Wisconsin Veterinary Diagnostic Laboratory
UNIVERSITY OF WISCONSIN–MADISON

Newsletter - Winter 2020

Message from the Director

Happy New Year from the WVDL, we wish you the best in 2020! We have been busy in the Madison and Barron laboratories and are excited to tell you about news and events in our quarterly newsletter. Please feel free to pass this on to other team members in your organization.

I would like to thank Alissa Grenawalt for her service on the WVDL Board of Directors, representing UW-Extension. Alissa served for 7 years on our Board and we are sad to see her go. Alissa will be replaced by Dr. Larry Baumann, who is an extension veterinarian and faculty member at UW-River Falls. We hope to see Alissa around at a county fair or other event and are excited to work with Dr. Baumann.

Every year we look at our operational costs and testing revenue to decide if, and by how much, fees should be increased. For FY21, which starts July 1, 2020, we will be raising our diagnostic testing fees by 3%. Not all fees are applicable to this increase, which is tied to the consumer price index and our costs of testing services. The WVDL is ~35% funded by state taxpayer dollars and the remaining majority of our operational budget is generated by diagnostic testing fees.

Below, you will find timely information on submission of EIA testing. The USDA has changed the requirements for the information on forms and we can help you get right information to ensure timely ELISA test turnaround. You will also find important updates and information about Johne's Disease testing, EHD AGID testing (for export purposes), and some information on diagnostics for my favorite pathogen, *Listeria monocytognes*.

We also have some important announcements about our Saturday hours and shipping information for Saturday delivery.

Thank you for taking the time to read our quarterly newsletter and please contact us at any time with questions.

Keith Poulsen
Director

Barron

Changes to EIA Test Requirements
The USDA has made changes to the EIA (Coggins) test requirements that affects owners, veterinarians and laboratories that conduct this test. These changes include a new version of the VS Form 10-11 (see Fig. 1 below). These requirements went into effect January 1, 2020. If the requirements are not met by submitting veterinarians, the WVDL will be forced to hold results until rectified.

If you are in need of forms or assistance, the WVDL will be happy to accommodate and assist you. Below is a list of new requirements the WVDL feels our clients should be made aware.

- VS Form 10-11 (Fig. 1 below) must be completed in its entirety. **All boxes on the form must be entered.**
  - If a box has information that is not applicable, please write “N/A”, none, strike a line through the box, or write “same as ….”. *No box on the form can be left blank*.
- The WVDL will continue to offer only the EIA ELISA test. If an EIA AGID test is needed, we will refer the testing to NVSL in Ames, Iowa.
- Please enter the county the equine resides in.
  - This is a new requirement from the USDA.
- Another area of emphasis is the National Accreditation Number. All submitting veterinarians are expected to obtain and maintain Category II veterinary accreditation status.
  - We are required to verify accreditation numbers on the USDA website annually. Please do not enter your state veterinary license.

All permanent markings, brands, tattoos, scars, etc. shown on the figures must be described in the boxes below the figure.

If you have any concerns or questions about filling out the new form, please contact us, we are more than willing to be of assistance. If we receive forms that are incomplete or do not meet the requirements, we will be contacting the submitting veterinarians to correct the issues so that results can be released as soon as possible. We appreciate your patience in adopting this new form and look forward to assisting you as needed.

FIGURE 1

GPO U. S. GOVERNMENT PRINTING OFFICE: 2018–404-689

USE TYPEWRITER OR PRINT CLEARLY - PRESS HARD - YOU ARE MAKING 5 COPIES

According to the Paperwork Reduction Act of 1995, an agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0579-0127. The time required to complete this information collection is estimated to average .083 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

OMB Approved
0579-0127

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

EQUINE INFECTIOUS ANEMIA TEST FORM

FORM SERIAL NUMBER
AA 226712

COMPLETETION OF ALL UNSHADED NUMBERED FIELDS IS REQUIRED FOR SUBMISSION, IF NONE WRITE "NONE" AND TYPE OR PRINT LEGIBLY.

