

Title: Development of reagents and serological assays for porcine deltacoronavirus (PDCoV) – NPB #14-184

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Scientific Abstract:

A novel porcine deltacoronavirus (PDCoV) was reported in China in 2012 and in the U.S. in February 2014. Since then, PDCoV has been identified in a number of U.S. states and linked with apparent clinical disease including acute diarrhea and vomiting in the absence of other identifiable pathogens. PDCoV is currently diagnosed by real time PCR and clinical symptoms along with elimination of other viral pathogens known to cause similar disease. No specific antibody-based reagents were available to assist in diagnosis of PDCoV, and limited serological capabilities were available to detect an antibody response to this virus. Therefore, the objectives of this study were to develop readily available reagents for detection of PDCoV antigen in diagnostic tests, such as virus isolation, immunohistochemistry and fluorescent antibody techniques, and to develop and optimize several serological assays including indirect ELISA, fluorescent microsphere immunoassay (FMIA), indirect fluorescent antibody (IFA) and virus neutralization assays.

The full-length nucleoprotein (NP) of PDCoV was cloned and expressed in *E. coli* as a 41 kDa polyhistidine fusion protein. This protein was purified by nickel-NTA affinity column chromatography and is recognized in Western blotting and ELISA by convalescent serum from infected pigs. Both denatured and refolded versions of this protein were used to immunize rabbits for hyperimmune serum. Rabbit hyperimmune sera specifically recognize the NP and can be used in indirect fluorescent antibody staining and immunohistochemical staining procedures for the detection of PDCoV antigen. These reagents have been widely distributed to assist with research studies and diagnosis of PDCoV.

An IFA test for PDCoV serology was first developed using cell culture adapted virus. Serum samples from swine herds with recent documentation of PDCoV infection and samples from expected naïve herds were used

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for initial assay optimization. For development of ELISA and FMIA test formats, a refolded version of the same NP described above was used as an antigen. The tests were optimized in a checkerboard fashion to reduce signal to noise ratios using samples of known status. Receiver operator characteristic (ROC) analysis was performed to establish assay cutoff values and assess diagnostic sensitivities and specificities. Both FMIA and ELISA showed seroconversion of challenged pigs between 8-14 DPI. In addition, neutralizing antibody responses in serum are being quantified using a prototype fluorescent focus neutralization (FFN) assay. These new diagnostic tests provide additional tools to aid in the control and surveillance of porcine deltacoronavirus outbreaks.