Wisconsin Veterinary Diagnostic Laboratory
UNIVERSITY OF WISCONSIN-MADISON

Newsletter - Winter 2018

Message From The Director

Hello from all of us here at WVDL and welcome to our January 2018 winter newsletter. In this edition you'll see a review of BVDV ear notch testing and other news from our Virology Section. You can also read about the challenges and solutions that our cervid CWD testing program in the Pathology Sciences Section has faced as a result of shortages of test kits from the manufacturer. We are pleased to introduce a new staff member at our Barron Laboratory, Jill Fries, and as always, have included other news and information from WVDL.

We'd like to take this opportunity to thank you for your business, wish you a happy and successful New Year.

Phil Bochsler
Director

Microbiology

New Serological Tests Available for Avian Diseases

The WVDL is happy to announce a collection of avian serological tests to be offered by the Barron location. These include:

1. **Infectious bronchitis virus (IBV) ELISA for $2.60** per sample which will be used to detect pre- and post-vaccination IBV antibody levels in chickens.
2. **Infectious bursal disease (IBD) virus ELISA for $2.60** per sample which will be used to detect pre- and post-vaccination IBD antibody levels in chickens.
3. **Newcastle disease virus (NDV) ELISA for $2.60** per sample which will be used to assess immune status and quantification of antibody levels to NDV to facilitate monitoring in large flocks. This new kit is for chicken sera. We have been running the kit for turkey sera, which is billed at the same price ($2.60/sample).
4. ***Mycoplasma gallisepticum* and *M. synoviae* (MG/MS) combination ELISA for $3.00** per sample which will be used to screen large numbers of chicken and turkey sera. To differentiate and confirm a positive between the two species, conventional hemagglutination-inhibition (HI) tests for MG and MS serological testing will be used. **The Barron WVDL will automatically run the HI confirmatory test at no additional cost to the client.**

These serological tests compliment the suite of serological tests already available including: Avian Encephalitis by ELISA, Avian Influenza by AGID, Avian Pneumovirus by ELISA, *Bordetella avium* by ELISA, Hemorragic Enteritis by ELISA, *Mycoplasma gallisepticum*, *M. meleagridis,* and *M. synoviae* by HI and Plate tests, Newcastle disease by HI, *Ornithobacterium rhinotracheale* (ORT) by ELISA, Paramyxovirus 2, 3, and 6 by HI, Reovirus by ELISA and *Salmonella* Pullorum Plate and Tube tests. These serological tests are part of a suite of serological, molecular and culture testing options for poultry. Please see our website for additional information about these testing options.

New Molecular Test Available for Bovine Pink Eye

In addition, the **WVDL has validated, and is offering starting January 1, a pink eye PCR panel that will include the following agents: *Moraxella bovis*, *Moraxella bovoculi*, *Mycoplasma bovoculi*, *Mycoplasma bovis* and Infectious Bovine Rhinotracheitis (IBR).** The **cost for this PCR Panel is $68 (both in-state and out-of-state fee) per sample.** We thank all the clients who submitted samples for the validation of this test. Please see our website (www.wvdl.wisc.edu) for the submission form titled: Pink Eye Diagnostics. Clients should use the M6 viral transport swab when requesting PCR only. However, if culture is needed, which would be necessary for the isolation of a bacterium for autogenous vaccine development or susceptibility testing, please send an Amies swab or similar culture media. Please contact the WVDL Supply Room by sending an email to supplyroom@wvdl.wisc.edu or calling for the purchase of swabs or transport media. Please note that the aerobic culture is only set up for *Moraxella bovis* and *Moraxella bovoculi* when requested. Additionally, the bacteriology section can add *Mycoplasma* culture for the isolation of *Mycoplasma bovis* and other hardy *Mycoplasma* species. However, the bacteriology section cannot culture *Mycoplasma bovoculi* at this time as it requires specific media that is not stocked. The molecular test is offered at the Madison location, but culture is located at both the Barron and Madison locations.