1. LABORATORY ACCESSION NUMBER (*for laboratory use only*)	2. DATE BLOOD DRAWN	3. TEST REQUESTED BY VETERINARIAN ☐ ELISA ☐ AGID

4. REASON FOR TESTING
☐ Interstate Movement ☐ Within State Use/Annual ☐ Change Ownership/Sale ☐ International Import/Export ☐ Illness/Clinical Suspect ☐ Investigation/Exposure

5. LOCATION OF EQUINE AT BLOOD DRAW (*ranch, farm, stable, or market*)	7. NAME AND ADDRESS OF OWNER
5a. NAME	7a. NAME
5b. PHYSICAL/STREET ADDRESS	7b. MAILING ADDRESS
5c. CITY, STATE, ZIP CODE	7c. CITY, STATE, ZIP CODE
5d. TELEPHONE NUMBER	6. COUNTY OF EQUINE AT BLOOD DRAW / 7d. TELEPHONE NUMBER

I CERTIFY I AM A CATEGORY II FEDERALLY ACCREDITED VETERINARIAN, AUTHORIZED IN THE STATE WHERE THE SAMPLE WAS OBTAINED, BY ME, FROM THE ANIMAL DESCRIBED BELOW.

8. ACCREDITED VETERINARIAN

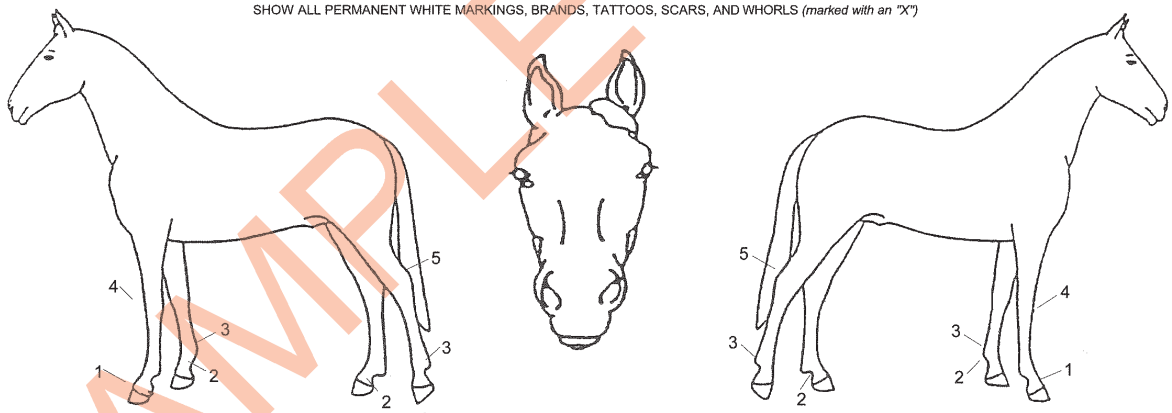
8a. VETERINARIAN NAME	8b. NATIONAL ACCREDITATION NUMBER	8c. VETERINARIAN SIGNATURE	8d. SIGNATURE DATE
8e. PHYSICAL/STREET ADDRESS OF VETERINARIAN	8f. CITY, STATE, ZIP CODE		8g. TELEPHONE NUMBER

9. Tube Number	10. Tag/Tattoo/Brand Number	11. Name of Animal	12. Color	13. Breed (*or species if not a horse*)	14. Age or DOB	15. Sex

M - Male Intact
F - Female Intact
G - Gelding
FS - Female Spayed

16. MICROCHIP, BREED, OR REGISTRATION NUMBER

SHOW ALL PERMANENT WHITE MARKINGS, BRANDS, TATTOOS, SCARS, AND WHORLS *(marked with an "X")*



1 - Coronet, 2 - Pastern, 3 - Fetlock, 4 - Carpus, 5 - Hock

REQUIRED: *NARRATIVE DESCRIPTION OF PERMANENT WHITE MARKINGS, BRANDS, TATTOOS, SCARS, AND WHORLS. (If none write none) Suggested nomenclature includes Heel, Heels, Coronet(1); Half Pastern, Pastern(2); Fetlock(3); Half Canon, Canon, Carpus/Hock(4/5) above Carpus/Hock*

