



# Veterinary Medical Diagnostic Laboratory

University of Missouri

August 2015 Newsletter

## Message from the Director

Dear clients, colleagues and supporters:

I hope you have had a great start to your summer. Life at the MU VMDL has been full of exciting activities aimed at protecting animal and public health and educating future veterinary professionals and scientists.

In 2015, the MU VMDL renewed its membership with National Animal Health Laboratory Network (NAHLN) and became a Tier I Lab of Food and Drug Administration Veterinary Response Network (FDA Vet-LRN). Our Avian section has been working with the State Veterinarian and USDA to combat and prevent Avian Influenza outbreaks in Missouri. The Molecular Biology section has been validating PCR assays to ensure accuracy of our results. The Bacteriology section has acquired a high-throughput MALDI Biotyper for accurate and rapid microbial identifications. The Toxicology/Chemistry section is actively helping producers solving problems associated with mycotoxin contamination. In March, three of our pathology residents passed the 2015 Phase I ACVP Certifying Exam. In May, our graduate students' research received second place in the 38th Annual Phi Zeta Research Day. Over the past year, several faculty and staff members have emerged as section heads, managers, and program coordinators. Because of their professional expertise and commitment to excellence, the MU VMDL is able to make great strides in diagnostic service, education, and research.

While there is much to be proud of, there is still much more work to be done. Our top priority is and will always be serving our clients. Thank you for choosing the MU VMDL as your diagnostic lab. Thank you for your support and suggestions that help us to enhance our programs.

Sincerely,  
Shuping Zhang  
Professor and Director



### *Inside this issue:*

- \* Business News
  - Testing Updates
  - New VMDL forms
  - New employees
- \* Featured Story:  
Avian Influenza

Check out our new recurring columns:

- \* "Out and About" - what have our VMDL members been up to?
- \* "How Timely" - Information on a disease relevant for the season.
- \* "I Spy...Featured Images"
- \* Sample Handling Tip of the Quarter

The MU Veterinary Medical Diagnostic Laboratory and Teaching Hospital will have a booth at the CVC meeting in Kansas City this August 29th - 31st. Hope to see you there!



MU Veterinary Medical Diagnostic Laboratory

# Testing Updates from the VMDL

The new VMDL Tests Offered/Fee Guide will be distributed and implemented August 1, 2015. Please expect to see minor changes in fees at that time.

## Clinical Pathology Testing News:

- \* **Progesterone testing is now offered daily** (Monday through Friday) while maintaining the current fee of \$13.00!
- \* **Cortisol pricing:** We are now offering a price break on multiple cortisol measurements per patient as follows:
  - Single cortisol = \$25.00
  - Two cortisol samples (ex. ACTH stimulation testing) = \$45.00
  - Three cortisol samples (ex. low dose dexamethasone suppression testing) = \$60.00
- \* **Reduction** in Total T4 (TT4) price to \$25.00 (TT4 for small animals only)
- \* New **Canine Total T4/TSH combo** is now available for \$45.00
- \* Of potential interest for **farm animal** patients: We are offering a **new electrolyte and mineral chemistry panel** including sodium, chloride, potassium, bicarbonate, calcium, magnesium, and anion gap for \$12.50
- \* Reconfigured **mini liver chemistry panel** to include glucose, BUN, albumin, total protein, globulins, cholesterol, ALT, ALP, GGT, and total bilirubin for \$21.00
- \* Although fecal parasite ova identification will remain the same, identification of arthropods and worms (mites, lice, ticks, larvae from tissue lesions or the environment, and adult forms of nematodes/trematodes/cestodes) will no longer be offered. If you have a need for this type of parasite identification, you may consider utilizing the Texas A&M Veterinary Medical Diagnostic Laboratory. Please consult their webpage or staff regarding sample submission. (<http://tvmdl.tamu.edu/tests/parasite-identification/>)



*Here are a few of the friendly folks in our clinical pathology laboratory.*

## Other News from the VMDL

### Simplified, Easier to Use VMDL Sample Submission Forms

Based on client feedback received directly through interviews at the 2015 Missouri Veterinary Medical Association meeting and through field surveys, the VMDL has developed new submission forms in order to better serve clients. The most popular client request was for the new submission forms to feature check boxes enabling its users to more quickly and efficiently navigate the form and indicate the system and/or clinical signs of interest for each case. This simplified format should encourage clients to provide a more detailed history which will better aid the laboratory in its diagnosis.

