Wisconsin Veterinary Diagnostic Laboratory

UNIVERSITY OF WISCONSIN–MADISON

Newsletter - Winter 2016

Message From the Director

We have had a busy year so far and we anticipate the testing case load to continue to rise in support of WVDL's mission of providing reliable testing and outstanding customer service to our clients. The number of tests performed at WVDL for fiscal year 2015 increased ~20% compared to the previous year, which is, on average, an increase of ~1900 tests per day!

News from the USDA and National Animal Health Laboratory Network (NAHLN). WVDL has been a core laboratory in the NAHLN since its inception in 2002. Recently, the USDA reorganized the NAHLN into numerical level designations. WVDL is is designated as a Level 1 laboratory. Level 1 diagnostic laboratories are tasked by USDA to provide surveillance testing for a number of economically high consequence diseases such as bovine spongiform encephalopathy and highly pathogenic avian influenza, Newcastle disease and classical swine fever virus.

Our next board of directors meeting is scheduled for 1pm, March 9th, 2016 at the

Madison Laboratory.

Phil Bochsler, DVM, PhD, DACVP
Director

Microbiology

Johne's Disease Detection ELISA kit has changed

There have been several changes in Johne's Disease antibody detection recently at the WVDL. Due to a significant increase in the variability of results given by the IDEXX kit when subsequent bovine samples are submitted, the WVDL has moved to using the ThermoFisher Prionics PARACHEK 2 kit for all bovine, ovine and caprine serum and plasma samples and bovine milk samples. We are happy to report that the USDA has given provisional approval for the use of the ThermoFisher Prionics PARACHEK 2 kit for export samples. This kit has provided more stable results for our clients who need this test for export purposes. The IDEXX kit will continue to be used as a backup test for Johne's Disease antibody detection. You can request this kit be used by indicating so upon submission.

The changes in the kits, used for the detection of Johne's Disease antibodies, can cause some confusion as an animal that once had a history of negative test results could become suspect or positive when serum, plasma, or milk from that animal are tested on a new kit. Each kit manufacturer develops a proprietary antigen that is used to bind antibodies that are specific to that antigen. Since IDEXX and Prionics use different antigens, it is possible that one serum, plasma, or milk sample could test positive with one kit, but be negative with the other. This is because the antibody in the sample may only bind the one antigen, but not the other. The only way to confirm if the animal is infected with *Mycobacterium avium* subspecies *paratuberculosis* is to send a fecal sample for direct PCR or liquid culture. Please see our website for more information on Johne's Disease detection.

Anaplasma cELISA kit antigen has changed

Recently, VMRD has released a new competitive enzyme-linked immunosorbent assay (cELISA) for the detection of *Anaplasma* antibodies. The previous kit used the recombinant major surface protein 5 (rMSP5) bound to the maltose binding protein (MBP) antigen and had a specificity of 97.8%. The new kit, which has been validated at the WVDL and was recently put into use, uses the rMSP5 protein bound to the glutathione *S*-transferase (GST) and has improved the specificity to 99.7%. This commercial rMSP5-GST cELISA is a faster and simpler assay with a higher specificity, comparable sensitivity, and improved resolution compared to the previous kit. See Chung, et al (2014) J of Vet Diagn Invest.

Tritrichomonas foetus qPCR to be run Mondays

The WVDL has seen an increased demand in the *Tritrichomonas foetus* quantitative PCR, which is a test used for the shipment of animals between states. The WVDL would like to remind clients interested in this test and the *Tritrichomonas foetus* culture that the genital washing should be incubated in a Biomed Diagnostic InPouch™ immediately after sampling and then sent to the WVDL within 48 hours. The test cannot be performed if the sample, in the InPouch™, is not received within 48 hours. Additionally, the InPouch™ must be incubated for 24 hours prior to DNA extraction and PCR. Therefore, we will be consolidating all samples received for

Tritrichomonas foetus qPCR testing and will perform the PCR on Mondays. All samples from the prior week will be tested on Mondays. If you are in need of a quicker turnaround time, please contact the WVDL for immediate testing options.