New Service Available for Saving Bacterial Isolates

To better serve our clients' needs for autogenous vaccine development, the **WVDL is offering an isolate stocking option for $20 per isolate.** In order to preserve isolates in the virulence state required for vaccine production, we will be freezing isolates on cryogenic beads at -80 ^{o}C till shipping for autogenous vaccine development at a private corporation of the client's choosing. *Therefore, we have added a 'check box' on the several bacteriology submission forms, including the general, milk, scours, pink eye, and bovine respiratory disease submission forms, for the stocking and shipping of isolates. Please use that 'check box' or write in notes on the submission form to bacteriology indicating your wishes to save a particular isolate or isolates.* If you know what private corporation you wish to have the isolates sent to please indicate that on the form as well. We understand, it is not always possible to know in advance of sample submission that an autogenous vaccine might be produced from a requested culture. **Please call or email the WVDL within 2 weeks of test finalization if you require an isolate to be saved and shipped for autogenous vaccine development.** Isolates will be stocked for $20 per isolate. Upon shipment of the isolate a shipping fee of $75 per accession will be charged. Only one shipping fee will be applied, but multiple isolates from that accession can be sent using that one shipping fee, but each isolate will be billed at $20 per isolate. Isolate stocking and shipping occurs at both the Barron and Madison locations.

New Molecular Test Available for *Prototheca zopfii* Type 2

The WVDL is proud to offer a **new PCR for the identification of *Prototheca zopfii* type 2 for $33 per sample.** This PCR is validated for milk, bulk tank milk, and pure culture isolates. Bedding such as sand and water can be submitted for testing since WVDL is currently validating the assay for these sample types. This PCR is used to confirm the presence of *Prototheca zopfii* type 2, which is the pathogenic type in comparison to type 1, which is considered an environmental isolate. This PCR compliments our current *Prototheca* culture option as well since growth of *Prototheca* species on specialized agar does not indicate if the pathogenic type is present. This PCR therefore can be used on primary samples or from a pure culture isolate to confirm or deny the presence of the pathogenic type. If you have obtained a positive *Prototheca* culture results and would like further identification of that isolate, please contact the WVDL within 5 working days of test finalization. Please use the Milk Submission form to request this test. This PCR is offered at the Madison location and run on Fridays with results reported the same day.

Reminder that CAE/OPP Tests Should Be Shipped To Barron Location

Caprine Arthritis Encephalitis (CAE) virus and Ovine Progressive Pneumonia (OPP) serology is performed only at the Barron location on Tuesday and Friday. To avoid delays in processing due to extra handling and shipping, please send CAE/OPP submissions directly to the Barron laboratory.

Update on ZACTRAN and ZUPREVO Susceptibility Testing

The Clinical and Laboratory Standards Institute (CLSI) set standards for gamithromycin (ZACTRAN®) and tildipirosin (ZUPREVO™), which allow for the reporting of resistant, susceptible and intermediate interpretations when the following bovine respiratory disease pathogens: *Mannheimia haemolytica, Pasteurella multocida,* and *Histophilus somni* are isolated. We have been using the Kirby Bauer disk diffusion method for several years to produce interpretations for these drugs. Only these isolates that have been identified to the genus and species level will be tested using the Kirby Bauer disk diffusion antimicrobial susceptibility testing method for susceptibility to these two antimicrobials. We want to remind our Barron clients that when these isolates are identified from bovine respiratory cultures there is a several day delay in reporting these two drug susceptibilities since the Kirby Bauer test is only run in Madison. We thank you for your continued patience.

Update on MDR *Salmonella* serotype Heidelberg Case Count

The WVDL and our state and federal collaborators would like to remind veterinarians and producers to use caution when working with animals with confirmed or suspected multidrug resistant (MDR) *Salmonella enterica* subspecies *enterica* serotype Heidelberg. To date, there are 54 confirmed human infections in 15 states (n=18 in Wisconsin; Figure 1), in which 35% (n=17) of infected people have been hospitalized and 15% (n=8) had invasive disease. Moreover, 33% (n=18) of ill persons were under the age of 5 years. There continues to be human infections with MDR *Salmonella* ser. Heidelberg (Figure 2) where 63% (n=34) reported having contact with cattle including ill dairy beef calves. The Center for Disease Control and Prevention (CDC) has updated their website and provide several documents related to advice and information for livestock handlers, veterinarians and healthcare providers. https://www.cdc.gov/salmonella/heidelberg-11-16/index.html

