test
Free T4	Decreased values are more specific for hypothyroidism than total T4 Generally less affected by drugs and non-thyroidal illness than total T4 Can assist in therapeutic monitoring of patients with hypothyroidism (especially if auto-antibodies cause difficulty with T4 evaluation)	Must be measured by equilibrium dialysis - more expensive test Can still be decreased in non-thyroidal illness or drug treatment (less so than T4)
Canine TSH	If high in the face of a low T4 or low fT4, primary hypothyroidism can usually be diagnosed Can assist in therapeutic	25 % of hypothyroid dogs have normal range TSH - therefore cannot rule out hypothyroidism Cont'd

	monitoring of patients with hypothyroidism (especially if auto-antibodies cause difficulty with T4 evaluation)	Can be increased by drugs and recovery from non-thyroidal illness Identifying secondary hypothyroidism not possible
TSH stimulation test	Remains gold standard test for diagnosis of hypothyroidism	Almost impossible to obtain TSH - medical grade out of production, chemical grade can cause anaphylactic reactions, recombinant human TSH may be available in future
TRH stimulation test	Main stimulatory test still possible to perform	Expensive - TRH can be difficult to obtain Administration can cause vomiting and other side effects Not felt to be any better than measurement of T4, fT4 &TSH to identify hypothyroidism
Auto-antibodies to thyroglobulin	Required as one of the tests for certification by the OFA May allow identification of immune-mediated thyroiditis	Requires cross-border shipping with sample handling and expense A negative result does not rule out thyroid pathology No assessment of thyroid function
Auto-antibodies to T3,T4	Provides explanation of unusual results on T3 and T4 testing Increases suspicion for immune-mediated thyroiditis	Requires cross-border shipping with sample handling and expense A negative result does not rule out thyroid pathology No assessment of thyroid function

Estrone Sulfate Test

Estrone sulfate is a hormone produced by the fetoplacental unit. It is an indication of pregnancy and of fetal well-being. Estrone sulfate rises rapidly in the blood 60-90 days after conception and persists until partuition. Samples for this test should be collected starting at 90 to 100 days after breeding. Although a crytorchid testicle may produce estrone sulfate, we recommend measuring testosterone before and after HCG stimulation.

- Blood should be collected into a red-topped tube and allowed to clot fully before centrifugation so that fibrin strands are not present in the supernatant.
- Following centrifugation, the serum should be removed from the clot as soon as possible and placed into a new red- topped tube for submission.
- At least 1.0 ml is required for measurement.
- Refrigerate serum as soon as possible; do not store at room temperature.
- Ship to the AVC Diagnostic Services Laboratory in a cool state (using gel-packs). If shipment to the AVC is delayed more than 24 hours after collection, the serum should be frozen and shipped in a frozen state. Diagnostic Services will forward the specimen to a reference facility.

Progesterone

- Blood should be collected into a red-topped tube and allowed to clot fully before centrifugation so that fibrin strands are not present in the supernatant. Following centrifugation, the serum should be removed from the clot as soon as possible and placed into a new red- topped tube for submission.
- At least 0.5 ml is required for measurement.
- Refrigerate serum as soon as possible; do not store at room temperature.
- Ship to the AVC Diagnostic Services Laboratory in a cool state (using gel-packs). If shipment to the AVC is delayed more than 24 hours after collection, the serum should be frozen and shipped in a frozen state.

Hematology

The quality of the tests and their interpretation is primarily dependent upon the quality of the submitted material. Keep the following important factors in mind when submitting samples.

1. Sampling prior to therapy is vital to the reliability of all types of samples.
2. Submission of a complete clinical history including physical examination findings, current medication, and time and date of obtaining the sample from the patient.
3. Submission of volumes in excess of requirement is ideal as it enables further investigation without resampling.

Sample Arrival Time

Samples should arrive at the laboratory before 3:30 pm to ensure that all requested tests can be completed that day. For samples that arrive after 3:30 pm some tests may be done that day but some will not be completed until the following day.

Emergency or "STAT" Requests

All requests for "STAT" service should be addressed to the laboratory. Consideration should be given to which individual tests are required "STAT" and which tests can be done at the standard work schedule.

Multiple Sample Submission

Diagnostic Cases

Requests involving more than 20 samples per case or per farm require a 24 hour notice.

Herd Profiles

The laboratory will be able to do profiles on herds. In view of the expense involved, it is important that one carefully selects the profiles and specific tests required.

Research Projects

Projects involving many repeated samples or large batches of samples should be discussed with the laboratory prior to sampling.

Hematology

Samples in lavender-topped (EDTA) tubes for CBC (complete blood count) should be forwarded to the laboratory as soon as possible. Samples that are two days old or more when received by the Diagnostic Services laboratories could be inaccurate. Always include air-dried, unfixed, non-refrigerated smears made at the time of sampling. Meaningful smears cannot be made from shipped EDTA blood. The routine hematology tests that can be performed on shipped blood samples are listed with appropriate information and comments.

TEST		SAMPLE REQUIREMENTS	COMMENTS
WBC	White Blood Cell count	Whole blood (EDTA) (lavender vial)	Gently invert and rotate vial immediately after collection.
RBC	Red Blood Cell count		
HgB	Hemoglobin		
Hct	Hematocrit	Small and Large Animal patients require **3 ml** of whole blood to complete all of the tests listed.	
MCV	Mean Cell Volume		Vial must be free of clots.
MCH	Mean Cell Hemoglobin		
MCHC	Mean Cell Hemoglobin Concentration.		Vials should be more than half full – preferably completely full.
RDW	Red Blood Cell Distribution Width		
Plts	Platelets		
Pct	Plateletcrit		
MPV	Mean Platelet Volume		
PDW	Platelet Distribution Width		
Retic	Reticulocyte (when applicable)		
HB	Heinz Body		
TP	Plasma Total Protein		
Fib	Fibrinogen (large animals only)		
WBC	Differential	2 freshly made AIR DRIED smears. Please see the following comments.	Avoid submitting blood smears in the same package with tissues in formalin - the fumes can cause alterations in the staining.
RBC	Morphology		

Preparation of blood smears:

1. Smears can be made directly from the venipuncture or from a well-mixed EDTA sample immediately after collection.
2. Smear preparation technique aids:
    a. Use clean glass slides with a bevel edge.
    b. Do not use too large a drop of blood.
    c. Smears should be made on a firm surface.
    d. Air dry rapidly by waving – DO NOT blow on smear OR expose to moisture.
    Do not refrigerate or stain.

Histopathology

General:

1. All specimens should be submitted in 10% neutral buffered formalin.
   10% phosphate buffered formalin fixation RECIPE (makes 1L):

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)	

2. Do not allow the formalin solution and/or fixed tissues to freeze.

3. Unless masses are very large (ie > 6 cm in greatest thickness), please do not section or incise samples. When submitting larger masses, then sectioning in half, or possibly in quarters may be necessary before placing samples in formalin.

4. Aquatic Specimens:
   a. The width of fin-fish and crustacean tissues should not exceed 1 cm, this will ensure proper fixation.
   b. Bivalve Shellfish - remove one valve from the animal and immerse whole animal in 10% formalin made up in filtered seawater (35 ppt).

5. Fix samples in adequate amounts of formalin. Ideally, the volume ratio of formalin to tissue would be 10:1. If this is not feasible, a minimum of 5-7:1 would suffice. Place tissue samples immediately in the large volume of formalin for 24 hours, then transfer to a smaller sample containing less formalin for shipping.
   a. Alternatively, following fixation in large volumes of formalin solution for 24 – 48 hours, tissues may be removed, wrapped in formalin-soaked gauze sponge, placed in a plastic bag, and sealed for shipment. This technique decreases the probability of spillage and leakage of formalin during mailing. Do not submit these samples completely dry, as this can create artifactual changes.

6. Ideally each sample should be submitted in a separate container labelled as to the anatomical location of excision.

7. Do not send samples in glass bottles (they often break, leaking formalin en route). Plastic screw cap bottles should be placed in a sealable, plastic bag. Include enough material to absorb the entire volume of formalin in case of breaks or spills.

8. Send submission sheets with clinical history, signalment and working diagnoses in a separate plastic bag.

9. **Digital images** of lesions are always welcome and heartily encouraged. These images may be printed off in clinic and attached to the submission form or may be emailed directly to Diagnostic Services (avcdiagnostics@upei.ca). When emailing images, please include the owner and patients name and what test you have requested so that these images can be forwarded to the persons working on your case.

Carcass, Whole Fish and Shellfish Submissions:

General Information

Whenever possible, submission of the entire carcass is preferred. Under ideal circumstances, the carcass should be submitted as soon as possible after death. If a period of time must elapse prior to submission (<3-4 days), the carcass should be kept cool but not frozen. If at all possible, please avoid freezing the carcass as this produces severe artifactual changes which can make interpretation of subtle lesions difficult to impossible. However, if you cannot get the carcass to the lab in this time period or cannot keep it cool, freezing of the carcass may be necessary. Please include a complete clinical history with all submissions. Please feel free to call and talk to a pathologist regarding case material prior to submission if you have questions regarding shipping, sampling or specific tests.

For Fish and Shellfish submission live animals are preferable if at all possible, please contact the Diagnostic Lab in advance to ensure proper holding facilities are available for live submissions.

Herd Health Problems

In cases involving a herd problem, especially neonatal diarrhea, the best specimen for submission is a live, acutely affected, untreated animal. If this option is considered, please call the lab prior to sending in the animal as arrangements of euthanasia will need to be made, which may entail an extra charge. When submission of a live animal is not possible, the next best submission would be samples obtained by the submitting veterinarian at the time of field necropsy. Prior to euthanasia, collect blood in red top and lavender top tubes.

