



Policies and Concept of Operations Recommendations for the Use of Agricultural Screening Tools During an Animal Disease Outbreak

Report from the Agricultural Screening Tools
Workshop IV.

May 15-16, 2012
Ames, Iowa

Agricultural Screening Tools Workshops

The Department of Homeland Security (DHS) National Center for Foreign Animal and Zoonotic Disease Defense (FAZD Center) convened an agricultural screening tools (AST) workshop on May 15-16, 2012 in Ames, IA. The overall goal of the workshop was to use the information and recommendations from the three previously held AST workshops to draft recommendations for developing concepts of operations for agricultural screening tools.

Workshop participants addressed the following focus areas:

- Use of lab-based diagnostic assays during an outbreak.
 - Foot and Mouth Disease (FMD) real-time polymerase chain reaction (RT-PCR) assay.
 - Use of bulk tank milk samples in RT-PCR for FMD detection.
 - 3 ABC FMD enzyme-linked immunosorbent assay (ELISA) to differentiate infected from vaccinated animals (DIVA).
 - Penside FMD assays (*e.g.*, lateral flow devices).
- Prioritization of samples.
 - Sample triage.
 - Prioritization.
- Biosafety Level (BSL) requirements.
 - Prioritizing use of laboratory space.
 - Processes appropriate for BSL2, BSL2+ and BSL3.
 - Defining BSL2 enhanced (BSL2+).
- Validating an assay during an outbreak.

- Responding to an emerging disease.
- Regulatory decisions.
- Performance factors (e.g., sensitivity, specificity).

This workshop was the fourth in a series of workshops sponsored by the FAZD Center focused on AST gaps, priorities and policy. Titles and objectives for each of the three previous workshops are listed below. Information regarding the gaps and findings are detailed later in this report and can be found on the FAZD Center website at fazd.tamu.edu.

- Protecting Agricultural Infrastructure: Defining the Needs and Requirements for Agricultural Screening Tools.
 - Define “Agricultural Screening Tools”.
 - Assess current status and availability.
 - Identify and prioritize gaps and requirements for future research.
 - Focused on FMD as a model for other high-consequence disease analysis.
- Enhancing Ag Resiliency: The Agricultural Industry Perspective of Utilizing Agricultural Screening Tools.
 - Obtain animal industry input into practical technologies and needs.
 - Prioritize needs and requirements for Ag screening tools in the context of business continuity.
 - Policy gap analysis.
 - Industry perspectives on Ag Screening Tools.
- Recommendations for Establishing Policies and Concepts of Operations Agricultural Screening Tools.
 - Gather input from scientific experts, regulatory officials, and stakeholder groups in preparation for drafting policies related

to the diagnosis of, and laboratory response to, foreign animal disease (FAD) outbreaks.

Executive Summary

This report describes the key findings, issues, and discussion points that arose during an agricultural screening tools workshop hosted by the FAZD Center in May 2012. Participants included 31 personnel representing the FAZD Center, DHS, USDA Animal and Plant Health Inspection Service (USDA-APHIS), USDA National Institute of Food and Agriculture (NIFA), NAHLN laboratories, the animal health industry, and state animal health organizations (full list in Appendix A).

Objectives

The objectives of this workshop were to:

- Build on knowledge and input from AST Workshops I-III.
- Establish recommendations for writing concepts of operations for the use of RT-PCR, ELISA and penside assays during an FMD outbreak.
- Establish recommendations for defining concept of operations (CONOPS) for prioritization of samples/reagents during an outbreak.
- Establish recommendations for the use of BSL-2, BSL2+, and BSL-3 space during a high-consequence disease (HCD) outbreak.
- Receive updates on procedures for handling select agent samples during an outbreak.
- Establish recommendations for processes to validate assays during an outbreak.

A large portion of the meeting was organized into small working groups. For each topic, the participants were divided into two groups: Red Group and Blue Group. After individual discussions,

members from each group reconvened and presented on their discussion/findings and summary recommendations were established for each topic. The discussion topics are presented below.

- Policy/CONOPS for Use of Diagnostic Assays During an Outbreak.
- Policy/CONOPS for Prioritization of Testing Samples Among Zones.
- BSL Requirements.
- Policy/CONOPS for Test Validation During an Outbreak.

At the end of the workshop, each panel presented its list of recommendations to all workshop attendees for new policies, projects, and additional steps to support laboratory preparedness for an FAD outbreak. This report summarizes the discussions and participants' recommendations from the workshop. Participants recommended a number of actions to assist in development of policies and for laboratory concepts of operations during an FAD outbreak. Primary recommendations from all topic areas include:

- Establish a protocol to enable leveraging of accredited veterinarians for sample collection during an outbreak.
- Develop protocols for situations in which an animal is demonstrating clinical signs but tests negative on a penside assay.
- As additional lateral flow or penside technologies become available an assay performance comparison should be performed and CONOPs developed accordingly.
- Develop a strategy for communicating with industry on how newly developed/deployed tests will be used during an outbreak.
- Review policy for triaging samples to determine who and how samples would be triaged and how that information is communicated to the NAHLN laboratories.