Small Ruminant Brucellosis testing using the Card Test has changed

For small ruminant sera samples, we will be using a different Rose Bengal antigen concentration for the Brucella Card Test. This change will increase the sensitivity for *Brucella melintensis*, but will also cross react with *B. abortus* and *B. suis*. Please order the same test (Brucella Card Test), but indicate the species on the submission form so the correct test is run.

Gamithromycin and tildipirosin added to current set of antimicrobials tested for susceptibility

New Clinical and Laboratory Standards Institute (CLSI) guidelines have been issued for determining susceptibility of a veterinary bacterial isolate to a particular antimicrobial compound. Because of these changes, the WVDL has added two new antimicrobials, gamithromycin (ZACTRAN®) and tildipirosin (ZUPREVO™), to the current set of antimicrobials used during susceptibility testing. Gamithromycin and tildipirosin have been approved for the treatment of the following bovine respiratory disease pathogens: *Mannheimia haemolytica, Pasteurella multocida,* and *Histophilus somni*. Only these isolates that have been identified to the genus and species level will be tested using the Kirby Bauer, disk diffusion antimicrobial susceptibility testing method for susceptibility to these two antimicrobials.

Increase in contamination during *Campylobacter fetus* subspecies *venerealis* culture

The WVDL has seen an increase in the amount of *Campylobacter fetus* cultures. This culture traditionally takes 3-4 days; however, some samples of genital washings, stored in Weybridge Transport Medium, are contaminated with high levels of normal flora, which can obscure *C. fetus* organisms and make reporting microbiological findings difficult leading to delays in result reporting. Weybridge Transport Medium contains many inhibitory compounds to eliminate normal flora and promote the growth of *C. fetus*. However, some normal flora are resistant and will overgrow in the Weybridge Transport Media. This overgrowth of normal flora bacteria could inhibit *C. fetus* from growing or obscuring its growth on the Skirrows media. In order to provide clients with the best and most accurate results in the least amount of time, we are asking clients to perform the following tasks to reduce the level of normal flora contamination in the genital washing:

1. Train and become proficient in taking genital washing samples,
2. Use sterile technique to the best of your ability,
3. Clean debris from the preputial orifice and clip preputial hairs to about one-half inch length,
4. Send the sample within 24-48 hours after collection.

The following timeline describes the steps needed to confirm a sample does not contain *C. fetus:*

1. The Weybridge Transport Medium is streaked on Skirrows medium and cultured for 3 days.
    a. Samples with the following will be reported as ‘none isolated’ within 3-4

working days of the culture being initiated:

1. Samples with little to no growth and have no suspect colonies
2. Samples with suspect colonies that are identified as not being *C. fetus.*

b. Samples with the following will require additional time:
    1. Samples with an overgrowth of contaminating normal flora (see image), must be filtered and recultured for 3 days.
        1. After the additional 3 day culture, the filtered sample may have little to no growth, or have suspect colonies identified to not be *C. fetus*, which will then be reported as 'NI with filtration'.
        2. However, after the additional 3 day culture, it is still possible that either a suspect colony must be recultured for 3 days prior to MALDI-TOF identification or PCR is needed, which adds 1-3 more working days till the results are finalized.
    2. Samples that have a colony that is suspect, but too small for MALDI-TOF identification, will be subcultured for 3 days and MALDI-TOF performed after culture.
    3. Samples that have a colony identified as *C. fetus* by MALDI-TOF are sent for PCR for subspecies identification. This adds 1-2 working days.
    4. Example of contaminated plate:

Contaminating normal flora can create a 3-8 day delay on the results. The WVDL will report *C. fetus* cultures that require additional work after the first incubation as still <pending> so clients know additional testing is needed. Samples that did not have *C. fetus* will be reported as 'none isolated' and those that had none isolated (NI) after filtration are reported as 'NI with filtration'. Please call the WVDL if you have any questions regarding the length of time a *C. fetus* culture is taking and we can assist clients in methods to reduce normal flora contamination and turn-around time.