17. HEAD	18. NECK AND BODY (*include coat color patterns, if any*)
19. LEFT FORELIMB	20. RIGHT FORELIMB
21. LEFT HINDLIMB	22. RIGHT HINDLIMB

FOR LABORATORY USE ONLY

23. EIA LABORATORY NAME	24. DATE SAMPLE RECEIVED	25. DATE RESULTS REPORTED	26. OFFICIAL TEST RESULT ☐ Negative ☐ Positive	27. TEST TYPE USED ☐ AGID ☐ ELISA
	28. LABORATORY REMARKS			
23a. CITY				
23b. STATE	29. SIGNATURE OF NVSL - APPROVED EIA TECHNICIAN		30. INTERIM RESULT REFERRED FOR CONFIRMATION ☐	

FALSIFICATION OF THIS FORM OR KNOWINGLY USING A FALSIFIED FORM IS A CRIMINAL OFFENSE AND MAY RESULT IN A FINE OF NOT MORE THAN $10,000 OR IMPRISONMENT FOR NOT MORE THAN 5 YEARS OR BOTH (18 U.S.C. SECTION 1001).

SAMPLE FORM

Avian Serology Antigen on Backorder

The WVDL is experiencing a delay in our order of the *Mycoplasma gallesepticum* (MG) plate agglutination antigen. The company that supplies the lab with the antigen currently has the product on backorder. We still have stock and hope to have enough antigen to keep performing the test until the order is

Microbiology

Uptick in the Number of Listeria Cases
Wisconsin and several other mid-western states had a record amount of precipitation in 2019 particularly in the spring and fall. This has caused many livestock operations to feed less than ideal hay, corn silage, haylage and baleage to cattle and small ruminants (sheep and goats). A veterinary practitioner from southern Wisconsin has reported to the WVDL that he is seeing a marked increase in clinical listeriosis in cattle, sheep and goats. Typically *Listeria monocytogenes* causes sporadic neurological disease is cattle, but it has the ability to cause herd outbreaks of neurological disease particularly in sheep and goats. Ideally, practitioners should submit both fresh and formalin fixed tissues from aborted fetuses and brain stem samples from animals that have died of neurological disease. Please completely fill out the WVDL General Submission Form when sending samples to the lab.

The WVDL has two diagnostic tests that can be used to test livestock for *Listeria monocytogenes*. The first is traditional bacteriological culture, including the screen, which is a 2-4 day culture using selective medias and the second is cold-enrichment culture, which utilizes the screening selective medias as well as a cold-enrichment broth that is subcultured weekly for four weeks. The cultures are performed on fetal stomach contents collected from aborted fetuses as well as brain stem samples collected from animals that have died of neurological (circling) disease. Additionally, the WVDL offers a *Listeria monocytogenes* specific real time PCR assay performed on feces, tissues and bacterial isolates. The *Listeria monocytogenes* real time PCR assay is very accurate and it also reduces the turn-around time for laboratory results, particularly for the cold enrichment culture.

Johne's Disease Diagnostics
Johne's disease continues to be a challenge with multiple options available for diagnostics, some of which are specific to the host species.

ELISA - Valuable diagnostic information can be gained from a quantitative interpretation of the Johne's ELISA. In general, the ELISA value is a measure of the concentration of serum antibodies to *Mycobacterium avium* subspecies paratuberculosis (MAP) the causative agent of Johne's disease. Generally, serum antibody levels increase as the infection progresses. Animals with higher ELISA values are more likely to be shedding the bacterium in milk and colostrum and be heavy fecal shedders than lower scored animals. High ELISA scored animals are also at increased risk of developing clinical Johne's disease. There are several commercial kits available for the detection of antibodies specific to MAP. The WVDL has validated the VMRD MAP Antibody Test Kit for bovine milk and sera as well as caprine sera. The WVDL is also offering the Zoetis SERELISA kit, which can be used upon request. It is important to remember that each kit manufacturer develops proprietary reagents such as antigen and conjugates (antibodies) that may not be the same. It is possible that one serum sample could test positive with one kit, but be negative with the other. This is because the antibody in the serum may only bind to the one antigen or antigenic site from one kit manufacturer, but not the other kit manufacturer's antigen. The only way to confirm if the animal is infected with MAP is to send a fecal sample for direct PCR or liquid culture.