- Initiate discussions between the NAHLN Program Office and state animal health officials (SAHOs) to determine steps needed to ensure, when necessary, movement of samples from states with a FAD to NAHLN laboratories within designated “free” states.
- Clinical and suspect cases, along with new species and contact premises should be considered highest priority samples for testing.
- Create standards for “BSL 2 enhanced” (BSL 2+) space to provide additional laboratory capability to perform higher-risk processes and allow for more efficient use of laboratory space within the network during a disease outbreak.
 - Samples collected early in an outbreak (after the index case) should be testing in BSL 3 laboratory space.
 - As the outbreak progresses and additional space is needed (beyond BSL 3), samples could be sent to labs that have BSL 2+ laboratory capabilities.
- Ensure that appropriate assays are available for all phases of an outbreak¹.
 - Validate current or new RT-PCR protocols for pooled samples to support herd-based testing, especially for beef cattle, sheep, and goats.
 - Evaluate potential for using 3ABC ELISA or other serologic tests with alternative matrices (bulk tank milk, oral fluids, meat juice and pooled blood samples).
 - Ensure that the SVANODIP FMD-Ag penside assay is only used during an outbreak when animals are demonstrating clinical signs consistent with disease.

Highlights of the 4th AST Workshop Discussions

Workshop Overview

The fourth agricultural screening tools workshop began with a presentation by Dr. Tammy Beckham, Director of the FAZD Center, describing workshop objectives and an overview of the past AST workshops, focusing on the recommendations developed at AST III in October 2011. Dr. Jon Zack, Preparedness and Incident Coordination Director for the USDA National Center for Animal Health Emergency Management (NCAHEM), presented on the Foreign Animal Disease Preparedness and Response Plan (FADPREP) documents and recommended practices during a foreign animal disease (FAD) outbreak.¹ Dr. Beckham then presented a review and status update of current AST projects funded by the DHS Science and Technology Directorate (DHS S&T) and performed by FAZD Center partners as well as the USDA APHIS NVSL.

The second day included an update on application of select agent regulations during an outbreak by Ms. Barbara Martin, Coordinator of the USDA National Animal Health Laboratory Network (NAHLN), and an overview of the test validation process to deploy a new assay during an outbreak, using swine influenza virus (SIV), as a recent example.

Participants were split into two groups (Red Group and Blue Group) for breakout discussions over both days. There were four of these sessions total (two on each day). After each session, the groups presented to all workshop attendees on their findings. A wrap-up session was held at the end of the second day to reiterate discussions from the workshop, establish recommendations and identify next steps.

Discussion Summaries

Discussion Topic #1: Policy/CONOPS for Use of Diagnostic Assays During an Outbreak

The purpose of Break-out Session 1 was to discuss and provide recommendations for the use of diagnostic assays during an outbreak. Assay priorities and gaps were previously identified in AST Workshops I-III. This focus group concentrated on development of recommendations for establishing concepts of operations for previously validated assays and/or assays under development. The Blue Group, led by Dr. Richard Breitmeyer, was tasked with discussing the FMD ELISA and FMD PCR. The Red Group was led by Dr. Beth Lautner. This group focused on the use of penside assays. Discussion and recommendations from each group are described below.

Discussion: Use of Laboratory-Based Testing Methods

The Blue Group discussed concepts of operations for currently validated assays and those under development during the course of an FMD outbreak. The performance characteristics of each assay were reviewed within the context of “fitness for purpose”, the various phases of an outbreak (as defined in FAD Prep documents¹; Table 1) as well as the validated sample type. Gaps identified by this group were noted and summarized at the end of this section for future action.

Table 1: Phases of FMD Response (adapted¹)
Heightened Alert Phase: FMD outbreak in either Canada or Mexico, but not US.
Phase I: From confirmation of the first case of FMD in the US until reasonable evidence to estimate outbreak extent.
Phase II: Surveillance and epidemiology provides timely evidence of outbreak extent to support decisions by Incident Command.
Phase III: Surveillance and epidemiology indicates FMD is under control. Plan implemented to recover disease-free status.
Phase IV: US declared free of FMD, possibly with vaccination.

Recommendations for Concepts of Operations and Assay Development Needs for Laboratory-Based Testing Methods

- The current RT-PCR FMD assay has been validated for oral swabs, vesicular fluid and epithelium (the “classic samples”).² This assay is robust, however, it is individual animal based.² In the event of an outbreak, an RT-PCR FMD assay validated against pooled sample matrices would be useful. Pooled samples will aid in rapid detection and assessment of herd status, as well as decreased laboratory processing times and overall increased testing capacity. This type of sample will also support business continuity and movement permitting during an outbreak. Pooled sample matrices could include bulk tank milk for the dairy industry and oral fluids for the swine industry, as well as pooled swabs. There is currently an effort underway to validate the RT-PCR FMD assay with bulk tank milk samples and likewise, ongoing work to do the same with swine oral fluid samples. Though the bulk tank milk test is based in part on the current RT-PCR FMD protocol used in NAHLN laboratories for “classic samples” (vesicular fluid or epithelia), the test is being validated for pooled (bulk tank) samples. The advantage of validating the assay for bulk milk samples is that the current RT-PCR FMD tests individual animal status whereas the bulk tank milk test has the potential to assess herd-based status. Validation of a molecular assay for use with oral fluid samples (depending on the performance characteristics) might also be used as a herd-based assay to allow for multiple types of surveillance, including targeted surveillance of animals with clinical signs (e.g., fever) and ability to prove disease freedom in the event of a disease outbreak.
- Currently, the 3ABC ELISA for FMD is considered the OIE standard for demonstrating proof of freedom from disease during an FMD outbreak. This assay is a herd-based test, capable of identifying vaccinated and infected animals within a herd. During the recovery phase, serology assays will be conducted in high numbers, and demonstrating negative results will be vital for meeting the OIE standards to for return to trade.
 - Sample matrices that might be appropriate for a serological assay with these characteristics were discussed. Bulk tank

milk, oral fluids, meat juice and pooled blood samples are all options for high throughput screening. Meat juice is the current sample matrix being used in the national Pseudorabies surveillance testing program. It is validated for use on individual animals but members of the Blue Group suggested its potential use as a pooled sample for serological surveillance in swine.

