













Diagnostic Services Laboratory, Atlantic Veterinary College

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Diagnostic Update



By Lisa Penney, Senior Chemist

The Toxicology and Analytical Services Laboratory (TASL), located within the Atlantic Veterinary College (AVC), provides a wide range of diagnostic tests. State-of-the-art instrumentation combined with knowledgeable and experienced staff members permit the extraction and analysis of an extensive array of compounds. The TASL was formed over twenty years ago as a centre of excellence in toxicology within the AVC. Since that time, the laboratory has expanded to include clients from other parts of Canada and the world.

The TASL works closely with veterinarians and other scientists to determine the cause of sickness, injury or death in a wide variety of animal species. Detecting abnormal mineral levels in tissues and serum, vitamin deficiencies and the presence of toxic agents such as lead and strychnine are among the tests conducted. The TASL also determines residual amounts of contaminants, such as pesticides and herbicides, in water and animal tissues.

Another area into which TASL has expanded is the analysis of products for the aquaculture industry. In recent years, aquaculture has emerged as an increasingly important supplier of fish, shellfish and aquatic plants in Canada

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and throughout the world. The rapid growth in this sector has been accompanied by an increase in the use of antibiotics and other compounds in fish farms, leading to concerns for consumer food safety. The TASL has developed methods for the extraction and quantitation of the active ingredients in medicated fish feeds, such as oxytetracycline, emamectin benzoate and florfenicol. Additionally, the laboratory can determine residual amounts of these drugs in fish flesh and marine sediment.

Above and beyond routine testing, the TASL has the expertise and capacity to develop and validate high quality analytical methods to suit client needs.





Staff members have extensive experience in this area and will work closely with customers to ensure that new methods meet or exceed requirements and expectations. The TASL is equipped with a full range of analytical instruments, including liquid chromatography - mass spectrometry, atomic absorption spectrometry, gas chromatography - mass spectrometry and high performance liquid chromatography. The laboratory also houses a range of equipment used for sample preparation and extraction.



The Toxicology and Analytical Services Laboratory (TASL) team. Left to right: Heather Briand, Dr. Collins Kamunde, Darlene Mahar, Lisa Penney and Orysia Dawydiak

In recent years, it has become possible to detect extremely minute quantities of an enormous range of compounds in a seemingly infinite number of sample matrices. The ability to "see" such small amounts of a chemical in everyday materials assists many areas of science, including food safety, environmental toxicology and veterinary diagnosis. The TASL combines employee proficiency and dedication with modern equipment to provide accurate and reliable results in a prompt and friendly manner. Quality results, timely answers and personalized service are the foundation of the Toxicology and Analytical Services Laboratory.

Submission of Urine Samples for Bacteriological Culture

By Lorraine Lund, Veterinary Laboratory Technologist and Anne Muckle, Veterinary Clinical Bacteriologist

The interpretation of results from a urine culture can be a frustrating experience for clinicians at times. From the perspective of a diagnostic veterinary laboratory, it is essential that the person submitting uses proper sample collection, storage and transport procedures in order to ensure meaningful urine culture results. If at all possible, a cystocentesis sample should be collected as catheterized and free-flow samples are more likely to be contaminated.

Opinion on the stability of urine samples is varied. However, storage at refrigerator temperature as soon as possible after collection and shipment on ice packs is recommended. Since quantitative culture is needed to determine the significance of the bacteria isolated, it is important that the numbers of viable bacteria in the urine do not change significantly. Refrigerated urine submitted in sterile red topped (serum) tubes is satisfactory.

The usual delay between the collection and receiving of samples at the Atlantic Veterinary College Diagnostic Services from off-Island clinics is one or two days. For urine samples collected on Friday and not shipped until the following Monday, we recommend sending a swab of the urine placed in transport medium along with the urine sample. The swab should be refrigerated. We process and culture both the urine and swab samples for a single laboratory fee and results usually correlate closely. A quantitative urinary culture result of >1,000 colony forming units (CFU)/ml is significant in both dogs and cats. Our urine culture protocol uses 1 microlitre and 10 microlitre calibrated inoculation loops, enabling detection of very low numbers of bacteria.

It is understandable that clinicians may be concerned about negative urine culture results when they thought an animal had an obvious bacterial urinary tract infection. Ideally, the collection, transport and storage of the sample should not negatively affect the recovery of common urinary tract bacterial pathogens of dogs and cats. Inhibition of bacterial growth as a result of recent antibiotic treatment may be a factor in some cases, and other conditions presenting with urinary tract disease symptoms should also be considered.

