The University of Georgia

College of Veterinary Medicine
Veterinary Diagnostic Laboratories
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We're on the Web!
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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 DVSc 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 DVSc, 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 also has 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.

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The University of Georgia College of Veterinary Medicine

Diagnostic Veterinary Matters

A newsletter from the Georgia Veterinary Diagnostic Laboratories

Volume 2, Issue 2 • Fall 2009

Georgia Veterinary Diagnostic Laboratories
Athens • Tifton

Note from the Directors

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.

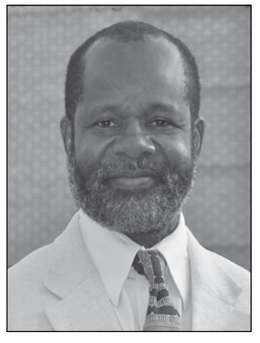
As you all know, economic times are tough, and the diagnostic laboratories are witnessing the same budget cuts and business income shortfalls that now plague multiple sectors nationwide. Additional budget cuts are expected, and we do not know how much this crisis will affect our services. We continue 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 at 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 the Athens Laboratory; the Tifton Laboratory will follow in the next few months.

Future upgrades to the website will include online bill pay and online submission of accessions for 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.



Jeremiah (Jerry) T. Saliki

Murray E. Hines II

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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 Dobbins award for Excellence in Service, which recognizes outstanding service to the people of the state of Georgia and the surrounding region.

4. **Dr. Jerry Saliki** was re-appointed to a third, three-year term (2010–2012) as editor-in-chief for the *Journal of Veterinary Diagnostic Investigation*. This is an international journal published by the American Association 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
- **Gulnaz Shaikh**: individual Coggins certification test
- **Serology staff**: Pseudorabies latex agglutination, Pseudorabies serum neutralization, Bluetongue, Bovine leukosis and Coggins AGID tests
- **Dr. Ellis** and **Dr. Miller**: Scrapie/Chronic wasting disease immunohistochemistry

Tifton Veterinary Diagnostic Investigational Laboratory

1. **Dr. Murray Hines II** was appointed permanent director of the Tifton Lab, effective October 1, 2009. He also was elected to the Executive Board of the International Association of Paratuberculosis (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 Kendrick, who retired in January.

4. **Ms. Debi Batten** has returned part-time as our afternoon receptionist. Ms. Batten was 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
- **Michele Farrar** and **Kristie Goins**: Bovine leukosis serology
- **Michelle Farrar, Kristie Goins, Dallas Ingram,** and **Julie Musgrove**: individual Coggins certification, Pseurodrabies serology, and Brucellosis card agglutination
- **Dallas Ingram**: Equine viral arteritis serology
- **Jill Johnson**: Johne's culture
- **Julie Musgrove**: Johne's serology (ELISA)

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.

A pathologist is on-call to receive and process necropsy submissions both Saturday and Sunday. If you need to contact the duty pathologist regarding a weekend necropsy, please call **706-207-3948**.

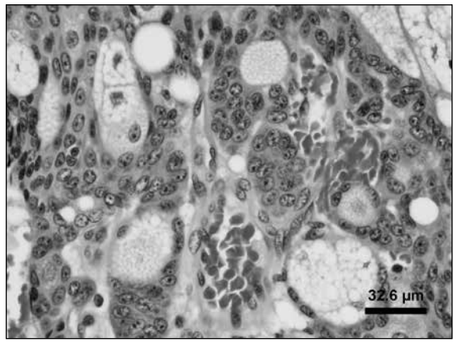
Science-at-a-Glance: Immunohistochemistry Basics & Diagnosis of Neoplasia

by Angela Ellis, DVM, PhD

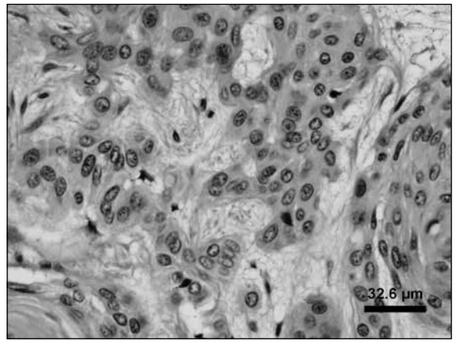
Immunohistochemistry has been available for many years, but is becoming much more widely used with advances in automated staining processes, available antibodies, and knowledge. The principle behind the technique is relatively simple: all cells have antigens, and the distribution of antigens is tissue-dependent.

