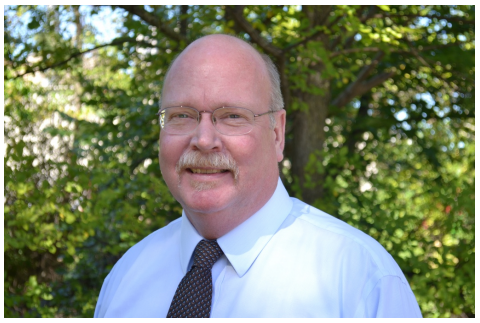
Wisconsin Veterinary Diagnostic Laboratory

UNIVERSITY OF WISCONSIN–MADISON

Newsletter - Fall 2015

Message From the Director



It has been my pleasure to be part of the WVDL's mission of providing outstanding service to our clients for the past fourteen years as a diagnostic pathologist and Section Chief of Pathology.

With Dr. Pete Vanderloo's retirement as Interim Director and departure this July, one of my charges as the new Director is to facilitate continuance of our mission and foster the atmosphere of continuous improvement.

WVDL has a great, multi-talented staff who are committed to excellence in our working role with veterinarians and animal health professionals, owners, agribusiness, and our partners at the University, State of Wisconsin, and the federal government. I'm fortunate to be part of this group. Our mission is clear, our commitment is high, and we look forward to working with you in the years to come.

Phil Bochsler, DVM, PhD, DACVP
Director

Microbiology

The WVDL is happy to announce a new test (test code: SALDUBEL) for the surveillance of animals infected with *Salmonella enterica* subspecies *enterica* serotype Dublin. The ThermoFisher PrioCHECK *Salmonella* Antibody ELISA Dublin kit detects *Salmonella* Dublin antibodies in serum and plasma and can be used as a biosecurity screen for purchasing animals or for surveillance in existing herds. The price is $12/sample and interpretation guides will be available at wvdl.wisc.edu.

The WVDL is now offering a new service for the maintenance and shipping of microbiological isolates (test code: ISOVACSHIP). Most clients utilize this service for the shipping of *Moraxella bovoculi* and *Moraxella bovis* isolates for autogenous vaccine development. If you believe a culture may identify an isolate that will require the WVDL to ship the isolate, please request this test code. Additionally, you can request the test code once the culture results are finalized. Please request the shipping test code within 48 hours upon finalization of the accession. The WVDL will contact the referring veterinarian for more information and will maintain the isolate for up to 2 weeks at which time the isolate will be shipped to the location specified by the veterinarian. This service is offered at both the Madison and Barron locations.

Recently, there have been several changes to Johne's Disease ELISA kits. The WVDL and NVSL were using the ThermoFisher Prionics PARACHEK kit, which was certified by the USDA for the detection of Johne's Disease in the US for regulatory purposes. However, in January 2015, the WVDL was informed by ThermoFisher that this kit would no longer be available. At this time, the WVDL validated the IDEXX Johne's Disease ELISA kit, which was also certified by the USDA. This kit and its associated cut-off values were used until June 25th, 2015, when IDEXX changed the cut-off values for the kit. New cut-off values were established for the IDEXX kit after June 25th, 2015, which are currently in use. This IDEXX kit is certified by the USDA, but has a lower cut-off to determine positivity than the prior IDEXX kit. The IDEXX Johne's Disease ELISA kit will be used for all bovine regulatory testing.

Additionally, ThermoFisher Prionics has released the PARACHEK 2 kit, which is not USDA certified, but is the only kit available for caprine and ovine Johne's Disease testing. The kit is certified by the European Union. This kit has been validated by the WVDL and will be used for testing caprine and ovine serum and plasma samples, and bovine milk samples for diagnostic cases.

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 antigen specific. Since IDEXX and Prionic's 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 bearing in mind that the organism is shed intermittently in feces. Please see our website for

more information on Johne's Disease detection.