Immunohistochemistry: We Have It - Do You Need It?

By Andrea Bourque, Veterinary Anatomic Pathologist

The Atlantic Veterinary College has recently purchased a new immunohistochemistry (IHC) autostainer. I know this may not be that exciting for everyone, but for pathologists working in Diagnostic Services, Christmas came early! This is a much anticipated piece of equipment which will allow us to perform tests in house that we have previously had to send out to referral laboratories. Once things are up and running, this will correlate to a quicker turnaround time and cheaper cost on the tests or markers we will be offering.

What is immunohistochemistry? Basically, IHC allows us to visualize an antigen of interest in tissue sections using specific antibody markers visible as a color change using light microscopy. These antigens may represent cell surface markers or receptors or proteins specific to certain cell types or different bacterial, viral or parasitic infectious agents.

Along with evaluating tissue architecture and cell morphology, pathologists use this technology routinely in veterinary and human medicine to assist with differentiating the cellular origin of different tumors. Many practitioners will have received biopsy reports with very general diagnoses like "poorly

differentiated sarcoma/carcinoma" or "poorly differentiated malignant tumor". These diagnoses are usually accompanied by statements such as "immunohistochemistry is required to make a more definitive diagnosis". Unfortunately, in some cases, there are limitations in using routine histology alone in how far pathologists can go and still make an accurate diagnosis. Mistakes in this regard could seriously affect the prognosis and treatment plan of some patients. An excellent



Immunohistochemistry (IHC) autostainer at Diagnostic Services, AVC

example of this are "round cell tumors". This is a catch-all, descriptive term which encompasses a group of tumors composed of round neoplastic cells (pathologists are not all that imaginative at times!). Neoplasms in this group vary from benign tumors, such as cutaneous histiocytomas and plasma cell tumors, to malignant neoplasms, such as lymphomas, histiocytic sarcomas, amelanotic melanomas and some mast cell tumors. Typical microscopic and clinical features affect how these differential diagnoses are ranked and make some tumors more likely than others.

However, differentiating between several of these tumors using histology alone is sometimes impossible and IHC is often the most effective tool to make a diagnosis.

Another common usage of IHC is in the diagnosis of infectious diseases in fixed tissues, especially in cases involving agents otherwise difficult or impossible to isolate. Examples of the most common agents we currently use IHC testing for include feline coronavirus, Leptospira sp., Mycoplasma bovis, Listeria monocytogenes, Bartonella sp., a variety of respiratory viruses and Rabies virus. We hope to offer many of these antibody markers in the future. We will be developing our IHC expertise this summer and hope to offer a panel of commonly used tumor markers by the fall of 2008. In the meantime, we will continue to use the valuable services of referral laboratories.

An Eye for an Eye: Ophthalmology Submissions for Histopathology

By Ines Walther, Veterinary Anatomic Pathology Resident and Andrea Bourque, Veterinary Anatomic Pathologist

In veterinary practice, the number of recognized ocular diseases is continually increasing, especially in dogs. The use of sophisticated ocular examination techniques such as ultrasound and magnetic resonance imaging, is allowing clinicians to detect subtle lesions. Responsible breeders of purebred dogs learning about inherited ocular disorders bring their concerns to veterinary practitioners. The purpose of this article is to provide clinicians with some practical information to optimize the information attainable from eyeball submissions (and perhaps make life a little less challenging for both clinicians and pathologists!).

Improper handling or preservation of any tissue can markedly impair the ability of the pathologist to provide an accurate diagnosis. This is especially true with ocular specimens. Artifactual changes which can mask important

subtle lesions, or worse, are identical to the lesions characteristic of some ocular disorders, are common with improper handling. Here are some helpful hints when submitting eyeballs:

- 1. A concise history with detailed results of ophthalmic examination, including a gross description and the location of any observed ocular lesion, must be provided. Lesions, especially when subtle, are frequently not obvious to the pathologist following fixation.
- 2. The age, breed, sex and any other pertinent clinical history should be included.
- 3. Globes should be removed quickly and gently and should include a portion of the optic nerve when possible.
- 4. For optimal fixation, the eyelids, extraocular muscles and orbital fat should be removed from the globe prior to prompt placement in fixative. The globe should be fixed intact and not opened or sliced.
- 5. Buffered 10% formalin is the most readily available and commonly used fixative. Formalin provides adequate fixation but penetration through the sclera can be prolonged. For optimal fixation of most feline and canine eyes, injecting $\sim 0.25 - 0.50$ ml of formalin into the vitreous using a 25 gauge needle through the sclera near the optic nerve just prior to immersion will provide good results. This will allow for rapid fixation of retinal tissues and help prevent retinal detachment, which is one of the most common artifactual changes seen histologically.
- 6. Place the globe in an appropriate volume of formalin. A 1:10 ratio of tissue: formalin is ideal.

