WVDL Summer Newsletter

August 2023

Wisconsin Veterinary Diagnostic Laboratory
UNIVERSITY OF WISCONSIN–MADISON

Message from the Director

Hello from the WVDL and from both our teams in the Barron and Madison locations. We hope your summer is going well, probably faster than we all would like, and that everyone can or has taken some time off to enjoy the summer weather. Thank you for taking the time to read our quarterly newsletter. As usual, we have much to report with changes and improvements to our testing catalogue. We are anticipating a busy fall season and are looking forward to working with our stakeholders and clients. Please let us know if you have any visitors coming to the World Dairy Expo that would like to stop by the WVDL or speak to someone from our team on the Expo grounds. Also, please save the date for Thursday, December 7th,

2023, for our annual bovine genetics meeting, which will be held at our Madison facility. This event is free and offers CE credits.

We have a few new faces in the WVDL, and I would like to specifically welcome Dr. Stephani Ruppert to our team. Dr. Ruppert is an anatomical pathologist, and we are very excited to have her at the WVDL! Stephanie is originally from Las Vegas and is finishing her year as a clinical instructor at the UW-SVM.

As always, please feel free to reach out with comments or questions. We always look forward to speaking with you.

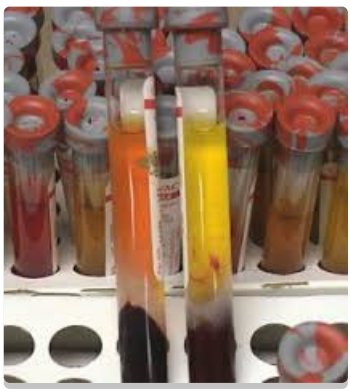
Enjoy the rest of your summer.

Keith

In this issue

Client Services Update

Reminder to avoid hemolysis in serum samples

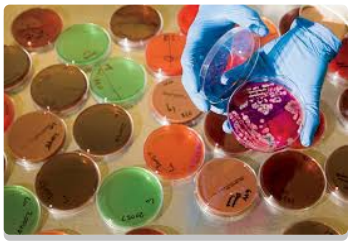


As we experience warmer weather, the WVDL would like to provide clients with some simple steps to take to avoid hemolysis of serum samples. To ensure optimal quality we highly recommend that collected whole blood be kept in tubes sitting upright at room temperature for a minimum of 30 minutes to a maximum of 60 minutes. For bovine samples, please leave tubes upright. For avian samples, leave tubes in a slanted position while clotting. This allows for the clotting factors to bind red blood cells through the serum. This timeframe allows the blood clot to form and minimizes the likelihood of any clotting factors remaining in the serum. If possible, samples may then be centrifuged and serum removed for submission. If centrifugation is not possible, samples should be chilled and

submitted directly to the laboratory for testing. **See more information below in the Serology Section for "Best Practices for Serum Submissions".**

Bacteriology Update

Changes to Antimicrobial Susceptibility Testing



As a reminder, the WVDL will continue to report less antimicrobials with interpreted categories as we continue to move to reporting only CLSI-approved antimicrobials. More details about why we are changing are described below.

In an effort to participate further in Antimicrobial Stewardship, the WVDL will be changing how we report antimicrobial susceptibility testing (AST). Antimicrobial Stewardship at the diagnostic laboratory level includes, "positively affecting clinical outcomes, help maintain antimicrobial effectiveness, assist clinicians in using antimicrobial agents safely, and minimizing the selection of resistant pathogens, laboratories must use a standardized, well-defined method for performing AST."1 The WVDL uses primarily the broth microdilution method, which quantitatively measures the *in vitro* activity of an antimicrobial agent against a particular bacterial pathogen. Antimicrobial agents, in a serial dilution, are prepared and mixed with a standardized suspension of the bacterium. The WVDL does not prepare these drug dilutions in-house, but rather relies on panels provided in 96-well plate formats by Trek Diagnostics (ThermoFisher Scientific). These are incubated and the minimum inhibitory concentration (MIC), which is the highest dilution (lowest concentration) of an antimicrobial drug that completely inhibits bacterial growth, is determined. Based on the MIC, the resistance, intermediate or susceptibility of an organism, from a particular host species and tissue to a particular antimicrobial is established using the Clinical and Laboratory Standards Institute (CLSI) breakpoints. A breakpoint is established by CLSI utilizing microbiological characteristics, pharmacokinetic-pharmacodynamic (PK/PD) parameters, and/or clinical outcome data. Veterinary-specific breakpoints were established with particular attention to the product label. The MIC for the particular pathogen-drug combination is used against the CLSI breakpoint established for that pathogen-drug combination to determine interpretative criteria which is susceptible, intermediate or resistant (see example). The CLSI guidelines also allow for >S as an interpretation which indicates 'not susceptible' and <R as an interpretation which indicates 'not resistant'.