Once each assay was evaluated in the appropriate context, Blue Group panel members discussed at what point/phase during an outbreak each of these tests should be used and how to focus limited resources (Table 2).

- It was agreed that the RT-PCR FMD assay could be utilized in all stages of an outbreak, or any time there are animals with clinical signs; however, validating assays using pooled samples will be critical for ensuring the most efficient use of time, personnel, and resources.
 - When considering the more discrete terms defined in the FAD PReP documents, RT-PCR was agreed upon to be the best choice for Phase I of an outbreak.
 - For Phase II, RT-PCR was recommended for clinically positive animals in an infected zone.
- The 3ABC FMD ELISA could also be used during each stage of an outbreak within the buffer and surveillance zones. The ultimate choice of what test to use will likely be determined by availability of reagents, resources and clinical situation; however, it was suggested that during later stages of an outbreak, a switch from RT-PCR FMD assay to 3ABC FMD ELISA will likely be warranted.
 - For buffer zones, 3ABC FMD ELISA was recommended for use with clinically negative animals, while RT-PCR was recommended for use in early detection.
- In surveillance zones, which would be the area of focus within an FMD free zone, pooled samples and herd-based assays were recommended.¹

Table 2: Diagnostic Test Recommendations for FMD Outbreak Phases I, II, III & IV				
	<u>Phase I.</u>	<u>Phase II.</u>	<u>Phase III.</u>	<u>Phase IV.</u>
<i>Infected Zone</i>	FMD RT-PCR	FMD RT-PCR	FMD RT-PCR	3ABC FMD ELISA for clinically negative; pooled samples and herd-based assays
<i>Buffer Zone</i>	FMD RT-PCR	3ABC FMD ELISA for clinically negative; RT-PCR for early detection	3ABC FMD ELISA for clinically negative; pooled samples and herd-based assays	3ABC FMD ELISA for clinically negative; pooled samples and herd-based assays
<i>Surveillance Zone</i>	FMD RT-PCR	3 ABC FMD ELISA for clinically negative; pooled samples and herd-based assays	3 ABC FMD ELISA for clinically negative; pooled samples and herd-based assays	3 ABC FMD ELISA for clinically negative; pooled samples and herd-based assays

Gaps

- Pooled matrices (complementary to the milk for dairy and oral fluids for swine) should be identified, developed and validated for beef cattle, goats and sheep.

- Research should be conducted to determine the feasibility of using pooled samples (bulk milk tank, oral fluids, pooled swabs) in serologic assays during an outbreak.
- Work should be performed to determine if meat juice samples can be pooled and used for surveillance of FADs in swine.
- Additional proficiency testing and certification for serologic and molecular based assays is needed.
- Research to generate enhanced processes for sample collection and inactivation in the field prior to shipment to the NAHLN laboratory is needed.
- Efficiency in processing and testing capacity could be gained with inactivated samples, allowing processing and testing in BSL-2+ instead of utilizing BSL-3 space.

In conclusion, workshop participants agree the most important considerations for how an assay will be utilized during an outbreak are fitness for purpose and fitness for use. Ensuring an assay has the recommended performance characteristics is critical, but determining the effectiveness of how to best deploy tools in the diagnostic arsenal is equally important.

Discussion: Use of Currently Available Penside Testing Technologies

The Red Group discussed penside assays that are commercially available as well as those in development.

Current penside technologies (e.g., SVANODIP[®] FMD-Ag lateral flow assay), allow for rapid screening when the animal has lesions consistent with FMD. Commercially available penside assays (lateral flow) do not perform consistently with all FMD serotypes. However, once the strain is characterized during an outbreak, a quick evaluation of performance of the assay should and can be conducted by FADDL.

Recommendations for Concepts of Operations for Currently Available Penside Testing Technologies (e.g., SVANODIP® FMD-Ag lateral flow assay)

- This assay should be utilized only during an outbreak when animals are demonstrating clinical signs consistent with disease for the sole purpose of triaging samples from animals with clinical signs in an affected zone.
 - This test will provide the ability to rapidly conduct screening tests on clinical lesions compatible with FMD in a known outbreak after the index case is confirmed.
- This assay could be utilized for helping laboratory personnel triage samples if personnel and reagent resources are limited.
- This assay could be utilized for conducting epidemiological traces.
- The lateral flow tests currently available are not appropriate for use in business continuity plans including movement of animals or for general surveillance and proof of freedom.
- Protocols for handling situations in which an animal is demonstrating clinical signs but test negative on this lateral flow assay must be developed.
- Implementation of currently available penside testing technologies should include:
 - Prior to use in the field, FADDL must confirm that the assay will detect outbreak strain.
 - The test should be evaluated for performance on the first 300 samples tested on the penside assay by submitting duplicate samples for PCR testing at the NAHLN lab.
 - If recommended, use is implemented as described above.
 - If animal has clinical signs but samples test negative on lateral flow assay, samples should be sent to the NVSL for further testing and additional samples should be collected from the herd and sent to NVSL for evaluation.

