

# 2023 NAHLN Farm Bill Funded Projects



U.S. DEPARTMENT OF AGRICULTURE

# NAHLN Farm Bill Showcase

## Presentations from 2022 (FY23, Cycle 4) NAHLN Farm Bill Funded Projects

Wednesday, November 6, 2024

1:00PM – 3:00PM CT

**Moderator: Kelli Almes**

|         |   |    |
|---------|---|----|
| 1:00 PM | Welcome – Dr. Beth Lautner<br>Associate Deputy Administrator, Diagnostics and Biologics, APHIS  |    |
| 1:05 PM | Regional Veterinary Workforce Development to Enhance Early Recognition of Newly-Detected, Re-Emerging, and Foreign Animal Diseases<br><i>Lisa A. Murphy, Taylor C. Chan, Susan Bender, Kevin Niedringhaus, Eman Anis, Robert Gibson, Colleen F. Monahan, James Trybus</i> ..... | 3  |
| 1:20 PM | Incorporation of a semi-automated PCR workflow to improve surge testing capacity and capability for small-midsized NAHLN laboratories<br><i>Yung-Yi Mosley</i> .....  | 4  |
| 1:35 PM | Development and Validation of Oxford Nanopore Technologies (ONT) Sequencing as a Molecular Point-of-Care Diagnostic (POCD) Assay<br><i>Mohamed El-Gazzar, Yuko Sato, Amro Hashish, Oliver Eulenstein, David Suarez</i> .....  | 6  |
| 1:50 PM | Increasing the diagnostic laboratory technical expertise of the Ohio ADDL<br><i>Dominika Jurkovic</i> .....   | 10 |
| 2:05 PM | Questions / Break   |    |
| 2:15 PM | Enhancing efficiency of field sample submissions to high throughput laboratories<br><i>Brian Ladman, Kimberly Isaacs</i> .....  | 11 |
| 2:30 PM | Increasing preparedness for an African Swine Fever outbreak by stockpiling primers and probe for NAHLN laboratories<br><i>Brianna LS Stenger, Tylise Graff, Haytham Ali, Jeff Strain</i> .....  | 13 |
| 2:45 PM | Questions / Comments / Closing  |    |

# NAHLN Farm Bill Showcase

## Proceedings from 2022 (FY23, Cycle 4) NAHLN Farm Bill Funded Projects

### 2022 NAHLN Farm Bill - Capacity Support

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| Enhancement of High-Capacity Testing for Transmissible Spongiform Encephalopathies<br><i>Kristy Pablonia, Terry Spraker, TSE Section Head</i> .....  | 14 |
| Improve Capability to Handle Surge Samples Through Purchasing High-Capacity Diagnostic Testing Equipment<br><i>Qirui Zhang, Melanie Prarat</i> .....   | 15 |
| Enhancing the Capacity of Animal Disease Research and Diagnostic Laboratory for Early Detection of Emerging and Foreign Animal Diseases Using High Throughput Sequencing<br><i>Sunil Mor</i> ..... | 16 |
| Diversifying Tennessee’s Diagnostic PCR Platforms and Increasing Surge Capacity<br><i>Adam Travis</i> .....  | 17 |
| Improving the Efficiency of FAD Sample Submissions through LIMS Enhancements<br><i>Adam Travis</i> .....   | 18 |
| Expanding Support to the NAHLN by Enhancing Sustainability of Testing Capacity<br><i>Jessica Walters, Anne Magee, Jennifer Carickhoff, Hailey Quercia</i> .....                                    | 19 |
| Expanding Support to the NAHLN by Improving Capability to Handle Surge Samples<br><i>Jessica Walters, Anne Magee, Jennifer Carickhoff, Hailey Quercia</i> .....                                    | 20 |

# **Regional Veterinary Workforce Development to Enhance Early Recognition of Newly-Detected, Re-Emerging, and Foreign Animal Diseases: Transboundary, Regional, Emerging and Novel Disease (TREND) Rounds**

Lisa A. Murphy, Taylor C. Chan, Susan Bender, Kevin Niedringhaus, Eman Anis, Robert Gibson, Colleen F. Monahan, James Trybus

University of Pennsylvania PADLS New Bolton Center  
Kennett Square, PA

## Project objectives:

- Establish virtual regional emerging and foreign animal disease training rounds and a case consultation network for veterinary laboratory diagnosticians, with an emphasis on trainees and early-career professionals.
- Provide annual in-person emerging and foreign animal disease training opportunities that encourage veterinary laboratory diagnosticians to interact with each other, practicing veterinarians, and state and federal agencies responsible for animal disease surveillance and response.
- Improve communications between regional veterinary diagnostic laboratories, practicing veterinarians, the National Animal Health Laboratory Network (NAHLN), and state and federal officials responsible for animal disease surveillance and response.

## Deliverables completed

- In the first year, the TREND rounds were established, with up to 40 attendees from 11 different institutions. The topics covered include HPAI, flaviviruses, rabies, unusual mortality events (UMEs), ASF and Classical Swine Fever (CSF), brucellosis, antimicrobial resistance, and tick-borne diseases. Subject matter experts included wildlife biologists, microbiologists, parasitologists, pathologists, public health officials and epidemiologists from 10 different institutions and regulatory bodies.

