

AUTOLAB SPR

Fast and sensitive detection of a virus infection in pigs

Surface Plasmon Resonance is a surface sensitive technique used to measure molecular interactions by the change of mass (directly correlated to the change of refractive index) at a sensitive surface. The technique has found its way in clinical analysis and medical research. This application note shows the use of the AUTOLAB ESPRIT instrument in the sensitive, fast and cost effective detection of viral infections in patient serum.

Viral infections can be detected by measuring antibodies against the virus in the patient's serum. Conventionally, this measurement is carried out in an Enzyme-Linked ImmunoSorbent Assay (ELISA). The ELISA procedure consists of extensive manual labour, labelling and detection steps. These procedures are time consuming (typically from 8 up to 20 hours per test) and costly. In contrast to ELISA, SPR measurements are label-free, real-time and, therefore, fast and less elaborate. This application note shows the detection procedure of a viral infection in porcine patients using the AUTOLAB ESPRIT.

Surface Plasmon resonance experiment.

A gold disk with a ProLinker B self assembled monolayer was used to immobilize a recombinant viral capsid protein (capsid, further referred to as PCV2 antigen). The protein is specific for the PCV2

virus that causes Postweaning Multisystemic Wasting Syndrome (PMWS) in pigs. The PCV2 antigen was immobilized in a 150 ng/mL concentration in 0.1 M acetate buffer pH 4.5 in a 30 minute incubation step. The surface was washed and blocked with 10 mM phosphate buffered saline (PBS) and 1% bovine serum albumin (BSA) respectively. Monoclonal antibody against the PVC2 antigen (raised in pig) was diluted 10 fold in serum to concentrations between 100 µg/mL and 1 ng/mL. These antibody dilutions were used to prepare a calibration curve to determine the antibody concentrations of patient serum samples. The calibration curve samples, the patient samples or PBS containing 1 mg/mL BSA (reference) were subsequently dispensed onto the sensor. The interaction was recorded for 30 minutes, followed by a wash step of 10 minutes with PBS. Each step of the assay was monitored in a real-time data plot (Figure. 1). The SPR test, including the immobilization, took 90 minutes.

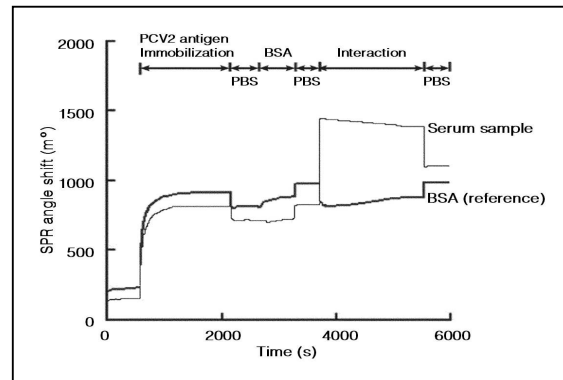


Figure 1. Real time measurement of immobilization of PCV2 antigen with subsequent addition of a sample (thin line) and a reference sample (BSA, bold line).

The angle shift values of all calibration curve and serum samples were calculated from the last PBS wash value of the sample minus the reference value. The angle shifts of the calibration curve samples were plotted against the antibody concentration in figure 2. The results show a linear relationship between the SPR angle shift and the antibody concentrations between 1 ng/mL and 100 µg/mL.

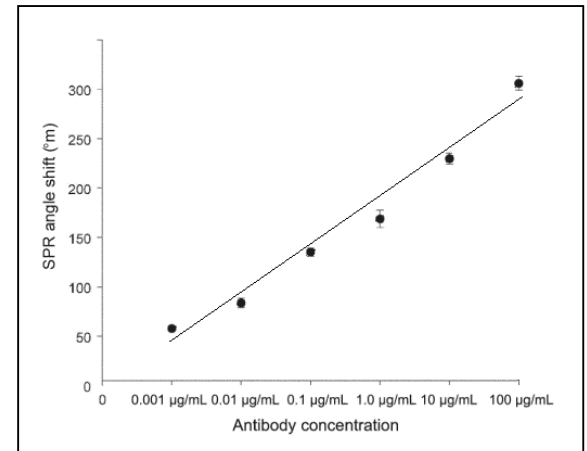


Figure 2. Concentration response curve. SPR angle shift caused by binding of various antibody concentrations on a PCV2 antigen sensitized gold disk.

