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Comparison of Surface-Plasmon-Resonance Biosensor Measurements and Western Blot Assay for Detection of GPE$^-$ Strain of Classical Swine Fever Virus by Using WH211 and WH303 Monoclonal Antibodies

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Abstract

Monoclonal antibodies WH211 and WH303 are in routine laboratory use for detecting Classical Swine Fever Virus (CSFV) or its major envelope E2 protein. While E2 is recognized as the most immunogenic protein, it is also the most variable and often not recognized by specific monoclonal against this protein. The aim of this study was to compare the detection sensitivity of WH211 and WH303 antibodies for CSFV GPE$^-$ strain via two well-known assays, Surface Plasmon Resonance Biosensor and Western Blot Assays.

Both WH211 and WH303 Abs which specifically known to detect E2 gene (gp55) of CSFV at different recognition sites (epitope) were used as ligands to detect the GPE$^-$ strain (analytes). GPE$^-$ strain showed interaction with monoclonal antibodies at highest dilution of 1:1000 (v/v) in SPR assay. At lowest dilution (1:10), the interaction of GPE$^-$ strain with immobilized monoclonal antibody WH211 showed more than two-fold increase (163.5 RU) than the interaction with monoclonal antibody WH303 (60.0 RU). This study documented profound preference for WH211 as the target for CSFV E2 gene by having higher sensitivity towards GPE$^-$ strain but with lesser affinity. E2 epitope of GPE$^-$ strain was found undetectable when blotted with WH303 at the investigated dilutions as compared to WH211. The findings indicated a 2500-fold higher SPR sensitivity in detection in comparison to Western Blot and the limit of detection of GPE$^-$ strain by Western Blot could not be achieved beyond 1:10 dilution of monoclonal antibodies.

Therefore, SPR approach could overcome the risk of GPE$^-$ vaccine strain from being invisible for identification. Although, WH303 was unable to recognize this strain by Western Blot, both WH211 and WH303 were applicable as a sensitive detection ligand for GPE$^-$ strain of CSFV using SPR analysis.

Keywords: Surface-Plasmon-Resonance; Western blot; GPE$^-$ strain; WH211; WH303

Introduction

In comparison of living and non-living organisms, viruses are the most unique. They replicate only in the availability of host. Based on their nano-morphological features, especially in small viruses such as Classical Swine Fever Virus (CSFV) with the size only between 40-60 nm in diameter, their effect on infected host cells could be used for indirect virus detection. CSFV is the causative agent for CSF disease. It leads to substantial economic losses in many parts of the world [1]. It causes acute contagious disease in pigs whereby it is characterized by high fever, multiple hemorrhages, and sometimes it induces chronic or clinically in apparent diseases [2]. CSFV within the Pestivirus genus and of the Flaviviridae family can be distinguished into 2 types of morphological changes in infected cells namely, Cytopathogenic (CP) and Noncytopathogenic (NCP). CP shown through the lysis of cultured cells while NCP did not cause any cell damage [3]. The live attenuated vaccine strain GPE$^-$ which was produced by multiple passages of the virulent CSFV ALD strain in cells of swine, bovine, and guinea pig origin, is NCP [4,5].

In Malaysia, the attenuated GPE$^-$ Japanese strain of CSFV has been used for vaccine preparation to prevent pigs from Classical swine fever disease [6]. Pigs inoculated with the E– strain (designated as GPE$^-$) did not develop such clinical symptoms as anorexia and pyrexia, and confer protective immunity either from CSFV infection or replication [5].

Different techniques of CSFV detections are being applied either at laboratory scale or at clinical level. Current reported methods are for antibody detection such as the Enzyme Linked Immunosorbent Assay (ELISA) [7,8], and immunochromatographic strip [9] used CSFV sera as the analyte. However, they were not solely meant for GPE$^-$ strain, unlike the study mentioned herein. Herein, this paper has no intention to question the efficiency of those commercial kits. The target in this study was Antigen. Unlike immunochromatographic strip and ELISA, SPR has additional features to track intensity of binding in real-time and has potential to detect targeted Antibody or Antigen simultaneously in a single chip depending on the experimental design.

Antigen detection by real-time reverse transcription polymerase chain reaction (real-time RT-PCR) [10] and Loop-Mediated Isothermal Amplification (LAMP) assay [11] can be faster and more sensitive but are limited by a high-risk of cross contamination.