## Deliverables yet to be completed

- In the second year, the goals are to open TREND rounds to additional agencies, institutions and accredited, practicing veterinarians, and organize in-person training opportunities.

## How the project benefits NAHLN

- Coordinated and collaborative workforce development among veterinary diagnostic laboratories will enhance early recognition and improve responses to outbreaks at the regional and national level. Focusing training, mentorship and networking opportunities for trainees and early-career professionals will improve retention within veterinary diagnostic laboratories. Ultimately, this project strives to strengthen the NAHLN and its partners in support of their missions to provide disease surveillance, respond quickly to disease events, and communicate with stakeholders and decision makers.



# **Incorporation of a Semi-Automated PCR Workflow to Improve Surge Testing Capacity and Capability for Small-Midsized NAHLN Laboratories**

Yung-Yi Mosley

University of Georgia - Tifton Veterinary Diagnostic Laboratory  
Tifton, GA

## **Project objective**

1. Work Plan Objective 1: To increase testing capability for surge samples in small-midsized NAHLN laboratories without increasing personnel, we will develop a semi-automated PCR testing workflow with ASSIST PLUS and MINI 96 (using ASF outbreak as a scenario).
2. Work Plan Objective 2: To add testing capacity and diversify the source of reagent and plastic supplies for magnetic bead-based extraction for small-midsized NAHLN laboratories, we will evaluate and incorporate IndiMag 48s into the semi-automated PCR testing workflow (using ASF outbreak as a scenario).
3. Work Plan Objective 3: To share the results and new workflow experience with other NAHLN laboratories, we will present the semi-automated workflow and IndiMag 48s verification data at AAVLD annual meeting and other NAHLN meetings such as the weekly response planning meeting.

## **Deliverables completed / Deliverables yet to be completed**

- Objective 1 – complete.

We have incorporated ASSIST PLUS and MINI 96 into the NAHLN ASF testing workflow with KingFisher Flex (MagMAX Pathogen RNA/DNA kit).

- Objective 2 - complete.

We have incorporated ASSIST PLUS and MINI 96 into the NAHLN ASF testing workflow with IndiMag 48s (IndiMag Pathogen prefilled cartridges).

However, during the process we found out that IndiMag cartridges (8 and 24 samples) do not fit perfectly on ASSIST PLUS platform due to small size difference in the footprint. The cartridge would move around slightly during robotic operation due to platform vibration resulting in misalignment of the tip and wells. A prototype adaptor was created using wooden tongue depressors. Five metal adaptors were ordered and have been received from UGA Instrument Design & Fabrication shop to solve this issue.

- Objective 3 – in progress

Dr. Yung-Yi Mosley presented at AAVLD Summer Virtual Mini Symposium on July 24, 2024 with the title of “Semi-automated workflow using Integra Assist Plus Pipetting Robot & Mini 96”.

Rebecca Cox, a graduate student supported by the grant will present at 2024 AAVLD/USAHA Annual Meeting in Nashville, TN with the title of “Semi-automated workflow for molecular testing of African Swine Fever using the 96- channel electronic pipettes and liquid handling system from Integra Biosciences”.

### **Additional objective – in progress**

We applied for a No Cost Extension to work on an additional objective which will carry out experiments and obtain data required to submit to NAHLN Methods Technical Working Group (MTWG) for dossiers evaluation to incorporate the additional instruments in the NAHLN workflow.

### **How the project benefits NAHLN**

This project aims to find solutions for small-midsized NAHLN laboratories to increase their testing capacity/capability for handling surge samples with smaller budget and lab space. Over half of the NAHLN labs are in the categories of level 1 branch labs (12), level 2 labs (22), level 3 labs (2) and two affiliate labs. These small-midsized NAHLN labs (total of 36) usually share less resources including budget, personnel and lab space. Nonetheless, they are key players in the network. To accelerate testing speed and improve testing capacity, incorporating automated liquid handling robots into the workflow can help achieve this goal. This project focuses on increasing the testing capacity for surge samples by incorporating testing equipment that are suited for small-midsized NAHLN laboratories. In addition, a compact magnetic particle processor, IndiMag 48s (INDICAL BIOSCIENCE) will be included for the semi-automated workflow. The addition of this equipment to the protocol can further diversify reagent and plastic source, especially during an outbreak scenario.

# **Development and Validation of Oxford Nanopore Technologies (ONT) Sequencing as a Molecular Point-of-Care Diagnostic (POCD) Assay**

Mohamed El-Gazzar, Yuko Sato, Amro Hashish, Oliver Eulenstein, David Suarez

College of Veterinary Medicine - Iowa State University  
Ames, IA

## **Project Overall Objective:**

The overall objective of this project is the customization and validation of ONT sequencing as a point-of-care diagnostic (POCD) tool to allow for the accurate and rapid identification and characterization of avian foreign animal diseases (FAD) – Avian Influenza and Newcastle disease viruses (AIV and vND), in the field or resource-limited setups. When developing ONT as a POCD, our group aims to meet the principles of affordability, sensitivity (no false negative), specificity (no false positive), user-friendly, equipment-free, and deliverable to end-users. This would greatly shorten the sample to results time, which would directly help in the early containment of foreign animal outbreaks.