Centrifuge the red-top tube, harvest the serum and save in another tube. Perform a complete post-mortem and submit specimens for microbiology and histopathology as indicated. Submit these samples and a complete history to the laboratory.

Euthanasia Policies

The post-mortem laboratory does not offer euthanasia services. The only exceptions are animals being submitted through AVC Farm Services and Aquatic Diagnostic services. Live animals must be brought in during normal working hours (8-4 pm, Mon to Fri). Submitters must call the post-mortem office (566-0864) prior to receiving these animals to make appropriate arrangements for euthanasia.

Cosmetic Necropsies, Animal Remains, and Disposal Service Policies

Cosmetic necropsies are not performed. The release of any remains from carcasses to owners is prohibited. Reasons for this policy include the possibility of disease transmission from the laboratory to either the owner or the owners' other animals, and misunderstandings by the owner about the condition of the returned carcass. If the submitting veterinarian wishes to perform a cosmetic post-mortem we will gladly do histopathology, microbiology, etc., for you.
Disposal services are limited to those animals necropsied at Diagnostic Services.

Insurance/Legal Necropsy Policies
It is the responsibility of the consignor to alert the laboratory to cases involving insurance or potential legal aspects to insure full documentation. There is an additional charge associated with these cases.

Legal cases are defined as those requiring lab personnel to maintain chain of evidence in order for any information obtained to be admissible as evidence in legal court proceedings.

Tissue Submissions:

1) Place tissues in fixative as soon as possible. Fixation is typically complete within 24-48 hrs for most tissues.

2) Please avoid crushing and squeezing samples to be sent for histopathology. Such trauma induces significant artifactual changes that can significantly alter the microscopic appearance of tissues and limit the pathologist's ability to interpret lesions.

3) Please do not use electocautery or lasers to remove samples for histology. These instruments "cook" the tissues and can obscure microscopic lesions.

4) Use wide-mouthed, leak-proof containers. Please **DO NOT USE GLASS JARS**. Also, please avoid using narrow necked containers. Fresh tissues are soft and pliable and can easily be put into a narrow-mouthed jar, but following fixation tissue becomes firm, rigid and cannot be easily removed.

5) **Provide thorough a clinical history**
    a. Record lesions, include size, extent, colour, consistency
    b. Report negative findings if relevant. An example could be the absence of gross brain lesions in an animal which had neurological signs.
    c. Report important treatment information (e.g. the dog is currently on antibiotics or steroids....) and important clinical findings (e.g. hypoalbuminemia, hypercalceimia, etc.).

6) **Tips and considerations on tissue submissions**: The quality of your diagnostic submission is critical in allowing the pathologist to recognize and accurately interpret lesions, and thus provide you and your client with a diagnosis. The following are some suggestions on how to handle many common, types of submissions:

    a) Eyes - please do not puncture or incise the eye.

    Remove all extraocular soft tissues (eyelids, muscles, etc.), leaving the orb bare, ideally with a small stump of optic nerve intact. If there is something of concern within these soft tissues that you would like examined, please submit them in a separate container.

    For a canine eyeball, inject approximately 1 ml of formalin into the eye, through the sclera, and then place the eye in formalin for fixation. For smaller feline eyeballs, approximately 0.5 ml of formalin will be sufficient, while larger equine eyeballs may require injection of 2 ml of formalin for adequate fixation

b) **Muscle biopsies**

When considering submitting muscle biopsies for evaluation, there are several factors to take into account. Diagnostic Services can perform routine histopathologic evaluation of formalin fixed muscle samples. For more specific histochemical and immunohistochemical testing, which generally requires evaluation of fresh (not frozen) muscle and possibly nerve samples, we would forward such samples to specialized neuromuscular laboratories for evaluation. Please call the lab before sampling, to make arrangements for the handling of these samples, to get appropriate submissions forms, to arrange for import permits if testing is to be forwarded to an American lab and to get a quote for costs, which vary depending on the tests required and the referring lab.

When considering performing muscle biopsy, the selection of which muscle to sample and the testing required depends on the clinical presentation and the presumptive/ or differential diagnoses that you are considering.

For clinical signs restricted to the **muscle mastication in dogs (i.e. masticatory muscle myositis - MMM**), the temporalis muscle is the optimal sampling site. Be sure to retract the more superficial caudoauricular/or frontalis muscle which lies just under the skin (and is not affected by MMM), and incise the thick fascia that covers the temporalis muscle to sample the underlying muscle. Often, this disease can be diagnosed by the detection of antibodies in the serum alone.

When clinical signs are generalized (usually in small animals) and a myopathic disorder is considered, proximal limb muscles, such as the vastus lateralis or biceps femoris from the pelvic limb and/or triceps muscle from the thoracic limb, are frequently suggested sampling sites.
In the horse, for the most commonly encountered muscular diseases, the gluteal or semimembranosus muscle are recommended sampling sites but this might vary depending on the disease suspected. For more information please check out this website : www.cvm.msu.edu/research/faculty-research/valberg-laboratory/for-veterinarians/obtaining-and-submitting-a-biopsy#information-on-submitting-a-sample.

Suggestions when sampling skeletal muscle:

a) Ideally, the sample should be 1 cm long, by 0.5 cm wide, and 0.5 cm deep and removed with as little trauma as possible (no cautery please)
b) The sample should be dissected along the long axis of the muscle so that the muscle fibers in the sample are oriented lengthwise
c) Fresh samples are the best samples in most instances, but when considering other differential diagnoses (tumors, etc.) or when there may be significant delays in getting fresh samples to the lab, taking an additional sample to be placed in formalin would be prudent.
d) **Fresh samples** on which all histochemical and immunohistochemical staining can be applied. **Please notify the lab before sending these samples**. Wrap the sample in saline moistened (not dripping wet) gauze and placed in a dry, water tight, specimen jar or red top tube. Keep the sample refrigerated. For overnight shipping to the lab, sandwich the sample between 2

cold packs and label the package "refrigerate upon arrival". Do not ship the samples Thursdays or Fridays or before long weekends/holidays.

e) **Formalin fixed samples -** if the sample is to be shipped immediately, place it directly in a water-tight container containing 10% buffered formalin. If the sample is to be shipped the following day, to minimize artifact associated with muscle contraction, immediately following sampling you can pin the muscle sample lengthwise to a piece of tongue depressor using 2 small bore needles, one positioned at each end of the sample. The following day, **prior to packaging for shipping REMOVE THE NEEDLES,** then send the sample in formalin (still attached to the tongue depressor), as you would any other biopsy sample.

Frequently used, referral veterinary neuromuscular labs include, but are not limited to:
UC Davis, Neuromuscular Disease Laboratory (www.vetneuromuscular.ucsd.edu)
Equine Neuromuscular Diagnostic Laboratory, Michigan State University, (www.cvm.msu.edu/research/faculty-research/valberg-laboratory)
Animal Health Laboratory, Guelph, Ontario (www.guelphlabservices.com)

Samples from an in-house Necropsy

a) Provide a thorough description of your post mortem findings
b) Submit sections of abnormal tissue and organs.
c) Submit samples of all major organs: lung, heart, liver, kidney
d) When performing necropsies in house, it is always wise to freeze and hold fresh (or unfixed) tissue samples from a variety of organs, so that further tests such as virology, bacteriology or toxicology may be carried out later, if necessary. Collecting and freezing stomach contents, when ingestion of a toxin may be suspected, is also prudent. When submitting formalized tissues indicate on your submission form whether frozen portions are being held.
e) The heart may be submitted in its entirety, particularly if a malformation is suspected.
f) Rinse the chambers with water and fill with 10% formalin.
g) Brains submitted for pathological examination may be submitted intact and immersed in 10 times volume of 10% formalin. Better fixation, and therefore examination, can be obtained by prefixing the entire brain for two days in a large container in 10% formalin and then shipping the entire brain in a smaller container with a small volume of 10% formalin is the preferred method.

Bone lesions – please provide a description of radiographic findings. Bone samples are generally hard and require time to soak in decalcification solution following fixation to soften sufficiently for subsequently trimming. Depending on the size of these lesions, this may take several days to weeks to complete, so be prepared for delays in receiving results in these cases.

Very small tissue samples (e.g. endoscopic biopsies, core needle biopsies etc.) - should be placed directly in special histology cassettes immediately following sampling, then the cassette is placed in formalin. These cassettes have small sieve like openings which allow formalin to enter but tissues cannot escape. This keeps these small samples safe and minimally traumatized. Please contact us should you like more information regarding these cassettes.

Hollow Organs: e.g. intestine, urinary bladder, uterus
Cut open longitudinally (with care) prior to placing in formalin to ensure fixation of mucosal surfaces

Tumour Submissions

Please indicate the size, location of the mass. Whenever possible, include information such as: How long has it been present? Is it increasing in size? Does it invade surrounding tissues? or does it have a capsule ?

Margin assessment - we highly recommend the use of surgical dyes to mark specific surgical margins. These dyes are relatively inexpensive, and different colors are available which can be used to identify different areas of interest. Black, yellow, purple and green are generally preferred. Unlike using sutures, these dyes do not cause tissue damage in areas of interest, and the dye remains in place following fixation and sectioning.

1) Ideally inking should be performed as soon as possible following resection (within 30 min at most) and **prior** to placement of tissue in formalin.
2) A cotton swab or wooden applicator should be used to place ink on specific areas of interest or true surgical margins (you should avoid submerging the whole sample in ink)
3) To prevent the ink from washing off, allow it dry 5-10 min before placing the tissue in formalin
4) For larger samples that will require sectioning, first ink tissues, allow it to dry and then section before placing them in formalin. This will help to minimize the dye from staining unimportant areas.