DIRECT PCR - At the WVDL, we use an in-house validated and NVSL proficiency tested Direct Fecal PCR that detects MAP extracted from fecal samples using three different genes including IS900, the most recognized gene target for MAP. An interpretation protocol has been established based on over 2,500 diagnostic samples of multiple different species in order to utilize these three targets to provide the most comprehensive MAP detection available. In order for a sample to be considered positive, two of the three targets must be detected in the multiplex PCR in which one must be IS900. The IS900 target cycle threshold (C_T) is the value that is reported on the result report since this target is the most sensitive due to being present in multiple copies in the genome. The validation completed at WVDL has shown that the combined use of these three targets in one assay is a reliable tool for MAP detection and limits cross-reactions from members of the *Mycobacterium avium* complex, which have been demonstrated by other researchers (Prendergast et al., 2018) as well as observed by the WVDL particularly from fecal samples from exotic species.

For a small subset of single samples (less than 0.01%), particularly for exotic samples, the interpretation using a three-target multiplex PCR could not accurately be determined as only one target was detected in the fecal sample. This data, combined with the animal histories, required us to create a new test interpretation of "**undetermined**". The test result of undetermined will be entered on a result report based on our validation criteria, and WVDL recommends retesting this animal within six months.

The test may be used in a herd Johne's Disease control program and may also be used as a primary diagnostic test for individual animals with clinical signs suggestive of Johne's disease. A not detected/undetermined result does not rule out the possibility that an animal is infected. A fecal sample from an animal shedding MAP in very low numbers may fall below the limits of detection for the test. Interpretation of individual animal's results should be done by the herd veterinarian in conjunction with a thorough consideration of on-farm management practices, herd history for Johne's disease and concurrent testing information gathered from several animals in the herd.

LIQUID CULTURE - At the WVDL, we use a culture system for the replication and detection of MAP. This test is mostly used for regulatory purposes and the WVDL recommends the Direct PCR to be used when the testing is not for a regulatory purpose. Other reasons the test may be used include: the submitter requires a live organism, the submitter suspects a member of the *Mycobacterium avium* complex that is not MAP, or if the submitter requires that the sample be cultured to confirm viability. For bovine samples, we allow for the submitter to determine if they would like a confirmatory PCR on all samples or just those samples that are positive post-culture. However, for all other species including goats, sheep and exotic animals, the PCR following liquid culture is required. Acid-fast staining is used to quantify the *Mycobacterium* species. Data supports that the PCR and liquid culture are approximately equal in sensitivity and some literature suggests that the PCR is more sensitive. However, liquid culture requires extensive incubation periods and is not ideal for diagnostic testing. Therefore, the WVDL recommends the direct fecal PCR rather than liquid culture.

The liquid culture system uses feces diluted in sterile water, followed by two overnight enrichments in broth with antibiotics to enrich and select for *Mycobacterium* species. Therefore, there is a three-day setup period prior to incubation. Samples are only setup on Monday, Tuesday and Wednesday. Afterwards, the sample is mixed with proprietary media, as described by the manufacturer, and incubated in capped bottles for 42-49 days for bovine samples and 49-56 days for caprine, ovine and other species samples. All positive samples are confirmed using acid-fast staining and PCR. The culture results are semi-quantitative for bovine samples only.

For both the Direct PCR and liquid culture, fecal samples can be pooled in groups of five of the same species. Pools are made at the laboratory and all positive pools are automatically run individually in order to identify the positive sample(s). Pools comprised of multiple days collected from the same animal will not be tested individually if the pool is positive unless specified by the submitter.

The Johne's Disease Diagnostic Submission Form has been modified to reflect the various options available. Please be sure to check the box for pooled whenever pooling is needed.

