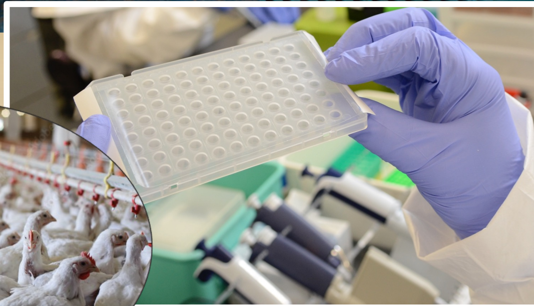
NAHLN Farm Bill Showcase



Abstracts from 2021 NAHLN Farm Bill (FY22) Funded Projects

NAHLN Farm Bill Showcase

Presentations from 2021 NAHLN Farm Bill Funded Projects

Wednesday, November 8, 2023
12:30PM – 3:30PM CT
Moderators: Christina Loiacono and Kelli Almes

12:30 PM Welcome -
Dr. Suelee Robbe-Austerman - Director, NVSL

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3:05 PM Questions / Comments

NAHLN Farm Bill Showcase

Presentations from 2021 NAHLN Farm Bill Funded Projects

Thursday, November 9, 2023
8:30AM – 11:30AM CT
Moderators: Kelli Almes and Stephen Cassle

8:30 AM Welcome -
Christina Loiacono – NAHLN Program Coordinator

8:35 AM Enhancing Avian Diagnostics Testing Capacity for the Detection and Differentiation of Avian Influenza Virus (AIV) and Newcastle Disease Virus (NDV) from Other Economically Significant Viral Respiratory Disease Agents by Multiplex Real-Time Reverse Transcription-Polymerase Chain Reaction (rRT-PCR) Assays
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9:20 AM Web-based Sample Submission for Foreign Animal Disease Investigations
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9:35 AM Enhancing Surge Capacity with a Streamlined Online Sample Submission Web Application / Increasing ASF Preparedness by Improving Diagnostic Submissions to the NAHLN Laboratory in Minnesota
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10:00 AM Data Exchange Between NAHLN Laboratories and Offices of State Animal Health Officials
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11:15 AM Questions / Comments / Closing

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NAHLN Farm Bill Showcase

Presentations from 2021 NAHLN Farm Bill Funded Projects

2021 NAHLN Farm Bill - Capacity Support

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Improving CVMDL Emergency Preparedness and Response: Developing Next-Generation Sequencing Capabilities

Guillermo R. Risatti, Zeinab Helal; Maureen Sims; Ji-Yeon Hyeon

Connecticut Veterinary Medical Diagnostic Laboratory, University of Connecticut, Storrs, CT

- **Project objective: Goal:** CVMDL's goal is to increase its emergency preparedness and response capacities by developing broader capabilities for the genetic characterization (WGS) of emergent pathogens. CVMDL will pursue **two objectives** for reaching the described goal.
- **Objective 1:** develop next generation sequencing (NGS) protocols to genetically characterize (WGS) emergent pathogens detected at CVMDL.
- **Objective 2:** develop and implement at CVMDL easy-to-use bioinformatics analysis pipelines for comprehensive and rapid analysis of sequenced pathogens.

Deliverables completed: the work performed by the CVMDL was able to meet the four milestones proposed in the project.

- M1: NAHLN Farm Bill 2021 funds awarded, and grant established at the University of Connecticut
- M2: Requested equipment installed at CVMDL.
- M3: Established protocol for processing tissue samples for NGS (Aim 1).
- M4: Developed bioinformatics tools (Aim 3).

Publications generated by the CVMDL team (participants of the project are underlined)

1. Zeinab H. Helal[a], Nina Francesca Soriano[a], Ji-Yeon Hyeon[b], Hyunjung Chun[a], Maureen Sims[a], Amelia Wheeler[a], Guillermo R. Risatti[a#]. Whole Genome Sequence of a Rabies lyssavirus (RABV) detected in an American black bear (Ursus americanus) in Connecticut, USA. Submitted to: Microbiology Resource Annoucement (ASM), under 1st review.
2. Hyeon JY, Helal ZH, Appel A, Tocco N, Hunt A, Lee DH, Risat t i GR. Whole genome sequencing and phylogenetic analysis of West Nile viruses from animals in New England, United States, 2021. Front Vet Sci. 2023 Apr 28;10:1085554. doi: 10.3389/fvets.2023.1085554. PMID: 37187933; PMCID: PMC10175668.
3. Chung DH, Helal Z, Desiato J, McGinnis H, Sims M, Hunt A, Kim J, Risat t i GR, Lee DH. Genome sequencing and analysis of the raccoon variant rabies lyssaviruses directly from clinical samples, Connecticut, 2017-2019. Front Vet Sci. 2022 Sep 23;9:1001204. doi: 10.3389/fvets.2022.1001204. PMID: 36213416; PMCID: PMC9539882.
4. Hyeon JY, Risatti GR, Helal ZH, McGinnis H, Sims M, Hunt A, Chung DH, Kim J, Desiato J, Lee DH. Whole Genome Sequencing and Phylogenetic Analysis of Rabies Viruses from Bats in Connecticut, USA, 2018-2019. Viruses. 2021 Dec 13;13(12):2500. doi: 10.3390/v13122500. PMID: 34960769; PMCID: PMC8704678.
5. Lee DH, Helal ZH, Kim J, Hunt A, Barbieri A, Tocco N, Frasca S Jr, Kerr K, Hyeon JY, Chung DH, Risat t i G. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in a Dog in Connecticut in February 2021. Viruses. 2021 Oct 23;13(11):2141. doi: 10.3390/v13112141. PMID: 34834948; PMCID: PMC8623824.

How the project benefits NAHLN: The project described here represented a measurable improvement for CVMDL. The laboratory can genetically characterize pathogens detected at CVMDL utilizing next-generation sequencing (NGS). This contribution is necessary for achieving one of CVMDL's strategic objectives: to provide support to the NAHLN whenever required.

Rapid Identification and Characterization of Avian Foreign Animal Diseases in a Single-Assay Using Nanopore Sequencing

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

Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA

Project Overall Objective:

Optimization of Oxford Nanopore Technologies (ONT), along with different enrichment methods to increase the sensitivity and accuracy of the assay. The ultimate goal of this project is to produce specific protocols for sample preparation, enrichment methods and establishing the assay limit of detection, aiming to allow ONT to be a frontline diagnostic tool for avian foreign animal diseases.

Project specific objectives:

#1. Evaluate the effect of different extraction and library preparation methods on the nucleic acid quality, quantity, read length, and effects on the ONT sequencing performance.
#2. Optimize and evaluate ONT ability to identify and characterize IAV-A and vND from pure isolates and clinical samples.
#3. Evaluate and optimize host nucleic acid depletion and target nucleic acid enrichment methods to improve the performance (sensitivity and accuracy) of the assay.
#4. Validation of Nanopore Sequencing limit of detection to that of qPCR and its accuracy of sequence typing to that of Sanger and Illumina sequencing.

Project completed deliverables:

Objective #1: comparison of four different commercial extraction kits using known positive clinical samples for Avian Influenza virus (AIV) and NewCastle disease virus (NDV).

Four commercially available extraction protocols were selected: (1) MagMAX™ Pathogen RNA/DNA Kit (Thermo Fisher Scientific); (2) QIAamp® Viral RNA Mini Handbook (QIAGEN; (3) TRIzol™ LS Reagent (Invitrogen); and (4) SwiftX™ Swabs (Xpedite Diagnostics). The extraction outcome was evaluated in quantity (total RNA concentration and number of viral copies), quality (purity), and sequence data generated with Nanopore Sequencing to compare the performance between the kits. Our results showed the outperformance of TRIzol™ in concentration results, and QIAamp® in purity results. Despite the highest concentration results, TRIzol™ showed inconsistent RT-qPCR detection rate, giving negative results from known positive samples. SwiftX™ Swabs kit showed the worst performance in all parameters. Even though MagMAX™ didn't outperform in any evaluated parameter, it showed a consistent good performance and better applicability. Therefore, MagMAX™ was selected as the optimum method for the further steps of the project.

Objective #2: for AIV trials, only clinical samples were included. For NDV, both clinical samples and isolates were utilized for the extraction and sequencing evaluation.

For AIV extraction and sequencing experiments, the sample types included multiple tissue types (lung, trachea, intestine, brain, kidney, heart) and swabs (spleen and air sac). For NDV, the clinical samples included oropharyngeal and drinker swabs, and multiple tissue homogenates (trachea, lung, liver, kidney, brain, and intestine). Additionally, Avian paramyxovirus isolates were included in the analysis.

Objective #3: Different host nucleic acid depletion and target nucleic acid enrichment methods were evaluated for the pre-processing step of AIV and NDV sequencing. In addition to the pre-processing, two different library

preparation chemistries from the ONT store were compared, the Rapid Barcoding kit, and the Ligation Sequencing kit with PCR Barcoding expansion.

For the host nucleic acid depletion, two commercially available DNases were evaluated (TURBO DNase, and Zymo DNase I). Additionally, host rRNA depletion was performed with one commercially available kit (NEBNext® rRNA Depletion Kit v2 (Human/Mouse/Rat)) and one custom-designed probe targeting the chicken rRNA and some poultry bacterial pathogens (Parris et.al., 2022). The enrichment of viral genome was achieved by applying different approaches. For AIV, an amplicon-based approach using Universal Primers for Influenza A Virus was performed, comparing two sets of primers (Zhou, et. al., 2009 and Mena et. al., 2016). Additionally, for AIV and NDV, a target-independent approach was implemented, a Sequence Independent Single Primer Amplification (SISPA) (Chrzastek et. al., 2017). Finally, a semi-target approach was developed based on the design of spiked primers (13 – 15 bp long) (Chiu, et. al., 2020) from a panel of representative sequences from NBCI genomes for AIV and NDV. Preliminary data showed lower diagnostic sensitivity of the sequence-independent approach, and the combination of the custom-designed rRNA depletion protocol with SISPA outperformed the other options in this category. The amplicon-based approach provided higher coverage and higher sensitivity, with both sets of primers performing similarly. Additionally, semi-target approach was utilized with slight modifications and provided coverage and sensitivity in-between the other two approaches. However, this approach carries a promise because given its potential to enrich for more than one target sequence at the same time during the reverse transcription step, we can keep a broad-spectrum enrichment and increased sensitivity working from clinical samples.

The library preparation chemistries will be evaluated regarding their applicability, total and viral-specific yield generated, sequence quality score and read length. Finally, the genome coverage and accuracy of the viral specific reads will be assessed. Preliminary data regarding the applicability of the kits 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 generation of shorter reads compared to the Ligation, that uses an end-preparation strategy for the samples' barcoding. Finally, the LSK provided a larger output, with a higher number of bases generated. Additional analysis will be performed regarding the reads' score, taxonomy classification and extraction of avian influenza reads for metrics calculation of genome coverage.

