

WVDL Quarterly Newsletter - Fall, 2017

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Wisconsin Veterinary Diagnostic Laboratory

UNIVERSITY OF WISCONSIN-MADISON

Newsletter - Fall 2017

Message From The Director

Friends and Colleagues,

Greetings from all of us at our Madison and Barron laboratories.

Samples received from all of you for testing this past Summer have kept us busy, which is what we appreciate. Not surprisingly and likely similar to your businesses, in order to get the work done our costs of doing business are gradually rising. Looking back, WVDL has not had a comprehensive price increase of our testing services in about four years, which is not sustainable.



As a result and as mentioned previously in our Summer newsletter, our Board of Directors considered this and has approved a price increase for most of our tests by four percent, effective this October 1. We will continue to strive to keep costs reasonable while producing the best results and service possible, and we appreciate your understanding, support, and continued business.

We hope you enjoy the newsletter, and Best Wishes,

Phil Bochsler
Director

Microbiology

EIA Testing

Clients submitting for Equine Infectious Anemia (EIA) Antibody ELISA testing will receive in-state pricing if either the owner or animal reside in Wisconsin. This test requires at least 1 mL of serum shipped on ice to the Barron WVDL. Testing is performed daily.

Snap Tests Discontinued

Due to low submissions, the Canine Snap 4DX and Equine Snap Lyme tests have been discontinued. If you have any questions, please contact the Barron WVDL.

Reminder of Resumed Barron Necropsy Services

A reminder that full necropsy services have resumed at the Barron laboratory. Please contact Dr. Holly Taylor in Barron if you are needing to submit an animal for necropsy.

Price Reduction for *Salmonella* Dublin ELISA

WVDL is pleased to announce that the cost of the *Salmonella* Dublin ELISA assay will be decreasing from our current in state cost of \$12 to \$9 and out of state cost of \$18 to \$13.50, due to an increase in sample volume and kit cost. This price decrease was effective as of September 1st which will begin to appear on October 1st bills. If there are any questions please feel free to contact the lab.

Johnes Antibody ELISA kit will Change

Microbiology Serology section has completed a comparison between 4 different Johnes antibody ELISA assays in an effort to provide the most reliable and cost affective results to our clients. Beginning around October 15th, WVDL will begin using the Zoetis Johnes antibody ELISA kit instead of the IDEXX kit. The Zoetis Johnes antibody ELISA kit has increased specificity and sensitivity. The change in kit manufacturer will not result in a change in cost and the test will be run on the regular Johnes ELISA schedule. Feel free to contact the lab with any questions.

Bacterial Identification by MALDI-TOF

The WVDL Bacteriology section is requesting that individuals submitting bacteria or yeast for identification using MALDI-TOF, please submit pure culture samples. Recently, we have received many culture plates with several isolates on a plate. Some plates have so much growth that colonies cannot be isolated from other colonies, particularly when swarming bacteria are present. Bacteria and fungi will continue to replicate during the shipping process, which can lead to difficulty isolating colonies from one another. Therefore, please subculture the colonies you would like to have identified. You may submit more than one subculture per culture plate using a clearly marked and gridded culture plate, but please make the subculture a pure isolate that can be easily subcultured. The WVDL requires a 12-18 hour old culture plate, so we will be streaking the submitted isolate for overnight culture and identification the next day. Each colony will receive a separate test code and cost. MALDI-TOF uses a protein profile that is produced by a target bacterium or yeast. It matches this protein profile to profiles stored in a library and identifies the bacterium or yeast by matching the profile to those in a library. The library contains pathogens and environmental bacteria and yeast. It is important to note that many genus and species of bacteria and yeast may be identified that were not reported prior to the use of MALDI-TOF. Additionally, some bacteria can give a result of 'no identification', which is an indicator that the MALDI-TOF library could not match the protein profile produced by the target microbe to those stored in its library. These microorganisms are typically environmental microbes. If you have any questions about your identification results, please contact the WVDL.

Shipping Reminders

Please remember to ship your samples using at least two physical barriers such as a milk vial encased by a sealed plastic bag. Additionally, the package should contain enough absorbent material to absorb all the liquid in the package. Samples needing to be sent chilled should have enough ice packs to maintain temperature and the submission form should be stored in plastic away from the ice packs. The WVDL provides free-of-charge milk collection flip-top, locking vials. Please contact the WVDL supply.room@wvd.wisc.edu to request milk vials and with large submissions these vials are ideal and encouraged.

