KANSAS STATE UNIVERSITY

Veterinary diagnostic assays available from K-State for collaboration

Contributors: Jianfa Bai, Ying Fang, Douglas Marthaler, Xuming Liu, Yanhau Li, Lalitha Peddireddi, Gary Anderson, Richard Hesse

Title: Multiplex real-time RT-PCR assay for the detection and differentiation of porcine rotavirus groups A, B and C strains with an internal control	**Ref #**: 2017-069
Summary: A multiplex real-time RT-PCR is developed and validated for the detection and differentiation of swine rotavirus groups A, B, and C strains. Swine rotavirus groups A, B, and C are major GI pathogens causing diarrhea in pigs. Previously, limited sequence information prohibited the development of accurate molecular diagnostic measures, especially for groups B and C rotaviruses in swine. KSVDL sequenced the full genomes of a number of group B and C strains leading to the development of this assay. It incorporates an internal control that monitors the extraction efficiencies and potential PCR inhibitions. The PCR amplification efficiencies are 105.9%, 103.2% and 101.6%, and correlation coefficients are 0.997, 0.997 and 0.998, respectively for groups A, B and C strains under multiplexed conditions. These data fit the general guidelines of 90-110% amplification efficiencies and >0.99 for correlation coefficients.	

Title: Development of sensitive and reliable diagnostic assay to detect atypical porcine pestivirus (APPV) in swine	**Ref #**: 2017-041
Summary: Based on current sequence information available (GenBank and KSVDL unpublished data) for APPV, a TaqMan-based real-time RT-PCR assay is designed targeting most conserved region(s) of the virus genome. This invention provides a sensitive and reliable PCR-based diagnostic assay for universal detection of genetically divergent APPV isolates. In addition, this invention can be automated for high throughput screening of clinical and surveillance samples.	

Title: Detection and differentiation of PCV3 from PCV2a, PCV2b and the highly prevalent PCV2d mutant strains	**Ref #:** 2017-042
Summary: KSVDL has identified a new species of circovirus, porcine circovirus type 3 (PCV3), from sows with clinical signs normally associated with porcine circovirus type 2 (PCV2) infection and in aborted fetuses. The fact that the sows and aborted fetuses were negative for PCV2, PRRS and SIV strongly suggests that PCV3 may be involved in causing the clinical signs and abortions. This invention will detect PCV3 strains and will differentiate it from PCV2 strains. *(**Note:** we are in negotiations with a vaccine company for vaccine & diagnostic rights to the PCV3 invention. They may be open to a diagnostic collaboration)*	

Title: Development of a TaqMan quantitative RT-PCR test for porcine parainfluenza virus 1 (PPIV1)	**Ref #:** 2017-043
Summary: PPIV1 has recently emerged in the USA, and the widespread presence of this virus in the US swine herd has been confirmed. A TaqMan qRT-PCR assay targeting the hemagglutinin gene of PPIV1 is designed based on all the sequences currently available in NCBI Genbank. This invention provides a molecular diagnostic assay for the detection of PPIV1 infection, epidemiological surveys, and outbreak investigations. In comparison with the conventional RT-PCR test, this invention has several advantages, including high diagnostic sensitivity, less time-consuming, automated process and low cost.	

Title: Multiplex real-time RT-PCR assay for simultaneous detection and differentiation of swine influenza C, D, and B viruses	**Ref #:** 2017-044
Summary: Swine influenza is a highly contagious, acute viral respiratory disease caused by influenza A, B, C, and D viruses (IAV, IBV, ICV, and IDV). IAV is the most common pathogen, and previously it was assumed to be the only influenza virus that could infect swine. Recent evidence demonstrate that IBV, ICV, and the newly discovered IDV can also infect swine. A high coverage, high-throughput, low-cost, and accurate multiplex one-step real-time RT-PCR assay has been developed. When compared with the previous uniplex real-time RT-PCR assays, this invention has several significant advantages, including higher coverage, higher diagnostic sensitivity and specificity, high-throughput, lower-cost, less time-consuming, and simultaneous multiplex detection and differentiation of IBV, ICV, and IDV.	

Title: Detection and differentiation of Seneca Valley virus (SVV) from foot-and-mouth disease virus (FMDV)	**Ref #:** 2017-045
Summary: SVV caused disease is clinically indistinguishable from economically important vesicular diseases such as FMD and because of this, clinical samples derived from reported cases of animals with vesicular disease need to be quickly analyzed. This new real-time PCR assay has the following advantages over older assays: designed with the most current sequencing data to ensure high coverage; multiplexed with a FMDV assay, so that one assay will detect and differentiate SVV and FMDV; an internal control will be included to ensure assay quality; and it will be validated for specificity, in particular with FMDV strains.	