Example: See chart below - *Escherichia coli* was isolated from a canine urine sample. The MIC for enrofloxacin was 0.25 µg/mL. Using the breakpoints listed below, the MIC for the isolate is categorized as susceptible because it is < 0.5 µg/mL.1

The CLSI guidelines are specific to a particular bacterium isolated from a particular host species' tissue. As an example, there are specific breakpoints for particular antimicrobial agents that have been established for bovine respiratory disease pathogens such as *Pasteurella multocida*, *Mannheimia haemolytica* and *Histophilus somni*. These breakpoints do not apply to these bacteria isolated from non-respiratory tissues from cattle. Additionally, these breakpoints do not apply to non-bovid species such that a *P. multocida* isolated from a cat would not get these same breakpoints. Therefore, the CLSI guidelines utilize a grouping system for interpretations of antimicrobial agents and their uses for veterinary pathogens.

- **Group A:** includes antimicrobial agents with VETERINARY-SPECIFIC breakpoints and interpretive categories that are considered appropriate for routine, primary testing for food and companion animals. These antimicrobial agents are considered first to report and use, and are preferred overusing those with human medical breakpoints. These Group A compounds have demonstrated an acceptable level of correlation between *in vitro* susceptibility test results and clinical outcome.

- **Group B:** includes antimicrobial agents with veterinary-specific breakpoints and interpretive categories but are considered antimicrobials that should only be tested and reported as 'drugs of last resort'. The Subcommittee on Veterinary Antimicrobial Susceptibility Testing (VAST) considers these antimicrobials to be 'drugs of last resort' and concern exists for selecting for resistance, which could be transferred from animals to humans. The veterinary laboratory can report these at their discretion but are mostly used as antimicrobial resistance monitoring.

- **Group C:** includes antimicrobial agents that use HUMAN medical breakpoints and interpretive categories. These agents may perform adequately, but outcomes for many veterinary applications have not been demonstrated. The veterinary laboratory can report these at their discretion.

- **Group D:** includes antimicrobial agents that are regulatory agency-approved for use in specific animal species. Although quality control data is available, these antimicrobial agents DO NOT have CLSI-approved veterinary-specific or human medical breakpoints or interpretive categories. These agents may be approved for use in other animal species and have veterinary-specific breakpoints in those animals. However, it is not recommended to use breakpoints set for a particular animal species to be applied to a different animal species. This is because there are differences in dosages and pharmacokinetics between animals, people and between animal species. Thus, these agents should be reported selectively before extra-label use agents (Group D), but after agents in Group B.

- **Group E:** includes antimicrobial agents that are NOT APPROVED but may be used in an extra-label manner per the Animal Medicinal Drug Use Clarification Act of 1994 (AMDUCA) guidelines in the US. These agents may be selective tested and reported and are often used for antimicrobial resistance monitoring. Group E may also include certain antimicrobial agents that are used only for a specific infection site (such as nitrofurantoin for treating urinary tract infections) in non-food-producing animals.1

See the chart for antimicrobial agents that could be considered for routine testing by veterinary microbiology laboratories here.