Swab Submission Reminder

When submitting swabs, please indicate from what location or sample type the swab was collected from. This allows for microbiologists to select the correct test for the best possible results. As an example, a swab of an absence might need both aerobic and anaerobic culture testing.

Free Pink Eye Testing

Microbiology Molecular section is currently in the process of validating a pink eye PCR panel that will include the following agents: *Moraxella bovis*, *Moraxella bovoculi*, *Mycoplasma bovoculi*, *Mycoplasma bovis* and Infectious Bovine Rhinotracheitis (IBR). We are currently in the process of collecting samples for the PCR validation and are asking our clients to submit eye swabs from animals showing signs of pink eye for bacterial culture (aerobic culture) and PCR at no cost (accession fee will be waived). We are offering this free testing until the end of 2017. There is a submission form available specifically for these cases that must be used if clients want to take advantage of the free testing. Please see our website (www.wvd.wisc.edu) for the submission form titled: Pink Eye PCR Panel Validation. We are requesting that duplicate swabs be submitted for testing; one swab in Amies media for bacterial culture and an additional swab in M6 media for PCR validation. Clients can use the swabs they currently stock, or they are welcome to contact the WVDL by sending an email to supplyroom@wvd.wisc.edu and requesting the Pink Eye PCR Panel Validation Swab Kit, which will include the appropriate swabs and a return label for shipping. If culture of the eye swab yields target

organisms and clients would like susceptibility testing and/or shipping of the target organisms for autogenous vaccine production, please request that on the submission form or call the WVDL Bacteriology Section. These will be offered at an additional cost to the client.

Moraxella Species Susceptibility Update

The WVDL will discontinue automatic susceptibility testing for *Moraxella* species obtained from eye swabs. Data from the WVDL (see below) and other sources indicate that *Moraxella* species are typically susceptible. Please indicate on the submission form or call the WVDL Bacteriology lab if drug resistance is suspected to add antimicrobial susceptibility testing.

Bovine Respiratory Disease Update

The leaves are changing color and the temperature is dropping. Bovine respiratory disease (BRD) risk increases as temperature decreases. The WVDL collects data on the submitted respiratory samples in order to trend what pathogens are being isolated and for antibiotic resistance. In 2016, the WVDL evaluated 1,418 samples for respiratory viruses and *Mycoplasma bovis* (Table 1). The greatest percentage of positive samples were for *Mycoplasma bovis* (29.8%) or bovine respiratory corona virus (19.9%). The bacteriology laboratory evaluated 1,918 samples (Table 2). The greatest percentage of positive cultures were for *Pasteurella multocida* (22.1%) or *Mannheimia haemolytica* (13.3%).

Table 1: Real time PCR positive samples for viruses and *Mycoplasma* (1418 submissions)

Respiratory Pathogen	No. Positive	% Positive	Change from 2015
Bovine respiratory syncytial virus	98	6.9	Increase of 1.9%
Bovine respiratory corona virus	282	19.9	Increase of 1.7%
Bovine viral diarrhea virus	26	1.8	Increase of 0.6%
Bovine herpes virus 1	32	2.3	Increase of 0.1%
<i>Mycoplasma bovis</i>	423	29.8	Increase of 12.5%

Table 2: Culture positive samples for bacterial BRD pathogens (excluding *Mycoplasma*, 1918 samples).

Bacterial Pathogen	No. Positive	% Positive	Change from 2015
<i>Histophilus somni</i>	149	7.8	Increase of 2.1%
<i>Pasteurella multocida</i>	424	22.1	Decrease of 2.2%
<i>Mannheimia haemolytica</i>	255	13.3	Decrease of 0.4%
<i>Bibersteinia trehalosi</i>	47	2.5	Increase of 0.5%
<i>Salmonella</i> species	102	5.3	Increase of 0.2%
<i>Gallibacterium anatis</i>	16	0.8	Decrease of 0.2%
<i>Trueperella pyogenes</i>	149	7.8	Increase of 2.4%
<i>Escherichia coli</i>	251	13.1	Increase of 5.2%