## **Project Specific Objectives:**

- #1. Evaluate the suitability and reliability of different ONT workflows for field application (RNA extraction, library preparation, and viral target enrichment).
- #2. The development of a Portable Standalone-Bioinformatics Pipeline (PSBP) for real-time data analysis on local computing resources independent of internet connection.
- #3. Assembly of Mobile-Sequencing Kit (MSK) and testing the newly validated protocols under field conditions (at farm level or resource-limited lab).
- #4. Head-to-head comparison of results from clinical samples submitted to one of NALHN labs and the newly validated POCD protocols.

## **Project Completed Deliverables:**

Objective #1: Evaluate the suitability and reliability of different ONT workflows for field application (RNA extraction, library preparation, and viral target enrichment).

### **A. Extraction comparison**

We compared four commercial extraction kits suitable for on-site application and used 10 known positive clinical samples for AIV. To mimic oropharyngeal swabs (the most common sample type for future on-site diagnosis of AIV), swabs from trachea (of necropsied birds) were collected and placed in 1 mL of phosphate buffer saline (PBS). Four commercially available extraction protocols were selected: (1) M1 Sample Prep Cartridge Kit for RNA 2.0 (Biomeme); (2) Quick-RNA™ Microprep Kit (ZYMO Research); (3) SwiftX™ DNA (Xpedite Diagnostics); and (4) SwiftX™ Swabs (Xpedite Diagnostics). These specific protocols were selected based on the ease of performing the extraction, with no requirement for complex equipment (only centrifuge, vortex, and/or heat block) and a short time to complete the procedure. The extraction outcome was evaluated in quantity for total RNA concentration and number of viral copies (qRT-PCR for AIV matrix gene).

All the kits were compared to our gold standard method - the MagMAX™ Pathogen RNA/DNA Kit (Thermo Fisher Scientific), which was previously evaluated by our group (Chaves et. al., 2024). All the methods performed well in terms of time spent (below 1 hour to extract up to 10 samples), with the SwiftX swabs the less time-consuming method, taking only 22 minutes for complete extraction. Our results showed the outperformance of SwiftX DNA in both total and viral-specific RNA concentration, overpassing the MagMAX™ in some of the samples tested. Followed by SwiftX DNA, the SwiftX Swabs showed excellent performance regarding AIV-CT value. The M1 Cartridge was excluded from future applications due to the extreme cost of the kit (\$605,00 per sample).

### B. Viral enrichment

For enrichment evaluation, we selected the amplicon-based approach for our POCD purposes – rapid and specific characterization of avian FAD. The motivation for our selection was based on a previous comparison between amplicon-based enrichment to a target-independent approach – the Sequence Independent Single-Primer Amplification (SISPA) (Chrzastek et. al., 2017), and a semi-targeted approach – a modified spiked primer designed based on Deng, et. al., 2020 principle, in combination with a conserved tail the primers. Our previous data showed higher sensitivity and less time spent during wet lab procedures for the amplicon-based enrichment. For AIV, we utilized the Universal Primers for the Influenza A Virus, (Mena et. al., 2016). For NDV targeted enrichment, two sets of tiling primers (Quick et al., 2017) were designed: the first set targeted specifically the fusion gene across different genotypes (of both classes I and II); the second set was designed to cover the entire genome of the virus. Data regarding NDV wet lab testing is still under preparation.

### C. Library preparation

Finally, the Rapid Barcoding kit (RBK114.96) was selected for library preparation based on our group's previous comparisons and the easiness/speed of the protocol. Data generated by our group showed that the Rapid Barcoding kit (RBK) is less expensive and less time-consuming compared to the Ligation Sequencing kit (LSK). Some disadvantages of the RBK are the fragmentation of the cDNA during the barcoding step and the generation of shorter reads compared to the Ligation, which uses an end-preparation strategy for the samples' barcoding. However, regarding sensitivity in pathogen recovery and accuracy of the sequence data, there was not much difference between the two library preparation kits.

### D. Pilot study

After extraction comparison, SwiftX Swabs and SwiftX DNA showed the best performance. They were selected for a pilot study using the MinION sequencer. 4 samples were extracted with the two Xpedit kits and the MagMAX and were enriched using the Influenza A Universal primers. Library was prepared using the RBK114.96 kit. Samples were loaded onto the MinION Mk1D and sequenced for 18 hours. Sequence analysis was performed using the EPI2ME software – flu workflow. Results showed a very low percentage of AIV reads generated by Swift DNA and Swift Swabs kit, in comparison to MagMAX.