Additional accomplishments

Poster presentation

- Iowa State University CVM Research Day (2023)
- Egg Industry Symposium (2022 and 2023)

Oral presentation

- Selected as a Lightning Speaker for Nanopore Community Meeting (Houston, TX December 2023)
- 2023 AAVLD Annual Meeting, National Harbor, Maryland (2023)
- AAAP 2023 Conference, Jacksonville, Florida
- 74th North Central Avian Disease Conference, Minneapolis, Minnesota (2023)

Awards

- Poster Presentation Award (2nd place) at CVM Research Day, Ames, Iowa (2023)
- AAVLD Student/Trainee Award at AAVLD Annual Meeting (2023)
- Graduate Travel stipend for the 2023 AAAP Conference
- Graduate Travel stipend for 74th North Central Avian Disease Conference
- 2023 AAAP Foundation Poultry Scholarship, American Association of Avian Pathologists (2023)

Project deliverables yet to be completed:

Objective #2: the inclusion of isolates for AIV sequencing comparison.
Objective #3: the comparison of different pre-processing techniques (host nucleic acid depletion, amplicon-based, and spiked primers) and library preparation approaches for NDV.
Objective #4: Establish the final workflow for ONT sequencing for poultry FAD and validation of the generated ONT data in comparison to Illumina and Sanger sequencing.

Project benefits to NAHLN:

This project aims to establish a comprehensive workflow for identifying and characterizing two Foreign Animal Diseases facing the poultry industry. By doing so, it will significantly reduce the time required for diagnosing Avian Influenza (AIV) and Newcastle Disease Virus (NDV), reducing it from days to weeks down to mere hours to a day. Furthermore, this approach eliminates the need for continuous PCR primer updates and virus isolation since the workflow is specifically designed for clinical samples.

References:

1. Zhou, Bin, et al. "Single-reaction genomic amplification accelerates sequencing and vaccine production for classical and Swine origin human influenza a viruses." *Journal of virology* 83.19 (2009): 10309-10313.
2. Ignacio Mena Martha I Nelson Francisco Quezada-Monroy Jayeeta Dutta Refugio Cortes-Fernández J Horacio Lara-Puente Felipa Castro-Peralta Luis F Cunha Nídia S Trovão Bernardo Lozano-Dubernard Andrew Rambaut Harm van Bakel Adolfo García-Sastre (2016) Origins of the 2009 H1N1 influenza pandemic in swine in Mexico eLife 5:e16777.
3. Chrzastek, Klaudia, et al. "Use of Sequence-Independent, Single-Primer-Amplification (SISPA) for rapid detection, identification, and characterization of avian RNA viruses." *Virology* 509 (2017): 159-166.
4. Deng, X., Achari, A., Federman, S. *et al.* Metagenomic sequencing with spiked primer enrichment for viral diagnostics and genomic surveillance. *Nat Microbiol* **5**, 443–454 (2020). https://doi.org/10.1038/s41564-019-0637-9

Validation of Automated High Throughput Testing for Diseases of High Consequence

Duan Loy, Bruce Brodersen, Aaron Sedivy

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Introduction

Many laboratories have acquired additional equipment and expertise for implementing automated high throughput testing for SARS-CoV-2. Laboratories have the capability to adapt this equipment to FAD testing, but adaptation of these instruments along with the extensive validation and verification of this approach is needed. Automation of laboratory testing reduces error in test performance and reduces the need for additional personnel while increasing testing capacity. Automation of different stages of test procedures allows laboratory personnel time to be repurposed to perform tasks that require manual attention. Automated liquid handlers and automated dispensers have the capacity to perform several critical test steps that reduce the need for manual liquid handling and can reduce the potential for human error. Additionally, they are critical to leverage low sample volume high test throughput solutions such as 384 well plate formats. Instruments with fixed tips and wash steps reduce the need for consumables, especially in times of tight supply chains. Programming and validation of the liquid handlers is required to perform the needed functions, such as needed movements for automation and performance of the desired actions for the test in question. Validation of the instrument, once test specific programming is completed, involves assuring cross contamination of specimens does not occur and there are comparable limits of detection to the prior method. Level of detection experiments are used with control specimens containing known concentrations of the target agent. Serial dilutions were performed to match the current limits of detection of the assay and placed on plates in a checkerboard pattern to assess cross contamination. Combined assessment of the performance of the liquid handling using these methods allows for comparison with manual methods. This project evaluated and validated the use of two liquid handling instruments applied to test processes using two avian NAHLN scope diseases (IAV-A and APMV-1) commonly ran in tandem during foreign animal disease investigations. A Tecan Fluent 780 that has barcoding capabilities was utilized for sample transfer from collection tubes to extraction plates, and a Tecan Fluent 480 was utilized to transfer extracted nucleic acid samples and positive and negative controls to plates containing PCR mastermix. A segment of the project also included evaluation of automated micro dispensing systems (Formulatrix Mantis) to rapidly dispense mastermix solution into plates. Additionally, the use of 384 well PCR plates was evaluated using these systems for evaluation in an advanced real time thermocycling platform (Thermo Quant Studio 7 Pro) (QS7).

Objectives:

Goal #1

Adapt current standard operating procedures (SOP-AV-0068) for testing of field specimens for viral pathogens of high consequence to poultry and livestock to automated liquid handling and reagent dispensing systems. This goal would apply specifically to highly pathogenic avian influenza virus.

Objective #1. Validate using the Tecan Fluent 780, an advanced liquid handling system that has the capability of reading barcoded tubes to create worksheets which transfer to the remainder of the automated testing system. This liquid handling system can transfer clinical samples to four 96 well plates for extraction in a matter of approximately 30-40 minutes.

Objective #2. Using the current specific operating procedures (SOPs) for testing of high consequence diseases, adapt the setup of PCR plates to an automated liquid handling system. Ninety-six well plates containing nucleic acid extracted from mock specimens will be used on the liquid handler and then transferred to PCR plates which are preloaded with master mix.

Objective #3. Validate loading PCR plates with master mix using an automated liquid micro dispensing system (Mantis, Formulatrix).

Objective #4. Perform cross contamination studies and limit of detection (LoD) studies using 96-well and 384-well plates to demonstrate equivalence to current SOP's using the QS7.

Deliverables Completed:

Objective 1: Evaluation of the Tecan Fluent 780 to transfer BHI media spiked with diluted positive extraction control (PEC) from collection tubes to 96 well extraction plate was completed. The validation process included evaluation for cross contamination during sample transfer while using fixed tips and a wash cycle. Samples evaluated included 10 negative samples and 10 containing PEC (IAV-A PEC, 204 ADV, NVSL) at a dilution of 1:10. This cross-contamination study design was also performed using the Fluent 480 instrument for the extracted nucleic acid transfer step. PCR was then conducted in both 96-well and 384-well formats. Comparison of analytical sensitivity and specificity demonstrated that the instrument and transfer protocol showed 100 % (30/30) agreement for both positive and negative samples in 96-well formats with mean Cq ± SD of 34.63 ± 0.46, 33.75 ± 0.58, 34.13 ± 0.51 for manual transfer, Fluent 780 and Fluent 480, respectively. All negative samples remained negative for IAV-A M target (n=60) and no contamination between wells was observed. Using the 384-well format, the result showed that overall agreement with the 96-well format was 100 % (30/30) in both positive and negative samples with Cq values of 32.66 ± 0.73 for the manual transfer and 31.75 ± 1.06 for Fluent 480. The results indicate that cross contamination was not observed using liquid handling in comparison with manual transfer methods.

Objective 2, 3, 4: Evaluation of the Tecan Fluent 480 liquid handler to transfer extracted nucleic acid from samples into master mix loaded plates was conducted. Limit of detection (LoD) and limit confirmation assessments were done in comparison with manual nucleic acid transfer. To test the equivalency of the Tecan Fluent 480 liquid handler and Formulatrix Mantis micro dispenser, LoD finding study was conducted using either the manual or automated protocols. Evaluation was done with mock extraction samples containing synthetic gBlock (3.4 x 10^5 copies) (Integrated DNA Technologies) nucleic acid of IAV-A gene fragments, which were spiked into elution buffer at concentrations of 10000, 1000, 100, 10, 1 and 0 copies/µl. Mock extraction samples were then RT-qPCR tested in triplicate using the approved NAHLN protocol. In the 96-well format, the RT-qPCR was tested on QS7 and ABI 7500 thermocyclers; in the 384-well format, the RT-qPCR was tested on an QS7 Pro thermocycler. Results showed the LoD of the IAV-A assay using the automated protocol (Tecan Fluent 480 and micro dispenser Mantis for sample processing in the 96-well format and 384-well format were confirmed to be 10 copies/µl or fewer of IAV-A M gene. Additionally, the LoD was evaluated for H5 Positive amplification control (PAC) (24.3 Cq ABI, 24.4 Cq QS7) and H5 MX PAC (23.9 Cq ABI, 24.2 Cq QS7) using the automated protocol for sample processing. The QS7 instrument was not different from the LoD of the manual transfer and dispensing process on ABI7500 at 1:100. However, The LoD for the H7 PAC (25.7 Cq ABI, 26.5 Cq QS7) using the automated protocol for sample processing on QS7 is 1:10 and the LoD of the manual process run on the ABI7500 was 1:100. The LoD of the H7 EA PAC using the automated protocol for sample processing on QS7 is 1:100, and the LoD of the Manual process on ABI7500 is 1:1000. The automated protocol demonstrated there was no loss in detection for the automated and/or high throughput plate formats when compared with manual transfer in 96 well plates for IAV-A M genes, and one dilution difference for the H7 targets was noted.

Goal #2

Workflows developed in Goal #1 should be transferrable to other tests for NAHLN scope diseases.

Cross-contamination studies were conducted according to objective 1 for APMV-1, using BHI spiked with PEC (205 ADV1402) at a ratio of 1:20 for APMV-1. The results showed that all negative samples remained negative

for APMV-1 target (n=60) and no contamination between wells was observed. Using the 384-well format, the result showed that overall agreement with the 96-well format was 100 % (30/30) in both positive and negative samples. The LoD was also evaluated for the APMV-1 assay using a similar method with synthetic gBlock nucleic acid sequences from the APMV-1 genome (3.48 x 10^5 copies/uL) and diluted to establish a standard curve for target concentration. The automated protocol for sample processing in the 96-well format and the 384 well format was confirmed to be 1000 copies/µl or fewer for APMV-1 gene target, which was equivalent to the manual procedure. The LoD of the vNDV assay was evaluated with the PAC control (24.5 Cq ABI, 25.17 Cq QS7) using the automated protocol for sample processing and run on the QS7 instrument. The LoD for the automated procedure was not different from the manual process run on ABI7500 at 1:100 dilution of the PAC. The protocol comparison demonstrated that there was no loss of detection for the automated and/or high throughput plate formats when compared with manual transfer in 96 well plates for APMV-1 and vNDV.

How the project benefits NAHLN

This project benefits NAHLN by providing evaluation of commercially available liquid handling solutions and higher capacity PCR instrumentation that could be utilized in the face of an outbreak that requires high throughput testing. Liquid handling and automation reduce the potential for human error and the use of fixed tips reduces the need for consumables, which may have limited supply during outbreaks. Speifically, the project provides liquid handling solutions along with processing scripts that enable rapid transfer of samples from tubes to extraction plates, extraction plates to PCR plates, and rapid dispensing of master mix into PCR plates, and workflows that support 384 well plates. Additionally, this provides validation data to support the equivalence of the QS7 instrument, which enables additional potential PCR instrumentation that can support 384 well plate formats. These scripts and instrument solutions provide a framework for utilizing these instruments more broadly for NAHLN scope diseases and they should be readily adaptable to different disease testing scenarios depending on the testing matrix. This evaluation provides support for the continued assessment of these instrument and methods using diagnostic samples or in outbreak scenarios to further diagnostic testing capacities and capabilities.