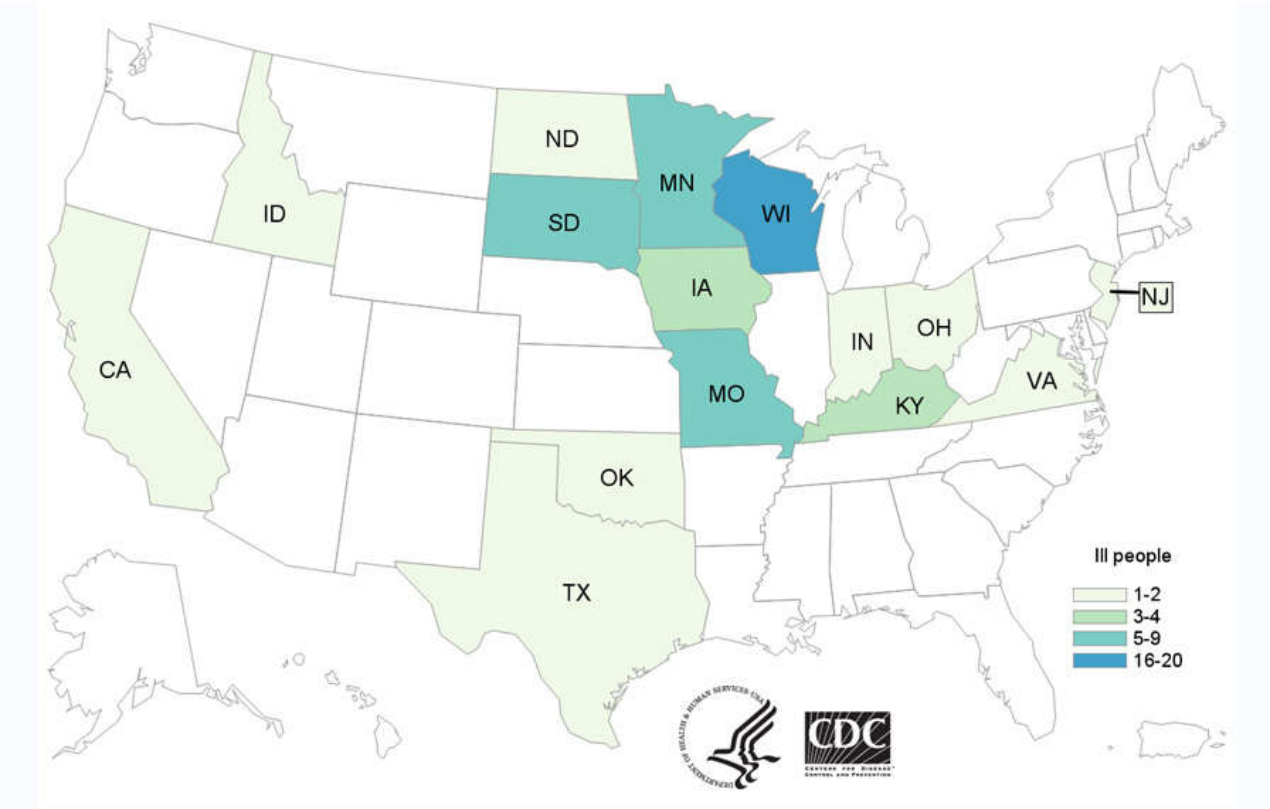

Figure 1: People Infected with the Outbreak Strains of *Salmonella* Heidelberg, by State of Residence, as of October 30, 2017 (n=54)