Previously reported real-time RT-PCR assays for discriminating wild-type CSFV from the Riems vaccine-strain have been established in the EU [12,13] and an assay for differentiating between wild type and the K-LOM vaccine-strain CSFV in Korea [14]. In China, a two-step real time RT-PCR assay to distinguish wild-type CSFV from the HCLV-strain vaccine based on nucleotide differences at the probe binding site and a one-step real time RT-PCR assay (wt-rRTPCR) using a Minor Groove Binding (MGB) probe for detection of mutations in wild-types have also been described [15]. However, it is unlikely that a universal detection assay could be used because different CSFV vaccine strains have been administered in different country. Despite all the invaluable tools for CSFV detection, studies related to GPE– strain of CSFV and its monoclonal antibodies namely WH211 and WH303 through Surface Plasmon Resonance (SPR) detection method has not been reported elsewhere.

An alternative and in vivo resemblance of detection approach can be achieved using SPR Biacore system. It allows label-free detection and real-time analysis of antibody-antigen interaction [16-18]. Since 1990s and up to this 21st era, studies using SPR technology as biosensor for the interactions of virus-antibody have covered scope related to viral epitope mapping [19], valency of antibody binding to virus particle [20], isotyping of antibody to virus [21], and affinities determination of antibody to whole virus [22].

As CSFV consists of several structural proteins namely E1, E2 and E^{rns}, in which E2 plays the most significant role as immunogenic determinant, E2 has been the target for this detection study [23]. Zeenathul et al. [1] reported a distinct substitution of two amino acids at positions 94 (R→G) and 97 (P→S) in the deduced E2 protein sequence of GPE$^-$ strain of CSFV. This work was initially established because of previous failure to detect E2 expression of GPE$^-$ strain during downstream research associated to recombinant virus development. This study aimed to observe the interaction between GPE$^-$ strain and the monoclonal antibodies (WH211 and WH303) against its E2 epitope using SPR biosensor detection and Western Blot.

Materials and Methods

Ligand and analyte

GPE$^-$ strain of CSFV (as analyte) was obtained from Malaysian Vaccine Pharmaceutical (MVP) Laboratory Facility (1 mg ml^{-1}). For other downstream application, the CSFV RNA was extracted and the concentration and the purity were determined by previously established method [1]. Two monoclonal antibodies (as ligands), WH211 (RAE0242) and WH303 (RAE0826) had been purchased from Weybridge Veterinary Laboratory Agency, United Kingdom (1 mg ml^{-1}). Both monoclonal antibodies were developed against E2 glycoprotein (envelope) of CSFV.

SPR detection system

SPR analysis was performed using Biacore 3000 (Uppsala, Sweden) and Research Grade Sensor Chip CM5, at an assay temperature of 25°C. HBS-EP buffer (0.010 mol l^{-1} HEPES pH 7.4 containing 0.015 mol l^{-1} NaCl, 0.003 mol l^{-1} EDTA and 0.005% surfactant P20 was used as the running buffer in the experiment. All buffers were filtered prior to use.

Immobilization pH scouting

A series of sodium acetate ranging from pH 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0 and 6.5 were prepared. Both monoclonal antibodies were diluted 1:10 in each solutions and pH scouting test was performed to find the appropriate immobilization buffer.

Immobilization

WH211 and WH303 monoclonal antibodies were diluted at 1:10 (v/v) in 0.010 mol l^{-1} sodium acetate of pH 5.0 and pH 4.0, respectively. It was then covalently coupled to a sensor chip CM5 via primary amine coupling. The procedure for immobilization was followed according to standard protocol. HBS-EP buffer was allowed to run at constant flow rate of 5 uL min^{-1} throughout the study. Then the sensor surface was activated with 1:1 N-hydroxysuccimide (NHS 115 mg ml^{-1}) and N-ethyl-N'-dimethylaminopropyl carbodiimide (EDC 115 mg ml^{-1}) solution. A volume of 100 ng µl^{-1} monoclonal antibody was injected soon after that for about 20 minutes with the flow rate of 10 µl min^{-1}. Flow cell 2 dedicated for immobilization of WH211 whereas WH303 was in the latter flow cell. Finally, 1 mol l^{-1} ethanolamine pH 8.5 of 5 µl min^{-1} was allowed to run for about 7 minutes. This was done to deactivate the excess reactive groups of the carboxylated matrix on the sensor chip.

Binding analysis

After immobilization of antibody, the GPE$^-$strain was injected onto both antibodies in different flow cells. It was diluted at 1:10, 1:100 and 1:1000 (v/v) in HBS-EP buffer which served as transport buffer. The virus was kept overnight in 4°C and centrifuged prior to use to allow complete homogenization. Total interaction time was 60 seconds with the flow rate of 20 µl min^{-1}. To check the sensitivity of the assay, the limit of detection (LOD) for this assay was calculated as below [24]:

LOD = 3 x SD, where SD is the standard deviation of blank sample

Regeneration analysis

The regeneration of the sensor chip surface was performed by injection of Glycine-HCl of various pH or just allowed the virus to dissociate in the running buffer. It was controlled by comparing baseline-resonance units before the test and after the washing procedure. For regeneration, the flow rate was set at 20 µl min^{-1} for 0.5 min^{-1}.