The low percentage of reads raised the hypothesis that the buffers used for extraction could have inhibited the PCR, and this idea was tested later. Our experiments confirmed that the SwiftX Swabs inhibited the PCR reaction, while SwiftX DNA did not inhibit the PCR. Further experiments will be performed to understand why the SwiftX DNA showed a low percentage of AIV reads, and optimization will take place to finalize the laboratory workflow for the POCD application.

Objective #2: The development of a Portable Standalone-Bioinformatics Pipeline (PSBP) for real-time data analysis on local computing resources independent of an internet connection.

In collaboration with a Bioinformatics group from Iowa State University and a recently hired Bioinformatics PhD student, our group is developing a custom GUI software to achieve the real-time analysis of AIV and NDV using Oxford Nanopore Technologies (ONT). The software will analyze ONT reads as they are generated by the sequencer, significantly reducing the time required to stop the run and directly obtain diagnostic results from clinical samples. In addition, this platform will incorporate a database containing representative sequences from each virus, enabling more accurate detection of the pathogens. The first steps of the process have already been done, and the software is working with internet connectivity. Future steps will include adapting the tool to run without an internet connection in the field.

### **Project Deliverables Yet to be Completed:**

Objective #1: conclude the wet lab comparison for POCD, optimizing the SwiftX DNA swabs, and running all the experiments with the NDV samples.

Objective #2: conclude the development of the GUI and establish it for offline application (independent of internet connection).

Objective #3: Assembly of Mobile-Sequencing Kit and test the protocol in the field/less equipped laboratories.

Objective #4: Establish the final workflow for ONT sequencing for poultry FAD for on-site application and validation of the generated ONT data compared to NALHN-certified laboratories.

We would like to draw your attention that our group aims to request a 1 year no-cost-extension. The reason being that the bioinformatics PhD student that previously committed to this project has quiet and we had to delay working on this project for the next recruiting cycle during which we have successfully recruited 2 new bioinformatics PhD students who are currently working on the project starting from summer 2024.

### **Project benefits to NAHLN:**

This project aims to develop a straightforward workflow and local bioinformatics pipeline for identifying and characterizing AIV and NDV on-site or in resource-limited settings. Early diagnosis in the field can accelerate containment measures during a FAD outbreak, ultimately reducing industry losses. Bringing sequencing technologies to the field and less-equipped laboratories is essential for democratizing access to genomic data. This approach enhances the system by delivering precise, actionable information that benefits producers and diagnosticians. Furthermore, the outcome of this project can be used not only for IAV or vNDV detection but also for any other foreign animal diseases.

### **References:**

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# **Increasing the Diagnostic Laboratory Technical Expertise of the Ohio ADDL**

Dominika Jurkovic

Ohio Department of Agriculture, Animal Disease Diagnostic Laboratory  
Reynoldsburg, OH

## **Project Objectives**

1. Develop improved BSL-2/2+ and BSL-3 Biosafety & Biosecurity training programs
2. Increase the number of staff approved for diagnostic laboratory testing
3. Increase staff with Quality System Management training and experience

## **Completed Deliverables**

ADDL staff successfully completed the BSL-3 Laboratory Training and the BSL-3 Laboratory Operations, Maintenance, and Verification trainings, which equipped them with the necessary skills and knowledge to handle high-containment laboratory environments safely and effectively. The laboratory has begun the process of revising the current standard operating procedures for the laboratory, which includes both BSL-2+ and BSL-3 protocols. Staff attended the 2024 annual ABSA conference to enhance their expertise in safety measures, optimize laboratory efficiency, and ensure compliance with the latest regulatory standards. Three staff members have completed the NALHD/AAVLD Quality Management Basic Training, with two also finishing the Advanced Training, equipping them with the knowledge and skills to develop, implement, and maintain a quality management system for the laboratory. The bacteriology team completed training in Contagious Equine Metritis, raising the number of certified analysts to four. This enhancement ensures sufficient coverage when cross-trained personnel are required for FAD outbreak testing.

## **Pending Deliverables**

Lab staff will participate in the ABSA annual conference, both virtually and in-person. This event provides an excellent opportunity for networking and gaining insights into the latest industry advancements. Revised biosafety protocols will be finalized, covering both BSL-2+ and BSL-3 standards, to ensure operations remain updated and efficient. Additionally, refresher and comprehensive training modules for new staff are being developed to maintain a knowledgeable and skilled workforce. ADDL staff will attend the upcoming AAVLD Accreditation Auditor Pool training and complete Principles and Practices of Biosafety Courses in 2025.

## **Benefits to NAHLN**

The proposal aligns with NAHLN's mission in ensuring effective emergency preparedness and response systems. By enhancing the expertise of the Ohio ADDL, the entire network of labs benefits from increased capability and knowledge sharing. This creates a more robust network that can respond more swiftly and accurately to emerging threats. This collaborative approach fosters a culture of continuous improvement and innovation, strengthening the network's overall ability to protect public health and agriculture.