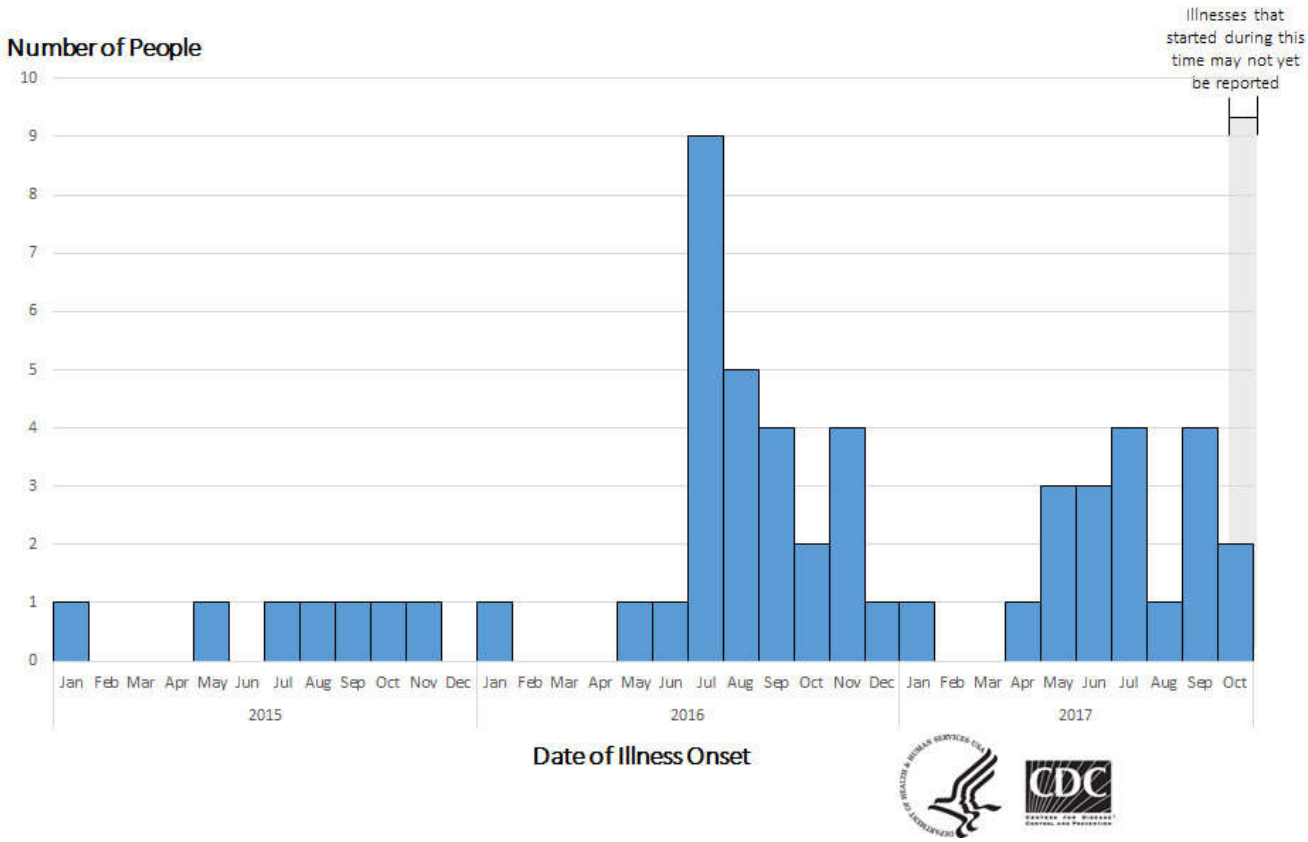

Figure 2: People Infected with the Outbreak Strains of *salmonella* Heidelberg, by Date of Illness Onset

The WVDL continues to isolate MDR *Salmonella* ser. Heidelberg from bovine tissues and feces. We would like to stress the importance of cleaning and disinfection when this MDR *Salmonella* has been identified on a premise. Preliminary evidence suggests that even after 1-2 years post identification of a MDR *Salmonella* ser. Heidelberg on a premise, we are able to culture *Salmonella* ser. Heidelberg from one or more locations on that premise. Below are the number of MDR *Salmonella* ser. Heidelberg cases that the WVDL has identified from 2009 to 2017 (U.S. and Wisconsin maps). As expected Wisconsin has the most number of isolates at 61 with Missouri at 33 (Figure 3). Additionally, Barron County (n=10) has the most isolates followed by Marinette and Shawano Counties (n=6 each) and Grant County (n=5) (Figure 4). The data here is a gross under estimation of the true burden of disease. The WVDL provides information about cleaning and disinfection protocols, bovine environmental sampling instructions, S*almonella* molecular and culture testing options and work flow and a *Salmonella* ser. Heidelberg interview questionnaire on our website in the Diagnostic Aids tab (http://www.wvdl.wisc.edu/index.php/diagnostic-aids/).