SDS-PAGE and Western blotting

In line with the interaction analysis, SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out by the method described by Laemmli [25], with 4% stacking gels and 12% resolving gels using a Bio-Rad mini gel apparatus. The separated CSFV proteins were either stained with Coomassie blue or analyzed by the use of Western Blots probed with monoclonal antibodies WH303 or WH211. Bound antibodies were detected by using horseradish peroxidase (HRP)-conjugated goat anti-mouse with ABTS peroxidase substrate system (KPL) according to the manufacturer's instructions.

Results

pH scouting for immobilization

Pre-concentration buffers are vital for the ligands to adsorb on the dextran matrix and ensure the immobilization procedure works effectively. As recommended, 0.010 mol l^{-1} sodium acetate is generally practical for all proteins. Both ligands (WH211 and WH303) were individually diluted in 0.010 mol l^{-1} sodium acetate buffers in pH ranged from 3.0 to 6.0. The optimum pH for immobilization of WH211 and WH303 were pH 5.0 and pH 4.0, respectively (Figure 1A and 1B).

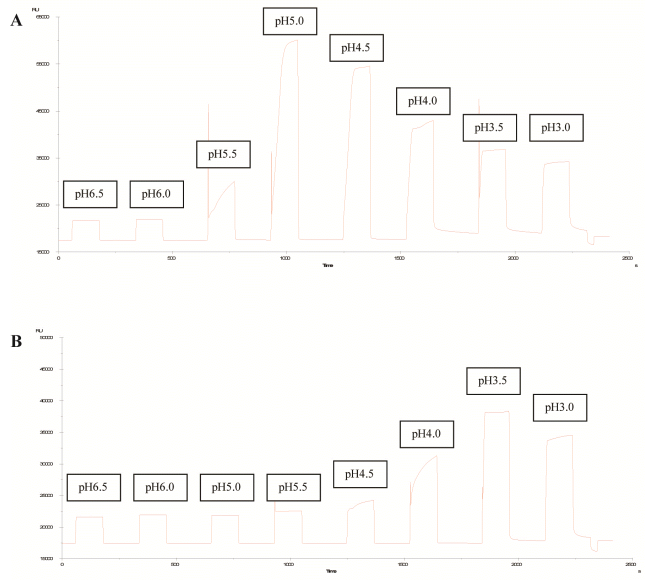

Figure 1: pH scouting for WH211 (A) on flow cell 2 and WH303 (B) on flow cell 3 of CM5 Sensor Chip. pH 5.0 and pH 4.0 were the optimum pH of immobilization buffer for WH211 and WH303 respectively.

At that pH, the respected antibodies showed the highest interaction towards the carboxylated matrix of the sensor chip and reached plateau. This indicates the ideal pre-concentration buffers prior to immobilization.

Ligand immobilization

In the study, direct immobilization was done using the common amine coupling method. Figure 2 showed immobilization of both monoclonal antibodies in flow cell 2 and 3 of the sensor chip. Flow cell 1 served as blank (not shown). Immobilization of WH211 gave 9366 response unit (RU) whereas RU for WH303 was 4276.

Affinity interaction analysis

Affinity interaction analysis between GPE$^-$strain (analyte) and both monoclonal antibodies can be seen in Figure 3, whereby 2 fold higher ligand interactions were found between GPE$^-$-WH 211 as compared to GPE$^-$-WH303. The contact time of analyte tested was very minimal to allow saturation point (plateau) which was absent at the interaction labelled by no. 3 in Figure 3.