Staff Spotlight: Kathleen Reed, Client Services

Where are you from? What high school and college did you graduate from? What are your degree(s) in?

- I grew up in Monona, Wis., and currently live in Maple Bluff. I attended Monona Grove High School and then UW-Madison where I earned a BS and MS in Biomedical Engineering. Currently I'm working on my Masters of Public Health here at UW-Madison.

- I'm not currently reading a book but the last one I read was the Potlikker Papers by John T Edge.

What is the one food that you will never bring yourself to eat?

- Lutefisk ... There are much better ways to eat catfish.

What is your favorite movie soundtrack?

- It is hard to pick so I'll say just about anything by John Williams.

What three flavors of ice cream would be on your triple scoop cone?

- Zanzibar Chocolate, Zoreo and This $&@! Just Got Serious from the Chocolate Shoppe.

When you were a child, what or who did you want to be when you grew up?

- When I was a kid, I wanted to grow up to be as tall as my uncle who is 6'4" ... I only made it to 5'1".

What pets did you have growing up?

- My family had two cats growing up. One was a typical cat and the other was more like a dog. My brother trained him to come when we called his name and he would sit up on his back legs to beg for bacon and lunch meat.

What skill would you like to master?

- I would like to learn how to play the piano, but I need to find the time. Maybe one day when I am not working, and going to school, and volunteering with the fire department

If you could be any animal in the world, what animal would you be and why?

- I would be a Halloween Moon Crab because they look amazing.

What is the favorite part of your job?

- My favorite part of working at WVDL are all of the amazing and interesting things I get to learn about animals and animal diseases. A close second is all of the quirky animal names that owners come up with.

Virology

AGID Testing Update
In summer 2019, the sole provider of USDA licensed manufacturer of Epizootic Hemorrhagic Disease (EHD) agar gel immunodiffusion (AGID) assays ceased business operations.To address this testing shortage, the WVDL-Virology section validated an Australian EHD AGID assay.

The Australian kit passed the WVDL validation process, however it is not a USDA approved kit. USDA is currently approving - the VMRD BTV AGID kit for use of EHD testing. This kit did not pass WVDL validation, and will be used in conjunction with the Australian EHD AGID kit upon specific request for export testing.

Based on your testing needs, the following EHD AGID test options are available. **Please be very specific when making your test request choices to avoid a delay in test set up.**

1. **Pre-purchase/screening** testing will be done with the Australian EHD AGID test kit only, to save costs. **The cost of this test is $7.50 and the test code is EHDAUSAGID.**
2. **Export** testing will be done with **both** the Australian test kit and VMRD test kit simultaneously to maintain turnaround time. Because the sensitivity of the VMRD kit is low, it is unlikely that we will have an Australian NEG and VMRD POS. However, it is likely that a bull could be Australian AGID POS and VMRD NEG. These bulls will be history checked and clients will be notified of the POSITIVE or WEAK POSITIVE result prior to finalization. RETEST REQUIRED may be used in this case, as appropriate. **The cost of this testing is $15 and the test code is EHDEXPAGID.**
3. Utilize our EHD SN for serology testing that does not require the AGID test method. **This test code is EHD1&2SN and is $12.85.**

In the meantime, we are exploring the potential for an EHD ELISA, which is an OIE approved method, and will be an acceptable option for serology testing of EHD.