# **Enhancing Efficiency of Field Sample Submissions to High Throughput Laboratories**

Brian Ladman, Kimberly Isaacs

University of Delaware Poultry Health System  
Georgetown, DE

## **Project Objective:**

To improve both the speed and accuracy of data gathered at the time of sample collection and submission for the Delmarva region and beyond. This project will create physical linkages between farm site metadata where samples are collected for surveillance or other purposes and the physical samples. This will be accomplished by adding the ability within the CoreOne for Labs (Trace First, Inc.) LIMS to allow users to create posters containing Federal premise identification numbers (PINs) including a QR codes for the passive collection of sample metadata. The QR code will take the submitter to a user friendly, mobile submission application that will capture farm location info embedded within the code. Finally, to construct a durable link between physical samples and sample metadata, the ability to create submission forms containing integrated barcodes will be added to CoreOne for Labs. The outlined workplan will enhance the ability of our lab system to receive testing associated data through standardization of IT processes and allowing us to send out complete and true sample metadata.

## **Deliverables Completed:**

The ability to create farm specific posters containing sample metadata required for HL7 messaging has been released to CoreOne for Labs users. The posters containing QR codes will facilitate rapid and accurate initiation of the sample collection and submission process.

## **Deliverables Outstanding:**

Printing and distribution of durable posters (plastic) to companies for installation near chicken farms and perhaps dairy farms.

The ability to create sample submission forms with barcode labels embedded within. The barcodes will link the samples to the submission forms, both physical and virtual. The barcodes will be randomly generated and distributed to clients in advance of submission.

The creation of the mobile sample submission webpage for field personnel.

## **How The Project Benefits NAHLN**

The NAHLN has been charged with the responsibility of ensuring network laboratories are prepared to rapidly respond to animal disease outbreak events. For a NAHLN laboratory to effectively meet this need, it must have at its disposal, tools that are designed to not only facilitate daily surveillance testing but also those that are designed to accommodate surge testing. The after-action-reviews of outbreak responses highlighted challenges

in acquiring and transmitting data associated with samples collected on-farm by poultry company service personnel or other field staff. Sample accessioning has been identified as one of the bottlenecks preventing labs from achieving their optimal capacity. Knowing precisely where and when samples are collected is critical to controlling disease spread and ensuring continuity of business for poultry operations both in and outside of control zones. Passive messaging data collection from the field would be ideal. The objectives in this proposal will improve both the speed and accuracy of data collected and submitted with samples destined for testing at NAHLN laboratories. This proposal aligns with NAHLN Funding priority 2 - Enhancing the ability to send and receive testing and testing associated data through standardization of IT processes.

# **Increasing Preparedness for African (and Classical) Swine Fever Outbreaks by Stockpiling Primers and Probe for NAHLN Laboratories**

Brianna LS Stenger<sup>1</sup>, Tylise Graff<sup>1</sup>, Haytham Ali<sup>2</sup>, Jeff Strain<sup>2</sup>

<sup>1</sup>North Dakota State University Veterinary Diagnostic Laboratory, <sup>2</sup>Thermo fisher  
Fargo, ND

## **Project Objectives:**

The project objects were: 1) Stockpile the primers and probes needed for at least 75,000 ASF/CSF reactions for NAHLN laboratories. 2) Verify the ready-to-use primers+probes “cocktail” performs as effectively as the individual primers and probes. 3) Provide NAHLN laboratories with the ASF and CSF primers and probes. A Method Verification study following MTWG guidelines to show that the primers+probe cocktails perform just as well as individually diluted primers and probes was completed and Dossier submitted. It was accepted by the MTWG and NAHLN leadership. An order has been placed for 125,000 single-plex ASF reactions and 25,000 CSF reactions for the ASF/CSF assay was placed. This means a total of 100,000 single-plex ASF reactions with 25,000 ASF/CSF reactions. Expected delivery is October 2024. (Labs will also be able to order more of these reagents if desired.) A -20°C Freezer with remote alarm contacts to store reagents was purchased and installed. Blanket planned deviations have been submitted to cover laboratories running the assays with primers+probe cocktails. Once deviations are approved and reagents arrive, NAHLN laboratories will be notified and be able request reagents be shipped or stored at the NDSU VDL until needed.

## **Project benefits to NAHLN:**

This project will enhance the readiness and sustainability of testing capacity across the NAHLN by developing and maintaining a stockpile of primers and probes for African and Classical Swine Fever PCR testing. This will reduce some of the financial burden on laboratories and reduce potential delays in testing response by having the primers and probe already manufactured. The supply of primers and probes will be stored and maintained by the NDSU-VDL until arrangements are made to distribute to NAHLN laboratories. A commercially manufactured primers+probes cocktail that includes the forward primers, reverse primers, and probes mixed together in one tube will also benefit NAHLN laboratories by: 1) eliminating errors due to improper dilutions; 2) reducing pipetting steps, tips, and tubes; 3) speeding up the master mix process; 4) increase quality control because a single lot can be used across laboratories.