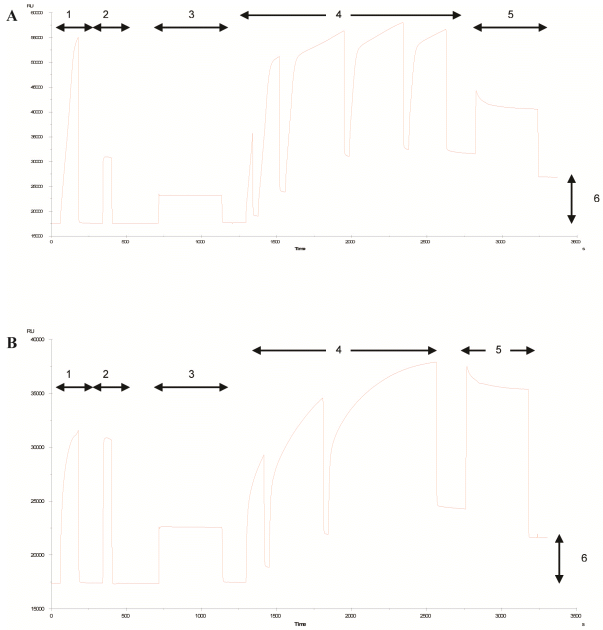

Figure 2: Amount of Ligand Immobilization. Response units of WH211 and WH303 on CM5 sensor chip were indicated by arrow 6 as response unit (RU) of 9366.4 (A) and 4276.1 (B). The steps in immobilization were presented using BIAevaluation software version 4.1. Arrows indicate: (1) target for immobilization level, (2) injection of running buffer, (3) activation of sensor chip's surface with amine coupling method, (4) injection of WH211 or WH303, (5) deactivation of excess reactive surface with ethanolamine, and (6) final amount of immobilized WH211 and WH303.

As expected, response towards WH211 was higher than WH303 when analytes in dilutions of 1:10 (v/v), 1:100 (v/v) and 1:1000 (v/v) ran over each ligand (Figure 4). Based on the formula mentioned in methods, the LOD was 1.5 RU, where the SD for blank was 0.5. Absolute RU estimates obtained from interaction of GPE$^-$ strain with WH211 and WH303 were statistically compared based on independent sample t-test using SAS software (SAS Inc., 2005). Figure 4 simplified the information from Figure 3.

Basically, Figure 4 showed that there was a significant difference between absolute RU estimates obtained from WH211 and WH303 after interaction with GPE$^-$ strain (t-value = 4.39, DF = 15, Pr ≥ t = 0.0001). Statistically it was proven that the RU obtained is independent of the monoclonal antibody immobilization factor. The statistic proved that the RU achieved for each monoclonal antibodies immobilization did not affect the binding analysis results (9366 RU for WH211 whereas 4276 RU for WH303). The GPE$^-$ strain responses to these monoclonal antibodies were analyzed in various dilutions in triplicates (1:10, 1:100 and 1:1000 v/v) (Figure 4) in order to investigate the lowest GPE$^-$ strain dilution for the binding analysis in SPR. At highest dilution (1:1000 v/v), GPE$^-$ strain responded at 3.5 RU on WH211 and 9.8 RU on WH303, respectively. This result showed the sensitivity of the chip for this particular assay.

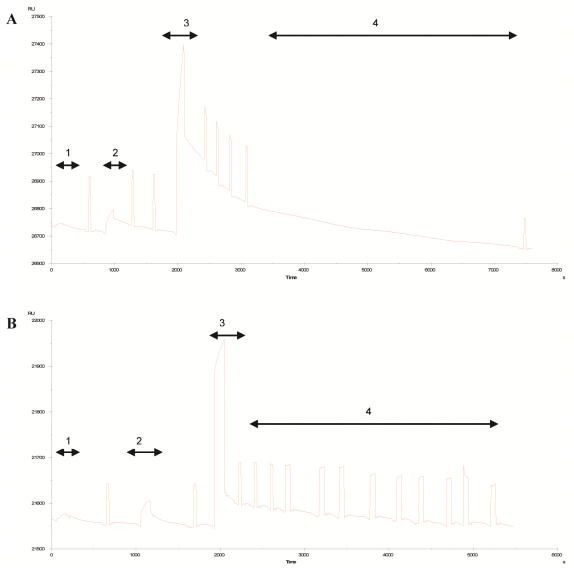

Figure 3: Affinity interaction analysis of GPE⁻ strain with WH211 (A) and WH303 (B). Arrows presented by BIAevaluation software version 4.1 indicated: GPE⁻ strain at dilutions of (1) 1:1000 (v/v) in HBS-EP buffer, (2) 1:100 (v/v) in HBS-EP buffer, (3) 1:10 (v/v) in HBS-EP buffer, and stabilization of (4) WH211 or WH303 coated-sensor surface using running buffer. Regeneration with HBS-EP buffer was performed after each binding event of virus (analyte) onto the antibody (ligand).

