RESEARCH ABSTRACT

SWINE HEALTH

Title: Novel multiplex diagnostic assays development for diagnosis of porcine respiratory disease complex – **NPB #11-037**

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Scientific Abstract:

Development of diagnostic tests that are able to rapid, simultaneously detect multiple pathogens offers an important tool for disease surveillance and control measurements. In this study, we have developed a multiplexed fluorescent microsphere immunoassay for simultaneously detection of specific antibodies in oral fluid and serum samples from animals infected with porcine reproductive and respiratory syndrome virus (PRRSV), swine influenza virus (SIV), and porcine circovirus (PCV2). Recombinant nucleocapsid proteins of PRRSV, SwIV, PCV2 were generated and used as antigen and covalently coupled to Luminex fluorescent microspheres. Based on an evaluation of 1444 oral fluid samples with known serostatus, the oral fluid-based multiplex FMIA achieved greater than 92.0% sensitivity and 80.4% specificity. In serum samples (n = 2484), the multiplex FMIAs reached greater than 98.2% sensitivity and 98.3% specificity. Time course studies showed that the assay can detect antibody responses to PRRSV, SIV and PCV2 as early as 14 days post infection and for greater than 90 days post infection in oral fluid and serum. For detection of acute infection, a monoclonal antibody-based PRRSV antigen capture multiplex FMIA has been explored. Using 113 nasal swab samples from experimentally infected pigs with SIV, the assay generated 98.6% sensitivity and 92.7% specificity for capturing SIV. However, the assay showed low sensitivity and specificity for PRRSV and PCV2. To meet the demanding of on-site field test, a sandwich immuno-chromatographic dipstick assay was developed to detect antibodies from animals infected with PRRSV, SIV and PCV2. The sandwich immunochromatographic dipstick assay was developed based on the high binding capacity of a specific pathogen antigen to swine antibodies, and the conjugation of swine immunoglobulin with colloidal gold nanoparticles as a color probe. Using nucleocapsid proteins (SIV, PCV2) and nsp7 (PRRSV) as antigens, dipstick assays were developed based on testing standard positive and negative control sera as well as a panel of field serum samples, and the diagnostic specificity and sensitivity were compared to the classical enzyme-linked immunosorbent assay (ELISA) or hemagglutination inhibition (HI) test. Based on the evaluation of 456 serum samples of known serostatus from pigs experimentally infected with either type I or type II PRRSV, the PRRSV dipstick assay showed an overall sensitivity and specificity of 95% and 96% respectively, in comparison with the IDEXX HerdCheck X3 ELISA. The inter-rater agreement (kappa value) between dipstick test and ELSA was 0.965. The SIV dipstick test achieved a sensitivity of 96% and a specificity of 99% using 177 field serum samples previously tested in HI assay, and the kappa value between dipstick and HI was 0.953. For PCV2 antibody detection, results from nucleocapsid protein-based dipstick test and ELIA were compared using 135 samples from experimentally infected animals. The sensitivity and specificity of PCV2 dipstick test were 88% and 92%, respectively. These multiplex assays are more suitable for large-scale field application on porcine respiratory disease surveillance and epidemiology studies. They present advantages of simplicity, rapidity and cost-effectiveness. The dipstick test is field deployable with a user-friendly format, which can be performed on-site in a swine farm by untrained personnel, while the multiplex FMIA will be used as a laboratory confirmation test for the accurate identification of various pathogens in PRDC.