

SWINE HEALTH

Title: *Negative cohort study plan for the estimation of diagnostic specificity of Two PCR assays for the detection of Classical Swine Fever, African Swine Fever and Foot and Mouth disease viruses in oral fluid samples”, NPB #17-220.*

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Institution: South Dakota State University

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Objectives: Objective 1: To validate the diagnostic specificity of the currently approved APHIS PCR protocols for detection of CSF, ASF, and FMD in oral fluids.

Objective 2: To validate the diagnostic specificity of non-USDA licensed, Tetracore commercial RT-PCR kits for detection of each disease- CSF, ASF, and FMD in oral fluids.

Materials & Methods:

South Dakota- Animal Disease Research & Diagnostic Laboratory tested samples from districts 3-6. Each of the laboratories tested 255 oral fluid samples for the three diseases of interest by both the APHIS approved NVSL PCR assays and the Tetracore commercial kits.

- District 3: Minnesota, Wisconsin, Michigan, Ohio, Indiana, Iowa, Illinois, Kentucky
- District 4: Missouri, Arkansas, Louisiana, Mississippi, Oklahoma, Texas.
- District 5: Montana, North Dakota, South Dakota, Nebraska, Kansas, Wyoming, Idaho
- District 6: Colorado, New Mexico, Utah, Arizona, Nevada, California, Oregon, Washington

These research results were submitted in fulfillment of checkoff-funded research projects. This report is published directly as submitted by the project's principal investigator. This report has not been peer-reviewed.

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Table 2: Sample allocation by State, VS district and testing laboratory

VS District	States (red states should be prioritized)	Testing Lab	Number of samples by lab
1 N=170	NC	NC IA MN	128 21 21
	VA		
	WV		
	PA		
	The rest (negligible swine production)		
2 N=170*	SC	NC	127
	TN- GA	IA	22
	AL -FL	MN	21
3 N=170	IA	SD IA MN	64 53 53
	MN		
	IL		
	OH		
	IN		
	MI		
	KY-WI		
4 N=170	OK	SD	64
	MO	IA	53
	TX	MN	53
	AR-LA-MS		
5 N=170	NE	SD IA MN	64 53 53
	KS		
	SD		
	ND-WY		
	MT-ID		
6 N=170	UT	SD IA MN	63 53 54
	CO		
	NM-AZ		
	NV-CA		
	OR-WA		
<p>*Option to reduce District 2 overall sampling in favor of other more densely populated districts (i.e. District 1) Each lab to test 255 samples for all three diseases by NAHLN PCR and commercial kit PCR States in red represent the top 16 market hog inventories nationwide</p>			

Tetracore kits using only controls supplied by Tetracore

Extraction:

SOP-DS-0074 Nucleic Acid Extraction using the MagMAX Pathogen RNA/DNA kit on a Magnetic Particle Processor.

PCR:

Tetracore kits for FMD, CSF, and ASF

INTERNAL CONTROLS:

- 6ul of Tetracore FMD/CSF IC plus 6ul of Tetracore ASF IC
 - Test were valid with Ct value less than 37.5

NVSL-NAHLN Protocol using controls supplied by FADDL

Extraction:

SOP-DS-0074 Nucleic Acid Extraction using the MagMAX Pathogen RNA/DNA kit on a Magnetic Particle Processor.

PCR:

SOP-DS-0080 Classical Swine Fever and Foot and Mouth Disease rRT-PCR Using the TaqMan® Fast Virus 1-Step Master Mix on the Applied Biosystems 7500 Real-time PCR Platform

SOP-DS-0071 Preparation, Performance, and Interpretation of the African Swine Fever rPCR Assay on the Applied Biosystems 7500 Real-time PCR System

INTERNAL CONTROLS:

- Xeno - 1ul for CSF/FMD and ASF
 - Test were valid with Ct value less than 40

Results: There were 255 oral fluid samples used for all 3 assays (FMD, CSF, ASF) with 2 different PCR protocols (NAHLN and Tetracore). The assays were compared between 3 different laboratories, but for the SDSU data, there were no false positive results, indicating the excellent specificity of the assay on “negative” samples for both the USDA-APHIS or Tetracore assays. All results were sent to the NAHLN office for analysis.

NAHLN Negative Cohort Study Data Collection Sheet													
Instructions: Use one row per animal tested for the Negative Cohort Study to record CT results for each applicable test and sample type. Acceptable samples- samples should be obtained from diagnostic submission or sampling of healthy animal. Any other, please specify in remarks.													
NAHLN Laboratory name	Sample State of orig	District (1-6)	Swine group (Grower or Breeder >5 mo of age)	Individual sample ID	Sample collection date	Sample received in the lab date	Sample tested date	NAHLN		Sample tested date	Tetracore		Remarks
								FMD	Xeno		FME	Xeno	
SDSU ADRDL	MN	3	Breeder	20392	10/20/2017	10/25/2017	1/28/2018	Not Detected	35.142	1/28/2018	Not Detected	43.2609	
SDSU ADRDL	AR	4	Breeder	20196	10/20/2017	10/23/2017	1/30/2018	Not Detected	33.5529	1/30/2018	Not Detected	Not Detected	
SDSU ADRDL	SD	5	Grower	18829	9/29/2017	10/2/2017	1/28/2018	Not Detected	35.0078	1/28/2018	Not Detected	Not Detected	
SDSU ADRDL	SD	5	Breeder	19042	10/2/2017	10/4/2017	1/28/2018	Not Detected	35.102	1/28/2018	Not Detected	Not Detected	
SDSU ADRDL	MN	3	Breeder	20392	10/20/2017	10/25/2017	5/17/2018	Not Detected	35.82	5/17/2018	Not Detected	36	re-run
SDSU ADRDL	AR	4	Breeder	20196	10/20/2017	10/23/2017	5/17/2018	Not Detected	36.72	5/17/2018	Not Detected	34.92	re-run
SDSU ADRDL	SD	5	Grower	18829	9/29/2017	10/2/2017	5/17/2018	Not Detected	35.83	5/17/2018	Not Detected	34.45	re-run
SDSU ADRDL	SD	5	Breeder	19042	10/2/2017	10/4/2017	5/17/2018	Not Detected	36.55	5/17/2018	Not Detected	36.56	re-run

Discussion: Explain your research results and include a summary of the results that is of immediate or future benefit to pork producers.

There is some variability among internal control (IC) values both for the NVSL-NAHLN protocol and for the Tetracore kits. The extraction method for oral fluids mandated for this project was minimally evaluated on a relatively small number of positive samples at FADDL to determine if the standard protocol would yield expected results. The variation in IC values seen in this study might be resolved with a simple centrifugation step of the oral fluid sample prior to extraction. The SDSU-ADRDL processes thousands of oral fluid samples on a monthly basis and all samples tested for viruses are centrifuged prior to extraction. SDSU-ADRDL also uses Tetracore PCR technology for PRRSV testing (at the same concentration used in this study for CSF/FMD) on oral fluids and does not see the variability in IC seen in this study. The major difference between the two protocols is the centrifugation step of the oral fluids prior to extraction.

Additional studies are on-going with the National USDA-NAHLN office and FADDL and aggregated results from other laboratories participating in the negative cohort study will be reported from Christina M. Loiacono, DVM, PhD, DACVP, Coordinator, National Animal Health Laboratory Network, USDA, APHIS, VS, NVSL. While the negative cohort is being completed, the USDA-NAHLN are working towards planning several phases of testing positive samples including a positive cohort.