Increasing Capacity to Handle Surge Samples Through Elimination of the Data Entry Step in the Laboratory

Jeremiah T. Saliki [1], Jeffrey Duke[2]

[1]Oklahoma Animal Disease Diagnostic Laboratory, Stillwater, OK, [2] University of Georgia

Objectives: Sample intake is a major bottleneck to managing high throughput in diagnostic laboratories. The goal of this project was to create intuitive and disease-specific online forms for use by people submitting single or multiple samples. The overarching goal is to increase the capabilities, capacity, and readiness of NAHLN laboratories to respond to diseases of economic or public health interest. This project involved the VetView laboratory information management system and included four objectives:

1. Improve usability of the VetView Portal User Interface by updating it to the ZK framework (https://www.zkoss.org). The ZK framework allows for dynamic content and rich interactive web design.
2. Design a multiple animal submission workflow, including features that make it easier to create lists of patients, specimens, and tests needed. Features include Excel file import, worksheet style layout to allow for patients and specimens to be created in an on-screen table with navigation like Excel or google sheets; barcode scanner support, and specimen labeling that can accept third-party sample ID and/or generate a printable label to uniquely track samples.
3. Create customizable NAHLN program disease specific submission forms that only ask and require the appropriate data for the submission. This will expedite the submission process and help to guarantee that the results can be messaged to NAHLN, with minimal oversight or training of accessioning staff.
4. Implement and test the new sample submission portal at five VetView/NAHLN Labs: GA-Athens, GA-Tifton, Kansas State, Missouri, and Oklahoma State. Each lab will work with the VetView team to set up and test the portal prior to recruiting and encouraging submitters to use the portal to submit cases electronically rather than using the current paper submission forms.

Status: Objectives 1 & 2 have been completed and released to laboratories for testing and implementation. Objective 3 is in development and is scheduled for release within 1 month. The disease specific template setup has been completed; creation of the dynamic forms based on the disease specific template is pending. Objective 4 has been partially completed at Oklahoma State where the electronic submission process has undergone successful beta testing and being routinely used by two beta testers. Testing and implementation of the portal is pending at the four other laboratories. A no-cost extension was sought and we are on track to complete the project by 2/29/2024.

Benefits to NAHLN: Nine NAHLN labs currently use VetView. Availability of this portal will increase testing throughput by alleviating the accession bottleneck. Data quality will improved by having submission forms tailored to disease specific requirements and having data entered directly by submitters. Improved data quality will also facilitate messaging test results to NAHLN. Finally, the new process will enhance scalability in times of outbreak by reducing the learning curve for submitters and accession staff in respect to disease required data elements and test coding.

Enhancing Expedited Bulk Sample Submission and Message Processing at the Oregon Veterinary Diagnostic Laboratory

Justin L. Sanders, Susie Strangfield, Matt McClain, Dawn Dirks, Donna Mulrooney, Michael McGrath, Brian McCluskey, Tammy Leimer

Oregon Veterinary Diagnostic Laboratory, Corvallis, OR

The objective of this project is to develop and implement enhancements to the LIMS in use at the Oregon Veterinary Diagnostic Laboratory (OVDL), CoreOne for Labs, that will allow OVDL to handle surge testing in the most efficient manner possible by reducing the need for duplicate data entry from sample submission to set up of the PCR instruments, and allowing for expedited reporting and messaging of results to the NAHLN. Project deliverables include: 1. The development of rapid submission templates for offline and online use – 100% complete, 2. The creation of a bulk messaging module for CoreOne – 100% complete, 3. the development of bidirectional HL7 interface to transfer data between the OVDL and Oregon Department of Agriculture – 100% complete, and 3. The development of a bidirectional interface between CoreOne and PCR instruments – 60% complete. This project benefits the NAHLN by by providing a streamlined interface for expedited reporting and messaging of results and minimizing errors due to duplicate data entry. The enhancements to CoreOne will be made available to all NAHLN member laboratories using CoreOne for Labs.

Enhancing OVDL Emergency Preparedness for Regulatory Testing Through Exercise and Inter-Agency Coordination

Christiane V. Löhr; Susan Tornquist, Dr. Kurt Williams; Andrew Dixon; Donna Mulrooney; Ryan Scholz

Oregon Veterinary Diagnostic Laboratory, Corvallis, OR

Objective 1: By the end of the project, the OVDL will have developed, implemented and trained on a preparedness plan for regulatory testing during emergencies.

1. Gap and needs analysis and development of an OVDL emergency preparedness plan
- **1.** Walk through of a FAD outbreak sample from Receiving to Reporting (PI, QA manager and Emergency management coordinator)
- **2**. Debrief walk through as low-level tabletop exercise with critical sections and personnel (PI, QA manager, Emergency management coordinator, supervisors in Receiving, Molecular Diagnostics, Necropsy, and Business Office)
- **3.** Develop an emergency preparedness plan (PI, QA manager, Emergency management coordinator, supervisors in Receiving (REC), Molecular Diagnostics (MOL), Necropsy (NEC), and Business Office (BUS))

2. Test, refine, and train on OVDL emergency preparedness plan
- **1.** Complete internal tabletop exercise with expanded personnel group (PI, Co-PI, project manager, QA manager, supervisors and staff representative from REC, NEC, MOL, BUS)
- **2.** Revise the emergency preparedness plan as needed and devise training program (PI, QA manager, Emergency management coordinator)
- **3.** Train on the emergency preparedness plan (OVDL all)

3. Functional emergency drill and integration of OVDL emergency plan into quality management program
- **1.** Complete OVDL-internal functional exercise with processing of dummy samples at expected volume (OVDL all)
- **2.** Revise emergency response plan and OVDL training strategy (PI, Co-PI, Co-I)
- **3.** Integration of the emergency response plan into the OVDL quality management program (QA manager, Emergency management coordinator)

Objective 2: By the end of the project, the OVDL will have tested and refined its cooperative emergency response preparedness for regulatory testing in the context of a statewide emergency response.

1. Integration of OVDL emergency preparedness plan into statewide emergency planning
- **a.** Host and participate in state-wide tabletop exercise (PI, Co-PI)
- **b.** Refine OVDL emergency response plan as needed (PI, Co-PI)
- **c.** Contribute to refinement of state-wide response plan (PI and Co-PI; primary responsible resting with ODA; see companion proposal)

Deliverables completed / Deliverables yet to be completed:

Project Timeline – Most objectives have been completed; some are still ongoing

OVDL Hazard Analysis – Completed
OVDL Internal TTX – Completed
Emergency Operations Plan Draft – Completed
ODA ASF Workshop – Completed
ODA ASF Multi-stakeholder TTX – Completed
HPAI/CWD Internal Function Exercise – Completed
Necropsy Functional Exercise – Completed
Bacteriology/Necropsy Walkthrough – Completed
Emergency Operations Plan Final Draft – Yet to be completed
OVDL Staff Training on Final Emergency Operations – Yet to be completed
OVDL Full-Scale Exercise – Yet to be completed

How the project benefits NAHLN: The project focuses on enhancing emergency preparedness of the Oregon Veterinary Diagnostic Laboratory (OVDL) within the Carlson College of Veterinary Medicine at Oregon State University, and offers several significant benefits to the National Animal Health Laboratory Network (NAHLN):

Capacity Building: The project involves iterative exercises and plan development, which allows the OVDL to build its capacity to handle a range of emergency situations and diseases. By developing and refining emergency preparedness plans, the OVDL not only becomes better equipped to respond to crises but also serves as a capacity-building model for other laboratories within NAHLN. This capacity building strengthens the overall readiness and resilience of NAHLN by promoting best practices in emergency response and preparedness, ultimately bolstering the network's effectiveness in safeguarding animal health and public safety on a national scale. This includes all of the Pacific Northwest, which is limited in its current capacity to manage diagnostic testing due to limited veterinary laboratories within the geographically and agriculturally diverse region.

Improved Response to Outbreaks: The OVDL, situated on the Pacific Coast and providing testing throughout Oregon and into Southern Washington, Norther California, Idaho, Montana, and Nevada, is one of only three diagnostic laboratories in the region capable of handling disease outbreaks of national importance. By enhancing emergency preparedness, the OVDL can respond more effectively and rapidly to disease outbreaks that may have national implications. This directly supports the NAHLN's mission in surveillance and early detection of animal diseases, especially those with the potential to spread across state lines and national borders (foreign animal diseases).

Inter-Agency Coordination: The project involves inter-agency coordination, which is essential for managing large-scale disease outbreaks. The ability of OVDL to work effectively within a statewide emergency response system benefits NAHLN by facilitating collaboration and information sharing between agencies. Specifically, between the OVDL and the Oregon Department of Agriculture. This, in turn, contributes to a more coordinated and effective response to animal health emergencies.

Quality Management and Training: The project's focus on developing, testing, and training on an emergency preparedness plan will result in a well-structured and organized response system with the laboratory. This will improve the capabilities of the OVDL and its employees.

In summary, the OVDL's project benefits NAHLN by improving the laboratory's preparedness for emergency management in veterinary medicine through introduction of innovative protocols, promoting cross-disciplinary collaboration, showcasing the integration of regulatory testing, offering training and education, and ultimately enhancing the sector's resilience in the face of animal health emergencies.

Increasing Regional Testing Capacity and Rapid Pathogen Characterization Capabilities at the Oregon Veterinary Diagnostic Laboratory

Justin L. Sanders, Donna Mulrooney

Oregon Veterinary Diagnostic Laboratory, Corvallis, OR

The objective of this project is to enhance the existing testing capabilities of the Oregon Veterinary Diagnostic Laboratory (OVDL) to support NAHLN-related disease surveillance and outbreak activities. The project deliverables include: 1. Incorporation of new testing equipment into the OVDL diagnostic testing operations – 50% complete, 2. Harmonization of laboratory protocols and streamlining of nucleic acid extraction procedures – 100% complete, 3. Implementation of rapid sequencing of pathogens – 40% complete. This project benefits the NAHLN by expanding the surge-testing capacity of the OVDL and providing additional capacity for pathogen characterization in the region.

Increased Capacity for Disease Testing with Digital Microscopy and Whole Slide Imaging for NAHLN

Keith Poulsen, Maggie Highland, Betsy Elsmo, Lorelei Clarke, Ashlee McDonald, Dan Barr

Wisconsin Veterinary Diagnostic Laboratory, Madison, WI

Objective 1: Increase the capacity of the Wisconsin Veterinary Diagnostic Laboratory as a NAHLN Level 1 Laboratory with the purchase and of validation digital microscopy for remote histopathology.

Enhance remote digital access and decrease histopathology turnaround time compared to traditional glass slides for remote pathologists, WVDL-Barron, and reference laboratories.

Objective 2: Improve the capability and turnaround time for reporting NAHLN scope diseases with digital microscopy, specifically for currently approved protocols for immunohistochemistry to diagnose chronic wasting disease.

