We are very pleased to present the second issue of the second volume of our re-established newsletter. The goal of these newsletters is to communicate with you about the status of the laboratory and our continuous effort to provide the highest level of service to veterinarians, animals, farmers, and pet owners of the state of Georgia.

As you all know, economic times are tough, and the diagnostic laboratories are witnessing the same budget cuts and business income shortfalls that now plague multiple sectors nationwide. Additional budget cuts are expected, and we do not know how much this crisis will affect our services. We continue to reduce costs without cutting services by putting technology to work and training personnel to operate more efficiently. However, due to rising operational costs, reduced state funding, mandated unpaid furlough days for all personnel, and potential additional budget reductions, the diagnostic laboratories are facing a difficult period.

Our modest and selective fee adjustments effected on July 1, 2009, will offset only a small portion of the budget shortfalls. Currently, there are no plans for additional fee increases, but should additional significant budget cuts occur, fee increases or a reduction in services may be considered. Due to the state-mandated unpaid furlough days and Christmas/New Year holidays, both laboratories will have skeleton crews working on the following 2009–2010 dates: October 30, November 25, December 24–January 4, March 8, and April 30. Though we hope to minimize the impact of these furloughs on our services, turnaround time may suffer as a result. We ask for your patience and understanding.

The quality of the results we deliver to you is critically important. We work hard to improve our services and reporting of results. Effective July 1, 2009, the Tifton Laboratory migrated to the UVIS laboratory information system that has been used by the Athens Laboratory since 2001. Some problems were encountered during the migration, but most issues have now been rectified and the few remaining issues will be corrected as soon as possible. We wish to thank you for your patience and continued support during this transition period.

Internet retrieval of case results and account information via the Diagnostic Laboratories’ Web site (www.vet.uga.edu/dlab) is now available for clients of both laboratories. In the face of serious budget shortfalls, these improvements are only possible because of the dedication and hard work of our faculty and staff. We remain confident that with your support and our ongoing efforts we will emerge from these trying economic times stronger and better prepared to continue serving your needs for accurate and affordable diagnostic services.

MEET THE FACULTY

Dr. Paula Krimer
Dr. Krimer received her DVM degree in 1990 from the University of Guelph, Canada. After two years of small animal practice in Toronto, she returned to the University of Guelph to receive her doctorate in veterinary science in clinical pathology in 2001. Her research for her DVSsc was on the diagnostic utility of corticosteroid-induced alkaline phosphatase in canine hyperadrenocorticism, which provided a strong grounding in epidemiology, reference intervals and study design.

After finishing her DVSsc, Krimer came to the UGA CVM pathology department for a one-year clinical instructorship. She was board certified by the American College of Veterinary Pathologists in 2002, and then worked for Antech Diagnostics for three years in New York City and Charlotte, N.C.

In 2007, she re-joined the UGA family as an assistant professor at the AVDL. She greatly enjoys teaching and has spearheaded valuable projects that improve laboratory administrative functioning and efficiency. Krimer serves as assistant editor for this newsletter, created computer programs for case allocation among pathologists, and initiated a spreadsheet for responding to frequently asked questions. She has also been very active in the American Society for Veterinary Clinical Pathology, serving as former chair of the ASVCP membership committee and as a current member of the development committee. Her research and diagnostic areas of interest include coagulation, hepatic disease, epidemiology, and Lyme’s disease.

Dr. Eloise Styer
Dr. Styer is a Public Service Associate at the TVDIL who developed and directs the laboratory’s electron microscopy section. She received her BS in biological sciences from Cornell University in 1967 and her MS and PhD in 1975 and 1978, respectively, in plant pathology/virology at the University of Maryland.

She joined the TVDIL in 1980 shortly after it inherited a 1960’s-era electron microscope. With that microscope, an ultramicrotome, and Styer’s expertise, the lab was then able to offer rapid diagnosis of viral infections through negative stain (direct) transmission electron microscopy (TEM).

Styer developed the well-equipped, full-service TEM laboratory that is now routinely used to identify a wide range of viruses in a variety of clinical and research samples. Styer was co- and principal investigator in several studies on proliferative gill disease (PGD) of channel catfish that identified the agent of PGD and earned her the 1994 Tifton Sigma Xi Association’s Outstanding Creative Research Award. Her presentation on the creative use of TEM approaches that resulted in the detection and characterization of new viruses of insects won an award from the Microscopy Association of America.