In the case of tumors, certain cell types express **unique markers** or antigens that can be used to identify their tissue of origin. Antibodies designed to specifically bind to those antigens are used to identify whether those antigens are present in a tissue section. Since antigens and antibodies are not visible on routine histologic sections, the antibody is bound, either directly or indirectly, to a colorimetric agent, or chromagen, which allows the pathologist to visualize the antigen if present.

Although the technique sounds relatively simple, there are many **potential pitfalls**. Antibodies may bind nonspecifically to other antigens, resulting in false-positive staining. If reagents are not properly washed off between steps, it may appear that the antibody has bound to the antigen when it has not, again resulting 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.



Above: Sebaceous epithelioma.
Below: Cornifying epithelioma. *Photos: Dr. Uriel Blas*



Immunohistochemical stains are based on markers expressed by **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.

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 may 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.

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, mesothelioma, chordoma, 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 actin 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, chordomas, chondrosarcomas, melanomas, liposarcomas, and synovial sarcomas.

Melan-A: This is expressed by melanocytes and is used to identify amelanotic 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).

Depending on the variability in the appearance of the skin lesions, **three to six biopsies** should be taken. In order to encourage submission of multiple biopsies for dermatopathology cases to the AVDL, the dermatopathology fee is fixed regardless of the number of biopsies submitted and the number of routine special stains required. As the lesions are chosen to biopsy, they are each marked (e.g., with a dot from a marker) and locally anesthetized with lidocaine injected subcutaneously. By the time the last lesion is anesthetized, the first lesion is sufficiently anesthetized to proceed with biopsy. The punch is centered over the lesion without any significant amount of normal skin included. **Only lesional skin** should be biopsied because the margin of a lesion is typically an evolving lesion so it is neither completely normal nor completely affected and consequently its diagnostic usefulness is usually limited. As downward pressure is placed on the biopsy punch, it is rotated in a single direction to prevent creating shear forces that would rupture delicate lesions such as pustules or vesicles. Pustules and vesicles tend to be easily disrupted and must be biopsied with a punch that is larger than the lesion or by excision with scalpel blade so that they remain intact. Once a vesicle (or bulla or pustule) is ruptured, its diagnostic usefulness has been destroyed. The biopsy should extend into the subcutaneous fat which, when using a punch, can be felt as a sudden easing in the amount of pressure required to rotate the punch.

A common artifact results from crushing the biopsy when picking it up with thumb forceps to transfer it to formalin. **Crush artifact** destroys any subtle changes in the skin and frequently makes it impossible to identify cell types in an inflammatory reaction, which limits the diagnostic usefulness of the specimen. A better means of transferring the biopsy to 10 percent buffered formalin is to pick it up by the deep subcutaneous fat using the needle with which the lidocaine was injected. Each biopsy should be placed in formalin as soon as it is collected, rather than taking all biopsies first and then fixing them all at once, because autolysis of skin occurs very rapidly. The changes associated with autolysis can mimic or interfere with subtle changes that are diagnostic for some conditions and make interpretation of the biopsy more difficult, if not impossible. If the skin is very thin, it can be placed on a small piece of cardboard or wooden tongue depressor (the subcutaneous tissue will adhere to the flat surface) to prevent folding of the biopsy prior to submerging it in formalin. A single suture can be placed at the biopsy site and healing is usually rapid and uncomplicated. Remember that each biopsy heals independently of other biopsies; so whether one biopsy or six are taken, healing occurs in the same amount of time.