Client Services

The WVDL is proud to partner with the **Dairy Calf and Heifer Association** again to offer interactive training on the UW Madison campus at WVDL and the UW SVM. This training is aimed at a variety of people from calf care takers to veterinarians. The meeting is scheduled for April 13th, 2016 from 3 to 6pm. The focus of this year's meeting will be calf

scours and fluid therapy basics for on farm use.

Dr. Don Sockett will be at the Professional Dairy Workers of Wisconsin (PDPW) conference on March 16th helping out with a necropsy wet lab. Stop by and say hello!

The 2016 WVDL Customer Service Survey will be sent out in March. The survey is electronically based and will look similar to 2015, but with some minor changes to streamline the process. We appreciate any and all input to improve the our diagnostic services to you.

Because the Port-a-cul anaerobic culture media was discontinued, we have switched to a new specialized anaerobic specimen collector for you anaerobic culture needs. The culture tube is called the "**BD BBL Vacutainer**" and can be found on the Microbiology Media Order Form. It is important to note for this new media should be used for the isolation of anaerobic organisms only and that Amies transport media should be used for aerobic organisms. is that separate swabs should be used for aerobic organisms in aerobic transport media.

Finally, you may have noticed changes to our website. Please contact us with comments and questions about the new look and functions of www.wvdl.wisc.edu.

Pathology-TSE-Chemisty

Changes in Bone Ash

Bone composition is critical to the health of animals. Two components, calcium and phosphorus, make up the largest share of mineral content and are important in building and maintaining strong bones. Bone is also a dynamic storage facility of these minerals, which are utilized in blood, tissues and milk production. Nutritional status can be assessed by determining the levels of these minerals and the amount of non-organic material as ash. Bone is cleaned, dried, defatted and ashed, then assayed for calcium and phosphorus by ICP-MS.

Select and collect ~10 cm section of mid-shaft of long bone, e.g. femur, humerus or rib. Indicate which bone was submitted for analysis. Keep bone refrigerated after collection and during sampling.

Spotlight on Poultry Pathology

At WVDL, we only occasionally see classic nutritional diseases, which are relatively uncommon due to well-balanced rations. Therefore, when these cases arise, there is usually an underlying error in feeding or other break down in husbandry. Identifying the primary source of the problem can be an interesting challenge.

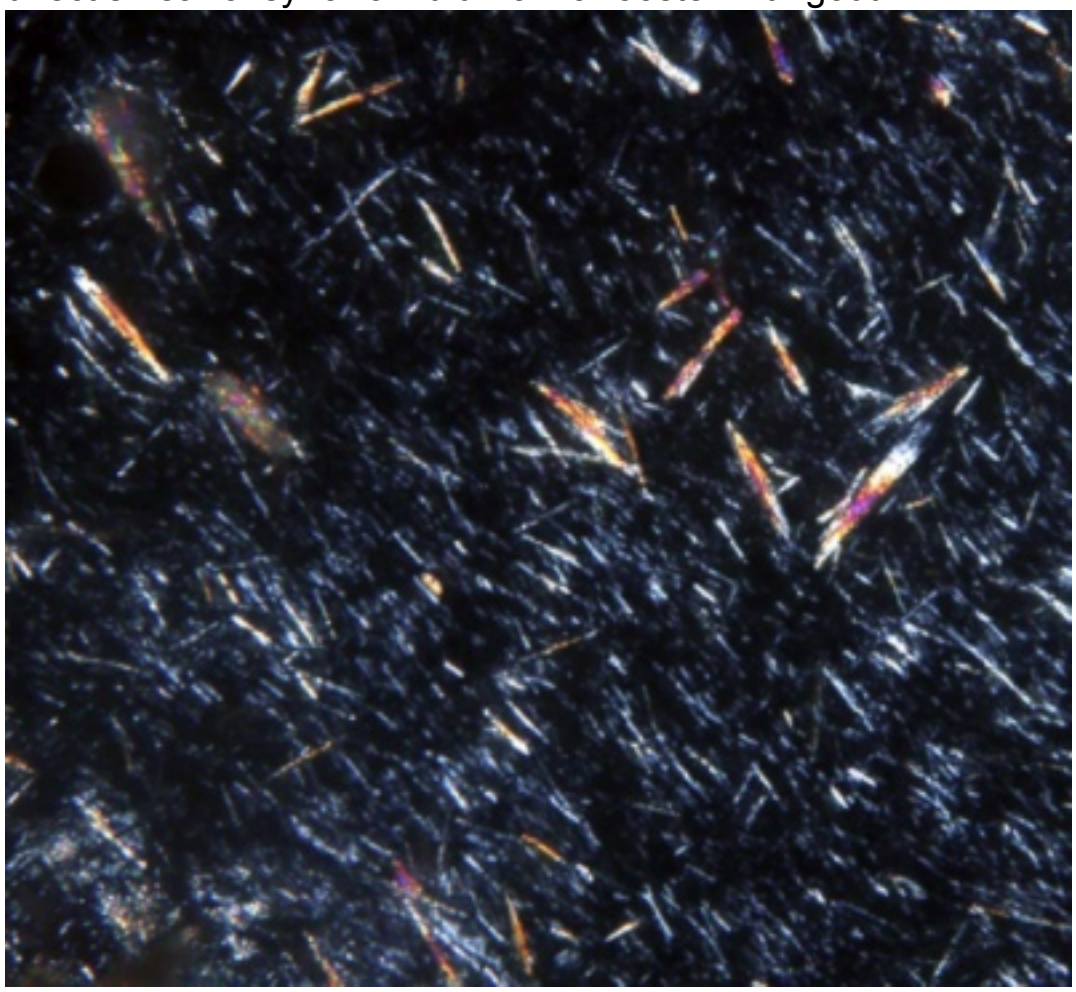
In recent months, WVDL has helped to diagnose and trouble-shoot various nutritional diseases—rickets in pheasants, vitamin E deficiency in chicks, and gout in a rooster.