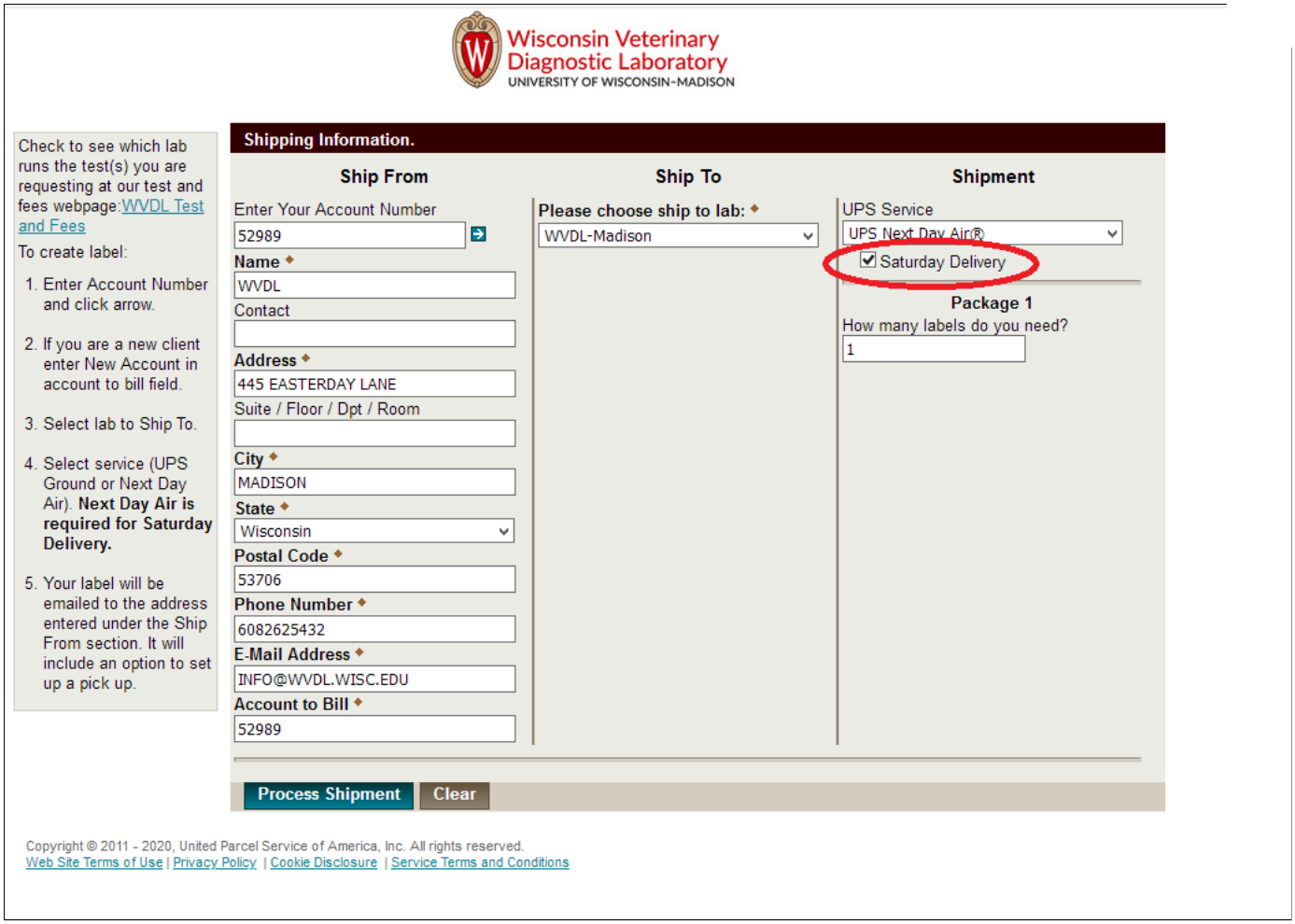
Client Services

Saturday Submissions
We now offer Saturday sample receiving hours at our Madison location. WVDL in Madison is open from 9 a.m. to 1 p.m. every Saturday for those wishing to ship samples for Saturday delivery. In-person drop offs, couriers, UPS and Fed Ex will be accepted. Use our UPS shipping program (see below) for a $30 flat rate for any size parcel from anywhere in the U.S. These hours can also be used to pick up supplies, media and testing kits.

- **Samples shipped to the lab need to be shipped for Next Day Air - Saturday Delivery.**
- **Regular ground or next day air shipping labels <u>will not</u> be delivered on Saturdays.**

We offer Saturday delivery shipping labels as part of our shipping program. To order these labels, log onto the WVDL/UPS Shipping label website here: https://www.wvdl.wisc.edu/index.php/shipping-information/

Follow instructions selecting Next Day Air for service and check the Saturday Delivery box. (see below)



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