- Currently available penside assays should be utilized in routine FADD investigations by FADDs & NAHLN laboratories to help determine performance prior to an outbreak.
- Training prior to implementation should include:
 - Training should begin in FADD and FADP schools.
 - NAHLN and FADDL should develop train the trainer materials for use with NAHLN laboratories and to be used within the incident command system (ICS).
 - Anyone using the test must be trained.
 - Training during an outbreak will be conducted by the lab liaison to the ICS in coordination with a NAHLN laboratory.
- There is a clear need to prioritize users of penside assays. Prioritized users of penside assays should include:
 - FADDs, FADPs, accredited veterinarians, State response core, Animal Health Teams, Accredited veterinarians in production systems.
 - Other potential users: FSIS inspectors/veterinarians.
- The group recommended control and distribution of the penside assays within the ICS.
- A protocol should be developed to have accredited veterinarian in a production system oversee field personnel if ICS utilizing the penside assay.

FAZD Center researchers at the Institute for Animal Health at Pirbright (IAH), in collaboration with scientists at PIADC, are currently developing a loop-mediated isothermal amplification (LAMP) FMD assay for penside use. The test would utilize a lateral flow format to simplify interpretation of results; however, this project is still in preliminary stages. The group discussed how to establish CONOPS and add this technology to the testing repertoire when it becomes available.

Recommendations for Concepts of Operations and Assay Development Needs for New Penside Testing Technologies

- An assay performance comparison should be conducted by assay developers and performance characteristics should be compared with currently available technology as concepts of operations are formed. (Attached as Appendix C). All data should be reviewed by a group such as the NAHLN Methods Technical Working Group and recommendations for use provided to USDA APHIS Veterinary Services (VS).

Rounding out the conversation on use of diagnostic tools, workshop participants briefly addressed the use of industry personnel, accredited veterinarians, and private laboratories as a way to increase sample collection and testing capacity. In addition, diagnostic capacity and results reporting was briefly discussed. Recommendations addressing these issues are outlined below.

- As new tests are validated and deployed, it is also critical for regulators to communicate to industry how those tests will be used. Processes should be developed to communicate with industry and ensure the right numbers and types of samples at the production facility.
- Additional discussions are warranted around the use of private laboratories during an FAD outbreak. A more specific plan should be created to address the use of private laboratories, as current NAHLN guidance lists use of external facilities as “if needed”.
- Real-time reporting of results and availability of data through a web-based reporting system should be addressed. This recommendation was also made by the NAHLN Methods Technical Working Group (NMTWG) in recent meetings and should be discussed by the NAHLN Coordinating Council.

Gaps

- Additional studies should be performed to determine if pooled epithelial samples could be utilized in currently available penside assays.

- Additional studies should be performed in order to have a greater understanding of how these tests perform in vaccinated animals with lesions.
- More sensitive/specific penside technologies/assays meeting internationally accepted standards for sensitivity and specificity are needed for FMD and other high consequence diseases. USDA APHIS VS prioritization of all diagnostic gaps based on stakeholder input and perceived risk must be performed so that the most critical gaps are determined and addressed.
- As new technologies are brought online, assay comparisons should be conducted by the developers and reviewed by the NAHLN Methods Technical Working Group. CONOPs will be developed by VS with input from stakeholders.

Discussion Topic #2: Prioritization of Testing Samples Among Zones

Break-out Session 2 focused on the prioritization of samples during an outbreak. The Blue Group was led by Dr. Patrick Webb and the Red Group led by Dr. Troy Bigelow. Both groups received the same charge to develop recommendations for prioritization of testing samples among outbreak zones, and the groups agreed that triaging samples must be a simple, efficient process. It was noted that the phase of the outbreak will largely influence the prioritization of samples, and that it may be more critical to prioritize based on “risk” rather than “zone”.

Discussion: Prioritization of Testing Samples Among Zones

Two primary premise types were identified, based on the FAD PReP documents and Secure Food Supply Plans: “at-risk” and “monitored.”

- At-risk premises are considered high-priority when moving/testing samples, assuming that animals in these areas have lesions; however, their risk is dependent on information from other areas. “At risk” premises contain susceptible animals, but none are currently displaying clinical signs associated with FMD.³

- Monitored premises are those that do not contain animals that are infected, exposed, or suspected to be infected.³ They remain a high-priority for testing as long as personnel are available to perform the tests. However, limited resources could slow movement of animals and/or products off uninfected premises.

It was noted that the goal of the Secure Food Supply Plans (e.g., SecureMilk, SecurePork) is to get as many premises to "monitored" status as possible, although this is a voluntary process. Premises enrolled in the business continuity efforts will facilitate a path for obtaining a historical log of negative data and ultimately give confidence that herds are negative, will continue to be negative, and animals/product able to be moved.