The pheasants had soft bones, pathologic fractures, and angular limb deformities due to dysplastic growth plate cartilage. Rickets is a disease of young, growing animals and can result from either vitamin D deficiency or calcium/phosphorus imbalance.

The chicks had very non-specific signs, including lack of coordination and balance. Vitamin E deficiency in chicks results in damage to small vessels in the brain. In poultry, the root cause of vitamin E deficiency is often degradation of vitamin E in the feed.

Regarding nutrition, roosters and hens have very different needs. This rooster was beautiful and in good shape; however, the air sacs and viscera had a frosty appearance and the synovial fluid was milky white! Deposited urate crystals account for the white appearance. Birds and reptiles excrete nitrogenous wastes as uric acid rather than ammonia. Dehydration and primary kidney disease can lead to gout, but the root cause in roosters is usually excessive dietary protein, such as the amount normally in a layer ration.

The image below shows rate crystals under microscopy with polarized light, which are in a direct smear of synovial fluid from a rooster with gout.



Barron Laboratory

Now no accession fee for Equine Infectious Anemia (EIA) testing! Test cost is $8.50 per sample for in state submissions. EIA testing is run daily at the WVDL, Barron Laboratory. Submissions received by noon will have same day testing.

*Reminder tubes should be clearly labeled with animal name and the name on the tube must match the name of the animal on the submission form. Samples should contain of 1ml serum, refrigerated and shipped with cold pack.

WVDL accepts three submission types for EIA testing:

1. Electronic submissions through the APHIS Veterinary Services Process Streaming (VSPS) database. VSPS provides free data repository for laboratory test submissions and results. An accredited veterinarian account with VSPS is required. Results are available via the VSPS portal by the end of the testing day.
2. Electronic submissions through Global Vet Link. Global Vet Link provides a real-time data repository for laboratory test submissions and results. A veterinary account with Global Vet Link is required. Test results are available by the end of the testing day via the Global Vet Link web site.
3. Official Federal VS Form 10-11. Test results are recorded on this form. Veterinarian and owner copies are placed in USPS mail the day after testing.

See our website at http://www.wvdl.wisc.edu/index.php/submission-guidelines/ for additional information about submission options.