Skin Biopsies:

Do not shave biopsy sites, as the hairs are useful guidelines for proper plane of section.

When investigating multifocal or generalized skin conditions, taking multiple skin punch biopsies or samples is recommended. Take samples from the most recently developing lesions and/or from the margins of more chronic lesions, including the adjacent more normal appearing skin where possible. Avoid sampling the center of very chronic, ulcerated lesions. If a bullous skin disease (pemphigus) is suspected the active lesion (such as an actual blister) is required for definitive histological diagnosis.

Providing a **thorough clinical history**, including signalment (including breed, sex, distribution of lesions, etc.) is vital in dermatology cases.

Flattening the biopsy on a piece of cardboard and floating it upside down in formalin (do **not** use needles to pin the samples), will result in a fixed tissue that can be properly orientated by the pathologist for sectioning.

Immunohistochemistry staining can be done on tissue blocks either in-house or sent to a referral laboratory. There is an additional fee for this testing.

Avian Submissions:

FOR THE DIAGNOSIS OF A DISEASE PROBLEM WITHIN A FLOCK
Submit 4 to 5 freshly dead birds that have died of the condition, preferably refrigerated and kept cool until they arrive at the laboratory. Time is important as small carcasses decompose quickly.

Submit 4 to 5 live, sick birds that are clinically affected with the condition. Take care not to select cull birds from the flock which do not represent the disease condition. These specimens should be shipped by express in containers suitable for live birds.

Suggested Standard Submissions from Aborted Fetuses:

Species	HISTOPATHOLOGY	BACTERIOLOGY	VIROLOGY	SEROLOGY
Bovine	Lung Heart Liver Kidney Spleen Adrenal Thymus Cotyledon	Lung Spleen/ Liver Cotyledon Intercotyledon area of placenta Abomasal contents Pericardial fluid	Liver Kidney Spleen	Paired sera
Porcine	Heart Lung Liver Kidney Spleen Thymus Placenta	Lung Spleen/ Liver Placenta Stomach contents Pericardial fluid	Liver Kidney	Paired sera
Ovine & Caprine	Lung Heart Liver Kidney Spleen Thymus Cotyledon	Spleen/ Liver Lung Placenta Abomasal contents	Liver Kidney	Paired sera
Equine	Thymus Lymph node Spleen Heart Lung Liver Kidney Placenta	Spleen/ Liver Lung Placenta Stomach contents	Lung Liver	Paired sera

Comments

Histopathology: Fix 5 mm thick portions of tissue in l0 volumes formalin per volume tissue; do not freeze if at all possible.
Bacteriology: Submit tissues in whirl-pack bags labelled "Bact", put each tissue in a separate bag. Fluids should be submitted in labelled red-top vacutainers. These specimens should be at refrigerator temperature but NOT FROZEN.
Virology: Submit tissues in whirl-pack bags labelled "viro", freezing is acceptable.
Serology: Collect serum at the time of abortion and 2-3 weeks later; remove serum from clot and store frozen at your clinic; paired sera can then be submitted as indicated. Never freeze clotted whole blood with the expectation of using it for serology later as this will interfere with serological tests.

Suggested Standard Submission for Neonatal Scours:

Species	HISTOPATHOLOGY	BACTERIOLOGY	VIROLOGY	PARASITOLOGY
Bovine	Abomasum Jejunum Ileum Colon Heart Lung Liver Kidney Spleen	Mesenteric Lymph node Jejunum Ileum	Jejunum Ileum Colon Feces	Ileal mucosal smear Feces
Porcine	Duodenum Mid-jejunum Ileum Colon Heart Lung Liver Kidney Spleen	Jejunum Ileum	Jejunum Ileum Feces	Ileal mucosal smear Feces

Comments

Histopathology: Fix 5 mm thick portions of tissue in l0 volumes of formalin per volume of tissue; do not freeze; either open intestine longitudinally or fill lumen with formalin and ligate ends.
Bacteriology: Submit each tissue in a separate, labelled whirl-pack bag. Smears for Gram stain may be made from a composite ileal/ jejunal swab (not the sections for culture) and air-dried.
Virology: Tissues may be submitted frozen, clearly labelled "Virology".
Parasitology: To make ileal mucosal smears transfer ileal mucosal scrapings to a glass slide and air dry. Fecal material should arrive at the laboratory in refrigerated condition (not frozen) as soon as possible after the sample is taken. Alternatively, feces maybe preserved in 2.5% potassium dichromate solution (l volume feces to l volume $K_2Cr_2O_7$ solution).

Histology – Research Submissions:

Test Name	Sample Submission	Procedure
Histology Block	Fixed tissue cassette	Processing and embedding
Histology Frozen Section	Fixed tissue	Prepare slide from cryostat section
Histology Mail – Large box Small box	Fixed tissue	Prepared slides. Returned in slide boxes.
Histology Processing	Fixed tissue in cassettes	Processing
Histology Processing and Stain (per cassette)	Fixed tissue in cassettes	Processing and stained with Hematoxylin and Eosin
Histology Recut	Tissue block	Recut Stained with Hematoxylin and Eosin
Histology Hematoxylin and Eosin Stain	Dry, unstained slide	Stained with Hematoxylin and Eosin
Histology Special Stain	Dry, unstained slide	Stained with a selection of 40 Special Stains Oil Red O and Silver available for a extra fee
Histology Unstained	Tissue block	Cut and returned unstained

-Call (902) 566-0883 for submission recommendations and scheduling.

Parasitology

Fecal Samples

Collection and Handling of Fecal Samples

a) Preferably, fecal samples should be collected from the rectum. If material is collected from the ground it should be from the top of a freshly passed deposit. Avoid deposit areas in contact with the ground. Care must be taken with samples collected from the ground to avoid doing fecal exams on neighbourhood or stray animals. It is advisable to collect only those samples which can be positively identified as relevant to the animal in question.
b) A minimum sample size is 5 g. Preferably, submit a "golf-ball" size sample in a plastic bag or a container that is airtight, watertight, and suitably robust. Gloves are not suitable primary containers.
c) All containers must be clearly and completely labelled: name, species of animal, date of collection.
d) Store the specimens in a refrigerator until shipping. DO NOT FREEZE FECAL SAMPLES. Samples should be at the laboratory within 12 hours if possible.
e) In cases involving a herd problem, ideally, every animal in the herd should be sampled. However for a reliable evaluation of a herd, sample 10-25% of individuals. When samples of individuals cannot be identified, such as in a feed lot situation, take random samples and clearly label them as such.
f) PLEASE DO NOT SUBMIT COMPOSITE SAMPLES.
g) Most parasites (excepting some protozoans) will still be detectable and easily identifiable in fecal samples examined 2 to 3 days after collection, if the samples have been refrigerated in the meantime. If more than 2 to 3 days may elapse between collection and examination (or the samples cannot be refrigerated), mix equal parts of 5% formalin and feces. This will prevent parasite development, especially the hatching of eggs. This procedure should not be used if the diagnostic technique depends on living parasites, such as the Baermann technique.

Feces, Direct Smears

These can be made by transferring a small sample of feces to a glass slide. Mix in a small amount of saline. Drop on a cover slip and examine directly under the microscope. This technique will reveal heavy infections of eggs and cysts (such as coccidiosis in poultry). It may also detect helminth eggs and larvae or protozoan trophozoites which typically do not float and therefore are not detected with standard fecal flotation techniques.

Feces, Visual Examination

Search macroscopically for large gravid segments of cestodes (e.g. *Dipylidium caninum* in dog and cat feces) or whole adult helminths (e.g. *Parascaris equorum* in horse feces).

Feces, Protozoal Diarrhea

Some developmental stages of these organisms (e.g. *Giardia* species, Trophozoites) are too fragile to withstand transport by courier. Testing ideally should be done at the practitioner's own laboratory, by examining saline wet-mounts of fresh warm feces. Delivery shortly after passage to the diagnostic laboratory is an alternative.

Feces, Baermann Technique

This technique separates first-stage larvae from feces. Organisms detected can include lungworm in sheep (*Mullerius capillaries)*, cattle (*Dictyocaulus viviparous)*, dogs (*Crenosoma vulpis)* and cats *(Aelurostrongylus abstrusus)*. This method can also detect *Angiostrongylus vasorum* and *Stronglyoides stercoralis* in dogs. Fresh feces should be collected to avoid confusion with free-living nematodes. Cattle feces should be collected from the rectum. Indicate on the submission form the requirement for a Baermann technique to be done.

An important note: The Baermann technique may also be used to separate lungworm larvae from lung tissue (e.g. *Muellerius capillaris* in sheep and goats).

Feces, Flotation

This technique will separate from feces various species of helminth eggs (e.g. *Ascaris suum* in pigs), and protozoan cysts (e.g. *Eimeria spp.* oocysts in sheep, *Giardia canis* cysts in dogs). Some helminth eggs (trematode, operculate cestode, various nematode), nematode larvae (*Dictyocaulus spp., Muellarius capillaris, Strongyloides stercoralis*) and protozoan trophozoites (*Giardia canis*) generally are not detected with this technique. If infection with any of these parasites is suspected a direct smear, sedimentation or Baermann technique should be requested. Indicate on the form the need for a fecal flotation to be done.

