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Development of Swine Influenza Virus Nucleic Acid Purification and Detection Workflow

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An integrated workflow consisting of high throughput nucleic acid purification and TaqMan® RT-PCR assays for detection and genotyping of Swine Influenza nucleic acid were developed and validated. The following data is under review to support Conditional License Applications filed with the USDA, Center for Veterinary Biologics.

Nucleic acid is purified using the MagMAX™ Nucleic Isolation technology and purified viral SIV RNA is detected and subtyped using the VetMAX™ Swine Influenza screening and subtyping kits. The Swine Influenza screening kit consists of the Swine Influenza A (SIV-A) TaqMan assay. The Swine Influenza A genotyping kit consists of SIV-H1/H3 and SIV-N1/N2 TaqMan assays. The SIV-A TaqMan assay targets multiple conserved genetic regions of the Influenza A matrix and nucleocapsid genome decreasing the likelihood of false negatives due to viral mutation. The assay is multiplexed with an internal control RNA, Xeno™RNA, to monitor nucleic acid purification efficiency, detect the presence of reaction inhibitors, and determine assay reagents functionality. The Swine Influenza A genotyping assays are two independent duplex assays targeting the hemagglutinin and neuraminidase gene respectively. The analytical sensitivity was determined to be 50 copies of SIV-A transcript RNA, 25 copies of H1 and H3, and 50 copies of N1 and N2, per 25ul TaqMan PCR assay.

The performance of the workflow was evaluated using field samples (n=58) of known SIV status; diverse reference SIV genotypes were included to ensure functionality of genotyping assays. RNA was isolated from swine nasal swabs, tonsil and lung tissue homogenates, virus, and cell lysate supernatant using MagMAX™ -96 Viral RNA Isolation kit. The purified RNA was utilized for TaqManRT-PCR assay employing the SIV screening assay on the Applied Biosystems 7500 Fast Real-Time PCR System. SIV-positive field samples were then tested with the genotyping assays. The SIV screening and genotyping results showed 100% concordance with secondary laboratory PCR results. All SIV reference genotypes (H1N1, H1N2, and H3N2) were correctly identified. The 100% field sample sensitivity and specificity as well as the superior analytical sensitivity demonstrate that this method provides an economical and rapid solution for SIV identification and typing.