Currently, the WVDL provides interpretations based on CLSI guidelines as well as some breakpoints supplied by Trek Diagnostics (ThermoFisher Scientific). The breakpoints supplied by Trek Diagnostics will no longer be used. The WVDL will solely be using breakpoints supplied by CLSI and will be reporting as found within the chart for antimicrobial agents on the WVDL website. Therefore, the WVDL will report mostly Group A and Group C antimicrobial agents based on the pathogen, what host species, and the location on that host species the pathogen was isolated from. On occasion, some Group B, D and E antimicrobials may be interpreted with an MIC and interpretive criteria based on CLSI Vet011 and Vet092 guidelines. An example is applying *M. haemolytica* breakpoints for bovine respiratory disease to other members of the *Pasteurellaceae* family is acceptable such as *Biberstienia* and *Gallibacterium* species.2 As well, the CLSI Vet09 extrapolates the *Staphylococcus aureus* breakpoints and interpretive criteria for bovine mastitis so that Gram-positive cocci (but not *Enterococcus*) can be

interpreted.2 Interpretations for bovine respiratory disease, metritis and mastitis have been extrapolated for camelid, caprine, cervid and ovine species. **Therefore, the WVDL would like to remind clients that there will be less antimicrobials reported with interpreted categories as we continue to move to reporting only CLSI-approved antimicrobials and improving the visual look of the reports.** Veterinarians can always contact the WVDL for more information regarding AST or if additional antimicrobial agent breakpoints are needed.

1CLSI Performance Standards for Antimicrobial Disk and Dilution Susceptibility tests for Bacteria Isolate from Animals. CLSI, Vet01, Edition 5.

2Understanding Susceptibility test Data as a Component of Antimicrobial Stewardship in Veterinary Setting. CLSI, Vet09, Edition 1.

Interpretive Category	Enrofloxacin Breakpoints (MIC, µg/mL)
Susceptible	≤ 0.5
Intermediate	1 – 2
Resistant	≥ 4

Molecular Diagnostic Update

Tritrichomonas foetus PCR Sampling Update

Trichomonad
(photo courtesy CDC)

The WVDL has recently validated a new *Tritrichomonas foetus* PCR assay which resulted in an increased sensitivity allowing for elimination of the pre-PCR overnight culture step, thus added flexibility for utilization of additional media for sample submission. **Starting September 1, 2023, samples submitted for PCR testing ONLY can be submitted in either 1.5 ml of saline or Lactated Ringer's solution or the traditional TF InPouch™.** Please review the UPDATED submission guidelines on the WVDL website here.

The WVDL recommends sending *Tritrichomonas foetus* PCR samples to the lab as soon as possible after sample collection; preferably within 24-48 hours after collection. Samples should be refrigerated if not ship after collection to reduced growth of bacterial/fungal contaminants. Shipping with ice pack is preferred, but may still be sent at room temperature. For PCR, we can pool up to 5 samples for more cost effective testing. However, pooling should not be used when fulfilling a regulatory testing requirement. The PCR assay is run Mondays, Wednesdays and Fridays.

The requirement for *Tritrichomonas foetus* **culture** remains unchanged, if culture and PCR are needed, 2 samples must be submitted. **A TF InPouch™ is required for culture testing**, and must be submitted at room temperature or with warm packs to maintain viability of the organisms. The Biomed Diagnostic TF Transport Tubes™ media is not recommended for culture or PCR. Please review the UPDATED submission guidelines on the WVDL website here.

Cryptosporidium parvum PCR Update

The WVDL has completed PCR improvements on the *Cryptosporidium parvum* assay. These improvements included a detailed investigation of the sampling process, nucleic acid recovery procedure, and the PCR assay itself, resulting in a significant improvement in the assay sensitivity. The improvement has already been implemented with no changes to the test requests or cost to our clients.