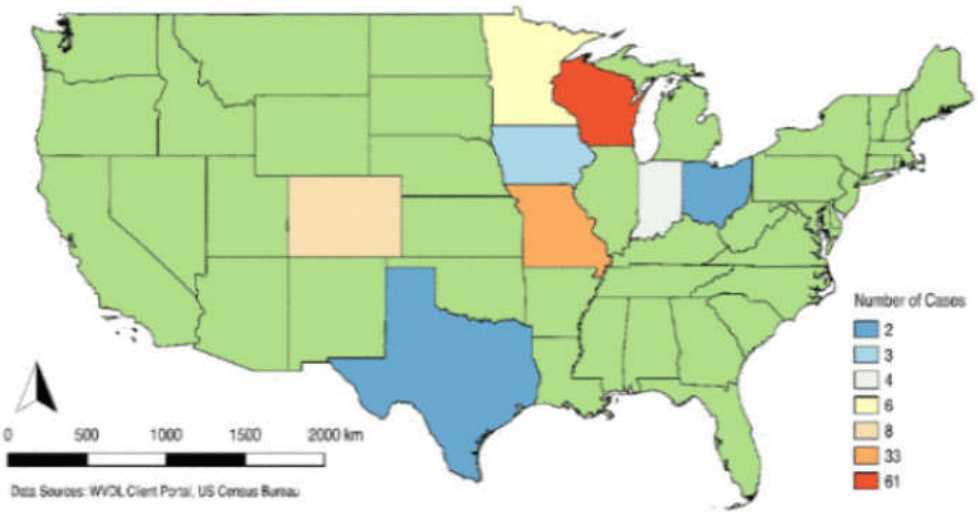

Figure 3: Map of *Salmonella* ser. Heidelberg isolates by State from 2009-2017.

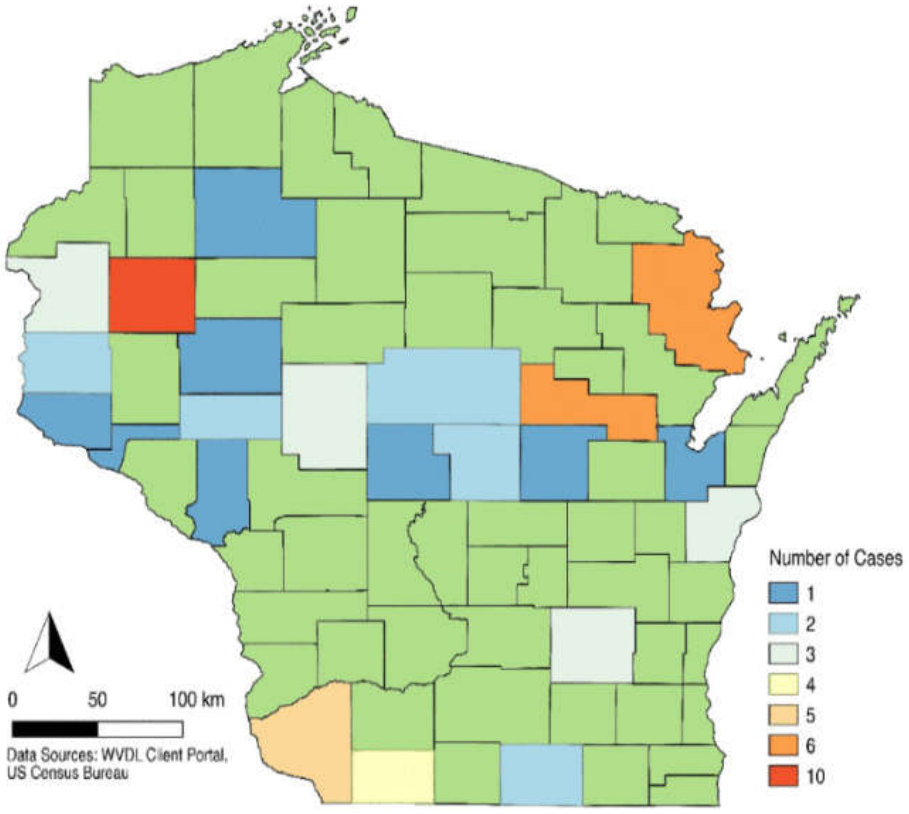

Figure 4: Map of *Salmonella* ser. Heidelberg isolates by Wisconsin County from 2009-2017.