Additionally, the VMDL has come up with grouped submission forms tailored to focus on specific species groups, including Avian, Equine, Food Animal, and Small Animal. Each of these forms will be available on-line for download from our website. Tests and services of particular interest are on each form for easy access and to streamline the submission process. Both the general and the species specific forms will be readily available for download and submission from our website. The paper submission form will still need to be mailed in with the sample. We look forward to serving you!

*Fred Williams III, DVM, Dipl. ACVP (Anatomic Pathology)*



### Meet the VMDL Team!

*Several new team members have joined us over the past two years without a proper introduction to you. Some of their names and voices may be familiar to you already, while others perform essential "behind the scenes" work.*

- \* Ameia Ferguson – Medical Laboratory Technician (ASCP)<sup>CM</sup> in Clinical Pathology
- \* Brett Jones - Medical Laboratory Technician (ASCP)<sup>CM</sup> in Clinical Pathology
- \* Susan Martin, MT (ASCP) – Quality Assurance Manager
- \* Debbie Sharpe – Senior Research/Laboratory Technician in PCR
- \* Latoyia Sly - Research Specialist II, Serology
- \* Floreisha Washington – Office Support and Veterinary Samples Assistant
- \* Michael Zhang, DVM, PhD, Dipl. ACVM– Associate Clinical Professor, Head of Serology



# Highly Pathogenic Avian Influenza in the Midwest



*Dr. Shaw is an anatomic pathologist who specializes in avian pathology. He is board-certified in both the American College of Veterinary Pathology and the American College of Poultry Veterinarians.*

Written by Dan Shaw, DVM, PhD

## ***History of the Outbreak***

The outbreak of H5N2 Highly Pathogenic Avian Influenza (HPAI) that has infected poultry operations in the Midwest began in British Columbia in early December 2014. It subsequently was found in a few backyard poultry flocks and some captive raptors in Washington, Oregon, and Idaho. It appeared in a commercial turkey flock and a commercial chicken flock in California in late January and early February of 2015, respectively. It is believed to have been carried by migrating waterfowl in the Pacific Flyway.

A flock of young commercial breeder turkey hens (26,310 turkeys) became infected with H5N2 HPAI near Willmar, MN, on March 4. The virus was then detected in 2 flocks of turkeys in Missouri: one in Moniteau County on March 9 (15,620 turkeys) and the other (13,850 turkeys) on March 10 in Jasper county. Then a flock on an Arkansas farm, which contained 40,020 commercial turkeys, was diagnosed with the disease. Overall, the affected flocks were noticed to be off feed and depressed, but the most striking finding was a sudden increase in death loss. There were few specific postmortem changes. The diagnosis of the disease was made at the Veterinary Diagnostic Laboratory of the Missouri Department of Agriculture in Springfield, MO, and confirmed at the National Veterinary Services Laboratory in Ames, Iowa, over the weekend of March 7-8. Within a few weeks the flocks were depopulated. The virus was later detected in a small back yard poultry flock in Lewis County, MO. No more incidents have occurred in Missouri to date.

## ***Cost of the Outbreak***

Meanwhile, the outbreak has taken a huge toll on poultry production in Minnesota, Iowa, Wisconsin, South Dakota, North Dakota, and Nebraska. The avian influenza virus has killed over 40 million turkeys and chickens. Minnesota is number 1 in turkey production in the US. It is in the top 20 states for egg production. From a report in the Minneapolis Star Tribune on May 26, 2015, about 10 percent of Minnesota's annual turkey production has been wiped out. The state also has lost 3.5 million hens at four egg-laying farms. In a University of Minnesota Extension analysis released on May 25 it was estimated the financial losses in Minnesota totaled \$309.9 million as of May 11. Direct losses from destruction of turkeys and egg-laying chicken flocks was around \$113 million. For every \$1 million in direct losses the study calculated the ripple effect of an additional estimated \$1.8 million in overall losses, including \$450,000 in wages. These amounts were expected to rise as more farms were affected and barns remained empty. In a report in the Des Moines Register on May 19 it was estimated by economists at Iowa State University that Iowa could face losses of just over \$600 million. Iowa is the top egg producing state and has over 57 million hens that produce over 14 billion eggs. The financial losses of producers will be partially offset by government and insurance compensation but these payments will not extend to ancillary industries that service poultry production.

