RESEARCH ABSTRACT

SWINE HEALTH

Title: Development and Evaluation of a Standardized Protocol for Utilization of Swine Oral fluids for PCR detection of High Consequence Pathogens: FMD, CSF, and ASF **- NPB #14-284 revised**

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Project summary: The primary objective of this project is to develop and validate a standard operating procedure (SOP) for the optimal purification of pathogen NA and quantitative PCR detection of high consequence Swine pathogens from swine oral fluids (SOF). This study employed the comparative use of three commercially available magnetic bead extraction kits (GeneReach, USA; Qiagen, Germany; and Life Technology, USA) and various SOF samples. Nucleic acid surrogates (provided by FADDL) for Foot and Mouth Disease (FMDv), Classical Swine Fever virus (CSFv) and African Swine Fever virus (ASFv) and live virus stocks were used for Influenza A (pH1N1), Porcine Reproductive and Respiratory Syndrome virus (PRRSV) and AFSV were utilized. Various concentrations of targets (surrogates, viruses) were spiked into SOF and extracted side by side using three automated extraction instruments (GeneReach Tacomini- 8 well, Qiagen BioSprint- 96 well and Life technology King Fisher 24 (BSL3)). Protocols utilized based on kit manufacture recommendations with experimental modifications included varying volume and dilution of SOF, varying kit components and using several pre-extraction treatments. Extractions performed in duplicate or triplicate, evaluated the effect of experimental parameters on the extraction efficiency via qPCR NA detection. PCR detection of Influenza A, FMDv, and CSFv were performed per National Veterinary Service Laboratory protocol utilizing two RT (reverse transcriptase)-qPCR master mixes (Life technologies-NVSL SOP reagents and Quanta Biosciences, USA, alternate). Quanta Biosciences qPCR mix (Fast mix II) was utilized for the detection of ASFv DNA (modified NVSL protocol). Detection of PRRSv RNA was performed using the Qiagen Virotype PRRSv kit. PCR was performed with the CFX 96 qPCR machine (Biorad, USA). Clinical samples tested with the developed protocol were SOF containing PRRSV and SIV positive and various previous qPCR tested negative samples (ISU VDL). For BSL 3 work, oral fluids collected from swine experimentally infected with Armenia 2007 strain of AFSV (BSL3 BRI-KSU) were used.