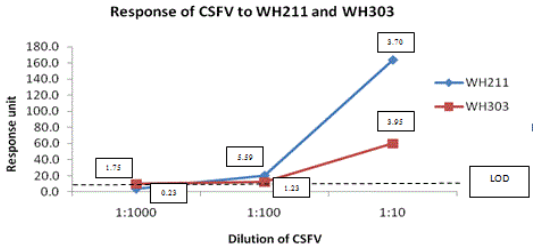

Figure 4: Response of virus in HBS-EP buffer to immobilized ligands. WH211 and WH303 monoclonal antibodies could detect various concentrations of virus [1:10 (v/v), 1:100 (v/v) and 1:1000 (v/v)]. The RUs obtained in the interaction of analyte with WH211 were as follows; 163.5 ± 3.70 RU (1:10), 20.1 ± 5.59 RU (1:100) and 3.5 ± 1.75 RU (1:1000) and towards WH303, were as follows; 60.0 ± 3.95 RU (1:10), 12.2 ± 1.23 RU (1:100) and 9.8 ± 0.23 RU (1:1000). Dotted lines indicate the limit of detection (LOD) of the assay. Standard deviation for the blank is 0.5 RU.

Regeneration analysis

Regeneration is crucial as it washes away the analyte from the immobilized ligand. In the study, the main regeneration buffer was the running buffer itself, which is HBS-EP. Unless facing a difficult condition, the regeneration buffer will be optimized using recommended buffer. Figure 5 showed five regeneration cycles for removing GPE⁻ strain from the immobilized monoclonal antibodies. More than 90% removal of GPE⁻ strain considered good for further analysis. Direct immobilization on CM5 sensor chip normally used low pH of Glycine-HCl for regeneration purpose since the covalent binding of antigen-antibody is easily broken [26]. However, few aspects such as native structure and physicochemical of the ligand remain vital for regeneration purpose [27].

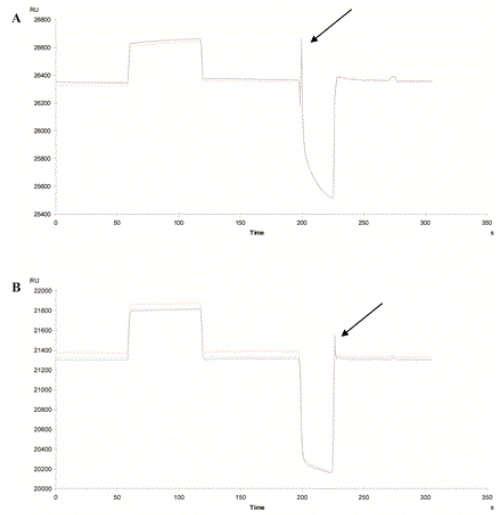

Figure 5: Five successive regeneration cycle. Regeneration using Glycine-HCl pH 2.5 (A) and pH 1.5 (B) to remove the captured virus by immobilized WH211 monoclonal antibody (A) and WH303 monoclonal antibody (B) respectively. Spikes were due to the changes of buffers (indicates with arrows).

SDS Page and Western blot analysis

E2 glycoprotein of GPE⁻ strain was probed with monoclonal antibody WH211 and WH303 at 10 µl (5:1 v/v), 5 µl (2.5:1 v/v), 2 µl (1:1 v/v), 0.2 µl (1:10 v/v), and 0.02 µl (1:100 v/v) through Western Blotting respectively. Western Blot analysis demonstrated the presence of 50 kDa E2 protein (Figure 6). This study considers WH211 as the internal control because the substitution of amino acids mentioned above did not affect WH211 epitope. The changes happened in the targeted epitope of WH303.

Discussion

Theoretically, SPR detection system using monoclonal antibody can be very specific in comparison to whole virus or any viral peptide. The reason is that the antibody's orientation is predictable, whereby either the heavy or the light chain of the antibody will be exposed upwards from the dextran surface allowing virus adsorption on it.

Previously, SPR approach has been used to measure the antibody (Ab) titers of CSFV in pig sera by using recombinant E2 protein (gp55 antigen of CSFV) as the ligand and found to be highly specific and sensitive [20]. They used a self-modified ProteoChip, coated with ProLinker™ in Autolab ESPRIT SPR system. The gp55 antigen of CSFV was immobilized in 0.10 mol l^{-1} acetate buffer of pH 4.5. In contrary,

the present study used monoclonal antibodies as the ligands to capture antigen, the GPE– strain (analyte).