Styer utilizes her botanical expertise to identify potentially toxic plants, algae and mushrooms submitted by practitioners or collected from the intestinal tract at necropsy. She also represents the TVDIL on UGA’s Institutional Animal Care and Use Committee.

TABLE OF CONTENTS
Note from the Directors 1
Personnel Highlights 2
At Your Service…on Saturdays! 3
What’s New? 4-7
Science-at-a-Glance features 4-7
Contact Us 8
Meet the Faculty 8
PERSONNEL HIGHLIGHTS

Athens Veterinary Diagnostic Laboratory

1. Dr. Uriel Blas-Machado was promoted to associate professor with tenure effective July 1, 2009.

2. Dr. Cathy Brown won the 2008 Outstanding Laboratory Service award, which is given to an individual who has provided excellence in laboratory support for field or hospital investigations.

3. Dr. Doris Miller won the 2009 Charles Dobbs award for Excellence in Service, which recognizes outstanding service to the people of the state of Georgia and the surrounding region.

4. Mr. Allen Bryant was elected to the Executive Board of the International Journal of Veterinary Laboratory Diagnosticians, the professional body that accredits veterinary laboratories, including the Athens and Tifton labs.

5. Dr. Susan Sanchez was promoted to full professor effective July 1, 2009.

6. The following staff members or groups successfully completed federally-administered proficiency tests:
   - Paula Bartlett, Sarah Bates, and Ingrid Fernandez: Avian influenza and Newcastle disease PCR
   - Terry Bennett and Pam Currin: Johne’s serology
   - Jennifer McClain: BSE (bovine spongiform encephalopathy or mad cow disease) ELISA
   - Guiniz Shaikh: individual Coggins certification test
   - Serology staff: Pseudorabies latex agglutination
     - Pseudorabies serum neutralization, Bluetongue, Bovine herpesvirus type 1, Equine viral arteritis serology
     - Equine viral arteritis serology
   - Dallas Ingram: Equine viral arteritis serology
   - Julie Johnson: Johne’s culture
   - Julie Musgrove: Johne’s serology (ELISA)

Tifton Veterinary Diagnostic Investigational Laboratory

1. Dr. Murray Hines II was appointed permanent director of the Tifton Lab. Dr. Hines II was elected to the Executive Board of the International Association of Paracoccidiosis (Johne’s disease) effective August 14, 2009.

2. Dr. Marcia Ilha was appointed as an assistant professor of anatomic veterinary pathology at the TVDIL effective May 1, 2009. Dr. Ilha obtained her DVM degree in 1999 from the Federal University of Santa Maria (UFSM) in Brazil, and completed her MSc at UFSM, Brazil, in 2001. She earned a graduate diploma in veterinary pathology from the University of Guelph, Canada. Dr. Ilha became a board-certified pathologist with the American College of Veterinary Pathologists in September 2009. Her interests are diagnostic pathology, applied research in diagnostic pathology, and pathology of wildlife species.

3. Mr. Allen Bryant joined our staff in custodial services on May 1, 2009. Mr. Bryant had been an employee of Coachmen Industries for over 20 years and replaces Gerald Brandrick, who retired in January.

4. Ms. Debi Batton has returned part-time as our afternoon receptionist. Ms. Batton is a previous employee of the TVDIL, but has for the last several years has been migrating with her husband, who is in the military.

5. The following staff members or groups successfully completed federally administered proficiency tests:
   - Mary Byrd, Kristie Goins, Dallas Ingram, Jill Johnson, and Julie Musgrove: PCR for Newcastle disease, Classical swine fever, and Foot-and-mouth disease
   - Mary Byrd, Kristie Goins, Dallas Ingram, and Julie Musgrove: Avian influenza PCR
   - Michelle Farrar: Bluetongue serology
   - Michele Farrar and Cindy Watson: Leptospirosis MAT
   - Michelle Farrar and Kristie Goins: Bovine leukaemia serology
   - Michelle Farrar, Kristie Goins, Dallas Ingram, and Julie Musgrove: individual Coggins certification, Pseudorabies serology, Brucellosis card agglutination
   - Dallas Ingram: Equine viral arteritis serology
   - Jill Johnson: Johne’s culture
   - Julie Musgrove: Johne’s serology (ELISA)

6. Improper dilution can result in false-positive staining. If reagents are not properly washed off between steps, it may appear that the antibody has bound to the antigen which has not, again, a single antigen in false-positive staining. Antibodies also have to be diluted to an optimum concentration prior to use. Improper dilution can result in over or under staining, resulting in either false positives or false negatives or equivocal results.