Submitting the biopsies properly is just as important as taking them. Successful diagnosis of skin disease requires collaboration between the clinician and pathologist. Skin biopsies taken for diagnosis of a disease condition (i.e., not tumors) should be submitted to a dermatopathologist. **Dermatopathology** is a specialized area of pathology that requires additional knowledge and an interest in clinical dermatology. The dermatopathologist not only evaluates the histologic changes in the biopsies, but attempts to correlate the histologic findings with signalment, history, and physical examination findings. Without this information, the pathologist can only provide a limited interpretation of the skin changes. For this reason, when submitting skin biopsies for histopathology, a **complete history** should be included. Signalment (age, breed, and sex) is important because certain breeds are predisposed to certain disease conditions (e.g., some diseases are congenital and occur in young animals). Other diseases are acquired and develop with age. Description of the clinical lesions, their distribution, presence or absence of pruritus, results of any other diagnostic tests performed, and treatment and response are all helpful in deciding which diseases are more likely than others. In many if not most cases, special stains are used (e.g., to examine the tissue for infectious agents).

The pathology report consists of a morphologic diagnosis which may or may not be a clinical diagnosis. In the majority of cases, the dermatopathologist also provides a comment in which the histologic changes are interpreted in light of the clinical information provided. In some instances, a definitive diagnosis may not be possible histologically, but the histologic changes can suggest a group of diseases and exclude other conditions. For example, histologic changes can be suggestive of allergic skin disease but they cannot indicate the specific allergy involved. In such instances, a dermatopathologist can suggest additional diagnostic tests to make the diagnosis.

Communication between the clinician and pathologist is the most important factor in successfully diagnosing skin disease. If the pathologist does not see changes consistent with the clinical description of the condition, (s)he should communicate this to the clinician. Likewise, if the clinician is confused about the pathology report, (s)he should not hesitate to contact the pathologist. In fact, when there is any question concerning collection or submission of specimens, a pathologist should be consulted. I would be happy to consult with a clinician before any biopsies are taken to be certain of choosing appropriate lesions, handling them correctly and requesting any additional diagnostic procedures that may be helpful in determining the cause of a skin condition. A small amount of time taken to establish communication between the clinician and pathologist may make the difference between a clinically useful skin biopsy and one that leaves the clinician, patient, and owner no better off than they were before a biopsy was performed.

What's New?

It's All Online!

1. **Results Online:** See all your finalized AVDL results online, dating back to July 2001. All you need to start viewing your results online is to call the laboratory (706-542-5568) to activate your account and receive login information.

   Already using the online system? Please provide us with feedback on how we can improve the system to better serve your needs. The TVDIL will be providing access to results of submissions to Tifton at a later date; however, the results database will only contain those submissions beginning on July 1, 2009.

   Both laboratories can e-mail, fax or mail results. If you wish to receive your report results by e-mail or to change your method of delivery, please phone or e-mail the appropriate laboratory to request the change.

   AVDL: 706-542-5568, athndlab@uga.edu
   TVDIL: 229-386-3340, dlab@uga.edu

2. **Account and Billing Information Online:** In August 2009, the AVDL implemented the second installment of our electronic services delivery upgrade: accounts online. See your account information, including all invoices, details of payments, as well as future invoice transactions. The next two installments, scheduled for implementation by the end of this year, are online credit payments and sample submissions using our online test catalog. Online sample submission will improve turnaround time and reduce clerical errors in processing.

3. **Coggins Online:** Together with GlobalVetLink, both diagnostic laboratories now offer a fast and easy online Coggins test submission and resulting system. Take advantage of this simple way of submitting samples and receiving your Coggins certificates. We encourage all equine practitioners to seriously consider using this system. To sign up visit **www.globalvetlink.com** or call **515-296-0681**.

New Tests

1. **Novel H1N1 and Endemic Influenza Testing:** Both Georgia laboratories now have the capability of testing for and differentiating between the novel and classic strains of 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 Reptile adenovirus in bearded dragons, snakes and other reptiles. Please submit 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.
   - A **Feline calicivirus** PCR is now available, and can be performed on conjunctival, nasal or oral swabs. This test is run twice a week, also on Wednesdays and Fridays.

3. **TDVIL**
   - Virology/Serology has added a new **Feline herpes virus** (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 and requires a minimum of 72 hours to complete. As toxicity of cells is a common problem in some samples, clients should allow sufficient time in case the test needs to be reset when preparing for export, sales or shipping. Care should be taken to ensure a clean sample, and serum should be shipped on ice. Contact Serology (229-386-3340) for more information on the EVA test.
   - Clinical Pathology is now performing **insulin testing** on serum samples. This test requires 0.5 ml of serum and is performed daily. Please contact Anita Merrill (229-386-3340) for additional information.