Free 24-Well Plates for the Collection of Ear notch Samples for BVD PCR Pool Testing

The WVDL is currently in the process of validating 24-well plates for ear notch collection. We are sending out these plates to clients to solicit feedback on ease of use for ear notch collection. The sample submission process will be similar to swabs samples that are collected in 96-well plates. Use of these plates will eliminate the need to label tubes and package the ears in individual tubes; thus, saving time on farm. One plate will hold 24 ear samples for one pooled PCR. With this new sampling method, we will still be able to test the ears individually if the pool result is positive to determine which animal in that pool is positive thus ensuring individual sample identification. Plates and lids are available at no charge by emailing supply.room@wvdl.wisc.edu. Please complete the submission form and survey sent via email when supplies are shipped so we may assess the ease of use and client interest in this new process.

Pathology Update

Chronic Wasting Disease (CWD) Confirmed in Florida

Florida becomes the 31st state to confirm Chronic Wasting Disease (CWD) after a 4-year-old white-tailed deer from Homes County tested positive.

Following confirmation of a positive test sample for chronic wasting disease (CWD) in Holmes County, the Florida Fish and Wildlife Conservation Commission (FWC) and Florida Department of Agriculture and Consumer Services (FDACS) have implemented management actions and an executive order to protect against the possible spread of CWD.

The FWC and its agency partners take CWD very seriously and have implemented a comprehensive response plan. As part of the plan, the FWC will collect samples from specific established zones to further assess the spread of the disease. The results from this initial sampling effort will inform resource managers so they can respond with appropriate management strategies.

CWD Testing Lab Employment Opportunities

WVDL now hiring seasonal staff to work in the CWD Testing Lab

Job Summary:
Chronic wasting disease (CWD) is a prion disease that continues to spread throughout Wisconsin and the nation. It affects deer, elk, reindeer, sika deer, and moose. CWD is fatal to animals and there are no treatments or vaccines. The team at WVDL provides services that diagnose CWD, which helps protect consumers and determine the spread of the disease. With the hunting season fast approaching, WVDL is temporarily increasing the size of their team to accommodate the workload.

Prior experience as a Microbiologist is not required but is helpful. WVDL is willing to train the right candidate to prepare and test for CWD. The responsibilities involve multiple aspects of TSE diagnostics including performing sample collection and preparation; testing with TSE plate assays and performing specialized test procedures to analyze specimens for the presence of prion pathogens. Familiarity and experience with tissue handling and preparation techniques, ELISA procedures, histotechnology, and immunohistochemistry are highly valued along with being detailed orientated. Specific experience with TSE testing is also a plus. This position will perform repetitive motions.

How to Apply:
To ensure consideration, complete applications must be received by no later than 11:55pm CDT on the assured consideration date. Applicants are asked to upload a resume and contact information for three professional references including a current or former supervisor.

Salary will be assigned within the appropriate range, commensurate with the candidate's qualifications and experience. Anticipated start date is on or after September 11, 2023.

The WVDL is committed to providing an environment of mutual respect, integrity, trust and accountability where employment is decided on the basis of qualifications, merit and business need. The WVDL believes that diversity and inclusion among our teammates is critical to our success as a lab, and we seek to recruit, develop, and retain the most talented people from a diverse candidate pool. We strive for continuous improvement by setting our goals high and expecting high standards using our core values.

Continuing Education for Pathology Sciences Microbiologists at the WVDL

Tori Smith, a WVDL Pathology Sciences Microbiologist and Histotechnician, will be attending educational and training events in the coming months. August 15th-17th, the National Animal Health Laboratory Network (NAHLN) and the USDA will be hosting their annual Quality Management Systems training. Through an interactive class environment, participants receive training on quality system requirements, the accreditation process, document control, internal auditing, and root cause analysis. Attendees will also conduct an audit and learn how to recognize non-conformances. These are all items that the WVDL Quality Team requires focus on in order to keep the quality of work at the WVDL at its best.