7. Immunohistochemical stains are based on markers expressed in normal cells. Although neoplastic cells typically express these same markers, anaplastic tumors can either lose or gain expression of normal markers. Many of these cell markers are expressed by a wide variety of cells and are used only to narrow down the list of differentials and not to make a specific diagnosis.

8. Cost of immunohistochemistry is variable and is dependent on the specific antibody or antibodies used. In cases where immunohistochemistry would be helpful, it will be suggested by the pathologist in the biopsy report. Immunohistochemistry is performed on formalin-fixed, paraffin-embedded tissues that have been archived for several years at the diagnostic laboratories after sample submission. Therefore, it is not usually necessary to submit additional tissues for immunohistochemistry as the originally submitted tissues will still be available for use.

At Your Service... on Saturdays!

The doors to the ADVL may be shut on weekends, but the work goes on! On Saturdays, staff members come in to read out pending bacterial culture results and receive mailed-in submissions.

The following are some of the most common immunohistochemical markers that we use:

Vimentin: This is the most ubiquitous intermediate filament in the body. It is expressed in mesenchymal cells including fibroblasts, myocytes, melanocytes, endothelium, adipocytes, chondrocytes, lymphocytes, and macrophages. It is typically used to verify that a tumor is of mesenchymal rather than epithelial origin.

Cytokeratins: This is actually a group of intermediate filaments of varying molecular weights. They are expressed in epithelial cells. Tumors that express cytokeratin include carcinomas, mesenchymal, chondroma, thymoma, synovial sarcoma, and meningioma. This stain often is used in conjunction with vimentin to differentiate between mesenchymal or epithelial origin in poorly differentiated tumors.

Desmin: This is normally expressed in skeletal and smooth muscle, and cardiac muscle. It can also be used to help identify leiomyomas/leiomyosarcomas and rhabdomyosarcomas. Other commonly used antibodies to specifically diagnosis leiomyoma/leiomyosarcoma or rhabdomyosarcoma are smooth muscle and skeletal muscle myosin, respectively.

S-100: This is a relatively nonspecific marker that stains cells of neural crest origin including glial cells, adipocytes, chondrocytes, and melanocytes. Tumors that are S-100 positive include schwannomas, neurofibromas, astrocytomas, oligodendrogliomas, nerve sheath tumors, choromas, chondrosarcomas, melanomas, liposarcomas, and synovial sarcomas.

Melan-A: This is expressed by melanocytes and is used to identify melanotic melanomas.

CD3: This is a marker for T-lymphocytes.

CD79: This is a marker for B-lymphocytes and plasma cells. CD3 and CD79 are typically used together either to verify that a round cell tumor is a lymphoma or to establish that a lymphoma is of B- or T-cell origin. Other antibodies commonly used in immunohistochemistry for detecting B-lymphocytes include CD20 and BLA 36.

CD18: This is a panleukocyte marker including lymphocytes and macrophages. It is often used to identify tumors of histiocytic origin but CD3 and CD79 often have to be used concurrently to rule out lymphoma. Substantial inflammation within a tumor can make interpretation difficult since normal inflammatory cells also express CD18.
MOORE MEDICAL (800-234-1464), and TW Medical veterinary supply (888-787-4483).

**What’s New?**

**1. Novel H1N1 and Endemic Influenza Testing:**

Both Virology/Serology has added a new **Novel H1N1 and Endemic Influenza** testing.

- **What’s New?**
  - **H1N1 Swine influenza virus (SIV) in pigs.** We are using the official protocol provided by the National Animal Health Laboratory Network. The only valid sample (both ante- and post-mortem) for this protocol is a nasal swab submitted in brain-heart infusion (BHI) broth. You may obtain BHI from our Bacteriology lab if you plan to submit any specimens for SIV H1N1 testing.
  - Both laboratories offer ELISA tests to differentiate serologically between H1N1 and H3N2 antibodies. About 1ml of serum is required for both ELISA tests.