## ***Transmission of the Disease and Associated Pathology***

Waterfowl are believed to be the reservoir of avian influenza viruses. Ducks can become infected and shed the virus without showing noticeable clinical signs. The virus is shed in respiratory secretions and feces. The virus is very delicate in the environment but it is protected by mucus and fecal material. Moist cool conditions help preserve the virus. It can survive for up to 7 days at 68° F. Due to the lipid membrane it is susceptible to a variety of disinfectants and solvents. It has been found that it takes a fairly large dose of the H5N2 HPAI virus to

infect domestic poultry. Once a chicken or turkey is infected the virus is deadly. At an egg laying farm in Wisconsin 40,000 hens died within 10 days of infection. Unlike past outbreaks of HPAI the currently circulating H5N2 is not associated with florid gross postmortem changes. There may be reddening of the trachea and lung and possibly cloudy watery fluid in the air sacs. Diagnosis requires detection of the virus using the specific PCR assay.



## ***Disease Prevention***

In Missouri and other states, there is great concern about the possibility of migrating waterfowl carrying the virus south and east in the fall. In 2012 Missouri ranked fourth in turkey production, ninth in broiler production, and fifteenth in egg production. Poultry and eggs ranked third in total agricultural sales in Missouri behind grain and cattle. The poultry industry in Missouri accounted for 15.7% of the total agricultural sales (over \$1.4 billion).

Bio-security is the most important element in prevention. This includes preventing contact with wild birds and their droppings, restricting visitors, restricting movement of poultry, sanitizing vehicles entering and leaving farms, etc. The use of vaccine is very controversial and highly regulated. Vaccination will not prevent infection but it does reduce the severity of clinical signs and level of virus shedding. The use of vaccination, however, causes problems with export of hatching eggs, chicks, and poultry products. Highly regulated use of vaccination is being considered by the USDA in the most severely affected areas in attempts to limit shedding and spread of the virus.

## ***Diagnosis and Disease Surveillance***

There are 2 methods of detecting evidence of exposure of a poultry flock to avian influenza (AI) virus. The PCR assay is the preferred method. It is very sensitive and detects the presence of the genetic material of the virus. Up to 11 tracheal or oropharyngeal swabs from gallinaceous poultry of the same species and from the same premises may be pooled in 1 of the 5.5 ml tubes. Three swabs can be placed in the 3 ml tubes. Sample collection from live or dead birds should be performed as follows: use synthetic or semi-synthetic swabs (e.g. polyester, rayon, nylon) with plastic handles to swab the oropharyngeal and choanal cleft areas. After swabbing the bird, insert the swab into the tube and swirl vigorously in the brain heart infusion broth. Remove the swab by pulling it to the top of the tube and squeeze the swab against the side of the tube to remove all liquid. Samples should be refrigerated and transported on a cold pack to arrive at the lab as soon as possible. Tubes of brain heart infusion broth tubes are available from the VMDL and the Missouri Department of Agriculture Animal Health Laboratories (Jefferson City and Springfield).

The second method that is available is serology using the agar gel immunodiffusion test (AGID). This test detects the presence of antibody.