Wildlife samples were listed as a lower priority due to perceived limited availability of resources, but it was noted that an examination of the domestic animal/wildlife interface would be helpful to understanding disease spread. If there was evidence of inter-species transmission to wildlife, these samples may become a higher priority.

Several questions were identified during this discussion, including whether ICS or the lab should be in charge of triaging samples. It was mentioned that each ICS will have a "lab liaison" that will play a critical role in interfacing with the NAHLN laboratories.

- Priority 1 samples, as determined by ICS, would "jump to the front of the line" for immediate testing. All other sampling would continue through the diagnostic testing process as usual.
- The group identified the conflict between regulators wanting surveillance information and producers wanting to move product as a critical rate-limiting step.
- An additional issue of high concern that should be addressed is the ability to move samples from state to state in the event that additional NAHLN capacity is needed.
 - States in the free zones might be willing to accept low-risk samples (routine or worried-well testing), but it will likely be

more difficult to convince them to take high-risk samples if local NAHLN laboratories become overwhelmed.

- The Laboratory Capacity Estimation Model (LCEM) will help in resource allocation; however, the decision ultimately resides with the state veterinarian and state diagnostic laboratory director.

Recommendations for Prioritization of Samples Among Zones

- The policy for triaging samples should be reviewed to determine who and how samples would be triaged and how that information would be communicated to the NAHLN laboratories.
- Discussions should be held between the state animal health officials (SAHOs) and the NAHLN Program Office to determine steps needed to ensure, when necessary, movement of samples from states with a FAD to NAHLN laboratories within designated “free” states.

Table 3: Recommended Sample Prioritization		
Highest Tier	Intermediate Tier	Lowest Tier
Clinical cases (animals with lesions)	Surveillance zone premises	Zoo animals/wildlife
Suspect cases (animals in contact with infected animals and/or suspected to be infected)	At-risk premises	Breeding stock
New species	Monitored premises	Exposition testing
Contact premises		Movement
		Worried-well

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Gaps

- Develop additional technologies for sample collection and preservation that will inactivate samples in the field, prior to shipment to NAHLN laboratories.

Discussion Topic #3: Biosafety Level (BSL) Requirements

The focus of Break-out Session 3 was determining how and when different biocontainment levels could be utilized for the various processes in sample testing. In addition, the group worked to define/establish “enhanced” BSL 2 requirements that might be useful in increasing capacity within NAHLN laboratories. In general, biosafety levels are designed to protect researchers and diagnosticians, as well as the environment. In order to more efficiently make use of biocontainment space that is available, the group performed a careful examination of processes that could be carried out at each level. In addition, the groups discussed the potential to “enhance” BSL 2 space within the NAHLN laboratories thereby creating standards for “BSL 2 enhanced” (BSL 2+) space. This enhanced containment level would provide additional space to perform higher-risk processes and allow for more efficient use of laboratory space within the network. During this meeting, a NAHLN-specific definition for “enhanced BSL 2+” space was developed. This definition will help mitigate risks and define handling sample types during an outbreak. It should be noted that this “enhanced containment level” would result in practical risk-reduction when performing processes at level 2+, but that “zero” risk does not exist.

Discussion: Biosafety Level Requirements

The Blue Group was led by Dr. Steve Hooser and the Red Group by Dr. Gary Anderson. Both groups compared current guidance for BSL 3 and BSL 2 laboratory space as defined in the CDC *Biosafety in Microbiological and Biomedical Laboratories (BMBL, 5th Edition)* to develop recommendations for a NAHLN-specific definition of the BSL 2+ requirements described below.⁴

Recommendations for Defining NAHLN-Specific BSL 2+, Above and Beyond Those Currently in Place for BSL 2 Laboratory Space

- Public access is not permitted (no clients/tours). However other lab workers may pass through the space.
- Hand washing is required after handling biohazardous materials and prior to exiting the lab. Foot, elbow, or electronic activation is recommended.
- Disposable clothing (wraparound or front closing laboratory coats) is required for all workers with potential exposure to hazardous materials. These items should be autoclaved before disposal.
- Class II A/B3 or Class II B2 biosafety cabinets are required for select (high risk) work with biohazardous agents based on risk assessment. Annual biosafety cabinet certification is required of all labs regardless of AAVLD certification status. All new personnel must train for use of this equipment and on an annual basis thereafter.
- Dedicated freezers must be posted with a biohazard sign and all containers must be labeled with contents. In an outbreak, labs may have to clear space and move non-outbreak materials to alternative freezers.
- Benchtop work is not recommended. However it may be permitted for use during an outbreak in alignment with risk assessment.
- Windows that open are not permitted. For labs with windows that can open, the windows must be locked or sealed during an outbreak response.
- Laboratories must be separated from the general public. Lab doors must be closed when doing work with biohazardous materials and have a "Restricted Access Only" sign.
- Laboratories are recommended to provide enhanced refresher training at the start of an FAD outbreak to supplement require annual training.

- Laboratories must define individual plans for handling suspect samples.
- All liquids must be captured and decontaminated prior to disposal.
- Lab workers must abstain from contact with poultry and livestock for 5 days following a high-risk animal pathogen outbreak response.
- Outbreak sample boxes must be opened in containment (*i.e.*, not in the lab's "receiving" section). If broken tubes are visible, the box must be moved and unpacked in a biosafety cabinet. Packing materials must be autoclaved or incinerated.