The WVDL will be discontinuing *Campylobacter jejuni/coli* culture for our Enteric Culture for bovine samples. This change is in response to literature that indicate this organism is a normal resident of both young and adult animals. The culture will continue to be included for canine, caprine, and other species' enteric cultures.

Client Services

We would like to welcome Chase Fritz and Emily Raasch to the Client Services team at WVDL. Emily will be working in client services in the morning and at the front desk in the afternoon. Chase is part of the Sample Receiving and Supply Room team.

Check out our PCR panels for detection and management of diarrhea on the farm. Scour Panels A, B, and C detect viral, bacterial, and parasitic pathogens in calves. Choose your panel based on age and clinical signs on the farm. Our doctoral staff can help as needed. We will also run *Salmonella* and *Cryptosporidium* PCR panels separately. Scour Panels A, B, and C detect viral, bacterial, and parasitic pathogens in calves. Chose your panel based on age and clinical signs on the farm. Our doctoral staff can help as needed. There are no out of state fees charged for PCR panels for detection of diarrheal diseases. We will also run *Salmonella* and *Cryptosporidium* PCR separately; out of state fees apply. Please see below for an extended explanation of *Salmonella* PCR and Bacteriology and information to help interpret test results.

The WVDL welcomed two Masters of Public Health Students, Dr. Janice Valenzuela and Masami Glines, over this past summer. Janice and Masami have been working with our *Salmonella* data over the past 10 years. Much of the data will be paired with Wisconsin State Laboratory of Hygiene and Department of Public Health data from previous MPH projects. Watch for publications and other information on our website. For a teaser: Our top 5 *Salmonella* serotypes from 2006 to 2015 are: 1. *S.* Dublin (by far), 2. *S.* Cerro, 3. *S*. Newport, 4. *S*. Montivideo, and 5. *S*. Kentucky.

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. Watch for advertisements for the DCHA meeting in April of 2016.

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

One retirement and two job changes* have partially changed our staff of diagnostic pathologists at the Madison laboratory.

-Kathleen Deering, DVM, MS will join our group in October, 2015.
-Raman Muthuswamy, BVSc, MS, PhD, Diplomate American College of Veterinary Pathologists, began work July, 2015.
-Christine Watson, MS, BVMS, MRCVS began work September, 2015.

All have experience working in a veterinary diagnostic laboratory setting, are very

knowledgeable, and are keen and ready to work with you. We continue to offer a range of necropsy, biopsy, TSE diagnostics, and Chem-Tox services and some nutritional analyses.

*(Drs. Delwyn Keane, Doug Lyman, and Phil Bochsler)

Barron Laboratory

Equine Infectious Anemia (EIA) submissions are tested daily at the WVDL, Barron Laboratory. Submissions received by noon will have same day testing. Samples should consist 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.

The WVDL Barron Laboratory will set up avian environmental, cloacal and hatchery samples daily for *Salmonella* isolation and identification. After collection, the samples should be protected from desiccation, light, and excessive temperatures and delivered to the laboratory within one day when possible. If a delay in shipping is anticipated, the samples should be refrigerated. 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.

Virology

The nationwide canine influenza outbreak has kept the virology section busy this summer. We collaborate very closely with Dr Sandra Newbury, director of the SVM Shelter medicine program. We have tested approximately 1300 samples with a 37% positivity rate. Because of this collaboration we have tested shelter samples primarily from the Chicago area but also from other states as the outbreak has spread. All positive samples tested thus far have been the H3N2 canine influenza strain. In a collaborative effort with Merck Animal health and scientists at Cornell and Princeton Universities, a map has been constructed that shows the spread of the outbreak. This map can be viewed by the public at the following link: https://ahdc.vet.cornell.edu/docs/CIV_Monitoring. There have only been two samples from Wisconsin that tested positive at our laboratory. Both were dogs

that had recently been boarded in Chicago. Because of the low incidence in Wisconsin we have changed our testing regimen from daily to Tuesday, Thursday and Friday. If an outbreak should occur in Wisconsin we will return to same day service.