# **Enhancement of High-Capacity Testing for Transmissible Spongiform Encephalopathies**

Kristy Pabilonia, Terry Spraker, TSE Section Head

Colorado State University Veterinary Diagnostic Laboratories  
Fort Collins, CO

## **Project Objective**

The Colorado State University Veterinary Diagnostic Laboratory (CSU VDL) in Fort Collins, CO participates in the NAHLN program as a Level 1 laboratory. The CSU VDL is very active in transmissible spongiform encephalopathy (TSE) testing and tests samples submitted from states across the country. The CSU VDL is approved to test for bovine spongiform encephalopathy (BSE), chronic wasting disease (CWD) and scrapie. The CSU VDL currently uses equipment installed in 2002 to conduct CWD and BSE ELISAs – the Bio-Rad NSP instrument. The current version of this instrument is outdated, the software no longer supported, and replacement parts no longer acquirable. All these variables contribute to the risk of halting testing if a component of the equipment or infrastructure fail. In order to proactively circumvent this risk, the goal of this project is to replace the outdated equipment with the most current equipment and software available, capable of integration into the CSU VDL network. In doing so, the CSU VDL would not only maintain our current testing capacity, but also facilitate high-capacity diagnostic testing at the highest quality.

## **Status of Deliverables**

The CSU VDL has worked with Bio-Rad over the past year to secure the newly developed NSP instrument. We also agreed to work with Bio-Rad and the NAHLN to validate the instrument, so that the instrument could be used in NAHLN laboratories. This project was funded in June 2023; however, there have been delays in obtaining the new NSP instrument due to delays in availability of the new equipment by Bio-Rad. The instrument is finally becoming available as of late 2024. We signed a non-disclosure agreement with Bio-Rad in the summer of 2024 that allowed us to obtain one of the first new NSP instruments, and we received the instrument in July 2024. To date, we have tested 120 CWD samples from mule deer, white-tailed deer and elk (21 positive and 99 negative) on both the new and old NSP instruments. All results have been comparable across both NSP instruments. We are continuing to conduct validation and will provide final results to the NAHLN.

## **Benefit to the NAHLN**

The CSU VDL has a high caseload of TSE testing. The old NSP instrument was very close to obsolete, and our CSU IT team was unable to continue supporting the software on this instrument. The new NSP will allow our lab and other TSE testing laboratories to have a functioning high-throughput instrument available. This allows us to provide the best possible results to our clients and ensures our compliance with NAHLN protocols. The instrument also provides our needed testing capacity and appropriate testing turnaround time for our clients. With the new NSP instrument, the CSU VDL will be able to maintain the highest quality diagnostic services for TSE testing.

# **Improve Capability to Handle Surge Samples Through Purchasing High-Capacity Diagnostic Testing Equipment**

Qirui Zhang, Melanie Prarat

Ohio Animal Disease Diagnostic Laboratory  
Reynoldsburg, OH

## **Project Objective**

The Ohio Animal Disease Diagnostic Laboratory (ADDL) is the only Level I NAHLN laboratory and AAVLD-accredited laboratory in Ohio. Both the foreign animal disease (FAD) sample volume for PCR testing including FMD/SVA, IAV-A, IAV-S, vND, ASF/CSF, and ISAV/VHS etc. and the routine samples for porcine/avian/bovine PCR testing at ADDL have been increasing year-over-year before and during the COVID-19 pandemic. The workload is currently increasing with the current Highly Pathogenic Avian Influenza (HPAI) national H5N1 incident. To accommodate the surging sample volume, we propose to improve our PCR testing capacity by purchasing one 7500 Fast real-time PCR system, one QuantStudio 5, and one Kingfish Flex (originally 4 of multi-channel pipettes). These instruments will increase ADDL capacity for FAD and routine sample testing, reduce the turn-around time, and fulfill our state, regional, and national stakeholder needs as part of the USDA's National Animal Health Laboratory Network.

## **Deliverables completed**

Our original work plan is to purchase one 7500 Fast, one QS5, and 4 multiple-channel pipettes to increase our capacity for surging test demands. Recently when procuring the quote, we were able to negotiate and secure a great deal with the company to use these funds for one 7500 Fast, one QS5, and one Kingfisher Flex, which normally will need at least another \$30,000 to buy all these three instruments.

The 4 multiple-channel pipettes (cost \$3202.2) were replaced by the Kingfisher Flex (cost much more than \$3202.2) as a bundle with the 7500 Fast and QS5. Thus, we can not only increase our capacity for PCR, but also the capacity for automatic nucleic acid purification at the same time.

No more funds from our current cooperative agreement are requested for this change. But we consider it as a success because we can use the same funds to increase both our capacity on PCR and automatic nucleic acid purification simultaneously. In this way we can get more samples tested for NAHLN during emergent outbreaks and for the daily routine tests in Ohio ADDL.

We happened to have the HPAI outbreak in Ohio Poultry farms since December 2023 and in one Dairy cow farm since March 2024 after we received these instruments. We were able to immediately put these instruments in use and they certainly increased our capacity when handling those extra-large volumes of samples for HPAI surveillance and monitoring during and after outbreak. Thus, the objectives for this grant are perfectly fulfilled at the right time.

