Blocking ELISA

Glutathione S-transferase fusion epitope-M201 (GST-201) was used as diagnostic antigens in the bELISA. Two complementary oligonucleotides containing the coding sequence for epitope-M201, flanked by restriction enzyme sites EcoRI and XhoI were hybridized and cloned into a pGEX-6P-1 (GE Healthcare Biosciences, Piscataway, NJ) at the corresponding restriction enzyme sites. Protein expression and purification were performed following the GST gene fusion system handbook [1].

The bELISA was performed in Immulon 2 HB 96-well microtiter plates (Thermo Scientific, Rochester, NY). The optimal concentration of purified GST-201 and HRP-conjugated MAb-201 used to develop the ELISA plate was experimentally determined by checkerboard titration. The GST-201 was diluted to the optimal concentration of 4 µg per 1 ml of carbonate buffer (0.1 M) then 100 µl diluted protein was pipetted to all wells of the plate. The plate was incubated at 4°C overnight. The following day, 250 µl blocking buffer [Tris buffered saline (TBS) containing 0.05% (v/v) tween-20 and 2% (w/v) bovine serum albumin] was pipetted into all wells of the plate and the plate was incubated for 1 h at room temperature with gentle agitation. The plates were washed 2 times with more than 300 µl TBS containing 0.05% (v/v) tween-20 (TBS-T20). Test and control samples were diluted 1:4 in blocking buffer and each samples were pipetted into 2 wells of the plate, 100 µl per well. Four wells of each plate were added with 100 µl blocking buffer only and were used as non-inhibition controls for calculation of percentage inhibition (PI). The plates were incubated for 1 h at room temperature. After that, the plates were washed 6 times with TBS-T20. HRP-conjugated MAb-201 was diluted 1:10,000 in blocking buffer and 100 µl of this was added to all wells of the plate, followed by incubation for 1 h at room temperature. The plates were washed 6 times with more than 300 µl TBS-T20.

For the final wash, the plate was washed with distilled water instead of TBS-T20. After that 100 µl ABTS peroxidase substrate (KPL, Gaithersburg, MD) was added to all wells of the plate. The plates were gently swirled and then incubated in a 37°C incubator. At 15 minutes, color development was stopped by the addition of 100 µl 1% SDS to all wells. The color development was quantified by reading at 405 nm with an EL800 microplate reader (BioTek Instruments Inc., Winooski, VT). The raw data were transferred into an Excel spreadsheet to calculate the PI using the following formula:

$$\mathrm{PI} = \left\{1 - \frac{\mathrm{OD\ sample}}{\mathrm{OD\ control\ (buffer\ only)}}\right\} \mathrm{x}\ 100$$

where OD represents optical density. Non-labeled MAb-201 was used as positive control. A single lot of serum samples obtained from pigs prior to PRRSV inoculation was used as negative control.

The optimal cut-off of the bELISA was determined by Receiver Operating Characteristic (ROC) analysis, using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com).

Reference

[1] Anomymous. GST gene fusion system. Amersham Pharmacia Biotech, Third Edition, Revision 2 1997;https://homes.bio.psu.edu/people/faculty/lai/lab/protocols/GST%20Gene%20Fusion%20System.pdf.