Additionally, the WVDL performs susceptibility testing using minimum inhibitor concentrations (Table 3). Notably, we observed significant reduction in susceptibility for *B. trehalosi* to gentamicin (3%) as compared to 2015. Additionally, we observed significant reduction in susceptibility for *P. multocida* to gentamicin (5%), neomycin (3%), and tylosin (3%) as compared to 2015. We observed significant reduction in susceptibility for *M. haemolytica* to clindamycin (2%) and for *H. somni* to chlortetracycline (11%), clindamycin (21%), neomycin (12%), oxytetracycline (5%), penicillin (5%), spectinomycin (8%), sulphadimethoxine (6%), tilmicosin (9%), tulathromycin (15%), and tylosin (9%) as compared to 2015. For *E. coli*, we observed a reduction in susceptibility to florfenicol (6%) and tiamulin (2%), while we observed a reduction for *Salmonella* species to florfenicol (4%), neomycin (5%), and TMP/Sulfa (7%) as compared to 2015. Though we noted reductions in susceptibility, many isolates remain susceptible to antimicrobials used to treat them.

Table 3: Antimicrobial susceptibility rates for bacterial BRD pathogens.

Bovine Respiratory Pathogens

Antimicrobial Agent	<i>Salm. spp</i>	<i>Past. mult.</i>	<i>Mann. haem.</i>	<i>H. somni</i>	<i>E. coli</i>	<i>Moraxella spp</i>	<i>Biber. trehalosi</i>
Ampicillin	64% (542)	100% (251)	90% (166)	99% (126)	46% (124)	100% (45)	68% (41)
Ceftiofur	N/A	100% (244)	100% (159)	98% (125)	61% (108)	N/A	N/A
Chlortetracycline	59% (542)	92% (251)	92% (166)	89% (126)	25% (124)	98% (45)	41% (41)
Clindamycin	0% (542)	0% (251)	1% (166)			8% (45)	0% (41)

				45% (126)	0% (124)		
Danofloxacin	N/A	100% (219)	100% (131)	N/A	N/A	N/A	N/A
Enrofloxacin	N/A	93% (244)	86% (159)	88% (125)	64% (52)	N/A	N/A
Florfenicol	43% (542)	96% (251)	95% (166)	94% (126)	10% (124)	95% (45)	83% (41)
Gentamicin	N/A	88% (251)	94% (108)	90% (69)	N/A	N/A	N/A
Neomycin	99% (542)	85% (251)	95% (166)	17% (126)	86% (124)	100% (45)	93% (41)
Oxytetracycline	82% (542)	39% (251)	78% (166)	17% (126)	67% (124)	98% (45)	80% (41)
Penicillin	59% (542)	60% (251)	73% (166)	55% (126)	23% (124)	86% (45)	20% (41)
Spectinomycin	0% (542)	95% (247)	70% (166)	79% (126)	0% (124)	0% (45)	0% (39)
Sulphadimethoxine	0% (542)	76% (251)	88% (166)	74% (126)	4%(124)	32.75% (45)	2% (41)
Tiamulin	42% (542)	48% (251)	69% (166)	43% (126)	38% (124)	77.75% (45)	68% (41)
Tilmicosin	0% (542)	51% (251)	92% (166)	95% (126)	0% (124)	100% (45)	71% (41)
Trimethoprim/ Sulfamethoxazole	N/A	78% (219)	90% (133)	93% (86)	N/A	N/A	N/A
Tulathromycin	0% (542)	70% (251)	85% (166)	80% (126)	0% (124)	81% (45)	78% (41)
Tylosin (Tartrate/ Base)	88% (542)	0% (241)	99% (166)	N/A	65% (124)	80% (40)	N/A

Staff Spotlight: Microbiologist Terra Nosbush



How long have you worked for WVDL? I started working at WVDL in Nov of 1999. Starting my 19th Year in November!

What do you do at WVDL? Microbiologist Senior in the Serology Department at the Barron Laboratory

What is the best part about your position? WVDL understands the importance of family. I have raised 3 children while working at WVDL. I have taken time off for births, several school activities, sicknesses, and other events. WVDL has always been very supportive of taking the time needed to be with family.

