University of Nebraska Lincoln

Diagnostic Center News

Fall 2009

University of Nebraska Veterinary Diagnostic Center

Co-Editors: Dr. D. Scott McVey & Mavis Seelmeyer

In This Issue:

Did You Know??

Taken from: http://www.freakyanimals.com/facts001.shtml

The last animal in the dictionary is the Zyzzyva, a tropical weevil.

There's a "meow" in the middle of homeowner!

The tallest dog on record was named Shamgret Danzas. He was 42 inches tall (at the shoulder!) and weighed 238 lbs.

Notes From the Director

Congratulations to Dr. David Steffen upon recognition by the Red Angus Association of America for outstanding service. Dr. Steffen has been recognized as a key diagnostic expert for congenital diseases of cattle.

We certainly want to welcome Dr. Seth Harris to the Nebraska VDC. Please see his brief bio on page 2.

The new laboratory information system is coming; we appreciate your responses to our request for preferred reporting methods. As we prepare to begin some of our testing phases, you may from time to time receive an E-mail or a fax from our laboratory that was not requested by your clinic. Again, this is just part of our testing phase, so please bear with us as we go through this process.

Our laboratory has been participating in the USDA NAHLN SIV H1N1 outbreak influenza surveillance program - please contact VDC staff about information regarding USDA surveillance program participation.

We now test bacteria for antimicrobial sensitivity by the broth dilution method using the TREK@ Sensititre plate system. The Sensititre system provides the only fully customizable system that is semi-automated and designed to cover most diagnostic testing needs in the veterinary laboratory. More than 240 antimicrobials, including 40 veterinary specific antimicrobials, are available for susceptibility testing of bacteria and yeasts, ensuring accurate first time results. Sensititre remains the system of choice for global surveillance programs, including NARMS (National Antimicrobial Resistance Monitoring System), coordinated via FDA-CVM, USDA, CDC. The advantages provided to our clients include rapid turn around and true MIC data and interpretation based on the Clinical and Laboratory Standards Institute's guidelines. Also, we will provide data for a very broad set of antimicrobials (broader than typically available through Kirby-Bauer testing). The disadvantage of this system will be increased costs – individual tests will cost $18.00. However, please contact the bacteriology laboratory to discuss strategies to minimize costs of AST tests and the need for testing in some cases. Also, please be sure to indicate on submission forms if you do not require a sensitivity test.

I would also like to remind our clients that typical sample retention is approximately two weeks from completion of work. Please contact the laboratory if you would like for us to retain specimens for a longer period of time and we will try to accommodate.

Please contact the laboratory if we can be of service or if you have any questions or suggestions!

- - - submitted by D. Scott McVey, DVM, PhD,
Director, Veterinary Diagnostic Center

NEW PATHOLOGIST AT VETERINARY DIAGNOSTIC CENTER

Dr. Seth Harris

Dr. Seth Harris is a new pathologist at the Veterinary Diagnostic Center. Dr. Harris received his DVM from Colorado State University and completed his Anatomic Pathology residency at Washington State University. He is originally from Tempe, Arizona. His wife is from a small town in western Iowa, so Dr. Harris and his family are thrilled to be in the Midwest and close to family again. Dr. Harris' hobbies include hunting, classic Mustangs, photography and spending time with his wife and children. Dr. Harris is a welcome addition to our staff. We know you will enjoy working with him!

PROPER CYTOLOGY SUBMISSIONS

Cytologies are a minimally invasive and rapid means of rendering a diagnosis or directing whether ancillary testing should be performed. They are commonly performed throughout veterinary medicine, yet minor problems with sampling technique can cause them to fail to give the needed information. Here are a few helpful hints designed to help you get a high quality specimen to increase your chance of rendering a diagnosis.

Most of the cytologies received are aspirates of masses or lymph nodes. The biggest obstacle in evaluation is when a single, poor quality specimen is submitted. To alleviate this problem, always send in multiple slides (four is a rough guideline) to get a representative sample from the mass. If you have a severely hemodiluted or poorly cellular specimen, more slides would be needed than if you have a cellular specimen. Soft masses and lymph nodes should be prepared similarly to a blood smear, while dense specimens should have a squash preparation done. The goal of the smear is to have a simple monolayer of cells on the slide, and to take care that the cells are not ruptured. With squash preparations, the weight of the slide is typically sufficient to develop a monolayer. After making the smear, allow it to air dry away from formalin fumes to prevent cell shrinkage and submit it unfixed. If you are not sure of the quality of your sample, stain and evaluate one slide, in-house before sending the specimen to the diagnostic laboratory. If you cannot identify intact cells, modify your technique to optimize the sample.

Fluids are another commonly received specimen. All shipped samples should be submitted in an EDTA tube if there is a concern about the sample clotting. The tubes should be chilled with an ice pack to prevent overgrowth of bacteria. While this yields a good sample for bacterial culture and total protein analysis, any cells that are present will likely swell during transport and become unidentifiable. Therefore, accompany your EDTA sample with a few freshly made air-dried slides.

Probably the least common specimen received is a direct impression of tissue. These can have a high diagnostic yield, but are often contaminated by excessive amounts of blood. Therefore, prior to making serial impression with a small (5-10 mm) piece of tissue, blot it on a paper towel to remove the blood.