Highly pathogenic avian influenza testing has been minimal since the last infected premises were cleaned in July. However, the USDA is predicting that more premises may be infected this fall. We have partnered with the Department of Agriculture and Consumer Protection and affected businesses to evaluate the laboratory response from the spring and find additional ways to improve our services for our stakeholders this fall.

On the aquatic front, a second paper authored by Anna Wilson-Rothering of the virology section was done in collaboration with Tony Goldberg, SVM as part of her Master's thesis. This publication demonstrates that the fish virus, viral hemorrhagic septicemia virus, circulates in lakes and may still be transmitting to naïve fish even though there are no large fish kills occurring.

Salmonella PCR and Bacteriology Testing at WVDL

Salmonella diagnostic testing can be challenging to interpret and apply to clinical or pathology samples. The WVDL has implemented changes to the workflow for the identification of *Salmonella enterica* subspecies *enterica* that utilize existing data from published literature and data collected at the WVDL to guide these changes.

Background:
The WVDL uses PCR to identify *Salmonella* nucleic acid and traditional culture methods to obtain live *Salmonella* isolates, which can be used for susceptibility testing. The WVDL offers a multiplex *Salmonella* PCR that identifies the *Salmonella enterica* subspecies *enterica* and *Salmonella enterica* subspecies *enterica* serotype (or serovar) Dublin, which is endemic in the cattle population. The *Salmonella* species PCR refers to the entire *Salmonella* genus and the assay uses conserved genes common to all *Salmonella* species. *Salmonella enterica* ser. Dublin PCR refers to only *S. enterica* ser. Dublin. Fecal, intestinal, and tissue samples are enriched in buffered peptone water (BPW) for more than 18 hours prior to PCR. Samples that yield a cycle threshold (C_T) value less than 36 will be considered a positive. Although the PCR may yield a positive result, a positive culture result from this same sample may be difficult to obtain. Internal data has demonstrated that C_T values of ≤ 35 for the *Salmonella* species have a higher success rate of retrieving a *Salmonella* isolate than C_T values greater than 35. However, for the *Salmonella* ser. Dublin PCR, culture rates are much less successful; a C_T of ≤ 32 for *Salmonella enterica* ser. Dublin PCR has a 60% culture rate. When a sample results in a high C_T value, it is recommended that additional samples from a particular animal or farm with appropriate risk factors or history of salmonellosis should be taken and tested to prove salmonellosis or obtain antimicrobial sensitivity patterns.

Testing Workflow:
The WVDL has implemented the following workflow to assist clients in obtaining the timeliest results. Diagnostic samples, which are enriched in BPW and are run for the *Salmonella* PCR panel, are automatically submitted for bacterial culture when the PCR C_T value is ≤ 35 for the *Salmonella* spp. PCR. Clients who do not want this testing done should notify bacteriology (608-262-5432 ext. 2115). Clients who would like culture

performed from a BPW sample C_T value was >35 should also notify bacteriology. Bacterial culture is initiated for samples that result in a C_T value of ≤ 35 for the *Salmonella* spp. PCR regardless of *S. enterica* ser. Dublin PCR C_T value. The purpose of the continued culture on a low C_T value for the *Salmonella* spp. with a *S. enterica* ser. Dublin PCR positive is to check for multiple serovars. If culture is successful, serotyping and sensitivity testing will automatically be performed at an additional charge. *Salmonella* serotyping can take multiple days to 3 weeks if the isolate is typeable. Non-typeable isolates can be sent to NVSL if requested. *Salmonella* spp. cultured from environmental samples will not routinely undergo antimicrobial sensitivity testing.