Samples can be appropriately shipped to the Barron Laboratory via our UPS shipping program. The UPS Ground method offers an affordable $7 flat rate option that will result in next day delivery to the Barron Laboratory from anywhere in WI.

See our website at http://www.wvdl.wisc.edu/index.php/shipping-information/ for additional information on our UPS shipping program.

See our website at http://www.wvdl.wisc.edu/index.php/shipping-information/ for additional information on our UPS shipping program.

Virology

BVDV Testing Reminders for Diagnostic cases

Reliable detection of bovine viral diarrhea virus (BVDV) is critical for maintaining the health of a herd. The choice of diagnostic assay as well as the sample type greatly influences the success of a BVDV control program. Several tests are offered at WVDL and it may help to provide reminders about the appropriate use of each test and the appropriate sample type.

Transient infections are characterized by low levels of virus so the leukocytes in a whole blood sample provide the best chance of finding BVDV. Because of these low viral loads, the individual real-time PCR assay on a whole blood sample is the most appropriate test. Virus isolation is another assay appropriate for transient infections but we advise against using it because of the time involved and the potential for maternal antibody interference.

Persistent infection (PI) can be detected by RT-PCR using individual or pooled samples. Whole blood, ear notches or nasal swabs can be used for animals less than 2 months old. Serum can be also be used once animals are older than 2 months. Pooling significantly reduces testing costs but is only possible because of the high viral load found in PI animals. Following a positive pool result, individual samples within a positive pool are tested to identify the positive animal(s). This will occur the following day. The C_T level of an individual PCR indicates PI status but the animal should be tested in 3-4 weeks to confirm. WVDL continues to offer the retest at no charge. A summary table of the tests offered at WVDL for diagnostic cases and recommended sample type is shown below.

Clients are reminded to include a fetal and dam ear notch for testing in abortion cases. Tissues tested by real-time PCR can have C_T levels in the 20's in severe acute infections. An ear notch can help distinguish between persistent infection and transient infection (usually ear notches are negative in a transient reaction).

BVDV Testing offered at WVDL for Diagnostic Purposes

	Recommended sample type	**Recommended Test**	**Inappropriate assay**
Transient (Acute)	Whole blood	Individual PCR	Antigen capture ELISA (ACE), Immunohistochemistry (IHC) Pooled PCR. These assays are not designed for the low viral load found in acute infections.
	Recommended sample type	**Recommended Test**	**Could also use:**

Persistent	Whole blood Ear notch Nasal swabs < 9 weeks Can use serum For calves >9 weeks old	PCR pool for screening herds or Individual PCR	Antigen capture ELISA (regulatory use only) (serum, EN) Virus isolation (serum, whole blood) These assays may be subject to maternal derived antibody interference

BVDV Testing Reminders for Regulatory Cases

WVDL offers antigen capture ELISA (ACE) for regulatory testing however we recommend using individual real-time PCR assays if the regulations permit. Animals < 3 months old may have systemic maternal antibody which may interfere with ACE. Real-time PCR avoids maternal antibody interference and is more sensitive and less expensive. A negative ACE test on an animal > 3 months of age indicates the animal is not a PI. However an ACE result does not provide information about transient infection status. ACE is not sensitive enough to detect low viral loads associated with transient infection. A proposed mechanism for acquisition of BVDV testicular infection is via transient infection. To avoid missing transient infections it is recommended to use a whole blood sample and real-time PCR.

March 1, 2016 Changes in virology schedule:

Neospora ELISA will be offered once a week on Tuesday
IBR SN will be offered once a week on Friday
BVDV Antigen Capture ELISA for regulatory testing will be offered once a week on Tuesday

New tests in Virology/ Molecular

The following real-time PCR assays are now available. These are not part of routine panels at this time so they should be requested as needed. Please consult with Dr Keith Poulsen or Dr Don Sockett for appropriate use.

Mink astrovirus
Bovine adenovirus 3 and 5
Breda virus

Facebook Twitter Website

Share Tweet Forward

www.wvdl.wisc.edu

Our mailing address is:
445 Easterday Lane, Madison, WI 53706
1521 E. Guy Ave, Barron, WI 54812