-------- submitted by Seth Harris, DVM, Veterinary Pathologist and
Elisa Salas, DVM, Resident Veterinary Pathologist

MICROBIOLOGY/PARASITOLOGY UPDATES

Fee Schedule Changes

You may have noticed a change in charges for some of our cultures. We have lowered the price of both the aerobic and anaerobic cultures to $5 and have added a charge of $10 for bacterial ID. This will reduce the cost for cultures where no pathogens are identified which is the largest percentage of cultures seen in the lab.

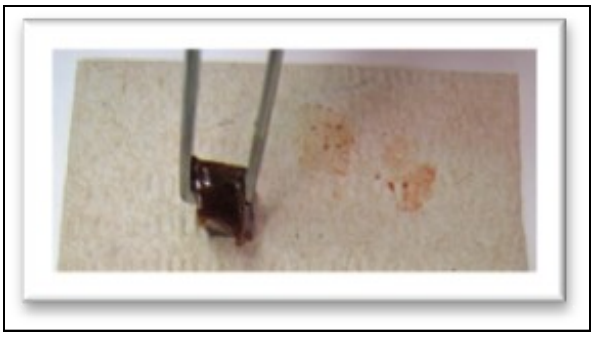
We have also reduced the price of milk cultures to $5. There are three choices form milk cultures now. Milk culture with ID, all pathogens will be identified and a susceptibility will be performed on each isolate (ID's will be charged at $10 each). Milk culture without ID, a colony count and number of colony types will be reported and no susceptibility with be performed. Staph aureus only culture, will report if Staph aureus is present and a susceptibility will be performed. Please indicate which culture you want on your submission form. The price of a susceptibility has increased to $18.

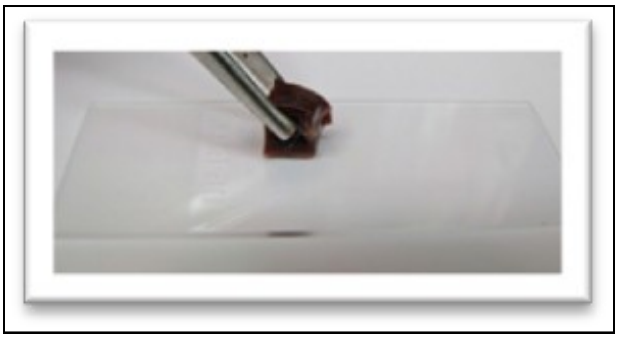
***Tritrichomonas foetus* Testing**

"The increasing use of real time polymerase chain reaction (pcr) assays in clinical diagnostic testing has been an interesting journey. Early in our in-house development of a real time pcr assay for *Tritrichomonas foetus* in preputial washes from bulls, we were very excited about the power of the test to detect very low numbers of the organism. However, these early studies were initially performed on dilutions of pristine, laboratory cultures of the organism. Some of our early validation tests were interpreted at a level to maximize sensitivity and therefore some false positives occurred. It became increasingly clear that further test refinement was necessary in terms of amplification chemistry, control strategies, instrument control and sample processing to maximize the combined sensitivity and specificity of the test. After multiple internal and external, collaborative studies under the leadership of Deb Royal and Jamie Bauman we feel confident that the assay is validated and reliable. This test is not perfect, but it is a valid tool for use in trichomoniasis diagnostics, with laboratory sensitivity and specificity above 90%. However, field studies indicate that the diagnostic power of testing may be in the range of 70% to 80%. This suggests that when testing bull batteries where infection has been previously diagnosed or where clinical problems and/or history suggest possible infection, multiple test strategies (with specimens collected at approximately three-week intervals) will likely be required identify all infected bulls. This is true with gel pcr tests, real time pcr tests or culture tests. We do plan to confirm suspect (high Ct) real time results with gel pcr because this provides added specificity and confidence in positive results.

There are several new studies on the stability of Tritrichomonas foetus during transport for PCR. These studies recommend incubation of the In-pouches at around 37°C for 24 hours and then freezing them for shipment. The incubation will increase the number of trich in the pouches and the freezing will then stop the growth of contaminating bacteria which can inhibit the PCR process. This will also make it easier for collection of samples at the end of the week, since once they are frozen there is no rush to get them to the lab. If you do not have an incubator, you can take a Styrofoam container and insert a microwaved ice pack (warm to the touch) or a hand warmer to create a make-shift incubator. Don't put the pouches directly on the heat source as they may over-heat. Once they have incubated for a day freeze the pouches and ship them to the lab on ice packs.

Please feel free to contact the VDC Microbiology staff or the Nebraska Veterinary Extension staff with questions regarding diagnostic methods and strategies."





Proper cytology technique for direct impression of tissue

University of Nebraska Veterinary Diagnostic

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The Nebraska Veterinary Diagnostic Center is accredited by the American Association of Veterinary Laboratory Diagnosticians

All regulatory testing for export is done in compliance with the code of federal regulations and technicians performing the test have been tested annually by the USDA through the National Veterinary Services Laboratories check-testing program. Additional assays within the lab regarding toxicology, microbiology and parasitology are assessed annually by check testing through the Veterinary Laboratory Association. Positive and negative control samples are included in all serologic and toxicologic testing protocols that require them.

Ancillary testing is reviewed by a single case coordinator who assures that test results are in agreement and any unusual test results are investigated to ensure that standard operating procedures are followed and that results can be replicated. Standard operating procedures are on file in each of the laboratories and available for inspection. A copy of our Quality Manual is available upon request.

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