Recommendations for the Use of High Containment/Enhanced Laboratory Capacity

- Samples collected early in an outbreak (after the index case) should be testing in BSL 3 laboratory space. With unlimited space, money and personnel, all samples would be handled in BSL 3. However that is not logistically possible during a disease outbreak in which a large number of samples are coming in to the laboratory on a daily basis.
- As the outbreak progresses and additional space is needed (beyond BSL 3), samples could be sent to labs that have BSL 2+ laboratory space. If enough BSL 3 space is available within the NAHLN network, it should be utilized to capacity prior to moving to BSL 2+ and for performing higher risk procedures, such as DNA extraction. Use lower containment space for lower risk activities, such as DNA amplification.
 - Each laboratory planning to define/utilize BSL 2+ space should perform a risk assessment and provide the assessment of planned activities to the NAHLN Program Office.

Gaps

- Enhance guidance in VS memo 580.4⁵ to include recommendations on sample handling and appropriate containment levels during an FAD outbreak.
- Additional discussions are needed between the state animal health officials (SAHOs) and the NAHLN Program Office to determine steps needed to ensure, when necessary, movement of samples from states with a FAD to NAHLN laboratories within designated “free” states.

Discussion Topic #4: Policy/CONOPs for Test Validation During an Outbreak

The two overarching themes of Break-out session 4 were: 1) determination of what is needed to deploy assays for regulatory decisions will depend on the type of response to an emerging agent; and 2) where possible, the US should consider adopting assays from other countries/entities to speed response capabilities. However, it was noted that assay performance characteristics on samples collected from the U.S, herd(s) would still need to be assessed. This validation would include at minimum a basic analytical assessment.

The Blue Group was led by Dr. Bev Schmitt. The Red Group was led by Dr. Tom Baldwin. Both groups came to similar conclusions regarding test validation requirements during an outbreak.

- Initial discussion focused on the lack of available assays for emerging diseases. There was consensus on the need for a “gold standard” for comparison. For diagnostic specificity, it is critical to differentiate a new and/or emerging disease from known ones.
- It was noted that specificity may be easier to determine quickly if significant numbers of negative samples are available, and that results regarding specificity/sensitivity values could be derived from early testing during an outbreak.

- To determine the threshold level for “calling positives”, it will be critical to know what the “significant titer” is for a serological assay and/or the “cutoff” values and thresholds are for molecular based assays.
 - This determination will be derived from the analytical specificity (bench validation) and would not have to be performed separately.
 - Validation procedures will be affected by outside factors such as rate the disease is spreading, trade implications, and consequences (e.g., indemnity, quarantine, depopulation).

The recommendation was made that supporting animal experiments, inter-laboratory comparisons (with the exception of an emerging disease with an assay available), and proficiency testing can be delayed in all instances when attempting to validate an assay during an outbreak. It was noted that it is important to monitor assay performance over time, especially when deploying quickly.

Recommendations for Policy and Concepts of Operations for Test Validation During an Outbreak

- Develop a policy regarding deployment of assays to be used for regulatory decisions for emerging agents.
 - While it is recognized that regulatory decisions will depend on the type of response to an emerging agent, a document listing of what should be considered before deployment of an assay should be prepared. This document should include information on analytical and diagnostic performance characteristics as well as potential animal studies and training and proficiency testing.
- The U.S. should consider adopting assays from other countries/entities to speed response capabilities.
 - Assay performance characteristics on samples collected from the U.S. herd(s) would still need to be assessed prior to

deployment of foreign assays. This validation would include, at minimum, a basic analytical assessment.

Information Updates

Select Agent Rule Requirements during an Outbreak

Ms. Barb Martin gave an update on previous discussions with the USDA National Center for Import and Export (NCIE) on how the Select Agent Rule (SAR) will be implemented during an FAD outbreak. Currently, the USDA NCIE has indicated that NAHLN can apply for an advance exemption to the Rule. An exemption request is being drafted by NAHLN for submission to NCIE. The exemption must address current requirements of the SAR including biosafety, security, incident response, sample storage, agent-specific training, and maintenance of records. In the past, APHIS has interpreted the SAR to mean that taking regulatory action results in a select agent designation, an action that will be defined by the index case.

Outcomes and Next Steps

The results and recommendations from this workshop will be utilized to form concepts of operations in a document similar to the FAD PReP manuals. This document will include a list of laboratory policies, guidance, and recommendations. This will be released through a similar process used for other NAHLN documents. It will also be important to implement a review process to ensure all recommendations are updated as new assays are deployed, policies implemented, and Secure Food Supply Plans come online.

References

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6. USC Environmental Health and Safety Department. Biosafety Levels (BSL): Requirements for Laboratory Work Practice, Equipment, and Facilities Matrix.