**2. AVDL**

- **The new pan adenovirus PCR** is capable of detecting any adenovirus and is particularly useful for exotics practitioners to detect Rattle adenovirus in bearded dragons, snakes, and other reptiles. Please submit a fluid from a cloacal wash, a cloacal swab, or fresh tissue such as liver or spleen. This test is run twice a week on Tuesdays and Thursdays.

- **Canine hepatitis virus** PCR detects Canine adenovirus 1 and 2 and can distinguish between them. Suitable samples for testing are a nasopharyngeal, conjunctival or throat swab, 1 ml tracheal wash, or 1 g feces. This test is run twice a week on Wednesdays and Fridays.

- **Feline calicivirus PCR** is now available, and can be performed on conjunctival, nasal or oral swabs. The submit is run twice a week, also on Wednesdays and Fridays.

**3. TDVL**

- **Virology/Serology** has added a new **Feline herpes virus** (FHV) test. **PCR** is now available, and can detect active FHV infection. Please submit 0.5 ml of serum (FHV) serum neutralization test for antibodies that can increase due to infection, exposure, and vaccination. **Acute and convalescent serum** is required for definitive diagnosis of active FHV infection. Please submit 0.5 ml of serum when requesting this test.

- **Equine viral arteritis (EVA)** testing is done on Fridays. **Canine adenovirus** testing is done on Fridays. **Canine parvovirus** testing is done on Fridays. **Canine hepatitis virus** PCR is run twice a week on Wednesdays and Fridays. **Canine parvovirus** PCR is run twice a week on Wednesdays and Fridays.

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Sample preservation and fixation:
If specimens from multiple sites or lesions are to be submitted, identify each specimen by site or size and place them in a separate container. Samples approximately 1 cm thick preserved in 10% buffered neutral formalin are the most appropriate for best preservation of tissue architecture and cellular detail. Some specimens should be fixed whole. When collecting smaller pieces from a large specimen, include specimens from areas representing lesions of different colors, consistencies, and apparently normal areas. Submit the whole neoplasms (if relatively small) from biopsies with surgical margins included. To avoid autolysis, fix specimens as soon as possible after collection. Avoid refrigerating or freezing specimens fixed in formalin. Formalin begins to freeze at about 40°F and may damage tissues. Addition of 1 ml of 95–100% ethanol to 9 ml of 10 percent buffered neutral formalin helps to prevent this. Fixing the specimen at least 24 hours before shipment may help prevent sample deterioration if the fixative is lost by leakage during shipment.

Specimens should be transported in just enough formalin to keep them moist after previous 24 hours fixation in an adequate volume of formalin. Avoid glass containers, which often break during shipment.

Fixing large specimens into narrow-mouthed bottles mechanically distorts and disrupts the tissue. Fresh tissue may easily be placed into narrow-mouthed bottles, but it is often difficult to remove after fixation and may require breaking the container, which is hazardous to laboratory personnel. Do not reuse specimen containers. This helps to avoid residual tissue from previous specimens and confusion from additional potentially incorrect patient information on the container.

Labeling the container and completing the submission form:
Label the container properly with owner’s and/or patient’s name, site of collection, veterinary practice/veterinarian, etc., for definite sample identification.

Clinical history complements microscopic evaluation and is crucial for full and accurate interpretation of the microscopic findings. Therefore, fully complete the submission form and specify each site on the body for each specimen if multiple tissues from different locations are submitted.

Indicating the age, species and sex of the patient involved is useful for obtaining a definitive diagnosis, as some lesions/diseases may be more common in or specific to a given breed, age, sex, or location on the body.

In summary, for better results:
1. Select a representative sample of the grossly observed lesion.
2. Use the appropriate punch (1 cm thick) representative specimen containing the active part of the lesion and apparently normal adjacent tissue.
3. Handle the specimens gently—avoid collection artifacts.
4. Preserve the specimen in adequate fixative (1:10 tissue:fixative ratio).
5. Label the container properly.
6. Fill the submission forms completely and provide the clinical history.

If you have further questions, please contact the Athens or Tifton Diagnostic Laboratories.