Finally, if you ever have any questions regarding how to submit ocular tissue or any other specimens, please feel free to call and talk to one of our Diagnostic Services pathologists, who will be glad to assist you.

Laboratory News

What's New in Diagnostic Services

By Shelley Burton, Veterinary Clinical Pathologist and Linda Ruschkowski, Veterinary Laboratory Technologist

Diagnostic Services is always a busy and fun place to work. Here are some recent happenings:

- Diagnostic Services sponsored a coffee break at the Atlantic Provinces Veterinary Conference (APVC) in Halifax on April 26, 2008. The turnout was great and Drs. Anne Muckle, Andrea Bourque, Barbara Horney, Cora Gilroy and Shelley Burton enjoyed visiting with colleagues and discussing our laboratory.
- The clinical pathology laboratory has received positive responses to the rapid electronic provision of interpretative comments on hematology, biochemistry and urinalysis data. We are pleased that our visiting clinical pathologist, Dr. Jim Bilenduke, stimulated the change last summer.
- In response to feedback received from our last client survey, the anatomic pathologists have made changes to how biopsy results are reported. This has significantly decreased the turnaround time on this service.
- We have said fond goodbyes to some people. Dr. Les Gabor (Anatomic Pathologist) and his family returned to Australia. Mr. Jim Carlsen (Post-

mortem) left to join the veterinary college in Calgary. Ms. Mada Coles (Bacteriology), Mr. Bob Maloney (Parasitology), Ms. Virginia Hanna (Post-mortem) and Ms. Dianne O'Connell (Histology) all retired. We have welcomed Drs. Shannon Martinson and Maria Forzan (Anatomic Pathologists) and Dr. Carolyn Legge (Anatomic Pathology resident).

 We are still in the midst of busy college renovations, entailing adjustments and cramped work spaces. Diagnostic Services thanks all our clients for their patience and good cheer until we are back in our improved space.

Staff Focus

Dr. Andrea Bourque

By Cora Gilroy, Veterinary Clinical Pathologist

Dr. Andrea Bourque is an integral part of the Atlantic Veterinary College (AVC) Diagnostic Services. Originally from Bathurst, New Brunswick, she completed a Bachelor of



Science from the Nova Scotia Agricultural College in 1992 and obtained her Doctor of Veterinary Medicine from the AVC in 1996. After time spent in private practice in the United States, Andrea returned to the AVC in 1999 for a 3 year residency in anatomic pathology. Andrea passed the certifying examination of the American College of Veterinary Pathologists, becoming a Diplomate in September of 2004. During both of her times of study at the AVC, Andrea moonlighted as a bartender, which added many great stories to her repertoire! Andrea has been a diagnostic anatomic pathologist with Diagnostic Services since the completion of her residency training and has also recently completed a Master of Veterinary Science from the AVC. Andrea's diverse responsibilities with Diagnostic Services entail all aspects of gross and microscopic anatomic pathology, administrative duties, training of pathology residents and instruction of senior veterinary students. Areas of special interest to Andrea include ocular pathology and immunohistochemistry; the latter is an exciting diagnostic tool new to AVC Diagnostic Services. Andrea is an outdoor enthusiast who enjoys camping, hiking and walking with her significant other, Jason, and their dogs, Sophie and Lexy.

Correction

Our apologies to Dianne O'Connell, whose name was incorrectly reported in our February 2008 issue on page 6 in the Spotlight on Histology. It should read Dianne O'Connell, not O'Connor.

Reader Feedback: The **Diagnostic Update** group invites comments or suggestions for future topics in the newsletter. Please submit your comments to *Dr. Cora Gilroy* (cgilroy@upei.ca), Diagnostic Services, Atlantic Veterinary College, UPEI, Charlottetown, PE, C1A 4P3 and they will be forwarded appropriately.