

## 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 #17-219**

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### Industry Summary:

The Iowa State University Veterinary Diagnostic Lab (ISU VDL) participated in a swine oral fluid negative cohort study (along with three other VDLs) that was coordinated and administered by the United States Department of Agriculture National Animal Health Lab Network (USDA NAHLN). The objectives of this study were to determine the diagnostic specificity of two different PCR assays for African Swine Fever (ASF), Classical Swine Fever (CSF), and Foot and Mouth Disease (FMD) in swine oral fluid samples obtained from swine herds located throughout the United States. Each of the four participating VDLs were responsible for collating samples and conducting ASF, CSF, and FMD PCR testing on a total of 255 swine oral fluid samples. This report serves summarize the findings of the 1,530 PCR assays conducted on 255 swine oral fluid samples at the ISU VDL. Each of the 255 swine oral fluid samples were tested by both the current (single-plex) ASF, CSF, and FMD PCR assays utilized across the USDA NAHLN as well as a set of single-plex ASF, CSF, and FMD PCR assays provided by a commercial PCR reagent manufacturer (Tetracore®). Thus, each swine oral fluid sample tested had six individual PCR assays performed on them. Albeit this project revealed the need for additional work and consideration to be given on the extraction methods used on oral fluid samples (most notably on the Tetracore® assays used in this study), no false positive PCR test results were observed across 6 PCR assays and total of 1,530 individual PCR assays conducted at the ISU VDL. These results suggest the ASF, CSF, and FMD PCR assays evaluated have an estimated diagnostic specificity of 100% on oral fluid samples from US swine. ISU VDL's contributions and results have been provided to the USDA NAHLN for collation with the results obtained from the other three VDLs that participated in this swine oral fluid negative cohort study. The USDA NAHLN will be providing the overall summary and findings of the study.

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**Keywords:** include at least 5 keywords

Diagnostic

Oral fluid

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CSF

FMD

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Cohort

NAHLN

### **Scientific Abstract:**

The Iowa State University Veterinary Diagnostic Lab (ISU VDL) participated in a swine oral fluid negative cohort study (along with three other VDLs) that was coordinated and administered by the United States Department of Agriculture National Animal Health Lab Network (USDA NAHLN). The objectives of this study were to determine the diagnostic specificity of two different PCR assays for African Swine Fever (ASF), Classical Swine Fever (CSF), and Foot and Mouth Disease (FMD) in swine oral fluid samples obtained from swine herds located throughout the United States. Each of the four participating VDLs were responsible for collating samples and conducting ASF, CSF, and FMD PCR testing on a total of 255 swine oral fluid samples. This report serves summarize the findings of the 1,530 PCR assays conducted on 255 swine oral fluid samples at the ISU VDL. Each of the 255 swine oral fluid samples were tested by both the current (single-plex) ASF, CSF, and FMD PCR assays utilized across the USDA NAHLN as well as a set of single-plex ASF, CSF, and FMD PCR assays provided by a commercial PCR reagent manufacturer (Tetracore®). Thus, each swine oral fluid sample tested had six individual PCR assays performed on them. Albeit this project revealed the need for additional work and consideration to be given on the extraction methods used on oral fluid samples (most notably on the Tetracore® assays used in this study), no false positive PCR test results were observed across 6 PCR assays and total of 1,530 individual PCR assays conducted at the ISU VDL. These results suggest the ASF, CSF, and FMD PCR assays evaluated have an estimated diagnostic specificity of 100% on oral fluid samples from US swine. ISU VDL's contributions and results have been provided to the USDA NAHLN for collation with the results obtained from the other three VDLs that participated in this swine oral fluid negative cohort study. The USDA NAHLN will be providing the overall summary and findings of the study.

### **Introduction:**

Foreign swine diseases such as CSF, ASF, and FMD could have devastating impacts to the US swine industry. Surveillance strategies to support freedom of disease for premises in the control area and for movement of animals and products in the event of an outbreak are imperative to a speedy recovery from an outbreak situation and to minimize trade disruptions. The validation of diagnostic tools for aggregate testing are needed to make efficient use of diagnostic and field resources in the midst of an outbreak. More specifically, the specificity of the assay, which is the proportion of true negative samples that will actually yield a negative test result, reflects how well an assay performs in a disease-free population. A highly specific test will minimize the fraction of false positive test results and consequently reduce the risk of having to investigate, trace back, and re-test any positive sample, which could have negative economic impacts for animal health programs and even impact trade. The validation of the specificity of the RT-PCRs that will be used for detection of CSF, ASF, and FMD in an outbreak situation will provide information on number of false positives test results that should be expected when testing a disease free population. In an outbreak situation, this becomes particularly important when the disease is in the eradication phase and/or the surveillance goal is to support freedom of disease. Additionally, having the potential availability of validated

commercial kits (even if only partially validated at this time) on the US swine population as an option to be utilized in a long-term outbreak situation is also desirable.

