DEVELOPMENT OF A QUANTIFICATION METHOD TO SPECIFIC PCV2 ANTI-ORF2 ANTIBODY USING A BLOCKING ELISA

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Introduction and Objectives

Post-weaning Multisystemic Wasting Syndrome (PMWS), a swine disease first identified in 1991, in Canada, has been observed since in the United States, Europe, and other Asian countries. Due to the different clinical forms of the PCV2 infections, this syndrome has been recently referred to PCVD (Porcine Circovirus Diseases). Vaccination is one method of controlling this disease. A complementary quantitative antibody test could be useful in achieving proper control of PCVD.

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. Nevertheless, the blocking format does not allow a straightforward approach for developing quantification methods because of the usual lack of linearity of these tests on a large range of antibody titers.

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 **re**create 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.

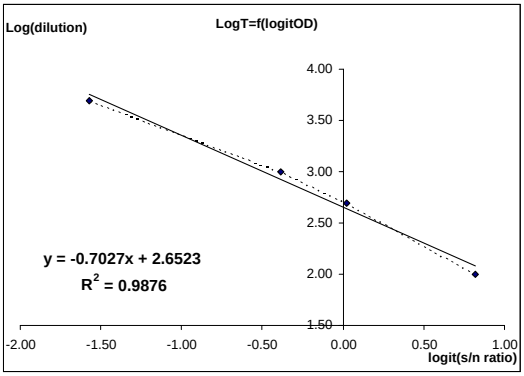
This model was determined within certain limits of dilution titers. Therefore to achieve a valid quantification method from negative to highly positive samples, different dilutions were selected and interpolation was calculated between results obtained from different wells.
The method developed was finally applied to 7 samples of known origin ranging from negative to strong positive samples to validate the model and assess robustness of the model.

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 r^2 of 0.988, a slope of β=-0.703 and an intersection of α=2.652 (graphic 1).



Graphic 1. Linear regression model between the Log of the titer and the logit of the s/n ratio

This linear model covered at least a range of a log (base 10) of titer. In order to have a quantitative method valid for serum ranging from negative status to high positive titer, an interpolation using different wells is needed. Inside the linear range, titers range from 100 to 1000 for s/n ratio respectively ranging from 0.895 to 0.242. An arbitrary decision was made to apply this results to the 1/1000 final dilution in the well. Derived from this decision, interpolation was calculated for the two next dilutions of 1:100 and 1:10000. A correcting factor (multiply by 10 or divide by 10) was applied to each of the titer results obtained within these wells and therefore linearity is respected between the three dilution wells. The final model was not limited on the lower bound of the 1:100 well and an arbitrary limit was fixed on the upper bound of the 1:10000 well. This limit corresponds to the very limit of linearity of the regression model (s/n=0.107). Titers obtained are expressed in ELISA units (EU).

The final model using three dilution wells was applied to determine titer levels of seven serum samples with known PCV2 status. Two PCV2 serum negative samples had titers lower than 200 EU. Intermediate samples had titers ranging from 1000 to 3000 EU while the two strong positive samples ranged from 5000 to 20000 EU.

Robustness observed with this model was satisfactory (CV < 10%).

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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