ELISA procedure

For comparison, antibody samples that were assessed in the SPR assay were also measured in a conventional ELISA: 96-well ELISA plates were coated with the PCV2 antigen (1µg/well, 60 min incubation), blocked with 1% skim milk in PBS and washed with PBS with 0.05% tween-20 (PBST, 60 min incubation). Serum samples were incubated for

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1 hour at 37°C. The plates were then washed three times with PBST. The bound antibody was detected using a secondary antibody raised in goat against porcine IgG, with a horse radish peroxidase (HRP) conjugate (60 min incubation). A HRP-substrate solution (Citric acid, 2,2'-azino-di-(3 ethyl-benzthiazoline sulfonic acid and H₂O₂)), was used to detect any bound secondary antibody. The plates were then developed in the dark for 15 minutes. The optical density (OD) of the sample wells was read out in an ELISA reader at 402 nm. These ODs were plotted against the SPR angle shifts for the same samples detected in the SPR assay (Figure 3). This plot shows a very strong correlation between the SPR and the ELISA results (Pearson correlation coefficient = 0.877, $p < 0.01$, for 70 samples).

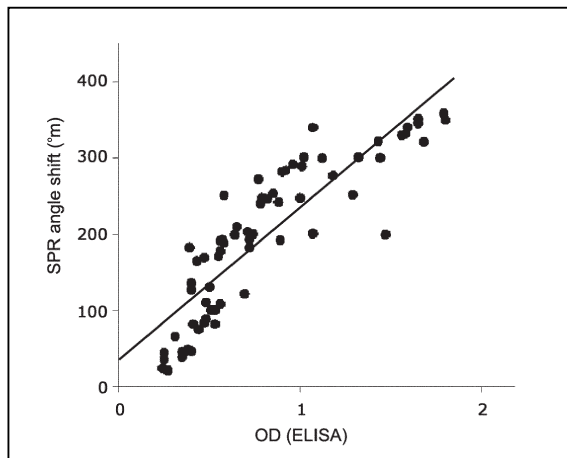


Figure 3. The correlation between PCV2 antibody titers as determined with Surface Plasmon Resonance using the AUTOLAB ESPRIT and a conventional enzyme linked immunosorbent assay (ELISA).

Conclusions

1) The SPR assay shows a linear relationship between the antibody concentration and the shift in angle, between 1 ng/mL and 100 µg/mL. Therefore, SPR presents a reliable assay to reveal PCV2 infections in pig serum.

2) The SPR and ELISA results showed a good correlation, indicating that SPR results are consistent with data from a conventional ELISA test.

3) The lowest concentration of antibody (1 ng/mL) in the calibration curve caused a 50 m° angle shift. All samples with angle shifts of 50 m° and higher are considered positive for the viral infection. 61 samples out of 70 tested positive in the SPR assay. In the ELISA test samples with an OD at least 0.3 above the negative controls were considered positive. In this test, 45 out of the same 70 samples tested positive. The SPR assay, therefore, appears to be the more sensitive method for screening for this viral infection.

ELISA is a reliable technique for the detection of viral infections. The SPR test proved to be consistent with the ELISA results. Moreover, the label-free and real-time character of the SPR measurement makes this test not only more sensitive than ELISA (1) but also faster, less elaborate and more cost effective.

This application note is adapted from: *Serodiagnostic comparison of enzyme-linked immunosorbent assay and surface Plasmon resonance for the detection of antibody to Porcine circovirus type 2.* (2006) Ho-Seong Cho, Tae-Jung Kim, Jae-Il Lee, Nam-Yong Park, The Canadian Journal of veterinary research , 70;263-268.

Reference

(1) Campagnolo C, Meyers KJ, Ryan T, Atkinson RC, Chen YT, Scanlan MJ, Ritter G, Old LJ, Batt CA (2004) J Biochem Biophys Methodes 61, 283-298