NOTE *Clinical Pathology*

Serodiagnostic Comparison between Two Methods, ELISA and Surface Plasmon Resonance for the Detection of Antibodies of Classical Swine Fever

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ABSTRACT. A protein chip based on surface plasmon resonance (SPR) was develpoped to measure the antibody (Ab) titers of classical swine fever virus (CSFV) using the recombinant gp55 protein as an antigen. The diagnostic potential of this SPR assay for detecting the Ab titers to CSFV gp55 was compared that of the enzyme-linked immunosorbent assay (ELISA) using 170 serum samples from 14 pig farms. The SPR assay was highly specific and sensitive, and there were no cross-reactions detected. There was a strong positive correlation between the SPR and ELISA titers (n=170, r=0.869, $p<0.01$). Therefore, the SPR label-free method is a valuable tool in the serodiagnosis of CSFV infection and determining Ab titers after vaccination.

KEY WORDS: classical swine fever, protein chip, surface plasmon resonance.

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Classical swine fever (CSF) is a highly contagious and often fatal disease in swine, affecting both the domestic and wild pig population [10]. Classical swine fever virus (CSFV) is the causative agent of CSF, and is a member of the genus *Pestivirus*, which belongs to the family *Flaviviridae* [14].

A specific and sensitive serological test for the detection of antibodies (Abs) to CSFV is needed for the surveillance and diagnosis of CSF [9]. There is need of development of more sensitive and specific assay for the detection of CSFV which can identify the animals infected within herd before it starts shedding of virus. Such test plays significant role in epidemiological and clinical applications [1, 3, 9]. Various methods have been developed to detect Abs against CSFV such as virus neutralization test or antibody (Ab) ELISA [3, 6, 9, 15]. Although these conventional methods are very reliable, they are time- and labor-intensive protocols. Surface plasmon resonance (SPR) is sensitive to changes in the thickness or refractive indices of biomaterials at the interface between a thin gold film and an ambient medium, and can characterize the biomolecular interactions in real time without labeling [11–13]. Briefly, light incident on a metal surface at a fiven angle of incidence can excite a surface-bound electromagnetic wave, a surface plasmon. By the binding of a biomolecule to the surface there will be change in refractive index, which will shift the angle of incidence at which the SPR excitation occurs. This shift in angle is tracked by monitoring the movement of the intensity minima of the reflected light with time using the Kretschmann configuration, and the binding event is presented as a sensorgram [16]. In this study, a protein chip based on SPR for measuring the Ab titers of CSFV in swine sera was developed, and its diagnostic efficacy was compared with that obtained using the conventional ELISA method.

A total of 170 swine serum samples were obtained randomly from both genders (various breeds and ages) at 14 swine farms. The sera were prepared with 1:20 dilutions and used for ELISA and SPR. All samples were analyzed for any reactivity against CSFV antigen by using a commercial ELISA kit (Jeno Biotech Inc., Chuncheon, Korea). The assays were performed according to the manufacturer's instructions. The optical density (OD) of the positive control was ≥ 0.5 and the OD of the negative control was 0.3. To validate the ELISA result, the value of a corrected positive control (CPC) was ≤0.3 (CPC=mean OD of positive control-mean OD of negative control). The ELISA results were analyzed by calculating the sample to positive ratio (S/P ratio) of a sample using the following formula, S/P ratio=(OD of sample-OD of NC)/CPC. Based on the S/P ratio, ≥0.14 was considered positive and <0.14 was considered negative. As the control for SPR assay, bovine viral diarrhea virus monoclonal Ab (Jeno Biotech Inc., Chunchon, Korea), porcine circovirus type 2 monoclonal Ab (Jeno Biotech Inc., Chuncheon, Korea) and normal porcine sera were used in this study.

The surface modification of a gold chip for the specific binding of antigens was used as described elsewhere [8]. Briefly, a patterned glass slide chip with a gold film (K-Mac, Daejeon, Korea) was used to prepare the gold chip. Prior to coating with ProLinker B (Proteogen, Seoul, Korea), the gold-coated glass slides were cleaned in a freshly prepared piranha solution (3:1 mixture of concentrated H_2SO_4 and 30 H_2O_2). After washing with deionized water, the glass slides were dried under a stream of N_2 gas. The gold chip was prepared by soaking a clean gold-coated chip in a 3 mM ProLinker B solution for 1 hr and rinsing it sequentially with $CHCl_3$, acetone, ethanol, and deionized water. The gold chip was inserted into an Autolab ESPRIT SPR system (Eco. Chemie, Uppsala, Netherlands), which

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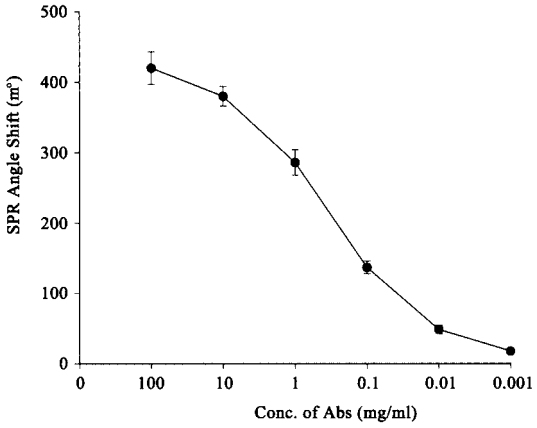

Fig. 1. The changes in the SPR angle shift by binding between the antigen immobilized Prolinker B and various concentrations of antibodies.