Appendix A: Workshop Participants

Name	Organization
Lowell Anderson	Assistant Area Veterinarian in Charge, Iowa, USDA-APHIS
Gary Anderson	Director, Kansas State Veterinary Diagnostic Laboratory
Tom Baldwin	Director, Utah Veterinary Diagnostic Laboratory
Tammy Beckham	Director, FAZD Center and Texas Veterinary Medical Diagnostic Laboratory (TVMDL)
Melissa Berquist	Associate Director, FAZD Center
Troy Bigelow	Swine Health Programs, USDA-APHIS
Richard Breitmeyer	Director, California Animal Health and Food Safety Laboratory System
Randy Crom	Veterinary Medical Officer, Emergency Management and Diagnostics, USDA-APHIS
Amaresh Das*	Microbiologist, Foreign Animal Disease Diagnostic Laboratory, USDA-APHIS
Art Davis	Director, Pathobiology Laboratory, USDA-APHIS
Larry Elsken	Global Vaccine Manager, Center for Veterinary Biologics, USDA-APHIS
Angela Ervin	Program Manager, Science and Technology Directorate, DHS
Larry Granger	Director, Centers for Epidemiology and Animal Health, USDA-APHIS
Heather Hirst	State Veterinarian, Delaware Department of Agriculture
Steve Hooser	Director, Indiana Animal Disease Diagnostic Laboratory
Elizabeth Lautner	Director, National Veterinary Services Laboratories, USDA-APHIS
Bill Layton	Administrator, Montana Veterinary Diagnostic Laboratory
Randall Levings	Scientific Advisor, Emergency Management and Diagnostics, USDA-APHIS
Christie Loiacono	Associate Coordinator, National Animal Health Laboratory Network, USDA-APHIS
Barb Martin	Coordinator, National Animal Health Laboratory Network, USDA-APHIS
Greg Mayer	Microbiologist, Foreign Animal Disease Diagnostic Laboratory, USDA-APHIS
Mike McIntosh*	Head, Proficiency and Validation Services, Foreign Animal Disease Diagnostic Laboratory, USDA-APHIS
Tom McKenna	Director, Wisconsin Veterinary Diagnostic Laboratory
Gene Niles	Director, Illinois Department of Agriculture

	Animal Disease Laboratory
Stacy Morris	Chief of Staff, FAZD Center and TVMDL
David Pyburn	Swine Health Programs, USDA-APHIS
Mark Robinson	National Program Leader, Division of Animal Systems, USDA-NIFA
Beverly Schmitt	Director, Diagnostic Virology Laboratory, USDA-APHIS
Mark Teachman	Director of Interagency Coordination, NCAHEM USDA-APHIS
Sarah Tomlinson	Associate Coordinator, National Animal Health Laboratory Network, USDA-APHIS
Patrick Webb	Swine Health Programs, National Pork Board
Jon Zack*	Director, Preparedness and Incident Coordination, NCAHEM, USDA-APHIS

* Participated via teleconference

Appendix B: Background and Follow-on from AST Workshops I-III

The initial agricultural screening tools workshop was held in November 2010. Goals for that workshop were to formulate a definition of the term “agricultural screening tool,” evaluate the current status of agricultural screening tools, and identify the gaps in and requirements for protecting U.S. agriculture. The workshop participants defined an agricultural screening tool as:

“A tool used to detect a potential disease or condition in an animal, group of animals, or animal product. The tool may be used in any phase of an outbreak response, and is not required to be confirmatory (diagnostic) in nature, but rather is intended for rapid initial detection.”

The second agricultural screening tools workshop was held in April 2011 and focused on industry perspectives for utilizing screening tools to protect the agricultural infrastructure. Accordingly, the group of participants included leaders from the beef, dairy, swine, sheep/goat, and poultry industries. The group was tasked with creating a prioritized list of recommendations for developing and using agricultural screening tools. As discussed and ranked during the second workshop, these priorities were to:

- Develop agricultural screening tools that can be used to permit movement of animals that do not have clinical signs of disease, especially during an outbreak or recovery period.
- Validate assays that are currently being used for PCR (polymerase chain reaction) and ELISA (enzyme-linked immunosorbent assay) testing for use with additional matrices, including:
 - Milk (such as from bulk milk tanks).
 - Oral fluids (such as from saliva-drenched ropes).
 - Meat juice.
 - Air and environmental samples.

- Blood, especially for testing for foot-and-mouth disease (FMD) virus.
- Validate pooling of samples to test for FADs, including:
 - Optimal pooling of swabs or similar specimens for poultry diseases.
 - Optimal pooling of animal blood and/or swab samples, especially for FMD detection.
- Develop simple, low-cost, field-deployable devices for nucleic acid extraction and/or amplification.
- Develop and validate serological tests for “disease free” testing and develop associated policies for using those tests.

AST IV was a natural follow on to AST III as both were focused on defining recommendations for policy development and use of agricultural screening tools.

In the third workshop, participants referenced recommendations and gaps identified in AST I & II and began to formulate recommendations for policies regarding the use of agricultural screening tools and laboratory concepts of operations during an FAD outbreak. In turn, these policies and concepts of operations would support the development and use of new agricultural screening tools. Specifically, this workshop focused on the use of diagnostic assays during an outbreak, laboratory operations during an outbreak, and laboratory sample and reagent prioritization. During the fourth workshop, participants continued to work towards establishing recommendations for policy development related to the following specific areas:

- Use of diagnostic assays during an outbreak of FMD.
 - PCR.
 - Penside testing technologies.
 - Serology.
- Prioritization of testing samples among zones.

- Biosafety level requirements.
 - Use of BSL-2/BSL-2+ and BSL-3 space during an outbreak.
- Test validation during an outbreak.