The WVDL continues to work closely with state and federal agents to make producers, veterinarians, physicians and other interested parties aware of this MDR zoonotic disease. WVDL staff have recently given presentations at: 1) the American Association of Veterinary Laboratory Diagnosticians (AAVLD) Annual Conference on October 15 in San Diego, CA, where Megin Nickels for the CDC also talked at this conference on this topic, 2) the Inform 2017 Conference on November 8 in Garden Grove, CA, where a USDA veterinarian also talked about preliminary data from a case control study we are participating in, 3) the One Health Symposium on November 10 in Madison, WI and 4) at the Conference of Research Workers in Animal Diseases (CRWAD) in Chicago, IL on December 5.

Staff Spotlight: Jill Fries, Barron Client Services Supervisor

Jill Fries was born and raised in Milwaukee, but spent every summer helping with her grandfather's vegetable farm, bait shop and The Accidental RV park on Slim Lake (southwest of Stone Lake, home of the world famous annual Cranberry Festival). She graduated from Carthage College with a degree in Geography and Sociology with an emphasis in Community Development. She has worked in a wide variety of fields for several non-profits; however, the last 21 years were in Public Health specifically Emergency Preparedness Management as it applies to public health. In that capacity, she spear headed more than a few emergency response activities as it applied to food borne diseases at several points of origin (originating farm, food importation, transportation, commercial processing and commercial serving).

Jill's husband, Chris, retired recently (September 29th) joined her in early December and they are looking forward to living under the same roof! Their three adult children are spread across the USA from Chefornak, Alaska, the Upper Peninsula of Michigan and Stevens Point, Wisconsin.

She is excited and looks forward to learning the ropes of the Barron lab. She is grateful for the warm welcome she has received from both the Barron and Madison staff. In her spare time, she enjoys resurrecting the family vegetable farm, growing and expanding flower gardens (she believes the world needs more flowers), sewing (fiber artist, garment maker and quilter), running and hiking with her dogs, and exploring the beautiful northwest Wisconsin region.

Click here for more info

Client Services

Client services hopes you and your families enjoyed the holiday season and we wish you the very best for 2018!

Our customer service survey will be distributed in February of 2018. We appreciate your time and thoughts to improve your WVDL customer service experience. The survey is administered through an online platform supported by the University of Wisconsin-Madison. Results are shared with the entire laboratory and our Board of Directors.

Time from sample collection to testing is important for reliable testing results. We have had this question from time to time, especially with ear notch samples for Bovine Viral Disease Virus (BVDV). Ideally, we like to see the samples within 72 hours of collection because the nucleic acid degrades significantly over time. This becomes a problem trying to pool samples to save money and we do not recommend storing ear notch samples to accumulate 24 for a pooled PCR test. At the laboratory, we store samples at -80°C, but that is not likely possible at the clinic or on farm. Please contact us with sample storage questions for BVDV or any other diagnostic test you may need.

Over the past few months we have seen more and more companion animal necropsies with private cremation. Please remember to send a signed submission form with your client or send it ahead of time via email (info@wvdl.wisc.edu or submissions@wvdl.wisc.edu) or fax. If you have any questions about cremation or necropsies please contact Brenda Anderson, our Business Services Manager. For biosecurity reasons, it is important that they understand we do not release remains, only cremains.

Equine Infectious Anemia - Barron Laboratory

- EIA Elisa tests are run daily at the WVDL, Barron Laboratory
- Samples should contain 1 ml of serum, refrigerated and shipped with cold pack
- Samples received by noon will have same day testing
- **No accession fee for EIA submissions**

The 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.

Virology

BVD sample Collection, submission and storage guidelines

WVDL receives thousands of ear notch samples each year. As a reminder, below are sample collection guidelines. Please see our website for complete details as well as guidelines for nasal swab collection for BVD PCR testing. http://www.wvdl.wisc.edu/wp-content/uploads/2016/12/BVD-PCR-Sample-Collection-Guidelines.pdf

BVD PCR Sample Collection Guidelines

1. Materials and Equipment Needed for Ear Notch Collecting:

a. Ear notching tool that yields a 1cm a side notch
b. Red Top blood tube: The preferred tube is a **Monoject**: 7ml draw, 16mm x 75mm.
c. Shipping container rack that holds individual tubes in slots.
d. Submission form: http://www.wvdl.wisc.edu/index.php/forms/ Click on the "Instructions for Excel Spreadsheet Template" and "Excel Spreadsheet for Electronic Submission".
e. Disinfectant for rinsing notching tool: 10% bleach (eg.100ml (3oz) bleach in 900ml, (27oz) water).

f. Clean rinse water: 3-5 gallon bucket. Change bucket water every 20-30 notches.
g. Use disposable gloves and wear clean coveralls.
h. Do not vaccinate or tattoo at the same time samples are taken.