Test the hypothesis that digital microscopy is faster than light microscopy with no loss in sensitivity or specificity using approved immunohistochemistry protocols for chronic wasting disease of cervids using WVDL's established sample library.

Test the hypothesis that digital microscopy has a shorter turnaround time than light microscopy with no loss in sensitivity or specificity using approved immunohistochemistry protocols for chronic wasting disease during captive and wild cervid harvests.

Deliverables completed:

Procure equipment: Purchase digital slide scanner and install in the WVDL histology lab. Configure associated software. Train technical staff.

Perform initial quality assessments: Perform validation for analyzing diagnostic slides (H&E, special stains, IHC) and TSE slides (IHC). Validate use of multiple screen types and settings to achieve the same results. Color calibrate computer monitors and laptops.

Test functionality: Optimize Eslide manager online slide management system to current WVDL workflow and case/slide naming specifications. Write new SOPs, following AAVLD guidelines, for use of digital pathology.

Retrospective case evaluation/test validation: Scan and assess quality of digitized archived CWD IHC slides. Pathologists interpret submissions by light microscopy (LM) and digital microscopy (DM); pathologists are blinded to case numbers and thus previous results for each slide in order to assess sensitivity and specificity between glass slide and digitized slide assessments.

Deliverables yet to be completed:

Increase test capacity and decrease turnaround time: Digitize diagnostic histopathology slides to be evaluated by pathologists working remotely or at the Barron Laboratory and for consultation on cases with internal or external pathologists. Compare turnaround time on cases that are completed by DM versus those completed by LM.

Prospective case evaluation: Digitize CWD IHC diagnostic slides during the 2023-2024 heightened CWD testing season and compare overall case turnaround time from prior years in which diagnostic interpretation

was limited to only LM.

Publish findings: Complete and submit findings to a peer-reviewed journal for review.

How the project benefits NAHLN

The identified need and project align with 2021 Priority 1: Increased capacity for disease testing focused on currently approved protocols. We will address Priority 1 by purchasing and validating digital microscopy (DM) as a method to rapidly distribute diagnostic images to pathologists working remotely and to our diagnostic lab located in Barron, WI. Barron is 250 miles from the Madison Laboratory, where tissues are processed for histopathology. We will also develop and validate methodology to assess and score chronic wasting disease (CWD) via the currently approved immunohistochemistry (IHC) protocols.

The immediate positive impacts of this equipment and validation will be to facilitate remote work and diagnostics by removing the requirement of a microscope and to decrease sample turnaround time by removing time required for shipping and handling. For NALHN Scope diseases, such as CWD, the WVDL has developed a national reputation for excellence and performs ~115,000 tests per year with ~800 IHC confirmations. Minimizing turnaround time for the ~20 states that submit samples is *critically important* for hunters to make informed decisions on consumption of harvested deer and states to manage their captive and wild cervid herds.

Scanned slides can be easily shared among several pathologists for consultation or training purposes and digital archives allow for collation of related case material. Digital microscopy also greatly enhances opportunities to develop standardized images for diagnostic training of NAHLN scope diseases, which the WVDL regularly handles. This proposal will address Priority 1 by building infrastructure to rapidly provide DM images for diagnostic cases in a foreign animal disease outbreak, such as African Swine Fever, where confirmation of histologic lesions is necessary at reference laboratories.

Validation of Real-time Reverse Transcription PCR (RT-qPCR) Assay for the Detection of Spring Viremia of Carp Virus (SVCV)

Prithvi Karki, Lijuan Zhou, Janet Warg, Tom Waltzek, Reddy Bommineni

Bronson Animal Disease Diagnostic Laboratory, Kissimmee, FL

1. **Objective:** Spring Viremia of Carp (SVC) is a reportable disease caused by rhabdovirus *(Rhabdovirus carpio)* frequently referred to as Spring Viremia of Carp Virus (SVCV). It has been listed as a notifiable disease by the World Organization for Animal Health (WOAH). It is a contagious viral disease mainly seen in farmed carp and outbreaks can cause substantial economic loss. Currently, the NAHLN-approved method for detection of SVCV involves virus isolation and confirmation via conventional PCR/ Electron microscopy. The process of virus isolation is time-consuming and requires specialized cell lines and technical expertise. A rapid detection method, RT-qPCR, directly from tissue is required to minimize that turnaround time. The objective of this project is to validate, optimize, and compare the previously published SVCV RT-qPCR assays listed below for suitability of surveillance and disease monitoring

Name of the assay	Reference
Yue assay	Yue et. al., 2008
Zhang assay	Zhang et. al., 2009
Q2N assay	Clouthier et. al., 2021
Q1G assay	Clouthier et. al., 2021

2. **Deliverables completed:**

2.1. Standardization of the assays:

2.1.1. Standardization and optimization of all the published assays were performed using the AgPath one-step kit on the Quantstudio 5 Realtime PCR system. During the initial standardization of the assays, different parameters such as buffer, annealing temperature, and primer/probe concentration were adjusted for optimization.

2.1.2. Triplicates of the finalized conditions were run successfully on all four published assays.

2.2. Specificity analysis of the primers and probes from previous publications:

2.2.1. A total of 362 SVC sequences, including 19 of SVC complete genomes were downloaded from NCBI GenBank. Sequence alignment was performed for the 19 SVC completed genomes.

The primer and probe sequences from 4 published assays were mapped to the aligned genomes and the number of SNP(s) for each primer or probe was indicated in the table below:

Isolates	Yue sequence (Glycoprotein gene)			Zhang assay (Glycoprotein gene)			Q2N assay (Nucleoprotein)			Q1G assay (Glycoprotein gene)		
	F1, 23bp	R1, 22bp	P1, 29bp	F2, 20bp	R2, 23bp	P2, 22bp	F3, 19bp	R3, 19bp	P3, 17bp	F4, 20bp	R4, 26bp	P4, 20bp
MT675953.1 - Heilongjiang, China	0	1 SNP (22)	1 SNP (25)	1 SNP (5)	3 SNPs (2, 5, 11)	0	0	0	0	0	0	0
MZ343157.1 - Mississippi river, USA	0	0	2 SNPs (2, 11)	1 SNP (5)	3 SNPs (2, 5, 11)	0	1 SNP (8)	0	0	0	0	0
MG663512.1 - South Korea	0	1 SNP (22)	1 SNP (24)	1 SNP (5)	3 SNPs (2, 5, 11)	0	0	0	0	0	0	0
MG663513.1 1 - South Korea	0	0	0	1 SNP (5)	3 SNPs (2, 5, 11)	1 SNP (4)	0	0	1 SNP (7)	0	0	0
MG663514.1 1 - South Korea	0	0	0	1 SNP (5)	3 SNPs (2, 5, 11)	1 SNP (4)	0	0	1 SNP (7)	0	0	0
KY475636.1 - Shanghai, China	0	0	1 SNP (12)	1 SNP (5)	3 SNPs (2, 5, 11)	0	0	0	0	0	0	0
KU230365.1 - Imported from HK,China	0	0	0	1 SNP (5)	3 SNPs (2, 5, 11)	0	0	0	0	1 SNP (12)	1 SNP (10)	0
KR012465.1 - Shanghai, China	0	0	0	1 SNP (5)	3 SNPs (2, 5, 11)	0	0	0	0	0	0	0
KR012466.1 - Shanghai, China	0	0	0	1 SNP (5)	3 SNPs (2, 5, 11)	1 SNP (19)	0	0	0	0	0	0
KR012467.1 - Shanghai, China	0	0	0	1 SNP (5)	3 SNPs (2, 5, 11)	0	0	0	0	0	0	0
KR012468.1 - Shanghai, China	0	1 SNP (4)	0	1 SNP (5)	3 SNPs (2, 5, 11)	0	0	0	0	1 SNP (12)	1 SNP (10)	0
KT321307.1 - Shanghai, China	0	0	1 SNP (12)	1 SNP (5)	4 SNPs (2, 5, 11, 22)	0	0	0	0	0	0	0
KJ513477.1 - Shanghai, China	0	0	1 SNP (25)	1 SNP (5)	3 SNPs (2, 5, 11)	0	0	0	0	0	0	0
EU177782.1 - Beijing, China	0	0	0	1 SNP (5)	5 SNPs (2, 5, 9, 11, 20)	0	0	0	0	0	0	0
DQ491000.1 - Shenzhen, China	0	0	0	1 SNP (5)	3 SNPs (2, 5, 11)	0	0	0	0	0	0	0
DQ097384.2 - Shenzhen, China	0	0	0	1 SNP (5)	3 SNPs (2, 5, 11)	0	0	0	0	0	0	0
AJ318079.1 - Germany	0	0	0	0	0	0	0	0	0	1 SNP (15)	3 SNPs (10, 14, 19)	0
NC_002803.1 - Finland	0	0	0	0	1 SNP (2)	0	0	0	0	1 SNP (15)	2 SNPs (10, 14)	0
U18101.2 - Finland	0	0	0	0	1 SNP (2)	0	0	0	0	1 SNP (15)	2 SNPs (10, 14)	0

Note: The numbers in the parentheses are the location of SNP in each primer or probe sequence

2.3. Assessing Analytical Sensitivity:

2.3.1. The RNA extracted from the SVC virus (source: NVSL ID 266921) was serially diluted from $1:10^1$ to $1:10^7$ and was used for the analytical sensitivity of all four assays.

Note: Zhang assay was excluded from this assessment since the primer/probe of this assay was not able to cover most of the isolates during in-silico analysis.

2.3.2. The Limit of detection (LOD) was up to 10^{-6} dilution and the PCR efficiency was 96.18, 94.42, and 100.1 for Yue, Q2N, and Q1G assay respectively.

2.3.3. The addition of internal control Escherichia coli bacteriophage MS2 did not affect the LOD in all three assays.

2.4. Assessing Analytical Specificity:

2.4.1. To assess the analytical specificity of the four published assays a total of 15 positive RNA samples (including two positive RNA from United Kingdom GeneBank ID AJ538067 and AJ538075.1) and two Sprivivirus RNA (PFRV UK9502* IV (Tench rhabdovirus) and PFRV A6V76 II (Grass carp rhabdovirus) were obtained from *National* Veterinary Services Laboratories (NVSL).

2.4.2. All published SVCV RT-qPCR assays (Yue assay, Zhang assay, Q2N assay, and Q1G assay) were run on the RNA obtained from NVSL. SVCV RNA was detected by all four published assays with varied Ct values and Sprivivirus RNA was "Not detected" confirming the specificity of these assays.

3. Deliverable yet to be completed:

3.1. Determine the diagnostic performance of the assay by testing around 50 source local fish samples. Since it is not feasible to obtain positive tissue samples spiking in positive RNA in tissue samples.