Both WH303 and WH211 are murine derived E2 specific monoclonal antibodies which are directed against epitopes at domain A (N'-terminal) of E2 envelope glycoprotein [28-31]. Serial deletions of this region precisely defined the epitope recognized by WH303 to be TAVSPTTLR (aa 829 to 837) of E2 (GenBank accession number X87939) [32]. Comparison of the sequences around the WH303-binding site among the E2 proteins of pestiviruses indicated that this motif is strongly conserved in CSFV strains but divergent among BVDV and BDV strains [32]. Of all the available CSFV strains, only GPE$^-$ vaccine strain has a modified TAV epitope with the amino acid sequences TTVSPTTLR (GenBank accession number D49533) [33]. These results provided a structural basis for the reactivity patterns of WH303 and also useful information for the design of a peptide containing this epitope for potential use in the detection and identification of CSFV. This linear epitope was also confirmed by Zhang et al. [34] using a phage display peptide library. Knowledge about the monoclonal antibody WH303 epitope has facilitated the identification of a novel virulence determinant within E2 [35] and has also led to the exploration of a WH303 epitope-based vaccine [36]. The SPR approach showed reactivity with both monoclonal antibodies, however, Western Blot managed to detect WH211 only. This phenomenon explains the existence of TTVSPTTLR epitope bears by the GPE$^-$ vaccine strain, which limits the binding of WH303 to the GPE– vaccine strain in the Western Blot.

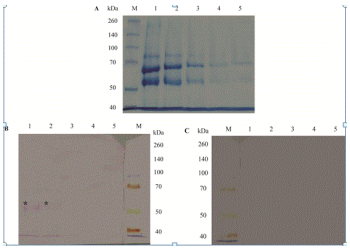

Figure 6: SDS-PAGE and Western Blot Analysis of E2 glycoprotein. Protein samples from GPE$^-$ strain were separated using SDS-PAGE and stained with Coomasie Blue (A). The dilutions of proteins tested were as follows: Lane 1-5: 10 ul (5:1 v/v), 5 ul (2.5:1 v/v), 2 ul (1:1 v/v), 0.2 ul (1:10 v/v), and 0.02 ul (1:100 v/v). E2 glycoproteins were probed with monoclonal antibody WH211 (B) and WH303 (C) through Western Blotting respectively. Western Blot analysis demonstrated the presence of bands with apparent molecular masses of 50 kDa, corresponding to E2 monomers (Weiland et al.), when probed with WH211 monoclonal antibody. No band was found when probed with WH303 monoclonal antibody.

In analytical chemistry, an often-used definition of detection limit is three times the standard deviation of the background (blank) noise [37]. The LOD is a general understanding to estimate the lowest analyte concentration that can be detected but not necessarily quantitated as an exact value in the assay. There is no standard or specific way to define specificity; however, the effort to make them meaningful to each other may help to determine sensitivity according to the purpose of the study and the instrument used. Using Influenza virus A as a model, Wang et al. [38] define detection limits into two parts (mass detection and mass detection limit per unit area) in Kretschmann configuration-based SPR microscopy by which they found the former is 1 ag and the latter is approximately 0.2 fg mm^{-2} [38]. Uttenthaler et al. [39] recorded the detection limits for African swine fever virus protein detection in quartz crystal microbalance sensor were 0.31 and 1 mg ml^{-1} in PBS and serum, respectively [39]. In this study, the LOD for GPE$^-$ vaccine strain was 1.5 RU. Hence, all the readings beyond this value were regarded as null and omit automatically.

In overall, although WH211- GPE$^-$ strain interaction gave higher response compared to that of WH303-GPE$^-$, the latter complexes showed higher affinity. Figure 3B demonstrated a long regeneration time for disrupting WH303- GPE$^-$strain interaction. Although the interaction of WH211-GPE$^-$ complex gave an apparently higher response compared to that of WH303-GPE$^-$, the latter complexes showed stronger bonding. The same phenomenon was also observed by Wang et al. [38], that the individual influenza viral particles tended to stay on the anti-influenza antibody surface for much longer time than on the PEG-coated surface, and eventually leave the surface attributed to the reversible binding event. The long regeneration time for disrupting WH303- GPE$^-$ interaction justified the affinity. Simultaneous GPE$^-$ strain detection using monoclonal antibody WH211 by Western Blot analysis required a minimal of 5 µl (2.5:1 v/v). In contrast, SPR detection only required 0.002 µl (1:1000 v/v), which is 2500 fold higher than Western Blot assay. To this extent, E2 epitope which was undetectable when probed with WH303 at the investigated dilutions by Western Blot can be readily detected by SPR even at 1:1000 v/v dilutions. Basically the workload and run time for both assays has a large loop of differences. The Biacore SPR system finished the entire works for approximately 2 hours whereas Western Blot required a one day job (including the SDS-PAGE works).

Each analysis has its own strength and applications. Therefore, there is no one single analysis that can serve all purpose. The potential applications of SPR method to distinguish the infected animals from vaccinated animals (Differentiation of Infection from Vaccination) (DIVA) can be achieved by investigating the binding strength and kinetics of associated antibodies with CSFV strains or vaccine strains used in particular region. For example, if the strong binding happens between WH303 and CSFV sample, it means the strain is either wild type or unlikely GPE strain. Nonetheless, if the strong binding occurs towards WH211, it means it is suggesting the GPE$^-$ vaccine strain. Other downstream assay such as High Resolution Melting (HRM) analysis which is a high throughput post-PCR analysis can support for screening genetic variance in vaccine and wild-type strains.