The USDA NAHLN partnered with the National Pork Board and the Swine Health Information Management Center and four swine interest VDLs (Iowa State University Veterinary Diagnostic Laboratory, North Carolina – Rollins Diagnostic Laboratory, South Dakota State University Animal Disease Research and Diagnostic Laboratory, and the University of Minnesota Veterinary Diagnostic Laboratory) to conduct a foreign animal disease negative cohort study on swine oral fluid samples obtained from swine herds located throughout the US. This report serves to report ISU VDL’s contributions and results obtained from the 1,530 PCR assays conducted on 255 swine oral fluid samples at the ISU VDL. The USDA NAHLN has received the results from the ISU VDL and will be providing a more comprehensive summary that collates the findings and results obtained from all the VDLs participating on this project.

### **Objectives:**

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

### **Materials & Methods:**

ISU VDL collected, aliquoted, and froze back 255 oral fluid samples originating from six pre-defined regions of the US for PCR testing. These regions are listed below for reference purposes.

Region 1: NC, VA, WV, PA, and remainder of states not listed below.

Region 2: SC, TN, GA, AL, FL

Region 3: IA, MN, IL, OH, IN, MI, KY, WI

Region 4: OK, MO, TX, AR, LA, MS

Region 5: NE, KS, SD, ND, WY, MT, ID

Region 6: UT, CO, NM, AZ, NV, CA, OR, WA

Each of the 255 oral fluid samples were tested by six individual PCR assays per protocols distributed to the ISU VDL by the USDA NAHLN. The six PCR assays used are listed below.

1. ASF PCR (USDA NAHLN - current assay used across the NAHLN)
2. CSF PCR (USDA NAHLN – current assay used across the NAHLN)
3. FMD PCR (USDA NAHLN – current assay used across the NAHLN)
4. ASF PCR (Tetracore®)
5. CSF PCR (Tetracore®)
6. FMD PCR (Tetracore®)

Re-testing was conducted on any tests results yielding an “Inconclusive” result due to the observance of an Internal Control Failure (ICF). Internal controls were used in all PCR assays and protocols conducted in efforts to avoid reporting false negative results.

ISU VDL’s test results were sent to the USDA NAHLN to be collated with the results obtained from the other participating VDLs.

## Results:

**Table 1. PCR Results from Initial Screening of Swine Oral Fluid Samples from Herds Located Throughout US.**

Agent	FMD		CSF		ASF	
	NAHLN	Tetracore	NAHLN	Tetracore	NAHLN	Tetracore
Assay						
Number of Samples	255	255	255	255	255	255
Number Positive	0	0	0	0	0	0
Number Negative	254	246	254	236	254	223
Number Inconclusive (Internal control failure)	1	9	1	19	1	32
% Internal Control Failure	0.4%	3.7%	0.4%	8.1%	0.4%	14.3%
% Specificity of PCR Assay	100%	100%	100%	100%	100%	100%

**Table 2. PCR Results on Swine Oral Fluid Samples Retested Due to Internal Control Failure on Initial Screening.**

Agent	FMD		CSF		ASF	
	NAHLN	Tetracore	NAHLN	Tetracore	NAHLN	Tetracore
Assay						
Number of Samples	1	9	1	19	1	32
Number Positive	0	0	0	0	0	0
Number Negative	1	9	1	19	1	32
Number Inconclusive (Internal control failure)	0	0	0	0	0	0
% Internal Control Failure	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
% Specificity of PCR Assay	100%	100%	100%	100%	100%	100%

**Table 3. Final PCR Test Results on Swine Oral Fluid Samples From Herds Located Throughout US.**

Agent	FMD		CSF		ASF	
	NAHLN	Tetracore	NAHLN	Tetracore	NAHLN	Tetracore
Assay						
Number of Samples	255	255	255	255	255	255
Number Positive	0	0	0	0	0	0
Number Negative	255	255	255	255	255	255
Number Inconclusive (Internal control failure)	0	0	0	0	0	0
% Internal Control Failure	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
% Specificity of PCR Assay	100%	100%	100%	100%	100%	100%

## Discussion:

Albeit this project revealed the need for additional work and consideration to be given on the extraction methods used on swine oral fluid samples (most notably on the Tetracore® assays utilized in this study), no false positive PCR test results were observed across 6 PCR assays and total of 1,530 individual PCR assays conducted at the ISU VDL. These results suggest the ASF, CSF, and FMD PCR assays evaluated have an estimated diagnostic specificity of 100% on oral fluid samples from US swine. ISU VDL's contributions and results have been provided to the USDA NAHLN for collation with the results obtained from the other three VDLs that participated in this swine oral fluid negative cohort study. The USDA NAHLN will be providing the overall summary and findings of the study.