The WVDL recommends the following tests depending on the age of the bovine animal:

<u>Calves < 4 months:</u> *Salmonella* PCR Panel; a C_T value of ≤ 35 for the *Salmonella* spp. PCR will automatically trigger bacterial culture. The PCR is suggested instead of bacterial culture because *Salmonella* ser. Dublin is difficult to culture.

<u>Adult animals and calves > 4 months:</u> *Salmonella* culture or *Salmonella* PCR Panel; a C_T value of ≤ 35 for the *Salmonella* spp. PCR will automatically trigger bacterial culture. While PCR is more sensitive than culture, starting with PCR will delay serotype results one day for positive samples that are able to be serotyped.

Interpretation:
A sample is positive for *Salmonella* spp. or *Salmonella* ser. Dublin PCR when the C_T ≤ 36. A C_T value between 36 and 40 is considered a weak positive, where little nucleic acid is found and further sampling or testing may be necessary.

Interpretation of PCR results requires that performance of the PCR assay is done with contamination control in mind at each step of extraction and amplification. At the WVDL, AAVLD approved PCR guidelines are followed to monitor and prevent environmental contamination. Proper sample acquisition requires practitioners to be cognizant of potential contamination from vaccines as well as other environmental sources such as clothing and gloves. *Salmonella* nucleic acid is typically prevalent in the environment on the modern dairy and poultry production facility. Use caution to not over interpret environmental samples submitted for PCR. Environmental samples should be cultured to confirm presence of viable pathogens.

The *Salmonella* PCR is multiplexed and both reactions are running in the same well at the same time. These two reactions have slightly different sensitivities. By design, the *S. enterica* ser. Dublin reaction is more sensitive. Therefore, it is possible that a reaction could be *S. enterica* ser. Dublin positive and *Salmonella* species negative. WVDL data shows that the above scenario occurs in ≤ 5% of all *Salmonella* PCR reactions when *S. enterica* ser. Dublin is also positive.

The *Salmonella enterica* ser. Dublin primers also detect *S. enterica* ser. Gallinarium and *S. enterica* ser. Pullorum *in silico*. The *Salmonella enterica* ser. Dublin PCR should not be requested on poultry cases.

Salmonella ser. Dublin positive PCR samples with *Salmonella* spp. PCR C_T ≥ 35 are not routinely cultured because *S. enterica* ser. Dublin can be quite challenging to grow and the antimicrobial sensitivity for the host adapted strain has not changed in the recent past

(Appendix 1). Data from WVDL shows that only enrofloxacin, gentamicin, and trimethoprim sulfa (TMS) should be considered as potential therapeutic antimicrobials: 15 of 27 isolates were susceptible to TMS. Trimethoprim sulfa is only available in oral formulation, which is appropriate for use in calves <2-3 weeks of age. Enrofloxacin is labeled for respiratory disease and in dairy animals less than 20 months of age only – off label use is illegal. Aminoglycosides are still part of a voluntary ban and carry 18-24 month slaughter withhold.

Campylobacter fetus subspecies *venerealis* Culture

The WVDL has seen an increase in the amount of *Campylobacter fetus* cultures. This culture traditionally takes 3 days; however, some samples of genital washings, stored in Weybridge Transport Medium, are contaminated with normal flora, which can obscure *C. fetus* organisms in culture, make reporting microbiological findings difficult and results delayed. 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 media. This overgrowth could inhibit *C. fetus* from growing or cover the Skirrows media, thereby hiding *C. fetus* colonies. 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 working days of the culture being initiated:

i.Samples with little to no growth and have no suspect colonies

ii.Samples with suspect colonies that are identified as not being *C. fetus*

    b. Samples with the following will require additional time:

i.Samples with an overgrowth of contaminating 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 1-2 more working days till the results are finalized.

ii.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.

iii.Samples that have a colony identified as *C. fetus* by MALDI-TOF are sent to PCR for subspecies identification. This adds 1-2 working days.
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 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.

www.wvdl.wisc.edu

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