Grading System

A high egg count may indicate a high number of parasites but a low number of eggs does not necessarily indicate a low number of parasites. A grading (non-quantitative) system is used as follows:

1-100 eggs on a slide is graded one plus +
101-300 eggs on a slide is graded two plus ++
301-greater on a slide is graded three plus +++

Blood Parasites

Canine Heartworm (*Dirofilaria immitis*)

a) ***Microfilaria I.D. (Knotts Test for Microfilaria Detection)***

The Knotts Test is used to detect and identify circulating microfilaria of *Dirofilaria immitis*. It is the only microfilarial test that allows differentiation between *D. immitis* and the non-pathogenic *Dipetalonema reconditum*. It can detect infected dogs as early as 6 months post-infection. Draw off at least 1 ml of venous blood (preferably 2 – 3 ml) into a vial containing either heparin or an EDTA (lavender top). Mix the blood and anticoagulant. Store sample in the refrigerator. Submit the sample to the diagnostic laboratory along with a complete history and time and date the sample was drawn. Due to the danger of a potentially fatal drug induced shock reaction in microfilaremic dogs given diethylcarbamazine (DEC), a microfilaria test is advised on all dogs prior to the use of DEC as a heartworm preventative. Be aware, however, that approximately 25-33% of the heartworm-infected dogs will not be detected with this test.

b) ***Heartworm Antigen Test***

This test is an ELISA serologic test that detects circulating adult worm antigen. Submit at least 1 ml of either serum or plasma (EDTA or heparin). The test detects infected dogs as early as 6.5 months post-infection (most reliably at 8 months post infection). If only one heartworm test is to be done (Knott's vs. Antigen Test), the antigen test is preferable to the microfilaria test. In rare instances, the antigen test can give a false negative result in a microfilaremic dog. Therefore, doing both tests offers the greatest diagnostic power to detect infection. Routine testing of dogs should begin in the month of April each year.

This test can also detect microfilaria in **cats.** If the animal is infected with only a few parasites, however, there could be insufficient antigen present to cause a detectable reaction. In these cases, the Heartworm antibody test is recommended and is a referred test.

Skin Scrapings, Mange Mites

a) This technique is used for lesions with minimal epidermal hyperplasia and lesions caused by deeply burrowing mites (e.g. *Sarcoptes, Notoedres*) or in hair follicles (e.g. *Demodex*). Dip a scalpel blade in mineral oil or glycerine. Using the blade, scrape the periphery of the lesions at right angles to the skin until pinpoint hemorrhaging occurs. The material collected on the scalpel blade should be pink in color. Put the scalpel blade with the oil and detritus into a sealed container, such as a small ointment jar or stoppered test tube.

Note: Do not use glycerine or mineral oil on samples destined for bacteriology or mycology. Separate samples should be collected for these procedures.

b) The following technique is used for lesions with marked epidermal hyperplasia and exfoliation and lesions caused by lice and superficially dwelling mites (e.g. *Chorioptes*). Scrape the dried exudate and debris into a small specimen jar.

c) Ear mites (e.g. *Otodectes*) can be found easily with an otoscope. They can be removed from the external ear with a cotton swab. Place the swab in a container and submit to the laboratory.

d) Poultry mites (e.g. *Dermanyssus gallinae*) do not remain on the host in daylight. The bird's environment must be examined. Search in bird nests, roosts, and nearby cracks and crevices in housing structures. Collect and contain specimens and submit to the laboratory for identification.

e) Some surface feeding mites (e.g. *Cheyletiella*) in dogs and other hosts can be collected by vigorously brushing the host over a plastic sheet. Mites and debris will accumulate on the sheet and can be transferred to a container.

Ectoparasites

a) Large slow moving ectoparasites, such as lice, keds, ticks and possibly fleas, can be collected with forceps or fingers. Gentle, steady traction, grasping the tick by its mouthparts as close to the skin as possible, can remove ticks with their mouthparts intact.
   Contain the specimen in a jar along with a paper towel soaked in water. Submit to the laboratory for identification.
b) Winged bloodsucking insects can be collected using a simple suction tube or vacuum cleaner fitted with an in-line filter or a chloroform tube placed over the parasite. Lice and occasionally fleas may be caught by this method.
c) Various parasites such as fleas and lice can be collected from recently killed or moribund small animals by placing the animal in a closed plastic or paper bag. The ectoparasite will leave the host and can be collected in the bag.
d) Examination of hairs or feathers can reveal nits (louse eggs) or bot fly eggs (e.g. *Hypoderma*, *Gastrophilus*).
e) Preserving large ectoparasites (e.g. ticks, fleas, keds, lice, dipterans) is needed for shipping. Specimens should be submitted in vials or sealed plastic bags with paper towels soaked in 70% ethanol as a preservative - **10% formalin should not be used**.
   For long-term storage utilize 70% ethanol / 5% glycerine.
f) To preserve small ectoparasites such as mites and mallophagan lice, **70% isopropyl alcohol** is used. Specimens placed in 70% ethanol usually become too brittle for processing and identification. Specimens should be shipped in small vials filled with preservative. It should be noted where specimens were collected on (or in) the host.
   Lots from different hosts should not be mixed.

Endoparasites

a) The following techniques for preserving large endoparasites for shipping. Specimens of cestodes and flukes should be fixed in buffered 10% formalin or in 70% ethanol. If flatworms are alive, they should be allowed to relax in tap water or saline prior to killing in hot (65^{o}C) water or formalin. The relaxation is necessary as contracted specimens are usually impossible to identify. Nematodes can be handled in a similar manner. After fixation in formalin, transfer to 70% ethanol / 5% glycerine for storage. It is important to be consistent in the use of fixation techniques as different methods can modify the morphological attributes of some helminths.
b) Lungworm larvae (e.g. *Muellerius capillaris* in sheep) in lung tissue, can be separated by the Baermann technique. Lung tissue must be submitted as soon as possible after collection because the lungworm larvae must be alive for this technique to work. For this same reason, the sample cannot be placed in preservative. Indicate on the submission form the requirement for a Baermann technique to be done.
c) Gut sections and scrapings may be submitted and are potentially useful to detect situations like nematodes infestations in ruminants. Gut sections with helminths should be fixed in 10% buffered formalin or 70% ethanol. Fixation of fresh material is desirable. Note on the submission form where in the alimentary tract the sections and/or scrapings were sampled. Each section of gut or each scraping should be submitted in individual containers to prevent mixing and confusion.
d) Eggs of pinworms (e.g. *Oxyuris equi* in horses) can be collected by placing scotch tape on the anal region and examining under a microscope.

Toxoplasma gondii – canine, feline, caprine, ovine
Neospora caninum – canine, bovine

Specimen Collection and Preparation
Collect a specimen of whole blood in a red-topped tube. Following clot retraction, centrifuge, aspirate the serum by pipette or syringe and place into a separate red-topped tube or plastic screw capped shipping vial. Ensure that all red blood cells are removed to prevent hemolysis during shipping. Usually about 0.3 ml of serum can be obtained from each l ml of clotted blood. Label the separated sample as serum and submit fresh if delivery within 48 hours is possible or frozen if longer transit time is likely. Serum can ALWAYS be frozen and stored before shipment. Do not freeze and thaw sera more than once. The tests require 1 ml of serum.

Do not use plasma; all testing with the kit must be performed on sera. Reactivity of the reagents with plasma is not clearly defined.

In keeping with good laboratory technique, do not use contaminated, grossly hemolyzed, lipemic or turbid specimens, which may indicate that the specimen has been exposed to deleterious conditions and/or substances.

Aquatic Samples

a) ***Myxobollus cerebrallis*** (whirling disease samples)

For population screening cranial cartilage and gill arches from a minimum of 60 fish should be submitted. Samples once collected should be frozen and shipped frozen to the Diagnostic Laboratory for analysis. Refer to suggested protocols at this website: (http://www.afs-fhs.org/bluebook/inspection-index.php)

b) Other Fish Parasites: Refer to protocols provided at this website:
(http://www.afs-fhs.org/bluebook/inspection-index.php)

Quality Assurance Program

The Veterinary Laboratory Association Quality Assurance Program® (VLA-QAP) located at the Atlantic Veterinary College is an external proficiency program offering a specially designed quality assurance test kit for veterinary laboratories. It provides a confidential means of comparing your laboratory's internal test results to those of your peers in the animal health field. It also provides a way to verify the successful execution of diagnostic methods, sample handling, and data collection within the laboratory. Quarterly results are tabulated and statistically generated to compare each user anonymously against all other user submissions on our secure web-based system.

Participants can choose any combination of the tests available to customize the program to meet the needs of their facility. Sample specimens are sent out four times per year for participants to analyze.

Areas of Testing

- **Terrestrial Bacteriology**
  - Provides 4 samples per year for culture and sensitivity.

- **Aquatic Bacteriology**
  - Provides 2 samples per year for culture and sensitivity.

- **Biochemistry/Toxicology**
  - Provides 8-10 samples per year for testing over 30 analytes, including toxicology analytes such as iron, copper, zinc, and selenium.

- **Endocrinology**
  - Provides 4 samples per year for analysis of 9 hormones.

- **Hematology**
  - Offers 4 or 8 simulated whole blood samples and 4 Wright-Giemsa stained slides for analysis and differentials.

- **Histopathology**
  - Provides 4 virtual slide cases a year of an H&E stained tissue preparation for morphological interpretation.

- **Cytology**
  - Provides 4 virtual slide cases per year of an H&E stained fluid and/or tissue preparation for morphological interpretation.

- **Parasitology**
  - Provides 4 samples per year of formalin fixed parasites and a virtual slide image for identification.

- **Serology**
  - Offers 8 companion animal samples per year for analysis.

- **Therapeutic Drug Monitoring**
  - Provides 4 samples per year for analysis of 3 different drugs; KBR, Phenobarbital, and Digoxin.

- **Urinalysis**
  - Provides 4 samples per year consisting of digital images and lyophilized urine for macroscopic and microscopic analysis.

  - **For more information, visit us at www.vlaqap.org**

Research

Diagnostic Services takes pride in our participation with numerous and various research endeavors.