What do you like to do in your free time? I enjoy spending time with my family, camping and trail riding with my horses. I also volunteer, with my horse, as part of the Blue Hills Mounted Search and Rescue group.

[Click here for more info](#)

Client Services

Client services in Madison has been busy as the WVDL had a 12% increase in diagnostic testing in the 2017 fiscal year, which ended in June. To better handle large testing cases and pre-acquisition cases, we are expanding our workspace into adjacent rooms for better utilization and sample processing flow.

Dr. Don Sockett has been busy with herd visits and as part of the *Salmonella* working group that is investigating the multistate zoonotic *Salmonella enterica Heidelberg* outbreak with the Wisconsin Department of Public Health, Department of Agriculture Trade and Consumer Protection, and Centers for Disease Control. He presented some of their findings at the American Association of Bovine Practitioners (AABP) national meeting in Omaha, NE on September 15th and will be presenting again at the American Association of Veterinary Laboratory Diagnosticians (AAVLD, our accrediting body) in October.

We would like to remind our clients about best practices of sample submission with fecal and respiratory swabs. This information is also in the Virology section news. Please do not send fecal samples, or any sample, tied off in gloves or obstetric sleeves. Gloves tend to leak, which creates contamination issues and makes a mess. With PCR, sample contamination is a much bigger problem than it was in the past due to the extremely high sensitivity of the test. WVDL will send your practice sample tubes free of charge to collect feces in. The vials (also for great for milk samples) are locking and sterile and can be ordered by completing this form also available on the WVDL website - [Veterinary Supply Order Form](#). Please only fill the vials halfway so they do not explode in transport or if frozen. Also, all PCR swab testing (respiratory or environmental) needs to be done with non-cotton, non-wood shaft swabs submitted in M6 viral media, BHI, or saline. Bacterial transport media is not accepted for PCR testing. Please contact us with questions.

Another important reminder surrounds our partnership with the Wisconsin State Laboratory of Hygiene (WSLH) for rabies testing of horses. Due to the risk of zoonotic infection of Eastern Equine Encephalitis (EEE), all horse brains will be tested for EEE via PCR at the WVDL prior to rabies testing at WSLH. All testing cases for equine brains are reported to the Wisconsin Department of Public Health to assess the exposure risk. High risk cases activate on-call personnel to expedite testing over weekends and holidays.

We welcome back student employees Cynthia Papantonatos, Payton Pritzl, and welcome new student employee Tony Kalb to our Sample Receiving group.

Website Upgrades - This fall we will be upgrading the "Diagnostic Aids" button on our website to be more user friendly. The button will be changed to "Diagnostic testing and disease resources" and the information will be separated by the type of testing instead of what section the test is run in. Contact information and disease resources will also be included and cross-referenced. Please contact us with questions and comments at anytime.

Virology

Sample Submission Guidelines

We have been noticing an increased number of sample submissions, for virology testing, submitted using inappropriate media and/or containers. *Here's a quick reminder for proper sample submission:*

- To maximize the diagnostic potential of your submitted **swab samples**, the WVDL recommends using M6 viral transport media (red arrow) or sterile saline for any PCR testing. Samples should be sent on ice, overnight to WVDL, using our low-cost UPS shipping. Aimes charcoal (yellow arrow) and Porta-cul agar gel media are best used for bacterial culture and antimicrobial sensitivity testing but in general, bacterial culture transport systems have inhibitory substances that may interfere with PCR testing. **Aimes and all other bacterial culture media will NO LONGER be accepted for PCR tests.** See <http://www.wvdl.wisc.edu/index.php/optimize-your-diagnostic-testing-using-the-best-transport-media/> for more information.
- Fecal samples** for Enteric PCR testing should be submitted, individually, in leak proof containers. Specifically, exam gloves **must not** be used for submission of fecal samples! This avoids leaky samples, prevents cross-contamination and ultimately keeps the testing cost down. Currently we provide the 2 oz. sterile locking vials, free of charge and they can be ordered here: <http://www.wvdl.wisc.edu/wp-content/uploads/2017/01/VETERINARYSUPPLIESORDER.pdf>
- Please contact WVDL for larger submission so we can provide racks and insulated shippers.



