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Development of a new ELISA test for detection of PRRS antibodies in swine oral fluids.

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Testing of swine oral fluids represents a convenient and cost-effective tool for disease monitoring and surveillance in commercial pig herds. Recently, detection of antibodies to PRRS in oral fluids using an overnight protocol modified from the serum IgG antibody ELISA (HerdChek® PRRS X3 ELISA, IDEXX Laboratories, Inc.) was reported by Kittawornrat *et al.* (J Vet Diagn Invest. 2012 Mar;24(2):262-9.). In this study, we describe the performance of a new ELISA test for same day detection of anti-PRRS IgG antibodies in swine oral fluids. The format of the new PRRS oral fluids ELISA maintains the same protocol workflow as the standard PRRS X3 kit, with the exception that: the sample incubation step is longer (2h vs. 30 min on X3), the original oral fluids sample input is 50µl, as opposed to 2.5µl for the serum assay, and the final dual absorbance read is 450-650nm instead of 650nm. An S/P ≥ 0.4 is considered a positive result. A comparison between the new protocol and the standard overnight protocol (SOP) using a set of reference standards consisting of pooled oral fluids from pigs vaccinated with type 2 PRRS MLV vaccine indicated 100% agreement between the two tests, with average S/P values 1.4 to 1.5-fold higher than SOP, indicating an enhanced sensitivity. The same results were obtained upon analysis of a set of experimentally inoculated pigs as well as field samples obtained from Iowa State University as part of an inter-laboratory reproducibility evaluation of SOP. Moreover, analysis of a temporal series of paired oral fluids and serum collected from individual boars experimentally infected with type I (strain D09-012131) or type II (strain MN-184; GenBank accession no. AY656992) PRRSV for up to 21 days post-infection (DPI) indicated no difference between the days to detection of anti-PRRSV antibodies in serum as in oral fluids, as well as a similar ability to detect both type I and II strains. Finally, an evaluation of pen-based oral fluid samples collected at various prevalences (0%,

4%, 12%, 20%, and 36%) of vaccinated pigs introduced at 14 days post-vaccination into pens of PRRS-negative pigs indicated that the new test detects anti-PRRS antibodies $\geq 96\%$ of all collection events in pens of at least 20% prevalence, and $\geq 85\%$ in pens of 12% prevalence. Taken together, these results describe a new sensitive test for anti-PRRS antibody detection aimed to support the emerging use oral fluids for easier and frequent surveillance and monitoring of pig herds.