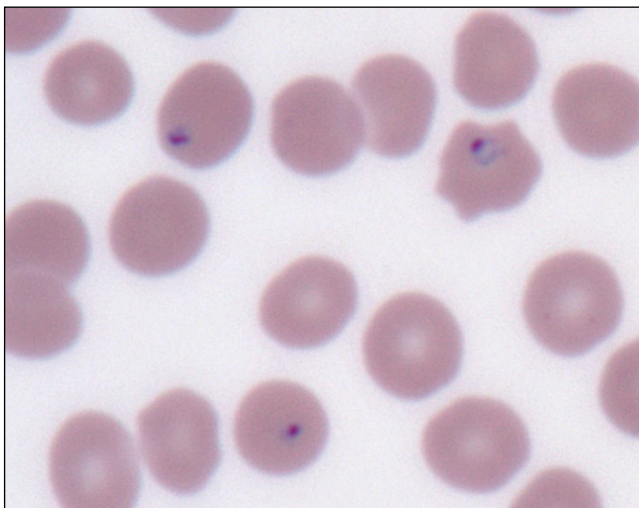
Since it takes 10 to 14 days for detectable antibodies to develop, this limits the utility of the test in an outbreak situation. It is useful for flock and regulatory monitoring. One-half ml. of serum per bird is required for this test. This can be obtained by drawing 1 ml. of blood from a wing vein. Place the blood into a red top tube. The tube should be laid at an angle to allow maximum surface area of the sample for 6 to 8 hours at room temperature. This will provide optimum clot retraction and serum yield. The clot can then be removed and the serum transported on ice to the lab for testing.

- ◆ *You can find more information on avian influenza by visiting the USDA, AVMA, and Missouri Department of Agriculture's websites.*
- ◆ *For questions regarding sample submissions to the MU VMDL, feel free to contact us directly.*

# “How Timely” - Information on *Cytauxzoon felis*

It finally feels like summer, and we all know that ticks (and fleas, and mosquitoes for that matter) are out in full force. Along with these creepy-crawlies comes a wide variety of unpleasant animal diseases. Many of these vector-borne diseases require serologic or molecular testing for definitive diagnosis. Some, however, can be spotted with nothing more than a stained blood smear, immersion oil, and a 100x objective on your microscope. We occasionally detect granulocytic rickettsial morulae in dogs that way (most often *Ehrlichia ewingii* in this region, although *Anaplasma phagocytophilum* would appear the same). The morulae in these diseases are usually found only in the acute phase of infection and therefore may be unobservable by the time a patient presents to your clinic.

In comparison, *Cytauxzoon felis* piroplasms are often (but not always) identifiable upon presentation of infected cats. Spring to early summer brings the highest number of cytauxzoonosis cases, but new cases can be identified through late summer and fall. As this is a disease that you may be able to definitively diagnose “in house” and there are still a few months of new cytauxzoonosis cases to come, here is a brief reminder of common clinical signs, CBC findings, and microscopic findings to help you reach a rapid diagnosis in this potent protozoal disease.



Three *Cytauxzoon felis* piroplasms within erythrocytes from an infected cat. Note their small size and eccentric purple region. Wright-Giemsa stain, 100x objective.

## CLINICAL SIGNS/HISTORY

- Potential exposure to ticks
- Rapid development of clinical signs
- Fever, anorexia, lethargy
- Icterus/jaundice
- Respiratory distress, abnormal vocalization
- Splenomegaly, lymphadenomegaly

## COMMON CBC FINDINGS

- Anemia – often mild to moderate and apparently non-regenerative to weakly regenerative
- Neutropenia or other evidence of inflammation. The presence of band neutrophilia (a left shift) and/or the presence of neutrophil toxicity are helpful markers of inflammation – both of which would require blood smear examination.
- Thrombocytopenia

## MICROSCOPIC APPEARANCE

- Intra-erythrocytic protozoa (piroplasms)
- Usually take up one-fourth to one-third the diameter of the erythrocyte
- Classically described as “signet ring-shaped”, but may be ovoid, round, teardrop, or amoeboid
- Pale center, thin dark border, dark purple-blue eccentric region



## “How Timely” - Information on *Cytauxzoon felis*

(continued from previous page) RBC piroplasms are not evident at presentation in all affected cats. Prior to this “erythrocytic” phase is the “tissue phase” of the disease. Large schizont-laden macrophages (macrophages filled with developing parasites) within blood vessels result in ischemic damage to many vital organs, resulting in the severe morbidity and relatively poor survival rate we associate with cytauxzoonosis. *Angela Royal, DVM, MS, Dipl. ACVP*