2017 West Nile Virus Surveillance

From May 1 to October 31, WVDL Virology performs surveillance testing of corvids (crows, ravens and blue jays) for West Nile Virus for the Wisconsin Department of Health Services and in cooperation with the USDA Wildlife Services. Corvids are particularly susceptible to WNV and therefore surveillance of corvid mortality can serve as an early alert system for health professionals that West Nile is present in an area. Individuals who find a sick or dead bird may call the dead bird hotline at 1-800-433-1610. If the bird is suitable for WNV testing, the hotline operator will coordinate with the finder and the local health department to send the bird to WVDL for testing. When a county has a positive result, that county is closed and there is no further testing for the year.

So far 2017 has been a banner year for West Nile. As of 9/12/17, WVDL has tested 108 corvids, 77 of which have been positive. We've tested birds from 60 of Wisconsin's 72 counties, and of these, 59 have had a positive test (98%), breaking the record of 54 positive counties in 2017.

[Statewide map of Wisconsin counties with positive WNV tests.](#)

WVDL also provides testing for WNV in horses in post-mortem cases. The pathologist submits CNS tissue for real-time PCR for a panel of agents including West Nile virus (WNV), equine herpes virus 1 (EHV-1), and Eastern equine encephalitis (EEE). Ante-mortem testing using a serum sample and IgM capture ELISA is available at the National Veterinary Services Laboratory (NVSL) in Ames, IA, for both EEE and WNV.

2017 Eastern Equine Encephalitis

Eastern Equine Encephalitis (EEE) – In July 2017, virology section microbiologists tested brains from two quarter horse yearlings. Both horses had CNS deficits and clinical signs for 1-3 days prior to death/euthanasia. Both were strongly EEE PCR positive. This was the first report of EEE in Wisconsin in 2017. As of 9/7/2017 there have been 10 cases of EEE in Wisconsin.

Test Updates

Bluetongue ELISA kits have recently changed to a new kit from VMRD. We have verified the new VMRD BTV cELISA V2 kit and continue to confirm any suspect or weak POSITIVE samples on the VDT cELISA BTV kit.

BVD SN testing – the virology section is moving towards performing the Bovine Viral Diarrhea serum neutralization assay once/week based on sample load. This will improve efficiency in the section work flow.

Staff Update

Dr. Kathy Kurth recently retired after 17+ years as the WVDL Virology Section Head. Kathy's contributions to WVDL are extensive. Kathy was instrumental in WVDL becoming a Level 1 NAHLN laboratory, which prepared WVDL for the Avian Influenza outbreak, numerous foreign animal disease investigations and provided opportunities for employees to train and assist other labs nationally and globally. Kathy led the laboratory in establishing molecular diagnostics testing, which has expanded to high throughput and streamlining testing efficiency. Dr. Kurth's expertise on test development led to numerous new tests which met the needs of clients and bull stud regulations. Additionally, Kathy has been a subject matter expert for countless clients, state and federal agencies and the bull stud industry. Kathy was also a mentor for undergraduate and graduate students, taught at SVM, is part of a large Next Generation Sequencing Grant and the Shelter Medicine program. Dr. Kurth will be greatly missed and we wish her well on her next endeavor.



Pathology Sciences

In recent years the WVDL has experienced an increasing number of requests for necropsy cases for possible litigation ("legal cases"). These types of cases require a significant investment of resources and time by all sectors of the lab in order to provide results of sufficient detail for legal proceedings. To be able to continue to provide this valuable service to the highest of standards, the WVDL will be implementing a new legal fee structure, effective October 1st, 2017.

Legal necropsy cases will cost a flat fee of \$800.00 per animal. In addition to the services and tests included in a standard necropsy, this fee also includes chain of custody documentation, photodocumentation, radiography with interpretation by a board certified radiologist, referral tests (such as toxicological screening), and routine disposal. Cremation services are not included in this fee. Reduced fees for legal cases may be assessed in multiple animal cases at the case coordinators' discretion.

Bottle necropsy submissions will be charged the current \$165.00 legal fee in addition to the corresponding bottle necropsy fee. Lastly, we are developing a new legal submission form that will be required for legal cases so that we have complete information to assist in defensible diagnostic testing.

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