In September, Tori will also be traveling to Baltimore, Maryland, to attend the National Society for Histotechnology (NSH) Convention. She will be presenting *"Histotechnicians in the Veterinary Diagnostic Laboratory"* at the convention. Most convention attendees work in the human medical field and Tori will be introducing participants to the tasks and tests completed at the veterinary diagnostic laboratory. She will also be attending numerous presentations on many topics ranging from bone

histology to immunohistochemistry optimization. She will be volunteering at the convention for the **2023 Career Day**, which allows volunteers to demonstrate tissue handling, embedding, cutting, staining, and cover slipping. In addition, volunteers will have the opportunity to work with local Baltimore High School students, teaching them about the realm of histology and what an average day of a histotechnician might be like.

As the incoming President of the Wisconsin Histology Society, Tori will end the convention by attending the House of Delegates (HOD) annual meeting along with two other Wisconsin delegates. The HOD is made up of representatives from each state in which NSH has active members. The delegates are responsible for amending Bylaws, approval of Honorary members, voting on recommendations by the Board, and creating committees.

These conventions provide great educational opportunities to our pathology sciences staff, not only through the knowledge learned by attending workshops, poster sessions, and vendor education, but through the connections and networking with other Histotechnicians from across the globe.

Serology Update

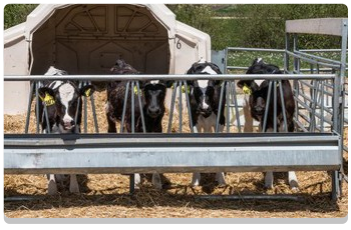
Changes to BLV AGID Testing

The WVDL has long used the BLV AGID kit from Veterinary Diagnostic Technology, Inc. (VDT; Wheat Ridge, CO). However, this company no longer produces the BLV AGID kit, and we are nearly ready to change manufacturers to the Innovative Diagnostics (ID) Vet BLV AGID kit. This kit performed similarly as the VDT kit, but with a slight increase in sensitivity. The kit change will occur approximately August 15, 2023, depending on the tests that are requested. We will use all of the VDT kit reagents prior to making the switch to ID Vet. Clients will not recognize any changes in their results when we switch kit manufacturers and are encouraged to contact the WVDL if there are any questions.

Changes to Bovine Serological Testing for Export Purposes

Starting the week of August 7, 2023, the EHD AGID will now be set up on Tuesdays rather than Mondays and the Q-Fever Complement Fixation (CF) Test will now be set up on Mondays rather than Tuesdays. These changes are to increase efficiency of these tests for our Export Clients. Please contact the WVDL if you have any additional questions or concerns.

Increase in positivity rate for the BLV cELISA (ID Vet) for cattle less than 9 months of age



Upon switching from the VMRD BLV ELISA kit to the ID Vet cELISA kit, the WVDL observed an increase in positive and inconclusive results. Upon analysis, we have found an increase in positive and inconclusive BLV cELISA results specific to cattle **under** the age of 9 months. For cattle **under** the age of 9 months, the positivity rate for BLV-specific antibodies is 2.3% (VMRD kit) and 17.3% (ID Vet kit). The WVDL is currently using ID Vet BLV cELISA and therefore, has an increased positivity rate for cattle **under** 9 months of age. However, for cattle over the age of 9 months, the positivity rate is very similar. Therefore, it is possible that the ID Vet

BLV cELISA kit is more sensitive for maternal antibodies. It is also possible that the sampling group has changed between 2022 and 2023. We want our clients to be aware of the increased positivity rate with bovine serum where the animal is **under** 9 months of age.

Changes to the avian encephalomyelitis (AE) ELISA test method for turkey sera

In 2014, the WVDL validated a test method modification to the AE chicken ELISA utilizing a conjugate composed of a turkey antibody to increase specificity for turkey sera. Unfortunately, the WVDL has observed lot-to-lot variation in the concentration of enzyme-linked turkey antibody within this conjugate. To ensure we continue to provide reliable results, a validation study of the AE chicken ELISA utilizing the manufacturer's test method with turkey sera was completed. This study confirmed turkey sera performed with 96% specificity and 100% sensitivity. Additionally, we confirmed that other diagnostic laboratories are utilizing the same AE chicken ELISA for turkey sera utilizing the manufacturer's kit components and procedure. As we complete this test method change, please note that the current report disclaimer stating that ***"This test is USDA licensed for use with chicken sera. It has not been approved for use with other avian sample types."***, is accurate and will remain but have assurance that the completed validation study leaves us confident turkey sera performs well on this assay. This method change will take place in August, at which time, a slight decrease in AE titer values for turkey's can be expected.