The University of Missouri has an ongoing study evaluating the diagnostic sensitivity of using lymph node and splenic aspirates to find infected mononuclear cells (schizont-laden macrophages) as compared with blood smear evaluation for infected RBC at the time of presentation to a veterinarian. ***We are actively seeking samples for this study.*** If are interested in learning more about this study, please do not hesitate to contact Dr. Erin Burton ([burtonen@missouri.edu](mailto:burtonen@missouri.edu)) or Dr. Leah Cohn ([CohnL@missouri.edu](mailto:CohnL@missouri.edu)), or call either at 573-882-7821.

## “Out and About” — What Have the VMDL Folks Been Up To Lately?

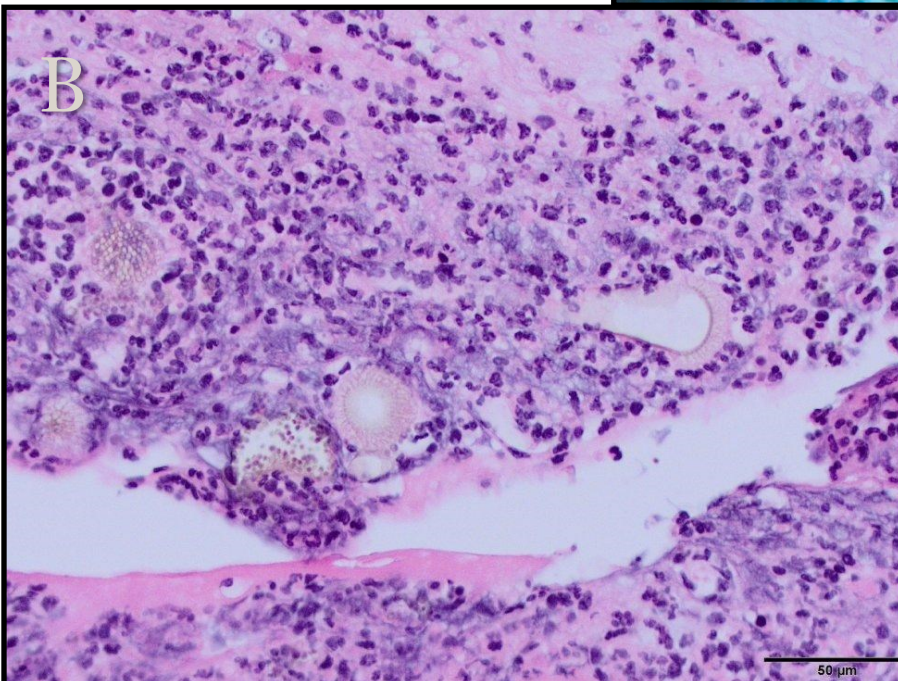
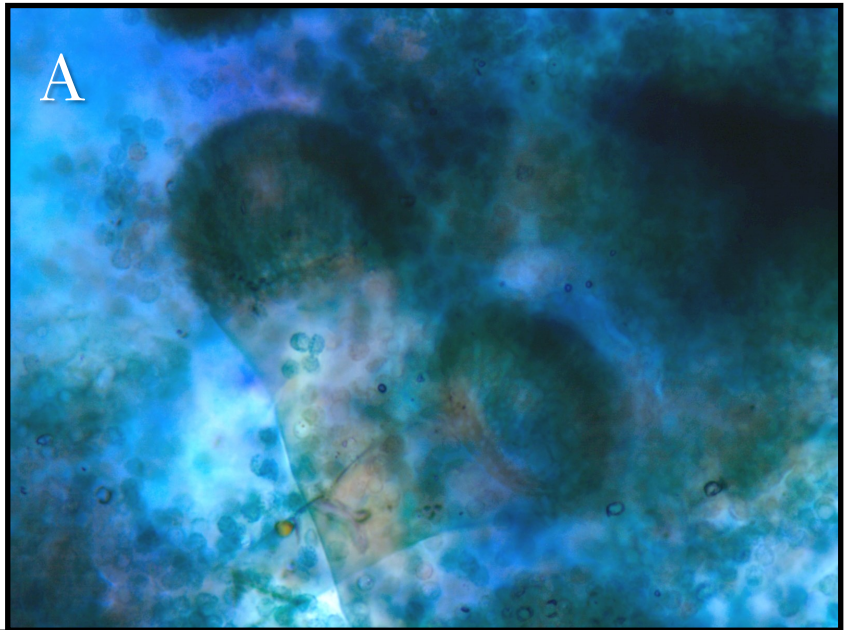
- National Animal Health Laboratory Network trip – Our Quality Assurance Manager, Susan Martin, MT (ASCP) and our Clinical Pathology Laboratory Supervisor, Cheryl Rojas, MT (ASCP), were awarded invitations to attend the 2015 NAHLN/AAVLD Quality Management System Training Program in August. This program is sure to inspire and guide us in our constant quest for quality.
- ASVCP On-line Cytology Rounds: Angela Royal (Clinical Pathologist) and Tara Piech (Clinical Pathology resident) hosted this webinar-style rounds session in June. Cytology slides are viewed on a microscope while the audio and digital camera video feeds are broadcast live to attendees across the globe.