High-priority recommendations identified from the third workshop included:

- Revise the NAHLN checklist to reflect the minimum general biosafety and biosecurity requirements for FAD sample processing and testing.
- Maintain an inventory of NAHLN laboratories with current Select Agent Program registration and approved agent.
- Establish procedures and processes for additional laboratories to conduct confirmatory serology testing during an FAD outbreak.
- Decide and communicate which samples/cases must be confirmed at NVSL following the index case, such as cases that:
 - Originate outside the established containment area.
 - Originate from a new species.
 - Occur in a new geographic area (region) and/or a new industry compartment.
 - Display a change in clinical presentation.
 - Suggest changes in the epidemiology of the disease.
- Revise the NAHLN Operational Plan to address the potential increase in the number of samples, and the risks of handling/testing those samples, at the beginning of an outbreak.
- Revise the NAHLN checklist to include a requirement for staff to have basic knowledge about the Select Agent Rule.
- Develop and communicate national surveillance plan options for the start of an outbreak.

- Explore the needs for regulatory authority regarding the use of penside FAD tests.
- Work with the NCIE to consider a “fast track” for Select Agent Program registration (or for temporary registration) based on the nature of an outbreak and the scientific need.
- Work with the NCIE to review the NAHLN checklist to see if laboratories can qualify for Select Agent Program exemption based on compliance with NAHLN standards.
- Establish a working group to develop a matrix or other tool to help prioritize diagnostic samples during an FAD outbreak.
- Educate stakeholders about the differences in high-priority and high-risk samples. For example, diagnostic samples collected for business continuity purposes may be high priority but not high risk, because they are presumed to be negative.
- Pursue an exemption from the Select Agent Rule only for official reporting to the Select Agent Program and to allow delayed disposal of presumptive positive samples.

Appendix C: Assay Matrix

Assay performance matrix comparing potential uses for currently available technology

	Target of Test		Status of Animal		Detection		Vaccination Status				Purpose of Testing During an Outbreak				
	Herd	Individual	Clinically Healthy	Clinical Signs	Antigen	Antibody	Vaccinated		Not Vaccinated		Confirmation	Indemnity	Business Continuity	Surveillance	Proof of Freedom
							Use assay?		Use assay?						
							Y	N	Y	N					
RT-PCR (swabs)		X		X	X					X 5	X 5	3 (early)	3 (early)	1	
Milk PCR	X		X		X		X	X		0	0	5 (product)	5	3	
Penside Lateral Flow Devices		X		X	X										
3 ABC ELISA		X	X (preferred screening)	X (preferred screening)		X	X	X		0	0	3 (late)	3 (late)	5	
RT-PCR (oral fluids)	X		X	X	X		X	X		0	0	X 5	5	3	
Meat Juice	X		X (surveillance)			X	X	X		0	0	0	5	5	

Appendix D: BSL Recommendations⁶

Recommendations for a NAHLN-specific definition of the BSL 2+ requirements

	BSL 2	BSL 2+ (Enhanced)	BSL 3
<i>Definition</i>	<i>Low Risk</i> (mild or low infectivity agents; e.g., low-path influenza viruses)	<i>Moderate to High Risk</i> (used for experiments with BSL 3 agenda authorized by IBS to be used in BSL 2 facilities)	<i>High Risk</i> (serious or potentially lethal; e.g., <i>M. tuberculosis</i> , <i>Brucella sp.</i>)
Recommendations			
Access	Public access	No public access; lab workers can pass through	No public access; lab workers cannot pass through
Hand-Washing	Required	Required (foot, elbow, or electronic activation recommended)	Required (foot, elbow, or electronic activation also required)
Clothing	Re-usable lab coats	Wraparound disposable clothing for all workers with potential exposure to biohazardous materials; should be autoclaved	Wraparound disposable clothing for all workers with potential exposure to biohazardous materials; should be autoclaved
Biosafety Cabinets	Class II A/B3 or Class II B2 required for all aerosol-generating work	Class II A/B3 or Class II B2 required for all biohazardous agents (based on risk assessment)	Class II A/B3 or Class II B2 required for all work
Biohazard Signs	Required anywhere BSL 2 materials are stored or BSL 2 work is conducted	Required anywhere BSL 2 or 3 materials are stored or BSL 2 or 3 work is conducted	Required anywhere BSL 2 or 3 materials are stored or BSL 2 or 3 work is conducted
Benchtop Work	Permitted	Not recommended, but may be permitted during an outbreak	Not permitted
Windows that open	Permitted with fly screens	Not permitted; windows must be locked and sealed during an outbreak	Not permitted; windows must be locked and sealed during an outbreak
Lab separated from general public	Not required	Required; lab doors must be closed when doing work with biohazardous materials and have a "Restricted Access Only" sign	Required; lab doors must be closed at all times and have a "Restricted Access Only" sign

<i>FMD Outbreak Situation</i>			
Opening box(es) containing samples	Not permitted	Permitted; visibly broken tubes must be opened in a biosafety cabinet; packing materials should be autoclaved and incinerated	Permitted; visibly broken tubes must be opened in a biosafety cabinet; packing materials should be autoclaved and incinerated
Laboratory plans	Defined by individual lab	Defined by individual lab (should include risk assessment)	Defined by individual lab (should include risk assessment)
Types of samples handled	None	Samples sent once outbreak has progressed (if there is insufficient space, money, and personnel)	Initial cases; used to capacity, then samples sent to BSL 2+
Procedures performed	None	Amplification for PCR testings	Extraction for PCR testing