In summary, WH211 was preferentially recommended for E2-based monoclonal antibody screening of GPE$^-$ strain due to its sensitivity and specificity in SPR assay.

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References

1. Zeenathul NA, Mohd-Azmi ML, Sheik Omar AR, Bahaman AR, Aini I, et al. (2009) Expression of E2 gene of CSFV and analysis of epitope diversity. J Vet Malaysia 21-25.
2. Greiser-Wilke I, Blome S, Moennig V (2007) Diagnostic methods for detection of Classical swine fever virus--status quo and new developments. Vaccine 25: 5524-5530.

3. Xu H, Hong HX, Zhang YM, Guo KK, Deng XM, et al. (2007) Cytopathic effect of classical swine fever virus NS3 protein on PK-15 cells. Intervirology 50: 433-438.
4. Aoki H, Sakoda Y, Nakamura S, Suzuki S, Fukusho A (2004) Cytopathogenicity of classical swine fever viruses that do not show the exaltation of Newcastle disease virus is associated with accumulation of NS3 in serum-free cultured cell lines. J Vet Med Sci 66: 161-167.
5. Tamura T, Sakoda Y, Yoshino F, Nomura T, Yamamoto N, et al. (2012) Selection of classical swine fever virus with enhanced pathogenicity reveals synergistic virulence determinants in E2 and NS4B. J Virol 86: 8602-8613.
6. Zeenathul NA (2004) Development of gene deleted recombinant Pseudorabies Virus expressing CSFV E2 protein. PhD dissertation, Universiti Putra Malaysia.
7. Batonick M, Loeffen W, Metwally SA, Mayr GA (2013) Identification and characterization of mimotopes of classical swine fever virus E2 glycoprotein using specific anti-E2 monoclonal antibodies. Virus Res 175: 12-19.
8. Li W, Mao L, Yanga L, Zhoub B, Jiang J (2013) Development and partial validation of a recombinant E2-based indirect ELISA for detection of specific IgM antibody responses against classical swine fever virus. J Virol Methods 191: 63– 68.
9. Ren H, Zhou S, Wen J, Zhan X, Liu W et al. (2014) Development of a One-Step Immunochromatographic Strip Test for Rapid Detection of Antibodies Against Classic Swine Fever. Frontier and Future Development of Information Technology in Medicine and Education, Lecture Notes in Electrical Engineering 269: 81-92.
10. Podgórska K, Kamieniecka K, Stadejek T, Pejsak Z (2012) Comparison of PCR methods for detection of classical swine fever virus and other pestiviruses. Pol J Vet Sci 15: 615-620.
11. Chowdry VK, Luo Y, Widen F, Qiu HJ, Shan H, et al. (2014) Development of a loop-mediated isothermal amplification assay combined with a lateral flow dipstick for rapid and simple detection of classical swine fever virus in the field. J Virol Methods 197: 14– 18.
12. Leifer I, Depner K, Blome S, Le Potier MF, Le Dimna M, et al. (2009) Differentiation of C-strain "Riems" or CP7_E2alf vaccinated animals from animals infected by classical swine fever virus field strains using real-time RT-PCR. J Virol Methods 158: 114-122.
13. Leifer I, Everett H, Hoffman B, Sosan O, Crooke H, et al. (2010) Escape of classical swine fever C-strain vaccine virus from detection by C-strain specific real-time RT-PCR caused by a point mutation in the primer-binding site. J Virol Methods 166: 98-100.
14. Cho HS, Park SJ, Park NY (2006a) Development of a reverse-transcription polymerase chain reaction assay with fluorogenic probes to discriminate Korean wild-type and vaccine isolates of Classical swine fever virus. Can J Vet Res. 70: 226–229.
15. Wen G, Zhang T, Yang J, Luo Q, Liao Y, et al. (2011) Evaluation of a real-time RT-PCR assay using minor groove binding probe for specific detection of Chinese wild-type classical swine fever virus. J Virol Methods 176: 96-102.
16. Jönsson U, Fägerstam L, Löfas S, Stenberg E, Karlsson R, et al. (1993) Introducing a biosensor based technology for real-time biospecific interaction analysis. Ann Biol Clin (Paris) 51: 19-26.
17. Karlsson R, Michaelsson A, Mattsson L (1991) Kinetic analysis of monoclonal antibody-antigen interactions with a new biosensor based analytical system. J Immunol Methods 145: 229-240.