- National meeting presentations: Tim Evans (Toxicology Section Head) presented four lectures at the AVMA meeting this July in Boston, with topics including mycotoxins, plant toxins, the roles pregnancy and lactation have on potential adverse effects of drugs and toxins, and a review of small animal intoxications highlighted by real case examples.
- Dr. Evans also has prepared three CE webinars for the MVMA this summer: Aflatoxins, Blue-green Algae, and Summer Updates for 2015.
- National meeting presentation - Bill Fales (Bacteriology Section Head) presented the following: Fales, WH, JW Bowman, IK Ganjam, Dae Young Kim, S. Schommer, MJ Calcutt and TR Reilly. 2014. Isolation and Identification of *Helcococcus ovis* from bovine joint fluid. 57th Annual Meeting of the American Association of Veterinary Laboratory Diagnosticians. Kansas City, MO.
- International meeting presentation - Bill Fales (Bacteriology Section Head) presented the following: Fales, WH, TR, Reilly, IK Ganjam and JW Bowman. 2015. Trends of Bacterial Antimicrobial Resistance Associated with Cattle Affected with the Bovine Respiratory Disease Complex (BRDC) in Missouri, USA. 17th World Association of Veterinary Laboratory Diagnosticians, Saskatoon, Canada.
- Make sure to stop by the VMDL and VMTH booth at the upcoming CVC Conference in Kansas City this August 29<sup>th</sup> to 31<sup>st</sup> - We're looking forward to seeing you!