Researchers are always welcome clients of Diagnostic Services. Our professional and technical staff can offer support and experience along with efficient testing and evaluation of specimens. We offer advanced automated technology customized for veterinary needs.

Past experiences have included research in all disciplines – Chemistry, Hematology, Microbiology, Parasitology, Virology, Toxicology and Histology – and has involved numerous aquatic, avian and terrestrial species including wildlife, companion animals, agricultural and laboratory animals. We accept projects from researchers in private industry, universities and/or government departments.

We encourage interested parties to contact us at 902-566-0863 to discuss potential projects and how our services may support your individual needs. Testing of interest to you that is not listed in our directory, may still be available upon request.

Therapeutic Drug Monitoring

Therapeutic drug monitoring can provide information useful in tailoring drug therapy to the individual patient. A clinical need for TDM may arise from a lack of therapeutic response, suspected drug toxicity, or the desire to confirm a therapeutic approach.

Diagnostic Services can arrange for analyses of serum or plasma concentrations of digoxin, gentamicin, phenobarbital, potassium bromide, procainamide, quinidine, theophylline and other therapeutic drugs along with interpretation of results by the clinical pharmacologist. Advance notification is required if test results are urgently needed.

Laboratory Requisition Forms

A Therapeutic Drug Monitoring Requisition Form is available from Diagnostic Services. **When submitting samples for analysis, all information requested on the form should be provided.** This is particularly true when requesting a consultation.

Assay Schedule

TDM test samples should arrive at Diagnostic Services before 10 AM on the day the assay is to be run.

i. Digixon……Referred - Daily
ii. Gentamicin…….. Referred - Daily
iii. Phenobarbital.……..Daily
iv. Potassium Bromide……Tuesday and Friday
v. Procainamide ……..Referred
vi. Keppra (Levitiracetam) …… Referred
vii. Zonisamide ……Referred

Other therapeutic drugs can be measured by special request with Diagnostic Services.

Drug	**Time to Steady Rate**	**Peak Sample**	**Recommended Sample Time**	**Trough Sample**	**Comments**
			* **		
Digoxin					
Dog, Cat	~ 7 days	2-5 hours	Peak if suspect toxicity; trough for lack of efficacy	Before next dose (or 8 hours after the last dose)	Use glass tubes preferably
Horse	~ 4 days				
Gentamicin					
Dog, Cat	~ 6 hrs	1 hour	Peak and trough	8-12 hours post-dose or before next dose	
Horse	~ 1 day	1 hour		8-12 hours post-dose	
Phenobarbital					
Dog, Cat	2 weeks	4-6 hours	Trough for routine monitoring; peak if toxicity is suspected	Before next dose (or 8-12 hours after last dose)	
Horse	~ 4 days				
Potassium Bromide	~ 3 months		Sampling anytime during dosing. Sample size: 1cc		
Keppra (Levitiracetam)	Never truly occurs in dogs and cats		Through sample can be measured within 1 week	Just prior to next dose (8hr post on 8hr dosing)	
Zonisamide	14 days	3 hours	Trough for routine monitoring.	Just prior to next dose (12hr post on 12 hr)	Test annually unless an issue arises
Thyroxine (T4)					
Dog, Cat	2-3 days	4-8 hours	See Endocrinology Section, page 25 for sampling times.		

* General Collection – Red-top vacutainer, spin down, and put in glass tube.

** Note: ***Do not use serum separator tubes for therapeutic drug monitoring***. The silicone gel can bind drugs and cause artificial decreases in concentrations.

Digoxin

Therapeutic Range

Serum or plasma concentrations, established for steady state **trough** samples:

Canine.....	1.2 – 3.8 nmol/L
Feline.....	1.2 – 2.6 nmol/L
Equine.....	0.6 – 2.6 nmol/L

Specimen Collection and Handling

1. Collect the sample immediately prior to the next dose if dosing BID. (If dosing SID, especially in cats, collect the sample 10 – 12 hours post digoxin administration).
2. Collect blood by venipuncture into a plain, red-topped tube, separate serum from cells. The required volume of serum is 0.5 ml.
3. Store and transport in glass containers only (cardiac glycosides absorbs to plastic).
4. Transport samples to the laboratory on ice; if a delay of greater than 24 hours is anticipated between sample collection and arrival at Diagnostic Services, the sample should be shipped frozen.

Gentamicin

Measurement of both peak and trough serum or plasma concentrations of gentamicin provides better assessment of drug efficacy and nephrotoxic potential.

Therapeutic Range

Serum or plasma concentrations:

	Peak	Trough
Canine.....	11 – 18 µmol/L	1.1 – 3.3 µmol/L

Toxic Concentrations

Serum or plasma concentrations:

	Peak	Trough
Canine.....	> 27 – 33 µmol/L	> 4.4 µmol/L
Equine.....	> 27 µmol/L	> 4.4 µmol/L

Specimen Collection and Handling

1. Collect blood sample 1 hour after IM or SQ dosing for peak determination or just before the next dose for trough determination.
2. Collect blood by venipuncture into plain, red-topped or heparinized tube. Separate serum or plasma from the cells and place into a plastic tube. The required volume of serum is 0.5 ml.
3. Store and transport in plastic containers only (gentamicin absorbs to glass).
4. Transport samples to the laboratory on ice; if a delay of greater than 24 hours is anticipated between sample collection and arrival at Diagnostic Services, the sample should be shipped frozen.

Phenobarbitol

The sampling time in relation to drug administration depends upon the therapeutic considerations. If seizures are not being controlled in the patient, **trough** sampling is best. If toxicity is suspected, **peak** sampling (4 to 6 hours after drug administration) is best. Alternatively, peak and trough sampling will allow for estimation of serum or plasma half-life and will facilitate the calculation of an adjusted dose of phenobarbital for the patient. For best assessment of steady state serum or plasma concentrations, at least two weeks should have elapsed since the last adjustment in the dosage regimen.

Therapeutic Range

Serum or plasma concentrations:

Canine..... 54 - 190 µmol/L
Feline..... 65 - 130 µmol/L

Specimen Collection and Handling

1. Select the desired dosage regimen and administer the drug until steady state is reached (approximately 16 days in canines).
2. Collect blood by venipuncture into plain, red-topped tube; separate serum from cells. The required volume of serum is 0.5 ml.
3. Store and transport in glass or plastic tube
4. Transport samples to the laboratory on ice; if a delay of greater than 24 hours is anticipated between sample collection and arrival at Diagnostic Services, the sample should be shipped frozen.

Potassium Bromide

Therapeutic Range

Serum or plasma concentrations:

Monotherapy…… 12.5 – 37.5 mmol/L
With Phenobarbital……. 12.5 – 31.3 mmol/L

Sample Collection and Handling

1. Collect a sample at any time during the day after the patient reaches steady state (~ 3 months).
2. Collect blood by venipuncture into a plain, red-topped tube. Separate serum from the cells (at least 1 ml serum is required).
3. Store and transport in glass or plastic tube.
4. Transport samples to the laboratory on ice; if a delay of greater than 24 hours is anticipated between sample collection and arrival at Diagnostic Services, the sample should be shipped frozen.
5. Avoid hemolysis and/ or lipemia as it interferes with the assay

Procainamide

Therapeutic Range

Serum or plasma concentrations:

Canine…….....…… 85 – 125 µmol/L

Toxic Concentrations

Serum or plasma concentrations:

Canine..…….. > 125 µmol/L

Sample Collection and Handling

1. Collect the sample immediately prior to the next dose for trough serum concentration.
2. Collect blood by venipuncture into plain, red-topped tube. Separate serum from cells. The required volume of serum is 0.5 ml.
3. Transport samples to the laboratory on ice; if a delay of greater than 24 hours is anticipated between sample collection and arrival at Diagnostic Services, the sample should be shipped frozen.

Keppra® - Levetiracetam

Minimum Sample Required: 0.5ml Serum or Plasma (heparinized). Do not use serum separator tubes.

Fasting recommended but not required. No ice needed.

Steady-state" never truly occurs for levetiracetam in dogs (and may not in cats) because the drug does not accumulate with each dose. Because minimal accumulation occurs, concentrations can be measured within days to 1 week of initiating a new dosing regimen.

Recommend a trough sample: Just prior to next dose (8hrs post on 8hr dosing). (Dr. McConkey, Pharmacologist, AVC).

If peak sampling is also wanted collect the peak sample: 2-4hrs post dose. Diagnostic Services will forward a submission form from Auburn University to include with your submission.

Zonisamide

Minimum Sample Required: 0.5ml Serum or Plasma (no serum separator tubes).

Fasting recommended but not required. No ice needed.

Recommend trough sample: Just prior to next dose (12hrs post on 12hr dosing). (Dr. McConkey, Pharmacologist, AVC). Testing 14 days after starting treatment or following a dosage change is suggested. Testing annually after the first test is sufficient, unless there is a problem.

If peak sampling is also wanted collect the peak sample: 3 hrs post dose. Diagnostic Services will forward a submission form from Auburn University to include with your submission.

Toxicology

Sampling Procedures for Toxicology

COMPOUND	SAMPLE REQUIREMENTS	QUANTITY
Metals:		
Arsenic	Anticoagulated Whole Blood (Heparin or EDTA)	2 ml
	Liver	25 g
Cadmium	Liver, Kidney	5 g
Cobalt	Aqueous	5 ml
Copper	Serum	2 ml
	Liver, Kidney	5 g
Iron	Liver, Kidney	5 g
Lead	Anticoagulated Whole Blood (Heparin or EDTA), Water (results in parts per billion)	1 ml
	Liver, Kidney, Rumen contents, Feces	5 g
Mercury	Anticoagulated whole blood – (Heparin or EDTA)	2 ml
Potassium	Aqueous	5 ml
Sodium	Aqueous	5 ml
Selenium	Serum,	1 ml
	Liver, Kidney	5 g
Zinc	Serum	2 ml
	Liver, Kidney	5 g

***Ensure samples are in suitable leak proof container. It is recommended to ship samples frozen with ice packs.**

Sampling Procedures for Toxicology

COMPOUND	SAMPLE REQUIREMENTS	QUANTITY
Vitamins:		
Vitamin A	Serum	3 ml
Vitamin E	Serum	3 ml
	Liver	5 g

For vitamin assays, serum should be removed from the clot as soon as the clot has formed; seal the tube tightly, avoiding light, and submit immediately, packed on ice or freeze at -15˚C, long-term storage at -75˚C.