18. Malmqvist M (1993) Biospecific interaction analysis using biosensor technology. Nature 361: 186-187.
19. Dubs MC, Altschuh D, Van Regenmortel MH (1992) Interaction between viruses and monoclonal antibodies studied by surface plasmon resonance. Immunol Lett 31: 59-64.
20. Cho HS, Park NY (2006b) Serodiagnostic comparison between two methods, ELISA and surface plasmon resonance for the detection of antibodies of classical swine fever. J Vet Med Sci 68: 1327-1329.
21. Abad LW, Neumann M, Tobias L, Obenauer-Kutner L, Jacobs S, et al. (2002) Development of a biosensor-based method for detection and isotyping of antibody responses to adenoviral-based gene therapy vectors. Anal Biochem 310: 107-113.
22. Dubs MC, Altschuh D, Van Regenmortel MH (1992) Mapping of viral epitopes with conformationally specific monoclonal antibodies using biosensor technology. J Chromatogr 597: 391-396.
23. Risatti GR, Callahan JD, Nelson WM, Borca MV (2003) Rapid detection of classical swine fever virus by a portable real-time reverse transcriptase PCR assay. J Clin Microbiol 41: 500-505.
24. Biacore AB (2001) Biacore® Concentration Analysis Handbook, Version AA. Biacore, AB, Uppsala, Sweden.
25. Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680-685.
26. Hardy SA, Dimmock NJ (2003) Valency of antibody binding to enveloped virus particles as determined by surface plasmon resonance. J Virol 77: 1649-1652.
27. Honari P, Allaudin ZN, Mohd Lila MA, Mustafa NH (2011) An approach towards optimal usage of immobilized sensor chips in Surface Plasmon Resonance based biosensor. African Journal of Biotechnology 10: 15795-15800.
28. van Rijn PA, van Gennip RG, de Meijer EJ, Moormann RJ (1992) A preliminary map of epitopes on envelope glycoprotein E1 of HCV strain Brescia. Vet Microbiol 33: 221-230.
29. van Rijn PA, van Gennip HG, de Meijer EJ, Moormann RJ (1993) Epitope mapping of envelope glycoprotein E1 of hog cholera virus strain Brescia. J Gen Virol 74 : 2053-2060.
30. van Rijn PA, Miedema GK, Wensvoort G, van Gennip HG, Moormann RJ (1994) Antigenic structure of envelope glycoprotein E1 of hog cholera virus. J Virol 68: 3934-3942.
31. Wensvoort G (1989) Topographical and functional mapping of epitopes on hog cholera virus with monoclonal antibodies. J Gen Virol 70 : 2865-2876.
32. Lin M, Lin F, Mallory M, Clavijo A (2000) Deletions of structural glycoprotein E2 of classical swine fever virus strain alfort/187 resolve a linear epitope of monoclonal antibody WH303 and the minimal N-terminal domain essential for binding immunoglobulin G antibodies of a pig hyperimmune serum. J Virol 74: 11619–11625.
33. Leifer I, Blome S, Blohm U, König P, Küster H, et al. (2012) Characterization of C-strain "Riems" TAV-epitope escape variants obtained through selective antibody pressure in cell culture. Vet Res 43: 33.
34. Zhang F, Yu M, Weiland E, Morrissy C, Zhang N, et al. (2006) Characterization of epitopes for neutralizing monoclonal antibodies to classical swine fever virus E2 and Erns using phage-displayed random peptide library. Arch Virol 151: 37-54.
35. Risatti GR, Holinka LG, Carrillo C, Kutish GF, Lu Z, et al. (2006) Identification of a novel virulence determinant within the E2 structural glycoprotein of classical swine fever virus. Virology 355: 94-101.
36. Liu S, Tu C, Wang C, Yu X, Wu J, et al. (2006) The protective immune response induced by B cell epitope of classical swine fever virus glycoprotein E2. J Virol Methods 134: 125-129.
37. Skoog DA, Holler FJ, Crouch SR (2007) Principles of Instrumental Analysis. 6th ed. Thomson Higher Education, Belmont, CA.
38. Wang S, Shan X, Patel U, Huang X, Lu J, et al. (2010) Label-free imaging, detection, and mass measurement of single viruses by surface plasmon resonance. Proc Natl Acad Sci U S A 107: 16028-16032.
39. Uttenthaler E, Koblinger C, Drost S (1998) Characterization of immobilization methods for African swine fever virus protein and antibodies with a piezoelectric immunosensor. Biosens Bioelectron 13: 1279–1286.