In a summary, this project funded us to purchase more instrument and certainly increased our capacity as the only Level I NAHLN laboratory in Ohio to handle the FAD testing for NAHLN not only in Ohio but also regionally in Midwest and nationally.

# **Enhancing the Capacity of Animal Disease Research and Diagnostic Laboratory for Early Detection of Emerging and Foreign Animal Diseases Using High Throughput Sequencing**

Sunil Mor

South Dakota State University, Animal Disease Research and Diagnostic Laboratory  
Brookings, SD

## **Project objective:**

Objective 1 - Improving samples preparation, nucleic acid extraction and library quantification for high throughput sequencing

Objective 2 - GridION for same day high throughput sequencing

## **Deliverables completed:**

- All proposed equipment have been installed and in use for diagnostic work mainly for clinical next generation sequencing.
- Oxford Nanopore GridION is currently being used for targeted whole genome sequencing of Avian Metapneumovirus subgroup B, Bovine Leukemia Virus, and Influenza A virus.
- GridION protocol has been optimized for realtime sequencing of disease investigation cases.

## **How the project benefits NAHLN:**

- Equipment supported from this funding enhanced capacity of Animal Disease Research and Diagnostic Laboratory (ADRDL) for detection of emerging pathogens.
- Recently in January 2024, we were able to detect first introduction of Avian Metapneumovirus subgroup B virus in US poultry. Later this was confirmed by NVSL and USDA declared it as an emerging disease.

# Diversifying Tennessee’s Diagnostic PCR Platforms and Increasing Surge Capacity

Adam Travis

C. E. Kord Animal Health Diagnostic Laboratory  
Nashville, TN

**Project Objective 1:** Procure and install a QuantStudio 5 (QS5) PCR system: The inclusion of a QS5 will maintain our surge capacity for HPAI while allowing us to continue performing ASF and FMD testing on more “routine” FAD investigations coming from our state’s swine plants. This also reduces our reliance on a single PCR platform (ABI 7500) in the event that a significant supply chain issue or end-of-life scenario arises for that platform.

**Deliverables:** The QS5 system has been procured and installed in the laboratory.

**Benefits:** Not only is the QS5 an additional PCR system that helps to diversify our available instrumentation and make us less reliant on a single amplification platform, we are also able to run routine assays on the QS5 and keep our ABI 7500 Fast instruments free to run NAHLN tests that have not yet been validated on the QS5 PCR system.

**Project Objective 2:** Implement NAHLN QuantStudio 5 diagnostic assays: During outbreak scenarios, network assistance may be required due to unanticipated personnel issues. Other proposals are being submitted that describe the laboratory’s involvement in a Southeastern cooperative cross-training exercise. Prior to or concurrent with that exercise, KAHDL will implement NAHLN scope testing (ASF/FMD) on this new diagnostic platform to most effectively cross-train visiting analysts, on whichever platform they routinely utilize at their home facility. Implementation of the QS5-specific procedures will also assist KAHDL in addressing ASF and FMD requests during times when HPAI surge capacity is at its peak.

**Deliverables:** The new QS5 has been validated for ASF, CSF, and FMD NAHLN protocols. We are currently working on validation packets to request a blanket deviation for AI, APMV, SIV, and FMD/SVA Multiplex NAHLN protocols.

**Benefits:** Due to recent news from Applied Biosystems about the sunseting of the ABI 7500 Fast PCR systems, the addition and validation of the QS5 PCR system has increased in importance. By planning ahead and diversifying our instrumentation early, we will be less impacted by this than if we had been caught completely unaware. We believe this also emphasizes the importance of the national network having multiple validated platforms for extraction and amplification so that the entire network is not vulnerable to this kind of change.



# Improving the Efficiency of FAD Sample Submissions through LIMS Enhancements

Adam Travis

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**Project Objective 1:** Notifier Enhancement – Delivery notifications: A previously funded NAHLN project from 2021 will be enhanced to allow sample submitters to receive notification when samples have arrived in the laboratory. This would allow regulatory officials, including APHIS personnel, to receive updates on sampling via autogenerated messages rather than emails with ever-changing recipient lists.

**Deliverables:** Our LIMS vendor, TraceFirst, has completed development of this feature. We recently onboarded a lab-dedicated IT specialist and with his help will be implementing this enhancement soon. We are collecting the names, phone numbers, and email addresses for everyone who should be notified for each FAD investigation and potential outbreak scenarios to configure the system. Previous tests have demonstrated that the Notifier is working as intended, and the feature has been made available to all laboratories utilizing CoreOne for Labs.

**Benefits:** The enhancements to the existing Notifier system will dramatically reduce or eliminate the need for sample tracking communications outside of the system. Our understanding is that the system can be expanded to include laboratories not currently utilizing CoreOne for Labs as their LIMS as well. Frequent, consistent messaging regarding sample movement is critical in outbreak situations, and this enhancement has the potential to streamline this network-wide.

**Project Objective 2:** User group meeting – On-site CoreOne for Labs user group meeting to share high volume accessioning strategies, identify pain points/areas for future enhancements, troubleshoot any ongoing messaging issues, and address other NAHLN priorities.