Science-at-a-Glance: Selection, Collection & Submission of Samples for Histopathology

By Moges Woldemeskel, DVM, PhD, DACVP

Histopathology is a powerful and inexpensive diagnostic method. Submission of the right specimen in the right fixative is crucial for microscopic evaluation of biopsy specimens and organ or tissue samples from necropsies. Properly selected, appropriately collected and preserved specimens are very helpful in establishing an accurate diagnosis.

Sample selection and collection:
During sample collection, a representative specimen of the gross lesion observed at necropsy or biopsy should be carefully selected. The specimen should include the **typical lesion, active margin, and adjacent unaffected/normal tissue**. Collecting cores of lesions, which may contain only necrotic debris, often renders the specimen unsuitable for diagnosis.

Handle the specimens **gently** to avoid compression artifacts, crushing or tearing by forceps (at collection). Improper handling mechanically disrupts tissue architecture and impairs microscopic evaluation. While performing skin punch biopsies, go perpendicular to the skin surface and deep enough to include the underlying subcutaneous fat. Avoid shaving, cleaning and scrubbing. Clipping hair may be sufficient. Very small samples removed by laser or electrocautery in most cases are unsuitable for histopathological evaluation and definitive diagnosis due to heat coagulation artifact (coagulation necrosis).

Occasionally there may be no apparent gross lesion at necropsy. If doubtful about which tissue to collect, include tissues from all major organs, intestines and brain (preferably whole brain). If a grossly visible lesion is found, include the adjacent normal appearing tissue during collection.

In collecting specimens from the **gastrointestinal tract** (gut), select at least three different intestinal sites of 1–2 cm (about 1 inch) in length. Ingesta in the gut may impair proper fixation. Use fixative to gently rinse the ingesta out of lumen (do not use water). Mucosa is the most commonly affected layer of the gastrointestinal tract and an intact mucosa is essential for evaluation. It also is prone to disruption during handling. Therefore, handle the tissue gently and make sure the formalin gains access to the intestinal lumen for adequate fixation.

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**.

Specimens approximately 1 cm thick preserved in 10% buffered neutral formalin are the most appropriate for best results. The specimen should be completely immersed in ample amounts of fixative. Saline solutions, ethanol or isopropanol should not be used as fixatives and can render the biopsy useless. A tissue to fixative ratio of 1:10 is the appropriate minimum volume (fixative 10 times the volume of the tissue).

Larger specimens (greater than 1–2 cm in diameter) should be cut into smaller slices of about 1 cm in diameter to facilitate easy penetration by the fixative. This renders better preservation of tissue architecture and cellular detail. Some specimens, such as **eye**, 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 neoplasm (if relatively small) from biopsies with surgical margins included.

To avoid autolysis, fix the specimen 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 percent 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.

Forcing large specimens into narrow-mouthed bottles mechanically distorts and disrupts the tissue. Fresh tissue may easily be placed into narrow-mouthed bottles, but are 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. Collect the appropriate size (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.

(continued page 5)

Carefully selected, appropriately collected and an adequately fixed representative specimen of a lesion with adequate clinical history are essential steps in obtaining a meaningful diagnosis.

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

Science-at-a-Glance: Diagnosis of Skin Disease — Skin Biopsies

by Pauline M. Rakich, DVM, PhD, DACVP

One of the most common reasons for bringing pets to a veterinarian is skin disease. In many cases, the diagnosis is obvious and treatment is straightforward and successful. However, diagnostic tests are necessary to determine the cause when lesions are unusual; suggestive of serious diseases that require expensive, dangerous, or long term treatment; or do not respond to treatment as expected. Skin biopsies are frequently the most direct means of making a diagnosis because they are relatively easy to do, rapid, cost-effective, and safe for the patient.