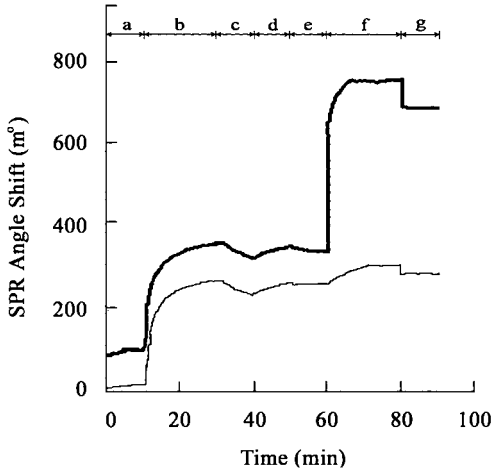

Fig. 2. Sensorgrams showing the immobilization of the antigen (150 *n*g/m*l*) and the subsequent immunoassay of the seurm sample (no.25, × 20, thick line) and 0.1 mg/m*l* bovine serum albumin (BSA) (thin line). (a) washing with 10 mM PBS, (b) gp55 antigen of classical swine fever virus, (c) washing with 10 mM phosphate buffered saline (PBS) (d) 1 mg/m*l* BSA (e) washing with 10 mM PBS (f) sample (g) washing with 10 mM PBS.

was operated at a constant flow rate of 1 μl/min and a temperature of 25°C. The gp55 antigen of CSFV was immobilized on the activated chip surface by injecting a 150 *n*g/m*l* solution of the antigen in a 0.1 M acetate buffer, pH 4.5, for 15 min. After injecting 1% bovine serum albumin (BSA) in phosphate buffered saline (PBS) for 10 min to block the chip surface, the solution containing the Ab or serum sample in the PBS buffer containing 1 mg/m*l* BSA was allowed to flow onto the chip for 15 min. Subsequently, a solution of 1 mM H_2O_2, 1 mM CN in 50 mM Tris buffer, pH 8.0, containing 10% ethylene glycol was injected onto the chip for 10 min. Each step of the immunoassay was monitored using the Autolab SPR system. *P* value <0.01 was considered significant. The detection limit was determined by examining serial dilutions of the monoclonal anti-CSFV (gp55) Ab (Jeno Biotech Inc., Chuncheon, Korea). The concentrations used were 10 fold dilutions ranging from 0.1 mg/m*l* to 1 *n*g/m*l*.

ELISA was used to determine CSFV specific Abs in pig sera. As a result, 170 samples (100%) were positive. The sample to positive ratio (S/P ratio) at least 0.14 above negative controls was considered as positive. The specificity was confirmed by performing SPR assay from monoclonal Abs of bovine viral diarrhea virus and porcine circovirus type 2 (Jeno Biotech Inc. Chuncheon, Korea) and normal porcine sera. The angle shift for SPR assay was not detected.

Figure 1 shows the relationship between the angle shift in the SPR as the Ab concentration. The figure shows that the shift in the SPR angle increased lineary with increasing gp55 Ab titers. The lowest detection limit for the protein chip based on SPR was 10^{-2} μg/m*l* Ab which corresponds to 50 m° (mean=50.7, SD=1.43) was considered positive. All 170 samples (100%) analyzed by SPR tested positive. These results suggest that protein chip based on SPR was 10 times as sensitive as ELISA, which supports a previous report suggesting that the assay protein chip based on SPR is much more sensitive than the standard ELISA [2, 7]. Figure 2 shows a sensorgram of one sample (No. 25). BSA was used as the negative control for the SPR assays, which were complete within 1.5 hr. The Pearson correlation coefficient was used to determine the correlation between the parameters (SPSS Base 12.0). A p-value (≤0.01 was considered significant. CSFV Abs levels in swine sera determined using SPR correlated (n=170, r=0.869, p<0.01) with the levels measured by ELISA (S/P range 0.36–1.13) (Fig. 3).

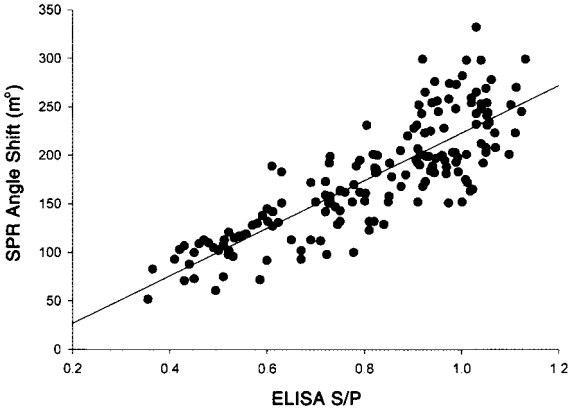

Fig. 3. Correlation between the antibody titers of classical swine fever virus in the swine sera determined by ELISA and SPR (Pearson correlation coefficient n=170, r=0.869, p<0.01).

The SPR assay was used successfully to evaluate CSFV gp55 Ab concentrations in pig sera. The comparative study showed that although ELISA is highy reliable, it is quite

time-consuming. This study found a good correlation between the results obtained from the SPR and the elisa measurements of the Ab levels.

Until now, most studies using the SPR assay detected the antigens or pathogen directly such as the foot and mouth disease virus [5], vaccinia virus, poliovirus, cowpea mosaic virus and tobacco mosaic virus [4] and *Legionella pneumophila* [13]. On the other hand, detection of the Ab using the SPR system has only rarely been carried out except for detecting tumor antigens [2]. Compared with ELISA, the protein chip based on SPR appears to be a fast (total 1.5 hr), valuable tool in the serodiagnosis of CSFV infection or Ab titers after vaccination.

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