Vitamin E in liver analysis is dependent on the condition of the tissue, degraded or liver in poor condition may produce unreliable results. Ensure tissue is kept frozen until it reaches the laboratory for testing, ship frozen with ice packs. Ensure sample is in a leak proof container.

Cholinesterase	Brain	Whole brain
Aquatic Residues:		
Oxytetracycline	^Feed	10 g
	^Salmon Flesh	100g
Emamectin	Feed	10 g
	Fillets	100 g
	Sediment	10 g
	Slice	10 g
*Florfenicol	Fillets	20 g
*Malachite Green	Feed	10 g
	Fillets	100 g

^ISO 17025 accredited test method

***Please contact lab before submitting samples. Development fee may be applied.**

- Ensure fillets and salmon flesh are kept frozen until they reach the laboratory for testing, ship frozen with ice packs. Samples must be packaged in a new or disinfected suitable primary container which has been disinfected on the outside and then placed in a suitable secondary container with enough absorbent material, and the outside disinfected.

Carotenoids:

**Astaxanthin	Feed	10 g
	Fillets	100 g
**Canthaxanthin	Feed	10 g
	Fillets	100 g

****Please contact lab before submitting samples.**

- **Ensure fillets are kept frozen until they reach the laboratory for testing, ship frozen with ice packs.** Ensure fillets and salmon flesh are kept frozen until they reach the laboratory for testing, ship frozen with ice packs. Samples must be packaged in a new or disinfected suitable primary container which has been disinfected on the outside and then placed in a suitable secondary container with enough absorbent material, and the outside disinfected.

Urinalysis

From both a chemical and cytological standpoint marked changes can occur in urine with standing and shipping. For the best possible results, urine should be examined immediately after collection.

If shipping urine for analysis, it is important to:

a) Collect fresh urine (5 – 10 ml) in a sterile **leak-proof container.**

b) **State method of collection** – catheterization, cystocentesis or freeflow.

c) A brief history should always accompany your specimen – e.g. if you suspect malignant cells or have seen an unidentifiable crystal or cell, antibiotic use etc.

d) Urines should be prepared for cytologic evaluation by splitting the sample into a red-topped tube and a conical tube. Store the red-topped tube in the refrigerator. Centrifuge the remaining urine in a conical tube. Dispose of the supernatant and prepare smears from the sediment. Allow the smears to air-dry and maintain them at room temperature. Submit both the intact urine and the air-dried, unstained smears for cytologic evaluation.

e) If bacterial culture is required, submit a urine swab also.

Urine Protein: Urine Creatinine (UP/UC)
Urine Cortisol: Urine Creatinine (UC/UC)

UP/UC and UC/UC ratios can be measured on 1 ml of urine collected via cystocentesis or clean free catch. After collection store the sample in the fridge or the freezer for periods longer than 2 days.

Virology

Regional Diagnostic Virology Services (RDVS) at AVC Diagnostic Services

General Information

The Regional Diagnostic Virology Services (RDVS) is a major component of AVC Diagnostic Services at the Atlantic Veterinary College. It was established and opened for service in 1988, two years after the Atlantic Veterinary College started. Prior to this, the diagnostic virology services for the Atlantic Region were being done by Agriculture and AgriFood Canada in Sackville, New Brunswick using traditional virology test, e.g. virus isolation. It has to be emphasized that the RDVS at AVC was developed and established through the financial support of the veterinary laboratories and agriculture department of the four Atlantic Provinces (New Brunswick, Newfoundland, Nova Scotia and Prince Edward Island) through grant and a fee for service arrangement until 2013 (25 years). The provincial laboratories remain strong clients of RDVS at AVC utilizing another agreement since 2013.

The RDVS initially offered diagnostic virology services for food animals (bovine, porcine, avian, caprine, ovine), equine and furbearing animals in the region as a part of the regional diagnostic agreement. It has since expanded to include companion animals and wildlife for practitioners and other clients in the region.

The RDVS offers a wide range of diagnostic virology tests for mammalian species from traditional methods such as virus isolation using cell culture to the established rapid tests such as negative staining electron microscopy, enzyme linked immunosorbent assay (ELISA), fluorescent antibody test (FAT) and molecular diagnostic test such as polymerase chain reaction (PCR), reverse-transcriptase PCR (RT-PCR), nested PCR (nPCR) and real time PCR (rt PCR). The request for traditional tests such as virus isolation (which has a long turnaround time, laborious and expensive) has gradually decreased in numbers and has been replaced by molecular diagnostic tests, FAT, and ELISA. However, virus isolation for mammalian species is still available for special cases (undiagnosed disease outbreaks, legal issues, etc.) for clients (pathologists and practitioners) in the region.

In 1993, the RDVS developed and established virology services for aquatic species initially in the Atlantic Region and has since expanded nationally and globally. The most popular test that was offered for this species was virus isolation using one to several cell lines (up to 5-7). The molecular diagnostic tests such as PCR, RT-PCR, nPCR and rt-PCR for specific virus(es) were also developed and established.

The RDVS is accredited by Canadian Food Inspection Agency (CFIA) for two tests-Equine Infectious Anemia and Enzootic Bovine Leukosis. In 2014, the RDVS laboratory achieved ISO/IEC 17025:2015 certification by the Standard Council of Canada.

The RDVS is a member of the Canadian Animal Health Surveillance Network (CAHSN) and actively performs tests mandated by CFIA and CAHSN.

Requirements for Sample Submission

All samples for virology must be submitted through a licensed veterinarian or provincial laboratories.
The submitter becomes responsible for test(s) requested and fees for each submission.
Samples received for testing become the property of AVC Diagnostic Services.
The RDVS Laboratory does not accept sample(s) submitted by the owner.

General Virology Submission Forms

There were three virology submission forms which can be downloaded and printed from the computer. They are as follows:

1) Food and Fur Bearing Animal Virology Submission Form
   http://files.upei.ca/avc/diagnosticservices/QA-F-101B-01_MC.pdf

2) Multiple Animal Virology Submission Form (for multiple tests and multiple samples)
   http://files.upei.ca/avc/diagnosticservices/QA-F-101D-01_MC.pdf

3) Companion Animal Virology Submission Form.
   http://files.upei.ca/avc/diagnosticservices/QA-F-101A-01_MC.pdf

Please fill out and complete the appropriate form. Written history should briefly be filled out in appropriate place in the form. Please use back of the form, if needed.

CFIA Regulatory Forms

The RDVS Laboratory is accredited to perform Equine Infectious Anemia (EIA) ELISA and Enzootic Bovine Leukosis (EBL) ELISA by CFIA. Only accredited veterinarians can collect and send samples for these tests.

The following official forms are required:

1. EIA ELISA (CFIA Form No. 3937)
2. BLV ELISA (CFIA Form No. 3841)

The accredited veterinarians can request these forms from their respective CFIA District Offices.

Please complete all necessary boxes in the form. Please ensure that the information including owner's information, animal location, reason for test and description of the horse is completed. Also, please ensure that the names of the accredited veterinarian, their signature, as well as the sampling date are included.

Specimen Selection and Collection

Clinical virology samples should be collected as soon as possible following the onset of illness. Please refer to the AVC Diagnostic Services Website where you will find a link to the "Detailed Guide in the Selection and Collection of Virology Specimen" http://files.upei.ca/avc/diagnosticservices/AVCDS_DetailedGuideSelectionCollectionVirologySpecimens.pdf . The table shows the available tests for specific virus(es), the recommended specimens for each test and information on the collection procedure and transport devices that can be used.

All specimens must be properly identified and labelled. The samples must be identified with Animal ID or laboratory or hospital reference number which is also present on the sample submission form.

Virus Isolation*:*

VI samples must be labelled with the origin of the specimens (e.g. nasal swabs, name of tissue or name of pooled tissues, etc.). The specimens for virus isolation should be collected aseptically and should be refrigerated promptly. Materials can be collected (using special swabs and special viral transport medium provided by the RDVS) from nasal sinuses, conjunctival surfaces or tissues. If samples cannot be sent within 24 hours (e.g. weekend), freeze at -70^{0}C to -80^{0}C. If this type of freezer is not available, store at refrigeration temperature (4^{0}C). Do not freeze specimens for virus isolation at -20^{0}C or regular freezer temperature.

Negative Staining Electron Microscopy*:*

The tracheal or bronchoalveolar washings should be placed in leak-proof container. For EM, 5-10 grams or 5-10 ml of feces or intestinal contents is required. If possible, feces should be collected directly from the animal. The intestine containing fluid materials should be tied on both ends before placing in a leak-proof container.

Molecular Diagnostic Test*:*

For molecular diagnostic tests, please use special swab and special viral transport medium (provided by the RDVS) for feces, intestinal content, nasal sinuses, conjunctival and laryngopharyngeal specimens. When submitting tissues, please collect as aseptically as possible and place it in a plastic bag before putting it in a leak-proof container.