3.2. Test pooled v/s individual spiked tissue samples to evaluate the analytical sensitivity of the assays.

3.3. SOP development for the assay.

4. Benefit to NAHLN:

4.1. Increased capacity for SVCV testing and disease surveillance via rapid RT-qPCR assays.

4.2. Validated protocols will be shared with NAHLN laboratories.

Enhancement of Laboratory Testing Capacity, Biosafety & Biosecurity Practices, and Communications Through Emergency Preparedness Exercises to Prepare for Outbreaks of African Swine Fever and Foot and Mouth Disease

Alex Nemethy, Leianna Tucker, Lijuan Zhou, Reddy Bommineni

Bronson Animal Disease Diagnostic Laboratory, Kissimmee, FL

Objectives: Florida is an essential border state for emerging diseases. Geographic location, subtropical weather, tourism, and agriculture commodities importation through our ports make Florida a gateway of diseases into the country. The Bronson Animal Disease Diagnostic Laboratory (BADDL) is a NAHLN Level-1 approved laboratory able to conduct testing for high-risk diseases such as African Swine Fever (ASF) and Foot and Mouth Disease (FMD). This project will utilize BADDL, other Florida Department of Agriculture & Consumer Services (FDACS) Animal Industry staff, and associated stakeholders to perform two 2-day joint exercises involving mock outbreaks of ASF and FMD. These exercises are designed to enhance laboratory emergency preparedness, by improving procedures, training, and communication.

Deliverables Completed

1. Designed and completed a 2-day ASF functional exercise. The exercise was designed to assess testing capacity, biosafety, biosecurity, and communication between BADDL and other stakeholders. A hot wash was completed at the end of the exercise and of the exercise and recommendations for improvements were collected.
2. Completed on-site Biosafety training covering understanding laboratory risks and demonstrating behaviors needed to mitigate those risks with proper biosafety features and PPE usage.
3. Key staff members participated in Animal Disease emergency response training.

Deliverables yet to be Completed.

1. Design and complete a 2-day FMD functional exercise. The FMD exercise will build on the lessons learned during the ASF exercise.
2. Complete on-site training covering BSL2 and BSL3 practices provided by Behavioral-Based Improvement Solutions.
3. Key staff members will complete introductory ICS training.

Benefits to NAHLN

The exercises and training included in this project enhance biosafety, biosecurity, and communication skills. Testing protocols under simulated outbreak conditions have helped BADDL assess testing capacity, communications, and workflows, ensuring more efficient emergency and outbreak response. Understating our capabilities and limitations allows us to provide more accurate testing estimates to the NAHLN. Lessons learned have improved BADDLs' decision-making processes and enhanced communication between all stakeholders contributing to early disease detection and a more effective response, safeguarding both Florida's animal industries and national agriculture.

Enhancing Avian Diagnostics Testing Capacity for the Detection and Differentiation of Avian Influenza Virus (AIV) and Newcastle Disease Virus (NDV) from Other Economically Significant Viral Respiratory Disease Agents by Multiplex Real-Time Reverse Transcription-Polymerase Chain Reaction (rRT-PCR) Assays

Hemant Naikare; Binu Velayudhan

University of Georgia Tifton Veterinary Diagnostic Laboratory, Tifton, GA

Project Objectives:

#1: To develop multiplex rRT-PCR assays that allows simultaneous, rapid and accurate detection and differentiation of both NDV and AIV targets with an exogenous extraction control, all in one tube.

#2: To develop a multiplex NDV matrix and velogenic F-gene rRT-PCR with an exogenous extraction control.

 # 3: To develop multiplex rRT-PCR assays that will maximize the efficiency for respiratory syndromic testing and include screening for infectious bronchitis virus (IBV) and infectious laryngotracheitis virus (ILTV).

Deliverables completed:

Objective 1:

1) Exogenous control (xeno) spiked known positive AIV matrix samples and xeno spiked known positive APMV-1 matrix were detected without loss of sensitivity compared to non-spiked individual known positive AIV matrix and non-spiked individual known positive APMV-1 matrix.

2) Xeno spiked known positives for AIV matrix and known positives for APMV-1 matrix were all detected without loss of sensitivity compared to non-spiked individual known positive AIV matrix and non-spiked individual known positive APMV-1 matrix.

3) Field sample evaluation is in progress with the above protocols.

Objective 2: Nothing to report; **Objective 3**: Nothing to report.

Comments: Progress on this project was halted due to delay in recruiting post-doctoral fellow during the COVID-19 crisis. We recruited Post-doctoral fellow on September 2nd, 2023, to work on this project. We intend to complete work on all objectives with one-year no cost extension deadline of February 28, 2025.

How the project benefits NAHLN:

The current approved NAHLN protocols for testing for AIV and AMPV-1 involves separate singleplex real-time reverse transcription polymerase chain reaction, runs with different cycling conditions, both with the same probe fluorophore labels, and both without an internal exogenous control (NVSL-SOP-0068, revised 2021). Our proposed objectives will incorporate assay control and allow for multiplexing which will save time and reagents when samples are to be tested for AIV and/or APMV-1. We evaluated the optimization of inclusion of an internal control for AIV matrix and APMV-1 matrix detection into a single multiplex rt-PCR test.

A poster was presented in 2023 AAVLD meeting.

ASF and CSF Negative Cohort Study - Partnering to Expand Testing Capacities, Support Further Evaluation and Validation of Four Commercially Available ASF and CSF PCR Assays, and Enhance Preparedness Across the NAHLN

Karen Krueger; Rodger Main, Jeff Zimmerman

Iowa State University Veterinary Diagnostic Laboratory, Ames, IA

Support from:

- South Dakota State University Animal Disease and Diagnostic Laboratory (SDSU ADRDL)
- University of Minnesota Veterinary Diagnostic Laboratory (UMN VDL)
- Tetracore, Inc.
- Thermo Fisher Scientific, Inc.

Funding from:

- ASF
  - National Animal Health Laboratory network - 2021 Farm Bill
- CSF
  - National Animal Health Monitoring Study on Swine
  - National Pork Board

Project Objective

The purpose of this study was to more fully evaluate the specificity of three different Classical Swine Fever Virus (CSFV) and African Swine Fever Virus (ASFV) PCR assays on two aggregate sample types (oral fluid and processing fluid) of high interest to the pork industry. The assays used included the NAHLN PCR protocols, Tetracore USDA Licensed ASFV DNA test Kit and CSFV RNA Test Kit, and Thermo Fisher Scientific African Swine Fever Virus (ASFV) Detection Kit and Classical Swine Fever (CSF) PCR assay. The goal of this study was to expand options for PCR testing to enhance preparedness of diagnostic laboratories by adding approved specimen types, PCR assays, and sample extraction options for this testing.

This project was a cooperative effort of NAHLN, FADDL, three NAHLN laboratories [South Dakota State University Animal Disease and Diagnostic Laboratory (SDSU ADRDL), University of Minnesota Veterinary Diagnostic Laboratory (UMN VDL), and Iowa State University Veterinary Diagnostic Laboratory (ISU VDL)]. Industry partners Tetracore and Thermo Fisher Scientific provided PCR assays for the testing.

Funding was provided by the USDA (NAHLN/2021 Farm Bill and NAHMS) and the National Pork Board.

Deliverables Completed

All testing was completed as described in the project proposal. The final outcomes are listed below.

- Testing of every sample in each laboratory generated a valid negative result (with a positive Internal Control result), yielding a specificity of 100% with this sample set.
- Laboratory-specific extractions were successfully utilized in this project.
- Data supports that these two aggregate sample types (oral fluid and processing fluid) can be successfully tested by the processes and assays evaluated in this study.

- A sample set that was generated for this study was also provided to Dr. Rahul Nelli for use in an additional Farm Bill project [SmartChip Real-Time PCR System significantly enhances the diagnostic laboratory preparedness for rapid detection of high-consequence foreign animal diseases (FADs)]. This provided a set of samples of two different specimen types verified to be negative for both CSFV and ASFV for Dr. Nelli's project.

The project was completed in the anticipated timeframe with no additional deliverables expected.

How project benefits NAHLN

This project provides support for expanding the PCR testing options of the NAHLN laboratories in the following ways:

- The commercial assays utilized in this project (Tetracore USDA Licensed ASFV DNA test Kit and CSFV RNA Test Kit, and Thermo Fisher Scientific African Swine Fever Virus (ASFV) Detection Kit and Classical Swine Fever (CSF) PCR assay) demonstrated a specificity of 100% on the samples used in this study. This information is crucial in the evaluation of alternative assays for use by NAHLN laboratories. Increasing options for PCR testing is critical, especially in the event of an outbreak and subsequent monitoring to assure clearance of the agent(s).
- Laboratories being able to use their routine extraction procedures would greatly enhance testing efficiencies, volume of testing, and turnaround time for results. Having one established workflow for routine and FAD testing, instead of separate processes for the two testing streams, would be very beneficial for dealing with the sample numbers that would be incurred during an outbreak and the subsequent testing that would be required.
- This study provides support for the use of two aggregate sample types not yet approved by NAHLN for testing of CSFV and ASFV by PCR. These specimen types are already routinely used for aggregate testing by the swine industry and approval by NAHLN would increase options for testing.

SmartChip Real-time PCR System Significantly Enhances the Diagnostic Laboratory Preparedness for Rapid Detection of High-Consequence Foreign Animal Diseases (FADs)

Rahul K. Nelli; Luis G. Giménez-Lirola; Phillip C. Gauger; Juan Carlos Mora-Díaz

Iowa State University Veterinary Diagnostic Laboratory, Ames, IA

OBJECTIVES

1. Work Plan Objective 1 - Purchase of equipment (03/01/2022 to 03/31/2022)
2. Work Plan Objective 2 - Installation of instruments (04/1/2022 to 06/30/2022)
3. Work Plan Objective 3 - Training lab personnel on the procured instruments (07/01/2022 to 09/30/2022)
4. Work Plan Objective 4 - Validating ISU-VDL approved qPCR assays for porcine respiratory (PRRS, IAV, and PCV2); enteric (PEDV, TGEV, PDCoV) and FADs (ASF, CSF, and FMD) proficiency panel. (10/01/2022 to 12/31/2022)

DELIVERABLES COMPLETED / DELIVERABLES YET TO BE COMPLETED

PURCHASED (objectives 1 and 2)

a) Thermo Scientific - Kingfisher Apex with 96 DW.
b) Agilent - Bravo Liquid Handler.
c) Takara - SmartChip Real-Time PCR system.