**Deliverables:** We have held a meeting with TVMDL and discussed meeting areas, possible dates, possible accommodations, and travel arrangements for the user group meeting. We have also compiled a list of labs we would like to invite. We are currently working on agenda items and picking a date for the meeting.

**Benefits:** This user group meeting will benefit NAHLN through any enhancements to our LIMS that could be added after the meeting. The labs will also be able to network and prepare to support one another should the need arise for network assistance during an outbreak response.

## **Expanding Support to the NAHLN by Enhancing Sustainability of Testing Capacity**

Jessica Walters, Anne Magee, Jennifer Carickhoff, Hailey Quercia

Virginia Department of Agriculture and Consumer Services, Harrisonburg Regional Animal Health Lab  
Harrisonburg, VA

**Project objectives:** Increase testing capacity in Foreign Animal Disease outbreak situations. Provide testing even in the face of shortages or supply chain issues. Assist with decision making at the state and federal level with regards to permitting and depopulation through zone surveillance.

**Deliverables completed:** Purchased and stored a stockpile of plates, tips, and kits to perform PCR testing for diseases such as HPAI, END, CSF, ASF, and FMD, and enhanced the sustainability of our testing capacity.

The Harrisonburg Regional Animal Health Laboratory is in the Shenandoah Valley region of Virginia, which corresponds to the highest density of poultry and dairy farms in the Commonwealth. Quick detection and control of foreign and emerging animal diseases is crucial not only to the welfare of the Commonwealth, where agriculture is the leading industry, but also to the nation overall given the contributions of Virginia to the food supply and export market. The ability to process samples from start to finish in a high-throughput manner is crucial for Disease Response Preparedness.

This Farm Bill Project allowed the Harrisonburg Regional Animal Health Laboratory to purchase a stockpile of plates, tips, and kits to perform PCR testing for diseases such as HPAI, END, CSF, ASF, and FMD. Having this inventory of supplies allows our laboratory to quickly respond to outbreaks of diseases of concern in the Commonwealth. We are prepared to run testing as soon as potential cases are identified, and run follow up testing in control zones. As part of the NAHLN, this stockpile of supplies will also allow us to aid other labs in the network, either through loaning supplies on short notice or aiding in performing testing.

This NAHLN Farm Bill Project enables the laboratory system to increase its ability to respond to outbreaks. We are very grateful for the contributions of this Farm Bill project which will help to safeguard the US agricultural industry.

# Expanding Support to the NAHLN by Improving Capability to Handle Surge Samples

Jessica Walters, Anne Magee, Jennifer Carickhoff, Hailey Quercia

Virginia Department of Agriculture and Consumer Services, Harrisonburg Regional Animal Health Lab  
Harrisonburg, VA

**Project objective:** Enhance the ability of the Virginia Department of Agriculture and Consumer Services Office of Laboratory Services Harrisonburg Laboratory to respond to animal disease outbreaks.

**Deliverables completed:** Kingfisher Flex Purification System procured, installed, and in use.

The Harrisonburg Regional Animal Health Laboratory is in the Shenandoah Valley region of Virginia, which corresponds to the highest density of poultry and dairy farms in the Commonwealth. This area is at high risk of a disease outbreak, as is the Commonwealth as a whole, given our ports and location within a major migratory pathway. In recent years this has been evidenced by outbreaks of HPAI and AMPV. Given this risk, acquisition of the KingFisher Flex Purification System benefits both this laboratory system and the NAHLN in several ways.

Prior to the purchase of this extraction unit, the lab depended on a single robotic extraction system. When this system has required repair or preventative maintenance testing has had to be halted. If the system were to go down during an outbreak this would have dire consequences, and Virginia would have to rely on neighboring NAHLN laboratories to aid in performing the testing. This would have increased turnaround time for response and the burden on other lab systems. By having two units, we have backup should one unit go down, allowing us to continue testing with minimal interruptions.

An additional benefit is that this acquisition increases the number of NAHLN testing units in the laboratory, increasing the capacity to meet testing needs in a surge situation. In the 2023 HPAI outbreak the system was able to respond and manage testing needs with one NAHLN unit. This was, however, an outbreak with only two infected commercial premises in a control zone of almost 200. If demand had been higher, it would have been more difficult to meet that need, as being able to extract only one plate at a time was a recognized bottle neck rate-limiting step.

Now with two NAHLN testing units we can elevate up to having two teams working simultaneously, doubling the testing that can be performed in a workday. This is crucial as we consider the challenges of HPAI to our poultry and dairy herds, as well as the concerns for ASF and other diseases entering the United States. With two units we can respond to larger outbreaks, and simultaneous challenges.

This NAHLN Farm Bill Project enables the laboratory system to step up its ability to respond to outbreaks. It also reduces the potential need to send excess testing to other members of the NAHLN and it gives us greater ability to aid our fellow members. We are very grateful for the contributions of this Farm Bill project which will help to safeguard the US agricultural industry.