Best Practice for Serum Submissions

- Collect blood for serum submissions in an appropriate tube with no additives (glass, glass serum separator, plastic – polystyrene or polypropylene) and allow to clot at room temperature for 30-60 minutes. This timeframe is essential for blood clot formation and minimizes the likelihood of any clotting factors remaining in the serum.
- Centrifuge blood, if able, and remove serum from the clot for submission. If centrifuging is not possible, collecting a greater volume of sample is recommended to ensure the laboratory is able to obtain an adequate volume of serum that is free of red blood cells.
- Protect blood samples from direct sunlight, extreme heat/freezing and vigorous mixing to prevent hemolysis and degradation of serum.
- Be mindful of extreme temperature shifts. On warm weather days, blood should be kept in a chilled cooler. Or on cold weather days, blood samples should be kept in a cooler with warm packs. Do not allow cold or warm packs to have direct contact with the samples.
- Avoid jostling, vibrating, or excessive movement of the blood tubes such as leaving the blood tubes on the vehicle's dashboard (which may also be hot) or on the floor.
- Excessive serum lipids (clotting factors) and hemolysis (lysed red blood cells) both have an interference effect on serology assays and will be rejected. Lipemic serum will appear thick/milky after centrifugation. Hemolyzed serum will appear dark red with little differentiation between the clot and serum. It will also leave a pink residue along the inside wall of the specimen tube when twirled. These samples should be discarded and a new sample shall be collected for testing.

Virology Update

BVDV virus isolation update

The WVDL performs virus isolation on serum and buffy coat samples for the detection of bovine viral diarrhea virus (BVDV). A buffy coat sample (collection of lymphocytes) is the required sample type from calves **less than six months of age** due to the presence of maternal antibodies in serum that may have the potential to clear virus giving a false negative result. The buffy coat sample submission is desirable for the following reasons:

1. BVDV is isolated more frequently from lymphocytes than serum.
2. During the acute phase of BVDV infection the virus can be isolated from lymphocytes for up to one week longer than with serum.
3. BVDV can usually be isolated from lymphocytes even if the animal has neutralizing antibodies to the virus.

A fresh sample is best for optimal buffy coat sample BVDV recovery. Hemolysis in older whole blood samples greatly reduces the buffy coat recovery rate. Viable virus particles in a sample are the key to the success of the virus isolation protocol. The virology section has recently completed several trials to determine a post-collection sample age cut-off for virus isolation and results demonstrated that viral viability is reduced as the samples aged. Therefore, **beginning 8/1/2023, we will reject samples if whole blood in EDTA is received > 7 days from the bleed date.** Due to the time-sensitivity to maintain sample freshness, we will need to have enough cells ready for inoculation upon receiving samples. **Advanced scheduling is REQUIRED when submitting greater than 20 samples at one time.** Please contact the virology section at least one week prior to submission.

CONTACT US

Facebook @wvdl_lab

Wisconsin Veterinary Diagnostic Laboratory Providing You With Reliable Results and Exceptional Customer Service

Wisconsin Veterinary Diagnostic Laboratory - Madison
445 Easterday Lane
Madison, WI 53706
Phone: 608. 262. 5432
Toll Free: 800. 608. 8387
Fax: 608. 504 .2594

Wisconsin Veterinary Diagnostic Laboratory - Barron
1521 E. Guy Avenue
Barron, WI 54812
Phone: 715-637-3151
Toll Free: 800-771-8387
Fax: 715-449-5052

445 Easterday Lane, Madison, ... info@wvdl.wisc.edu
1.800.608.8387 wvdl.wisc.edu/