TRAINING AND MENTORING (objective 3)

The research generated from this project supported high-throughput RT-rtPCR assays and liquid handling instrumentation.

a) Trained staff – 2
b) Training students
   - Summer students – 2
   - Masters students – 3
   - PhD students – 1
   - More potential students and staff will be trained in 2024

VALIDATION OF qPCR ASSAYS

a) Streamlined highthroughput workflow method established for SmartChip Real-time PCR System to perform RT-rtPCR assay for pathogen detection
b) Verified the comparability of NVSL assays for ASF, CSF, and FMD (SOP-1201.02, SOP-0643.07, SOP-0646.05) on SmartChip Real-time PCR System using newly streamlined workflow.
c) We tested the negative cohort samples as part of another NAHLN-funded study (***ASF PCR Negative Cohort Study – Partnering to expand testing capacities, support further evaluation and validation of two commercially available ASF PCR assays, and enhance preparedness across the NAHLN***), in which oral fluid (n=92) and processing fluid (n=184) samples were collected from different farm sites across the United States between April- May 2022 and previously confirmed negative at Iowa State University-Veterinary Diagnostic Laboratory (ISU-VDL). As expected, all 276 negative cohort samples tested negative on SmartChip, which aligns with the 96-well RT-rtPCR assays.
d) We proposed to validate porcine respiratory (PRRS, IAV, and PCV2) and enteric panels (PEDV, TGEV, and PDCoV) using the SmartChip real-time PCR system. We partnered with ThermoFisher to custom design VetMAX™ PRRSV 3.0, Gold SIV, and PEDV/TGEV/SDCoV Kits for SmartChip Real-time PCR System use.

e) Early studies indicate that PRRS virus RNA can be detected in blood swabs, oral fluids, processing fluids, serum, tongue tips, and lung tissues using VetMAX™ PRRSV 3.0 on SmartChip Real-time PCR System.
f) No non-specific detection in Tris EDTA buffer, elution buffer, and nuclease-free water has been observed, even with strong positive samples loaded adjacent to the negative wells. However, further validation is required using large sample sizes and more SmartChips.
g) In addition, the variability in results from Influenza A, PEDV, TGEV, and PDCoV requires further validation in terms of homogenizing RT-rtPCR assay conditions, sample volume, sample viscosity, and sample extraction protocols.
h) Our team is securing additional funds to complete more rigorous comparability studies to validate using porcine respiratory (PRRS, IAV, and PCV2) and enteric (PEDV, TGEV, PDCoV) panels in various sample types to match 96-well based assays.

RESEARCH OUTPUTS

a) Accepted Abstracts
   - 2023 AAVLD, Poster presentation **(Best Poster Award);** "Evaluating the potential of high-throughput SmartChip Real-time PCR system for high-volume testing for Foreign Animal Diseases."
   - 2023 NAPRRS/NC229 International Conference of Swine Viral Diseases (ICSVD); Oral and Poster presentation **(Student Travel Award)** "Evaluation of the High-Volume Testing Potential of SmartChip Real-time PCR System for the detection of Foreign Animal Diseases"
   - 2024 CRWAD, Oral presentation **(Student Travel Award);** "High volume testing for foreign animal diseases using SmartChip real-time PCR system."

b) Preparing more than two manuscripts in molecular diagnostics
c) Master thesis under preparation

GRANTS

Secured > 1 million grants using this state-of-the-art instrumentation

a) USDA APHIS American Rescue Plan: SARS-CoV-2 in Animals project, " Evaluation of cost-efficient, high-throughput detection of the SARS-CoV-2 and its variants for animal testing, monitoring, and surveillance in susceptible animal species."
b) Iowa Livestock Health Advisory Council (ILHAC) for the project "*E. coli* genotyping using SmartChip Real-time PCR System- an affordable alternative to gel-based PCR."

COLLABORATIONS

- **with wildlife veterinarians**
  a) Iowa Department of Natural Resources
  b) Iowa Wildlife Center
  c) Omaha's Henry Doorly Zoo and Aquarium
- **with other researchers**
  a) National Genomics Center for Wildlife and Fish Conservation (USDA Forest Service), Montana.
  b) Michigan State University
- **Ongoing discussions with industry partners**

HOW THE PROJECT BENEFITS NAHLN

1. This project significantly increased the molecular testing capacity for foreign animal disease outbreaks and emerging pathogens of economic significance for the Iowa State University Veterinary Diagnostic Laboratory (Level 1 NAHLN laboratory).

2. Based on NAHLN Sustained Laboratory Capacity - July 2022 guidelines, the SmartChip real-time PCR system, together with liquid handlers and Kingfisher extraction units, can enhance three times the ideal maximum capacity of a laboratory.

Required units	***Required items/personel***	***Time taken per run***	***Samples processed***
4	Kingfisher (KF)	30 mins	96 samples/unit, i.e. 384 samples in total
1	Bravo liquid handler	10 mins	384 samples + 12 (ASF/CSF/FMD) in triplicates
1	SmartChip Nano dispenser	80 mins	384 samples + 12 (ASF/CSF/FMD) in triplicates
1	SmartChip real time PCR system	60 mins	384 samples + 12 (ASF/CSF/FMD) in triplicates
	Total time	**180 mins (3hrs)**	**384 samples screened for ASF/CSF/FMD**
2	Staff	8 hrs shift	~1000 samples

3. The project strengthened the future testing needs associated with potentially zoonotic pathogens involving humans and animals.
4. The project meets the NAHLN Strategic Priority 1- Disease Detection, Outbreak Response, and Surveillance.
   a. Early detection, rapid response, and appropriate recovery of a high-consequence animal disease.
   b. Working with Vendors to develop enhanced access to materials needed during an outbreak.
   c. SmartChip real-time PCR System has the potential for AMR surveillance.
5. This used existing resources from another 2021 NAHLN-funded project, "***ASF PCR Negative Cohort Study – Partnering to expand testing capacities, support further evaluation and validation of two commercially available ASF PCR assays, and enhance preparedness across the NAHLN,"*** to validate SmartChip Real-time PCR system. This saved some additional resources for NAHLN to validate this novel high-throughput screening tool for ASF preparedness.
6. We are working towards submitting NAHLN protocol deviation for FAD surge testing using SmartChip real-time PCR systems.

Web-based Sample Submission for Foreign Animal Disease Investigations

Steve Bolin, Kimberly Dodd, David Korcal

Michigan State University Veterinary Diagnostic Laboratory, Lansing, MI

Project objective:

The objective of this project is to help address the bottle neck of manual data entry by creating a web-based submission process for foreign animal disease investigations or outbreaks managed by state and federal agencies. The goal for the Michigan State University Veterinary Diagnostic Laboratory is to increase our capacity and capability in responding to a foreign animal disease through the capture of appropriate data, a higher quality of data, and timely and accurate messaging of the diagnostic result to state and federal agencies through web-based submission by foreign animal disease diagnosticians.

Deliverables Completed:

1. Essential data elements required for foreign animal disease submissions were identified.
2. An inbound foreign system interface has been developed and validated with the support of our LIMS vendor Cerner.
3. An app has been developed to collect the essential elements of a required foreign animal disease submissions.

Deliverables yet to be completed:

1. Development of a secure client portal and identification of hosting options for deployment of the web application. Delays encountered in the university procurement process has slowed this process. A third-party vendor has been identified and a contract is currently being negotiated.

How the project benefits NAHLN:

During the recent highly pathogenic avian influenza outbreak, the application was utilized in-house with great success. The application reduced data entry time and decreased errors. These improvements increased laboratory capacity by eliminating the receiving bottle neck while reducing error rate. These improvements have greatly improved the laboratory's NAHLN messaging and reduced rework. By improving the laboratory submission process, the NAHLN will benefit with increase laboratory capacity and more accurate messaging.

Enhancing Surge Capacity with a Streamlined Online Sample Submission Web Application

Albert Rovira

University of Minnesota Veterinary Diagnostic Laboratory, St Paul, MN

Project objective:

The goal of this proposal is to develop a new web based submission platform for clients to use for submitting samples for diagnostic testing at the VDL.
Specific objectives are:
1. Incorporate input on laboratory functions and workflow into the design of the new application to ensure laboratory operations are considered.
2. Incorporate input from clients and regulatory officials to ensure the information required and the user interface needs are met.
3. Design, program and test a web based application that integrates effectively with the VDL LIMS.

Deliverables completed / Deliverables yet to be completed:

The VDL has a standing IT steering committee made up of faculty, staff and IT personnel from the laboratory that has provided input on information needs for laboratory functions. The committee has met on four occasions to monitor the progress of this project. A subcommittee composed of faculty, IT analysts, VDL staff and a programmer has been formed specifically for this project. The subcommittee has been meeting on a bi-weekly basis to advance in the design of the online submission tool. The current online submission forms were used as a starting template but have been significantly improved. The design phase is completed and we are working on the programming phase. The subcommittee has received input from different laboratory sections including Laboratory Receiving section, Admin section, companion animal pathologists and food animal pathologists. Input from submitting clients and producers to learn what their needs and preferences are for information to include in the submission forms has been obtained by site visits and comments channeled through the pathologists.

A subcontracted IT firm is in charge of programming. Draft versions of three different submission forms have been designed and are being programmed. Draft versions of the submission report generated after a submission is ordered were created and shared with internal users for feedback. Work is ongoing on designing the data tables that will feed the new online submission form so that a working prototype can be presented to Beta testers.

How the project benefits NAHLN:

This project addresses the following NAHLN priority: Increased capacity to handle surge samples through improving sample entry and tracking, Laboratory Information Management Systems (LIMS) enhancement, and electronic messaging of results

Increasing ASF Preparedness by Improving Diagnostic Submissions to the NAHLN Laboratory in Minnesota

Albert Rovira

University of Minnesota Veterinary Diagnostic Laboratory, St Paul, MN

Project objective:

The Goal of this proposal is to improve the preparedness of swine producers and veterinarians in Minnesota against ASF by providing the resources they need to produce excellent sample submissions to the MNVDL. Specific objectives are:

Objective 1: To create or compile training materials on ASF, regulatory testing, sample collection and sample submission

Objective 2: To train veterinarians and producers on ASF, regulatory testing, sample collection and sample submission

Objective 3: To evaluate the impact of diagnostic submission training

Deliverables completed / Deliverables yet to be completed:

A sample submission specialist position was opened. Three candidates applied, one was interviewed and was hired. ASF materials available at numerous websites such as USDA, SPS, AASV, FAO, EFSA and others were compiled and evaluated. Regulatory testing materials from USDA and internal sources were compiled and evaluated. Sample collection materials from SPS website and from internal sources were compiled and evaluated. Sample submission materials from SPS website and from internal sources were compiled and evaluated.

To determine the target population of clinics and production companies, a list of clinics and production companies was produced based on the volume of samples submitted to the VDL. The list was checked for completeness by the VDL director and swine diagnosticians. To deliver training on ASF, regulatory testing, sample collection and sample submission, clinics have been approached and in person visits to five of them have been completed. Training was delivered during these visits and a specific plan to improve laboratory submissions has been developed in each case. For one of the clinics, which is responsible for one of the largest volumes of submissions to the VDL, a comprehensive analysis of their submissions was performed and provided as feedback for improvement in 2022, and again in the Spring of 2023, May of 2023 and June of 2023. Submission data from all clinics has been analyzed and summarized in preparation for site visits. Additional clinic visits will be performed in November/December 2023.

How the project benefits NAHLN:

This project addresses the following NAHLN priority: Enhanced laboratory emergency preparedness by developing local and regional cooperation for regulatory testing through targeted outreach and exercise.

Data Exchange Between NAHLN Laboratories and Offices of State Animal Health Officials

Amy Swinford[1] , Michael McGrath[2], Tammy Leimer[2], Brian McCluskey[2], Angela Lackie[3]

[1]Texas A&M Veterinary Medical Diagnostic Laboratory, College Station, TX, [2] Trace First, [3]Texas Animal Health Center

Project objective - The objective of this project was to build a connection between the CoreOne for Labs laboratory information management system and the CoreOne Animal Health Information management system used by the Texas A&M, Texas Veterinary Medical Diagnostic Laboratory and the Texas Animal Health Commission respectively.

