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Development of a quantification method to specific Anti-ORF2 antibody using a blocking ELISA

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Introduction and Objectives
The objective of the study was to develop a quantitative serum antibody test for PCV2 in swine with a commercially available test (SERELISA® PCV2 Ab Mono Blocking, Synbiotics Corporation). This test is based on a blocking Enzyme-Linked ImmunoSorbent Assay (ELISA) which allows specific detection of anti-ORF2 antibodies against PCV2.

Material and methods
Results of the test are expressed as sample to negative control optical density (OD) ratio corrected by the positive control OD and referred as s/n ratio. The linear range of the bELISA was determined by conducting the assay with the s/n ratio of a positive reference sample at different dilutions starting at 1:10 (increasing by 2 and 10 dilution factors) and 1:50 (increasing by 10 dilution factors) in order to recreate a panel of samples ranging from strong positive to weak positive.

After graphical analysis, determination coefficients (r²) were calculated for different models with variable transformations for s/n ratio and the dilution of titer. Transformations were analyzed for the relationship between titer (T), 1/T, and log T and s/n ratio (sn), 1/sn, log sn and logit sn.

This model was determined within certain limits of dilution titers. Therefore to achieve a valid quantification method from negative to highly positive samples, different dilutions were selected and interpolation was calculated between results obtained from different wells. Determinations of coefficients and regression equations were calculated using R version 2.4.1. ANOVA for the robustness study was performed using the same statistical computing software.

Results
Linear s/n ratio values ranging from 0.11 to 0.93 were determined using the reference PCV2 positive serum sample at different dilutions. Comparing seven different regression models correlating different s/n ratio and titer functions, the best model was achieved utilizing the Log of titer and the logit of s/n ratio. This model was linear with an r² of 0.988, a slope of β=-0.703 and an intersection of α=2.652.

Discussion and Conclusions
This model provides a quantitative method for specific detection of anti-ORF2 antibodies against PCV2. The linearity and the robustness have been proven to be effective using three wells in a blocking ELISA. This innovative method provides a quantitative method for a blocking PCV2 ELISA and therefore allows the detection of a specific antibody subpopulation. This method is independent of the seroneutralizing properties of the targeted antibody subpopulation and therefore a nice alternative to Seroneutralizing Tests.

This standardized quantitative test is a tool that will lead to a breakthrough in the understanding of the PCVD epidemiology and be utilized to assess PCV2 control measures such as vaccination.

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