2. *Collection Procedure:*

a. Label Red Top collection tubes with the animal ID's and sequence number's (#1 through number in submission).
b. Dip notching tool in disinfectant then ALWAYS rinse away disinfectant with copious quantities of clean water. Caution: Residual disinfectant on the notching tool will yield false negative results, therefore thorough rinsing with clean water is required!!
c. Take a proper size ear notch from a clean portion of the ear (see below). For comparison, a triangle notch size is superimposed on a nickel. Place notch into a labeled, dry, red top collection tube (no formalin, other liquid or separator gel; do not use snap-cap milk tubes or whirl-pac bags). **Caution:** Collected ear notch **MUST** be free of contaminating dirt, feces, tattoo ink or BVD vaccine.

Best location for taking ear notch sample.

Required size of ear notch, ~1cm per side

BVD sample storage and shipping guidelines

Store collected ear notches for a maximum of 72 hours at refrigerator temperatures and ship over night to the WVDL on cold packs. This allows for a Friday collection and Monday shipping. Collection and storage of ear notches in clinics or on farms over the course of weeks or months causes sample degradation, making our testing invalid. Poor quality samples will be rejected.

Test Development

The WVDL Virology section is in the process of verifying several porcine assays per the request of DATCP and WPPA. This is in response to an impending update to Wisconsin's animal health rules to require farms to test for Porcine Reproductive and Respiratory Syndrome (PRRS) and Swine Enteric Coronavirus (SECD). We plan to offer these assays in early 2018. The following assays in development are:

- Porcine Reproductive and Respiratory Syndrome (PRRS) ELISA on oral fluids
- PRRS PCR on oral fluids
- Porcine Epidemic Diarrhea Virus (PEDV) PCR, multiple sample types
- Transmissible Gastroenteritis Virus (TGEV) PCR, multiple sample types
- Swine Delta Coronavirus (SDCoV) PCR, multiple sample types

Other test improvements include optimizing current PCR tests, improving work flow and process in virus isolation and molecular testing as well as integrating robotics for high throughput testing (automation of 384 platform).

National Animal Health Laboratory Network (NAHLN) testing

The NAHLN supports U.S. animal agriculture by developing and increasing the capabilities and capacities of a national veterinary diagnostic laboratory network to support early detection, rapid response, and appropriate recovery from high-consequence animal diseases. It is a nationally coordinated network and partnership of Federal, State, and university-associated animal health laboratories. NAHLN testing and work spans all sections of WVDL. In the virology section, the following molecular tests and numbers were performed for the fiscal year:

Target	Number of samples tested
Avian Influenza Virus	642
Avian Paramyxovirus	147
Swine Influenza Virus	35
Foot and Mouth Disease Virus	324
Other Influenza testing (feline, canine, mink) due to shelter outbreaks	5,442

Staffing

In the past quarter, there have been a few staffing changes to the virology section. We welcomed Emma Sweet and Josh TeSlaa as microbiologists into the section. We also were very sad to see Emily Paulson and Rachael Zinn leave. They will be greatly missed, however we are able to have Emily back as a veterinary student employee this Winter.

Pathology Sciences

CWD ELISA Test Kit Shortage effects WVDL

We have experienced an increase in the turn-around time for our CWD ELISA test results. This is due to the national shortage of testing kits supplied by Bio-rad. There has been a 100% increase in the number of animals tested in the US this year compared to 2016. This coupled with export licensing complications from France led to a limited number of test kits available in the US. Bio-rad worked diligently to produce more test kits in France, but the US still experienced several weeks of delays.

We received our shipment of CWD ELISA test kits on Friday, December 8th and tested 3,100 samples in the first 48 hours. Since that time we have tested 7,000 samples and have nearly cleared our back logged samples. Our goal is to return to our 3 day turn-around time after the holidays. Thank you for being patient during this difficult situation.

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www.wvdl.wisc.edu

Our mailing address is:
445 Easterday Lane

Madison, WI 53706
info@wvdl.wisc.edu

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