Deliverables completed / Deliverables yet to be completed - All deliverables have been completed and this interchange of data is now operational. Deliverables included:

Enumerate results sent from the lab to SAHO partners	There are a significant number of streams of result data which are sent by the Lab to SAHO's office(s). This milestone will exhaustively capture the information on these streams.

Allow retrieval and display of lab data	This is to allow CoreOne users at SAHO offices to, directly from within the app, access lab data for which they are authorized.

API for result access	This milestone will see the addition of functionality to CoreOne for Labs which will allow programmatic access to result data. Said access will be provided in conformance with strict access control and security standards.
Develop CoreOne Analytics datasets and dashboards	CoreOne Analytics datasets and dashboards will be developed that support programmatic access to result data.

How the project benefits NAHLN - The rapid delivery of diagnostic test results to animal health officials is critical in initiating a rapid, efficient and effective response to a disease occurrence. The lack of standard communication protocols surrounding delivery of these results often creates information vacuums with concomitant delays in disease response and miscommunication. The development of a direct link from the NAHLN laboratory to the State Animal Health Official's office will significantly increase the speed and accuracy of test results to animal health officials responsible for responding to animal health events.It also reduces the burden on the laboratory to provide the reports and, because it pulls the data directly from the Labs system, it is always accurate and not subject to the SAHO having out-of-date information. With the roll-out of the National List of Reportable Animal Diseases (NLRAD), this proposal supports the NLRAD's purpose to "have consistent animal disease reporting across the United States and to help animal health officials protect the U.S. agriculture infrastructure."

Notification of Priority and Emergency Disease Test Results

Kevin Snekvik[1] , Michael McGrath[2] , Tammy Leimer[2] , Brian McCluskey[2]

[1]Washington Animal Disease Diagnostic Laboratory, Pullman, WA, [2]Trace First

Project objective – The first objective of this proposal is the defining of triggers that initiates a workflow allowing reliable and immediate notification of relevant parties. Many of these triggers are likely to be similar to those identified by the National List of Reportable Animal Diseases (NLRAD) but other triggers that will initiate the notification of State Animal Health officials will also be developed. A suitably authorized user will be able to create/edit a trigger and will be able to build rules which must be met for the trigger to be considered to have been executed. In the initial version to which this proposal relates, the user will be able to define trigger parameters based on the following criteria:

- Test which was ordered
- Test Result - value match
- Test Result - pick list
- State of submission
- Date range

The second objective of this proposal is the creation of a dedicated and highly secure module (NOTIFIER) that will be responsible for executing the workflows and sending notifications when trigger events occur within the CoreOne for Labs system. The messaging standard developed within CoreOne for Labs to transmit the workflow messages to the NOTIFIER will be made available to all interested laboratories so that they can use the NOTIFIER for notification of other State animal health authorities. The Notifier Command Console will allow authorized users to view the status of the workflow execution. For audit purposes, highly detailed transaction records are retained with timestamps to show when notifications were sent and, in the case of the messages being "verified", the verification event will be recorded too. The standardized messages to the NOTIFIER will support more rapid and accurate test result delivery to regulatory officials and those responsible for emergency response actions. This improves the capability of the NAHLN overall to respond to emergency or high consequence disease events. The Notifier will be capable of sending notifications to any person/party; this could include State Animal Health Official's offices and staff, USDA and other related parties.

The last objective of this proposal is to build within the NOTIFIER a comprehensive, printable report of the notifications generated and their delivery status. An 'audit trail' of who is notified and when is critical in emergency response. This will improve the NAHLN reporting aspect of emergency response.

Deliverables completed / Deliverables yet to be completed - All deliverables have been completed and the NOTIFIER system is operational. Deliverables included:

Defining triggers that initiates a workflow	This will involve listing the events that can occur in a LIMS that would cause a NOTIFIER notification workflow to commence. It

	is important that the parameters within the LIMS which can be used to trigger NOTIFIER workflows are clearly understood; it is imperative that the necessary level of granularity is supported. For events and/or disease types not currently supported by NAHLN, open XML standards will need to be defined and published

Develop NOTIFER module in CoreOne for Labs	When asked by a LIMS to execute a NOTIFIER workflow, it is imperative that the NOTIFIER system architecture and infrastructure are high-availability. This milestone will ensure a robust design and will also incorporate mandatory adherence to FIPS140-2 security standards. When building workflows, the following elements will be supported: • Send email • Send verified email • Send SMS/text message • Send verified SMS/text message • Place outbound phone call • Place verified outbound phone call • Include event in daily digest • Include event in weekly digest • Wait for a period of time A user who is creating or editing a workflow can include unlimited numbers of elements from the previous list.

Incident Report	We will build a function in the Notifier to provide a comprehensive, printable, report of the notifications generated and their delivery status (where known).

How the project benefits NAHLN - The rapid delivery of diagnostic test results to animal health officials is critical in initiating a rapid, efficient and effective response to a disease occurrence. The lack of standard communication protocols surrounding delivery of these results often creates information vacuums with concomitant delays in disease response and miscommunication. This proposal extends delivery of test results considered of high consequence (not just FAD results as required by NAHLN) by State animal health officials directly from the laboratory. Delivery by text message, email, and telephone call will afford rapid and accurate delivery of results with a concomitant rapid and accurate response by State and Federal animal health officials.

The messaging standard created for CoreOne for Labs to transmit "Start Workflow" messages to the NOTIFIER will be an open standard so that other laboratories not using the CoreOne for Labs LIMS can also use the NOTIFIER for notification of test results to State animal health officials.

Enhancing Rapid Detection of Emerging Aquatic Diseases for Deployability AP22VSD & B000C012

Chrissy Eckstrand, Brandi Torrevillas, Becca Wolking, Kevin Snekvik

Washington Animal Disease Diagnostic Laboratory, Pullman, WA

Project objective: *The overarching goal of this project was to enhance WADDL laboratory capacity for rapid aquatic pathogen detection, genomic sequencing, and characterization by testing and integrating multiple novel molecular technologies using known high consequence pathogens.*

Deliverables completed:

Milestone 1.1: Sample collection, confirmation of viral infection, and pathology

An experimental infection of Atlantic salmon and Koi infected with VHSV and KHV, respectively, was completed with collection of formalin-fixed and fresh/frozen samples in collaboration with NVSL.For amplifying virus in culture, KF1 cells were purchased from ATCC and attempted to establish this line in our Aquatic Animal Health section, however the commercially obtained cell line was not viable. Live cells were imported from a collaborator, and these have been proliferating better, but still slowly.

Milestone 1.2a Sample prep. assessment

Several methods for ablation of background genomic material were tested including low-speed gradient centrifugation, ultracentrifugation, mechanical filtration, and enzymatic host depletion. Only enzymatic host depletion achieved significant improvement of viral genome capture. Preliminary experiments were successful achieving up to 2000x increase in number of virus reads per sequencing run.

Milestone 1.2b: Library prep. assessment

Throughout the course of this project, Oxford Nanopore Technologies (ONT) released several new library preparation kits with significant upgrades to kit and sequencing flow cell chemistry. While waiting for kit and sequencing chemistries to settle, we focused on using the Agilent Bioanalyzer, Qubit assays, and Nanodrop spectrophotometry to guide loading concentrations for library input and loading onto flow cells in order to improve throughput. We also utilized two Flongle adapters to run Flongle flow cells, a smaller, lower-yield disposable unit which comes at a tenth of the cost compared to MinION flow cells. We demonstrated that after host depletion, a singleplex library run on a Flongle was sufficient to achieve whole genome coverage of a 5.5kb parvovirus. These smaller flow cells are stable for only 4 weeks instead of 12, but the reduced cost and smaller reagent use may offer a substantial savings.

Milestone 1.3: Evaluation of automated library prep. systems

In January 2023 we swapped our Voltrax for the new Voltrax V2B device and received the new “blue” cartridges and upgraded library prep reagent kit (VSK-VSK004). We successfully used this device to prepare a number of libraries (as well as unsuccessfully, several times which led to loss of precious samples). Our team appreciates the ease of use especially for new users and the library quality is very good.

We have also begun programming our Opentrons OT-2 robot to perform components of our cDNA synthesis and library prep protocol. The Opentrons protocol library has scripts available for performing

ONT's ligation sequencing library prep but breaks it down into two steps which could be combined into one process given our throughput is closer to 8 samples than 96. This requires the entire process to be scripted in-house.

Milestone 1.4: Barcoding

Due to the kit chemistry upgrade, we have not yet begun testing multiplexing with the manual ligation sequencing library prep kit. We tested the Voltrax VSK-VMK002 multiplex kit on the automated library prep device with high molecular weight lambda phage DNA at two concentrations (high/low) and cDNAs generated from purified viral RNA obtained from ATCC at two concentrations.

Milestone 1.5: Adaptive sequencing

Adaptive Sampling is the ability to compare basecalled reads to a reference sequence to allow the sequencer to decide whether to keep or reject strands of DNA during sequencing. The goal is to either enrich sequences of interest or deplete off-target sequences but comes at the cost of higher pore attrition rates. So far, we have gathered data suggesting that while targeted enrichment using adaptive sampling can increase number of on-target bases sequenced, the rarity of viral templates in libraries prepared from tissue extracts (typically 0.001-0.0001% of all fragments) makes this extremely challenging. For that reason, we shifted to focus on reducing background nucleic acids prior to library prep.

Deliverables to be completed:

While we have made significant progress in many of the milestones, there are multiple milestones and end deliverables pending completion (as described above). WADDL is working diligently to complete the project within the coming quarters.

How the project benefits NAHLN:

This project directly benefits NAHLN in that it has significantly increased laboratory capacity preparedness to rapidly detect and characterize novel pathogens in aquatic species. WADDL is uniquely suited for the detection of aquatic pathogens as it has a well-established aquatic diagnostics section interfacing with the large fisheries of the Pacific Northwest. This project has allowed for the acquisition of new technologies, fine-tuning and optimization of complex steps in pathogen discover, and significantly advanced the technical sequencing expertise of our lab.

An Automated Antibiotic Sensitivity Testing System for Electronic Data Management and Sharing the Data with NAHLN

Erfan Chowdhury

Thompson Bishop Sparks State Diagnostic Laboratory, Auburn, AL

Thompson Bishop Spark's State Diagnostic Laboratory (TBSSDL) in Alabama, a level 1 laboratory in the United States Department of Agriculture National Animal Health Laboratory Network (USDA NAHLN), aims to share its antibiotic sensitivity testing (AST) data with NAHLN's antimicrobial resistance (AMR) pilot project. A common AST platform that is used by the participant laboratories to obtain the data for this NAHLN project is a commercially available automated system, Sensititre™. However, until recently, we used to perform Kirby Bauer disk diffusion method for our AST procedure in the Bacteriology Laboratory at TBSSDL. Therefore, in this project, we requested a grant for a fully automated AST technology, Sensititre™ ARIS HiQ AST System with Sensititre™ SWIN™ Software, and associated supplies. To accomplish the goal of this project, the lab was awarded $198,900.00 through 2020 Farm Bill grant. A summary of project activities and its potential benefits to the NAHLN are presented below.