Serology:

A minimum of 1.0 ml of serum is needed for a single test. For more than one test, a minimum of 2.0 ml is needed. Collect whole blood in red-top vacutainers. Please note that 10.0 ml of blood can generate 3-5 ml serum. Allow the samples to clot at room temperature. Release the clot using a sterile applicator stick, spin down and transfer the serum supernatant to a sterile tube. If a centrifuge is not available, remove the clot and set aside the serum.

For serodiagnosis, paired serum samples are required. The first sample is collected at the onset of illness and the convalescent is collected 2-3 weeks later. Acute sample(s) should be stored at -20C while waiting for the convalescent samples.

There are situations where the submitting of a single samples is necessary. Please specify the reason(s) in your submission form, e.g. sale, animal has died, determination of vaccination titer, etc.

Paired serum samples must be labelled as acute (A) or convalescent (C).

Fluorescent Antibody Test*:*

The specimens should be refrigerated or frozen very soon after collection. Place these in sterile plastic bags or leak-proof containers. The name of the organ(s) must be marked in the container. Around 3 to 5 cm tissue(s) should be collected. For smaller animals and birds, include the whole organs,

Special Virology Swabs and Viral Transport Medium

These special supplies can be requested from RDVS Laboratory. The cost is $3.00 per unit. If requested, the RDVS can send these to appropriate clinic or laboratory using courier collect.

Packing and Shipment of Specimens for Virology Testing

Leak-proof primary containers with feces (reinforced with tape, if needed), leak-proof primary containers with tissues, tubes containing viral transport medium and tube(s) with blood or serum should be placed in a suitable secondary container such as sealed plastic bags after disinfecting the outside of the primary container. Multiple tissues can be placed in the same bag, as long as they are from the same animal and there is sufficient absorbent material to absorb in case of leaks. Disinfect the outside of the secondary leak-proof container before final packaging.

The specimen(s) should be placed in a refrigerated container (Styrofoam box) with ice packs and should be surrounded with sufficient absorbent material to absorb in case of leaks.

Reference Intervals

Biochemistry – Reference Intervals

	Units	Canine	Feline	Bovine	Equine	Porcine	Ovine
Sodium	mmol/L	144-151	149-156	135-151	135-148	140-150	143-151
Potassium	mmol/L	3.9-5.3	3.3-5.2	3.9-5.9	3.0-5.0	4.7-7.1	4.6-7.0
Chloride	mmol/L	105-117	112-122	96-110	98-110	100-105	102-116
Calcium	mmol/L	2.02-2.91	2.28-2.85	2.11-2.75	2.80-3.44	1.80-2.90	2.30-2.86
Phosphorus	mmol/L	0.84-1.83	0.94-1.95	1.08-2.76	1.00-1.80	1.30-3.55	0.82-2.66
Magnesium	mmol/L	0.70-1.16	0.74-1.12	0.80-1.32	0.74-1.02	0.78-1.60	0.90-1.26
Urea	mmol/L	2.8-9.8	6.4-11.8	3.0-7.5	3.5-7.0	3.0-8.5	2.0-10.0
Creatinine	µmol/L	54-122	67-157	40-148	78-143	90-240	69-105
Glucose	mmol/L	4.0-6.3	3.2-6.7	1.8-3.8	3.6-5.6	3.6-5.3	1.2-3.6
Cholesterol	mmol/L	3.87-8.39	1.80-5.83	2.0-3.4	1.20-4.60	-	-
Total Bilirubin	µmol/L	0-4	0-4	0-30	4-102	0-4	0-5
Direct Bilirubin	µmol/L	0-4	0-4	0-3	0-7	0-4	0-5
ALP	IU/L	18-113	14-49	0- 121	95-233	10-400	66-158
CK	IU/L	44-249	58-489	0- 350	0- 500	0- 500	0- 350
AST	IU/L	18-55	11-44	46-118	197-429	25-57	48-128
ALT	IU/L	13-69	34-90	-	10-23	34-58	-
GGT	IU/L	0-7	0-6	0-31	0- 25	0- 25	0- 79
Total Protein	g/L	56-71	65-84	66-78	60-77	34-60	61-81
Albumin	g/L	30-36	25-37	23-43	25-36	18-22	27-39
Globulin	g/L	25-38	33-54				
A/G Ratio	-	0.7-1.5	0.50-0.90	0.66-1.30	0.60-1.50	0.60-1.50	0.54-1.22
Amylase	IU/L	324-1005	639-1364	-	-	-	-
Lipase	IU/L	78-583	16-139	-	-	-	-
SDH	IU/L	0-10	0-18	4-25	1-15	1-10	5-28

Blood Gases:

pH	-	7.31-7.42	7.24-7.40	7.35-7.50	7.20-7.55	7.30-7.50	7.32-7.50
pCO_2 venous	mmHg	34-45	25-35	34-45	38-46	35-45	35-45
pO_2 arterial	mmHg	80-110	80-110	80-110	80-110	80-110	80-110
HCO_3	mmol/L	24±4	24±4	24±4	24±4	24±4	24±4
Base Excess	-	0±3	0±3	0±3	0±3	0±3	0±3

Biochemistry and Coagulation Reference Intervals

	Units	Canine	Feline	Bovine	Equine	Porcine	Ovine
Protein Electrophoresis (agarose gel)							
Albumin	g/L	24-40	26-44	24-36	29-38	19-24	28-34
Alpha 1	g/L	2-4	6.5-9	-	7-13	9-12	2-6
Alpha 2	g/L	4-9	6.5-9	7-12	7-13	-	2-6
Beta	g/L	13-17	10-15	6-12	4-12	8-11	3-7
Gamma	g/L	4-8	12-27	16-32	9-15	3-7	7-13
Iron	µmol/L	14.5-22	14.5-38	14.5-29	14.5-25	14.5-36	29-40
TIBC	µmol/L	All species 44.8-62.7					
Bile Acids							
(pre)	µmol/L	0- 7	0- 7				
(post)	µmol/L	0- 15	0- 15				
Osmolarity	mOsm/kg	285-315	285-315	285-315	285-315	285-315	285-315
Fibrinogen	g/L	-	-	< 6.0	< 5.0	-	-
PT Range	seconds	6.1-8.0	8.4-14.7	N/A	N/A	N/A	N/A
PTT Range	seconds	8.8-14.8	10.8-16.9	N/A	N/A	N/A	N/A

Hematology – Reference Intervals

	Units	Canine	Feline	Bovine	Equine	Porcine	Ovine
HgB	G/L	135-198	89-156	80-150	110-190	100-160	80-160
Hct	L/L	0.40-0.56	0.28-0.44	0.24-0.46	0.32-0.52	0.32-0.50	0.24-0.50
RBC	X 10^{12}/L	5.7-8.4	6.4-11.5	5.0-10.0	6.5-12.5	5.0-8.0	8.0-16.0
MCV	FL	64-75	35-52	40-60	34-58	50-68	23-48
MCH	Pg	22-25	12-17	11-17	11-19	16.6-22.0	9.0-12.0
MCHC	G/L	334-357	310-381	300-360	310-370	300-340	310-380
			Occasional				
Reticulocytes	%	0-1 %	0-1.2%	0 - 0.5%	0%	0-1%	0%
	X 10^9/L	0-85	0-85				
WBC	X 10^9/L	5.4-14.3	4.7-17.0	4.0-12.0	5.5-12.5	11.0-22.0	4.0-12.0
Segmented Neutrophils	X 10^9/L	2.8-10.1	2.2-9.5	0.6-4.0	2.7-6.7	3.1-10.4	0.7-??
Bands Neutrophils	X 10^9/L	0.0-0.3	0.0-0.1	0.0-0.12	0.0-0.1	0.0-0.88	Rare
Eosinophils	X 10^9/L	0.0-1.5	0.0-1.5	0.0-2.4	0.0-0.93	0.06-2.42	0.0-1.0
Basophils	X 10^9/L	0.0-0.1	0.0-0.2	0.0-0.2	0.0-0.17	0.0-0.44	0.0-0.3
Lymphocytes	X 10^9/L	0.9-4.6	0.5-7.5	2.5-7.5	1.5-5.5	4.29-13.6	2.0-9.0
Monocytes	X 10^9/L	0.1-1.4	0.0-0.6	0.03-0.84	0.0-0.8	0.22-2.2	0.0-0.75
Platelets	X 10^9/L	218-470	306-517	100-800	100-600	310-510	250-750

Endocrine - Reference Intervals

Test	Units	Canine	Feline	Bovine	Equine
Cortisol					
Baseline	nmol/L	14-180	13-110	10-140	70-180
Post-ACTH	nmol/L	231-571 (2hrs post)	163-409-1hr 115-507-2hr		2-3x Baseline at 8hrs
Post Dexa	nmol/L	<35 (8hrs Post Dex at 0.01mg/kg IV)	<35 (8hrs Post Dex at 0.1mg/kg IV)		<30 (19 hrs Post Dex at 0.04 mg/kg IM)
Estrone Sulfate					
Pregnant	ng/ml				> 20 (> 90 days)
Non-Pregnant/ True Gelding	ng/ml				< 5 (> 90 days)
Stallion/ Cryptorchid	ng/ml				> 10
Progesterone					
Diestrus (luteal)	ng/ml	< 1	5-15	> 3.0	7-20
LH Peak	ng/ml	1.0 - 3.5	-	-	-
Estrus (follicular)	ng/ml	3.5 – 12.0	< 1.0	< 1.0	< 1.0
Pregnant	ng/ml	> 12	5-15	> 3.0	7-20
T3 (triiodothyronine)					
Baseline	nmol/L	0.6-2.4	0.3-1.7	0.8-1.9	0.9-3.1
T4 (thyroxine)					
Baseline	nmol/L	15-52	8-40	-	13-35
Therapeutic Range		32-58	8-25		
Testosterone	ng/ml	1.0-7.0 male <0.4 bitch or neutered male	1.0-6.0 (intact male)		1.0-4.0 Stallion, breeding season < 1.0 Stallion, non-Breeding Season < 0.3 Mares, Geldings
TSH	ng/ml	< 0.50			
Urine Cortisol/ Creatinine Ratio		0.5-17.7 x10e^{-6}	0.5-17.7 x10e^{-6}		