To maximize the information obtained from histopathology there are some basics to follow. Most importantly, unless a lesion is very small, **multiple biopsies** are much more likely to provide a diagnosis than a single biopsy; because of this, the AVDL has a **single charge** for a patient's dermatology biopsy regardless of the number of skin punches submitted.

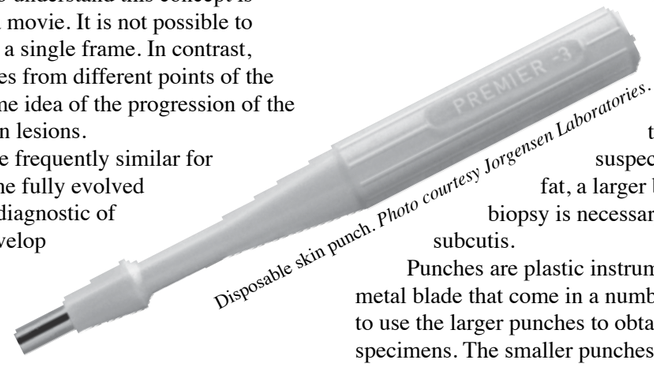
A single lesion is rarely representative of the entire disease process. Since skin lesions are in various stages of progression at any single time, biopsying multiple lesions makes it possible to determine the disease course and make a diagnosis. The easiest way to understand this concept is to imagine a still photo from a movie. It is not possible to learn the plot of a movie from a single frame. In contrast, by viewing multiple still frames from different points of the movie, it is possible to get some idea of the progression of the action. The same is true of skin lesions.

The very earliest lesions are frequently similar for a number of conditions. It is the fully evolved primary lesions that are most diagnostic of the condition because they develop spontaneously as a direct reflection of the disease condition. **Primary lesions** include macules, patches, papules, wheals, vesicles, bullae, pustules, plaques, and nodules. With time, these primary lesions regress, degenerate, or become traumatized, evolving into secondary lesions which are less diagnostic because they can develop from multiple primary lesions. These **secondary lesions** include epidermal collarettes, scars, excoriations, erosions, ulcers, fissures, lichenification, and calluses. Some lesions are not as easily categorized as primary or secondary and may be either.

If the skin condition is characterized only by these lesions, they may be the primary lesions and biopsy could be diagnostic. These include alopecia, scales, crusts, comedones, and pigmentary changes. Since some disease conditions wax and wane, it is better to postpone biopsies until primary lesions develop rather than taking biopsies of regressing or healing lesions. If the animal has been treated with **corticosteroids**, treatment should be discontinued (for two to three weeks for oral steroids and four to six weeks for injected steroids) prior to taking biopsies so that the anti-inflammatory effects of the steroid will not alter the histologic appearance of the lesions. Biopsy of as many primary lesions as possible is most likely to yield a diagnosis.

In general, physical restraint and local anesthesia are all that is required to take skin biopsies. The skin **should not be cleaned** or prepared in any manner because surface material may be diagnostic and thus it should not be disturbed at all. If the hair is long and interferes with the biopsy, it can be trimmed down to the skin surface with scissors.

The easiest means of obtaining skin biopsies is with punches. However, punches are not suitable for obtaining samples of subcutaneous lesions because they do not extend deep enough to obtain sufficient tissue to adequately evaluate a subcutaneous lesion. If the predominant lesion is suspected to be in the subcutaneous fat, a larger biopsy such as a wedge biopsy is necessary to adequately examine the subcutis.



Disposable skin punch. *Photo courtesy Jorgensen Laboratories.*

Punches are plastic instruments with a sharp round metal blade that come in a number of sizes, and it is important to use the larger punches to obtain the most diagnostic specimens. The smaller punches (4 mm) should be reserved only for biopsying facial lesions of cats and small dogs because they provide such a limited sample. For lesions involving other areas, 6 or 8 mm punches should be used.

Although the difference in size seems small, the larger biopsies provide significantly more material for histologic examination without producing a skin lesion that takes any longer to heal. A single punch can be used to obtain all of the biopsies from a patient. The punches are disposable and supplied individually packaged in quantities of 25 or 50 for about $2 each. They can be bought from a variety of sources, including HSB Veterinary Supply, Inc. (800-526-8981),

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