Within the first 2 quarters of the project, a Sensititre™ ARIS HiQ AST System with associated software and supplies were purchased from the Thermo Fisher Scientific (TFS) through Alabama Department of Finance.After receiving the equipment, four laboratory personnel were trained on the equipment by representatives from TFS. Subsequently, the equipment was tested through internal and external verification approaches. In an in-house verification, isolates from 25 clinical cases were tested side by side using the Sensititre™ ARIS HiQ AST System and Kirby-Bauer disk diffusion method. Eight isolates which were tested by the Sensititre™ at TBSSDL were also sent to the National Veterinary Services Laboratories (NVSL) for external verification. The equipment was successfully verified with approximately 98% agreement in both cases. After the verification, the laboratory has been using this Sensititre™ ARIS HiQ AST System for AST on bacterial isolates from clinical and necropsy specimens since October of 2021.

Addition of the Sensititre™ System to the Bacteriology Section has substantially improved AST in TBSSDL. In the past, with the Kirby Bauer disk diffusion method, results were limited by available interpretation criteria from Clinical and Laboratory Standards Institute (CLSI). Adding the minimal inhibitory concentration (MIC) method through Sensititre™ has increased the number of available antimicrobial drugs to be tested; additionally, the MIC method has allowed the lab to test for more patient-organism combinations. This automated platform has also enhanced our capacities to electronically manage AST data. The equipment has enhanced computer software to interpret susceptibility breakpoints. After reading, all results are saved, which can be used to analyze test results for atypical patterns and unusual resistance phenotypes.

Finally, the availability of the Sensititre™ System has let the TBSSDL to participate in the NAHLN AMR Pilot Project. Since January of 2022 the laboratory has been sharing its AST data with the NAHLN. We anticipate, the AST data from TBSSDL will be an excellent addition to the NAHLN's project

Enhance Capacity for Faster Identification of Bacterial Foreign Animal Diseases at the Thompson Bishop Sparks State Diagnostic Laboratory

Erfan Chowdhury

Thompson Bishop Sparks State Diagnostic Laboratory, Auburn, AL

Thompson Bishop Sparks State Diagnostic Laboratory (TBSSDL) in Alabama is a level 1 laboratory in the National Animal Health Laboratory Network (NAHLN). The lab is committed to assisting in the Foreign Animal Disease (FAD) response strategies of the United States Department of Agriculture Animal Health and Plant Inspection Services (USDA APHIS) by providing rapid and accurate diagnostic and surveillance supports for animal diseases in Alabama. However, until recently, the Bacteriology laboratory at TBSSDL was mostly depended on a series of traditional biochemical methods to identify bacterial and fungal pathogens. With these conventional methods, 3-4 days and sometimes even longer time is required for full identification of a bacterial pathogen. Therefore, in this project, we requested grant for a Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) to enhance our capacity for rapid and accurate identification of bacterial and fungal animal diseases, including exotic and emerging diseases. To accomplish the goal of this project, the lab was awarded $227,025.00 through 2020 Farm Bill grant. A summary of project activities and its potential benefits to the NAHLN are presented below.

Within the first 3 quarters of the project, a MALDI Biotyper® Sirius GP System was purchased from the Bruker, Germany, through Alabama State Purchase Department. After receiving the equipment, 5 lab personnel were trained on the equipment by Bruker representatives. Subsequently, the equipment was tested through internal and external verification approaches. In the in-house verification, initially the equipment was tested to identify 21 ATCC bacterial organisms and 7 ATCC yeasts. Subsequently, 50 clinical cases were tested side by sides using the MALDI Biotyper® Sirius GP System, OmniLog ID System, and RapID system. As an external verification, six isolates identified with the MALDI Biotyper at TBSSDL were sent to the National Veterinary Services Laboratories (NVSL) for further verification. In all cases, the equipment was successfully verified with an approximately 98% agreements. After a successful verification, the lab has been using the MALDI Biotyper® as the primary tool for bacterial and yeast identification since September of 2021.

Addition of the MALDI Biotyper® Sirius GP System to the Bacteriology Section has allowed the TBSSDL to accelerate its bacterial identification process from 3 to 4 days to 1 to 2 days. Moreover, MALDI Biotyper® contains spectral data of about 7000 reference strains in the database. We have also included special libraries for animal bacterial pathogens of bioterrorism importance and *Mycobacteria* on our system. Thus, this technology would allow us to identify significant animal bacterial pathogens that could be missed with conventional biochemical procedures. As such, we anticipate that this rapid diagnostic platform will let the TBSSDL to play better roles in assisting the USDA APHIS in its FAD response strategies. Moreover, in the past, sometimes conventional methods could not provide enough information to identify an isolate conclusively. As such, we had to ship all questionable isolates to the National Veterinary Service Laboratory (NVSL) at Ames in Iowa for identification. Thus, with the availability of this platform, we also anticipate that we would likely send fewer routine cultures to NVSL, which would shorten the turnaround time to our clients and reduce the testing burden of NVSL.

Overall, with the availability of the MALDI Biotyper® Sirius GP System, the TBSSDL is currently well-equipped for providing rapid and accurate diagnostic services for bacterial and fungal animal diseases in Alabama and to play better roles in the FAD response strategies of the USDA APHIS.

Enhancing Emergency Preparedness at Three NAHLN Laboratories through professional BSL-3 Training

Kristy Farmer

Thompson Bishop Sparks State Diagnostic Laboratory, Auburn, AL

Project Objectives:

- Have the University of California, Irvine School of Medicine (UCI-SoM) BSL-3 training program provide a week-long intensive BSL-3 training, with a focus on risk assessment and management, on site for 12 analysts.
- Invite 4 staff members from the Mississippi Veterinary Research & Diagnostic Laboratory System (MVRDLS, Pearl, MS) and 4 staff members from the Bronson Animal Disease Diagnostic Laboratory (BADDL, Kissimmee, FL) to participate with 4 staff members from Thompson Bishop Sparks State Diagnostic Laboratory (TBSSDL, Auburn, AL).
- Purchase a "plug and play" portable autoclave for the BSLL-3 suite at TBSSDL to improve waste management procedures.

Deliverables completed:

- The UCI-SoM BSL-3 training program performed a weeklong training at TBSSDL April 18-22, 2022, for 12 people from the 3 NAHLN member laboratories. Training focused on risk assessment, biocontainment, decontamination procedures, donning and doffing appropriate PPE, and emergency preparedness. The training was held in TBSSDL's classroom and in the lab's BSL-3 laboratory with practical demonstrations, exercises, and quizzes.
- After a consultation with UC-Irvine trainers, a Heidolph 85L, portable autoclave was purchased for the TBSSDL BSL-3 suite.

How the project benefits NAHLN:

Having twelve people, from three separate NAHLN laboratories, professionally trained in BSL-3 procedures including risk assessment, waste management, and entry and exit procedures will increase our Biosecurity and biocontainment at these three labs during an FAD event. In addition, having an autoclave located inside the BSL-3 suite at the TBSSDL will enhance biocontainment procedures for waste management.

Increase Capacity for Testing for Chronic Wasting Disease (CWD)

Udeni Balasuriya; Mariano Carossino

Louisiana Animal Disease Diagnostic Laboratory, Baton Rouge, LA

Project Objective: Chronic Wasting Disease (CWD) is a transmissible encephalopathy of cervids caused by prions. White-tailed deer are susceptible, and Louisiana has a substantial population of deer in the state. The Louisiana Department of Wildlife and Fisheries (LDWF) and the Louisiana Animal Disease Diagnostic Laboratory (LADDL) have a joint interest in diseases that affect white-tailed deer, including CWD. LADDL currently has a surveillance program for CWD utilizing the NVSL-approved immunohistochemistry (IHC) protocol. To enhance the capacity for testing deer within this surveillance program, LADDL will purchase additional automated Leica BOND-MAX instruments for staining, a gross station for tissue trimming, and improve sample entry and tracking using the Leica CEREBRO Sample Tracking and Workflow Management system. These upgrades to the current system will more than triple the CWD surveillance testing capacity and efficiency in the state of Louisiana.

Deliverables completed: The following purchase and installations of equipment have been completed: the PMT Scientific Prodigy Elevating Gross Station with the small and large Pegboards, the Formalin Dispensing Station, the Leica Biosystems BOND-MAX Automated IHC Staining system and the Leica CEREBRO Sample Tracking and Workflow Management system. Training on the use of the equipment has been completed. All equipment is operational and in use, however, a few software integration-related items are currently being addressed between LADDL, LSU IT, USALIMS, and Leica CEREBRO.

How the Project Benefits NAHLN: LADDL is participating in a 5-year contract/collaboration with the Louisiana Department of Wildlife and Fisheries and with the Louisiana Department of Agriculture for testing of CWD in both free-range and farmed deer in Louisiana. CWD testing is also offered to out-of- state clients. The funding for this project enabled the laboratory to triple the capacity for surveillance and Immunohistochemistry Diagnostic testing of CWD which is a NAHLN agent- specific assay. The first case of CWD was detected in Louisiana in 2022 using the IHC assay. With this enhancement, LADDL plans to process and test up to 8,000 deer specimens in the future. The efficiency of testing has been enabled by the installation of the automated sample tracking system and the IHC Staining system. It is imperative to continue to monitor the free-ranging and farmed deer for any new CWD cases. Currently, definitive diagnosis is based on Immunohistochemistry staining of the obex area of the brain stem or the medial retropharyngeal lymph nodes for the prion protein. LADDL can participate in this testing with the help of a fully equipment Histology/Immunohistochemistry laboratory with qualified histotechnicians and Board-Certified Pathologists.

Enhancement of Chronic Wasting Disease (CWD) Testing Capacity

Lanny Pace; Alejandro Banda

Mississippi Veterinary Research and Diagnostic Laboratory, Pearl, MS

PROJECT OBJECTIVE: Objective 1. Enhance the testing capacity and mitigate the risk of delayed chronic wasting disease (CWD) test results in the event of equipment failure and increase the diagnostic laboratory testing capacity for CWD in Mississippi.

DELIVERABLES COMPLETED: The following equipment has been purchased, installed, calibrated and it is ready to use:

a) iMark™ Microplate Absorbance Readers (2)
b) 620 nm Filters (2)
c) Model 1575 Immunowash Microplate Washer
d) DW40 Deepwell washer
e) Heating Block (1 Deepwell Plate Position) (2)
f) Precellys Evolution (2)
g) Block for 1 DW plate REF (2)
h) TeSeE NSP Purif System REF
i) NSP Waste Container
j) FSD Computer HP5810
k) iQ-Check Prep Barcode Reader

HOW THE PROJECT BENEFITS NAHLN: This project aligns well with NAHLN goals because is aimed to increase capacity for chronic wasting disease testing focused on currently approved protocols. The Mississippi Veterinary Research and Diagnostic laboratory (MVRDL) has been supporting the state's chronic wasting disease (CWD) surveillance since 2018 when the first CWD case in MS was confirmed by NVSL. MVRDL is approved by the NAHLN for CWD ELISA and has been conducting CWD testing to assist the MS Board of Animal Health (MBAH) and the MS Department of Wildlife, Fisheries, and Parks (MDWFP) in CWD surveillance and control. The resources obtained by this project will be focused to mitigate the risk of not being able to perform CWD testing due to equipment failure and to increase the diagnostic laboratory testing capacity for CWD in Mississippi and surrounding states.