Toxicology – Animal Mineral Levels

BOVINE
Values in ppm Wet Weight

ARSENIC

	Liver	Kidney	Heparinized Blood
Normal	0.004-0.40	0.018-0.40	0.03-0.05
High	1.0-50	1.5-5.0	
Toxic - acute	2.0-15	3.5-38	0.17-6.7
- chronic	7.0-100	5.0-53	

CADMIUM

	Liver	Kidney
Normal	0.02-1.0	0.05-1.5
High	1.4-9.0	5.0-36
Toxic - chronic	50-160	100-250
- acute	> 50	> 200

COPPER

	Liver	Kidney	Serum (No Selenium Supplement)	Serum (Selenium Supplement)
Deficient	0.5-10.0	1.0-5.0	0.02-1.00	0.10-1.20
Marginal	5.0-25.0	3.0-5.5	0.55-1.20	0.30-1.20
Adequate	25.0-100	4.0-6.0	0.8-1.5	0.32-1.20
High	200-550	5.0-7.0	2.5-4.0	0.50-2.50
Toxic	250-800	10-122	4.0-11.0	0.60-10.0

	Liver - Fetal
Deficient	2.0-20
Marginal	20-30
Adequate	30-150
High	200

Toxicology – Animal Mineral Levels continued

BOVINE
Values in ppm Wet Weight

LEAD

	Liver	Kidney	Heparinized Blood	Hair *	Bone *
Normal	0.1-1.0	0.2-2.0	0.01-0.2	0.5-5	1.0-7.0
High	2-10	3-20	0.30-0.40	3.5-90	30-75
Toxic	5-300	5-700	0.35-32	10-100	30-100

* ppm dry weight

SELENIUM

	Liver	Kidney	Serum or Plasma (No Supplement)	Serum or Plasma (Se Supplement)
Deficient	0.02-0.17	0.18-0.40	0.002-0.025	
Marginal	0.12-0.25	0.40-1.00	0.030-0.060	0.020-0.060
Adequate	0.25-0.50	1.00-1.50	0.080-0.300	0.025-0.150
High	0.75-1.25	2.00-2.50	2.5-3.5	0.800-3.500
Toxic - chronic	1.25-7.00	2.50-5.00	3.5-4.1	
- acute	7.00-47.0	1.00-8.00		

ZINC

	Liver	Kidney	Serum
Deficient	<20-40	16-20	0.20-0.40
Marginal	25-40	16-20	0.50-0.60
Adequate	25-100	18-25	0.80-1.40
High	300-500	50-140	2.00-5.00
Toxic	120-500	130-480	3.00-15.0

Toxicology – Animal Mineral Levels continued

CANINE
Values in ppm Wet Weight

ARSENIC

	Liver	Kidney
Normal	< 0.2	< 0.2
Toxic	> 10.0	> 10.2

CADMIUM

	Liver	Kidney
Normal	0.037	0.12-0.18
High	1.0-7.0	4.0-17.0
Toxic		> 200

COPPER

	Liver	Kidney	Serum
Deficient	< 20	< 5.0	< 0.20
Normal	30.0-100	5.0-15.0	0.20-0.80
Toxic	400-3000	> 20	

LEAD

	Liver	Kidney	Heparinized Blood	Hair *
Normal	0.1-3.5	0.1-2.5	0.01-0.10	0-88
High	3.6-5.0	5.0-10.0	0.30-0.80	0-87
Toxic	50-200	10-50	0.60-7.4	> 88
				* ppm dry weight

ZINC

	Liver	Kidney	Serum
Deficient	< 15	< 8	0.20-2.00
Adequate	30-70	16-30	0.70-2.00
Toxic	369	295	10.0-32.0

Toxicology – Animal Mineral Levels continued

EQUINE
Values in ppm Wet Weight

ARSENIC

	Liver	Kidney
Normal	< 0.4	< 0.4
High	1.0-5.0	
Toxic	7.0-15	> 10.0

CADMIUM

	Liver	Kidney
Normal	0.01-5.0	0.05-10.0
High	15-30	4.2-100
Toxic	> 80	75-170

COPPER

	Liver	Kidney	Serum
Deficient	< 3.5	< 4.0	0.60-0.80
Adequate	4.0-7.5	7.3-9.3	0.85-2.00
High	1000-1500	30-50	

LEAD

	Liver	Kidney	Heparinized Blood	Brain	Bone*
Normal	0.08-1.4	0.03-1.30	0.04-0.25	0.5	3.0-4.0
High	3.0-5.0	3.0-5.0	0.30-0.60	1.0-5.0	8.9-40
Toxic - chronic	4.0-50	5.0-140	0.33-140	3.0-30	40-350
- acute	10-500	20-200	0.60-2.5		

* ppm dry weight

Toxicology – Animal Mineral Levels continued

EQUINE
Values in ppm Wet Weight

SELENIUM

	Liver	**Kidney**	**Serum**
Deficient	0.14-0.20	0.14-1.10	0.008-0.053
Marginal	0.20-0.30	0.35	0.053-0.120
Adequate	0.30-1.00	0.70-2.00	0.140-0.250
High			0.350-1.000
Toxic - chronic	10.0		1.400-2.000
- acute	> 2.5	> 2.0	

ZINC

	Liver	**Kidney**	**Serum**
Deficient			< 0.50
Adequate	40-125	20-50	0.60-1.70
High	160-500	65-150	1.60-3.50
Toxic	1300-1900	295-580	1.00-3.50

Toxicology – Animal Mineral Levels continued

PORCINE
Values in ppm Wet Weight

ARSENIC

	Liver	Kidney	Heparinized Blood
Normal	0.003-0.20	0.03-0.10	0.01

CADMIUM

	Liver	Kidney
Normal	0.04-5.0	0.15-0.99
High	3.0-30.0	2.0-50
Toxic	> 13	> 270

COPPER

	Liver	Kidney	Serum
Deficient	0.30-1.02	2.0-4.0	0.09-0.40
Marginal	4.0-7.0	4.0-7.0	0.40-1.50
Adequate	5.0-25.0	7.0-10.0	1.30-3.00
High	15-200	12.0-25.0	1.70-3.00
Toxic	150-15000	300-1200	4.5-77

SELENIUM

	Liver	Kidney	Serum
Deficient	0.03-0.10	0.40-0.77	0.005-0.060
Marginal	0.12-0.25	0.77-1.10	0.060-0.100
Adequate	0.40-1.20	1.50-2.90	0.140-0.300
High	1.50-12.0	3.00-18.0	0.400-0.800
Toxic	3.00-120	3.80-90.0	0.500-2.360

ZINC

	Liver	Kidney	Serum
Deficient	9.6-25		0.18-0.25
Marginal	25-35		0.40-0.80
Adequate	40-90	15-30	0.70-1.50
High	> 200		
Toxic	500-3100	190-367	1.40-2.80

Toxicology – Animal Mineral Levels continued

OVINE
Values in ppm Wet Weight

ARSENIC

	Liver	Kidney	Heparinized Blood
Normal	0.01-0.20	0.01-0.30	0.01-0.08
High	4.0-8.0	1.0-6.0	0.04-0.50
Toxic	10.0-50.0	10.0-40.0	5.0-14.5

CADMIUM

	Liver	Kidney
Normal	0.02-1.40	0.06-0.48
High	2.0-20.0	4.0-12.0
Toxic	50-600	50-400

COPPER

	Liver	Kidney	Serum
Deficient	0.50-4.0	3.0-4.0	0.10-1.00
Marginal	5.0-20	4.0-5.0	0.40-1.00
Adequate	25-100	4.0-5.5	0.70-2.0
High	100-500	4.0-10	1.00-5.00
Toxic	250-1000	18-260	3.3-20

LEAD

	Liver	Kidney	Heparinized Blood	Brain	Bone (Tibia)*
Normal	0.03-0.80	0.10-0.80	0.02-0.25	0.1-0.5	1.0-3.0
High	5-25	5-100	0.70-0.90	1.2-2.0	10-40
Toxic	10-100	5-200	1.0-5.0		> 70

* ppm dry weight

Toxicology – Animal Mineral Levels continued

OVINE
Values in ppm Wet Weight

SELENIUM

	Liver	**Kidney**	**Serum**
Deficient	0.01-0.10	0.046-0.600	0.006-0.030
Marginal	0.15-0.25	0.70-1.10	0.030-0.060
Adequate	0.25-1.50	0.90-3.00	0.080-0.500
High	2.00-10.0	4.00-6.00	
Toxic	15.0-30.0	6.00-15.00	> 3.0
Toxic – injected (IM)	3.6-18.2	3.39-8.70	

ZINC

	Liver	**Kidney**	**Serum**
Deficient	20-30	15-30	0.22-0.45
Marginal			0.40-0.80
Adequate	30-75	20-40	0.80-1.20
High	100-400	50-1000	4.0-5.0
Toxic	> 400	240-1600	30.0-50.0

NOTE:

a. Tissue mineral levels are quoted from Mineral Levels in Animal Health, Diagnostic Data by Robert Puls, 3rd printing, 1990.

b. Conversion of units between wet and dry weight:

Units in dry weight: $\frac{\%\ \text{dry matter}}{100}$ = units in wet weight

Units in wet weight: $\frac{100}{\%\ \text{dry matter}}$ = units in dry weight