## “I Spy....Featured Images!”

**S**ampling technique and processing are quite different for cytology and biopsy specimens, but sometimes the similarity in results is striking! **Figure A** shows a 100x objective cytologic view of an impression smear from what appeared to be a diphtheritic membrane in the middle meatus of a horse with chronic, intermittent, hemorrhagic, unilateral nasal discharge. In dense areas of the cytology slides innumerable fungal hyphae were found, some with attached “fruiting bodies” as seen in this image (conidiophores with attached phialides and conidia). The slender hyphae with parallel walls, septae, and dichotomous branching at approximately 45 degree angles observed in this sample suggested an *Aspergillus* species, although *not* the common *Aspergillus fumigatus* given the presence of pigmented conidia. **Figure B** shows a 40x objective view of the same fruiting bodies within a biopsy specimen of this diphtheritic membrane. Again, fungal morphology suggests *Aspergillus spp* as a likely culprit and pigmented fruiting bodies were evident. Thank you to Dr. Mrad at Mid-Rivers Equine Centre for submitting this (pathologically-speaking) fabulous case!

### Figure A: Cytology sample

Image of pigmented fungal fruiting bodies from a nasal lesion in a horse. Wright-Giemsa stain, 100x objective.



### Figure B: Biopsy sample

Image of pigmented fungal fruiting bodies from the same nasal lesion in a horse. H&E stain, 40x objective.



# "I Spy....Featured Images!"

## *A little more on differences between cytology and histopathology...*

Fine needle aspiration or impression smears of solid tissue lesions for cytologic examination (clinical pathology) and biopsy of lesions for histologic examination (anatomic pathology) are complementary procedures. Each has its strengths and limitations as highlighted below.

### Cytology

\* Comparatively, cytologic examination allows for better assessment of individual cell detail. This facilitates often straightforward diagnoses of the round cell tumors, general categorization of neoplastic populations as epithelial or mesenchymal and assessment of their likelihood of malignancy, classification of the type of inflammation that may be present, and identification of a wide variety of infectious organisms (bacteria, fungi, protozoa, algae, etc.). For ideal staining and visualization of cytomorphologic detail, the cells from a fine needle aspiration sample must be spread into a thin monolayer on a slide. The compromise then for the ability to evaluate intricate individual cell detail is the loss of most tissue architecture.



*Angela Royal, DVM, MS, Dipl. ACVP (Clinical Pathology), left, and Gayle Johnson, DVM, PhD, Dipl. ACVP (Anatomic Pathology), right*

### Histopathology

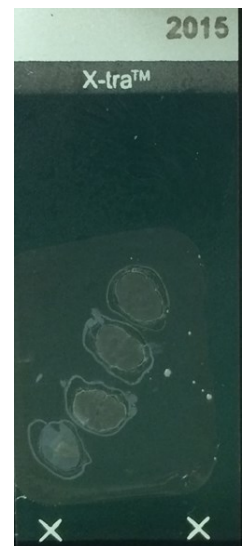
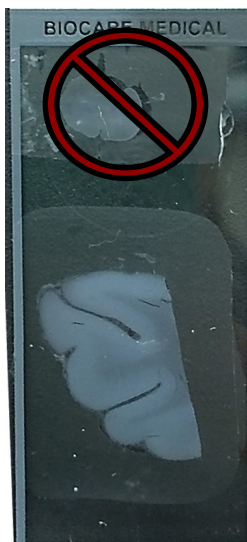
\* Biopsy specimens are formalin-fixed, embedded in paraffin, thinly sectioned with a microtome, placed onto slides, and stained. This process allows for ideal evaluation of tissue architecture, an essential part of diagnosing specific types of epithelial and mesenchymal neoplasms and grading neoplasms when indicated. Additionally, some neoplasms can only be accurately identified as malignant based on their abnormal tissue architecture (including aggressive invasion into adjacent normal tissue), and some inflammatory or tissue repair processes can result in cellular atypia which is confounding upon cytologic examination but easier to recognize in light of tissue architecture. Fine cytoplasmic features, however, can be lost, and evaluation of cell detail is limited to one slice through the tissue rather than visualization of whole intact cells.

## Sample Handling Tip of the Quarter

### How to submit precut slides for IHC

Submit the sample as you would for routine histopathology, do not alter the unstained slide. Please place tissue on the bottom half of the positively charged slide, if possible (as seen on the right), allowing us to add a control as needed. Submit 2 slides per block per antibody requested. If you have any questions, please feel free to contact us. Thanks!

Histology Lab



# New VMDL Courier Address Replaces Old Address

The VMDL has a new courier street delivery address. The previous E. Rollins address has been eliminated and replaced with the new courier address below:

**Veterinary Medical Diagnostic Laboratory**  
810 E. Campus Loop  
Columbia, MO 65211

Kindly make note of this change and submit future samples to the address above. The P.O. Box address below is an alternative shipping option for clients that would prefer to ship U.S. mail.

**Veterinary Medical Diagnostic Laboratory**  
P.O. Box 6023  
Columbia, MO 65205

**Veterinary Medical Diagnostic Laboratory**  
810 E. Campus Loop  
Columbia, MO 65211

## Contact Us

Give us a call for more information about our services and products

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(573) 882-6811

Visit us on the web at  
[vmdl.missouri.edu](http://vmdl.missouri.edu)

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