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Original article

Surface plasmon resonance analysis for detecting non-structural protein 1 of dengue virus in Indonesia

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ABSTRACT

Dengue is an acute febrile disease caused by dengue virus (DENV) that is transmitted by *Aedes* sp., which causes serious health conditions in many countries. Non-structural protein 1 (NS1) is a co-factor for the RNA replication of this virus, which represents a new strategy for the identification of dengue. Prompt and accurate laboratory diagnosis of this infection is required to assist in patient triage and management, as well as prevent the spread of this infection. In the present study, we tested the potential of surface plasmon resonance (SPR) as a diagnostic tool for dengue infections. NS1 antigen protein was used as an analyte that targets anti-NS1 antibodies, with their interaction resulting in a change in the refractive index. In comparison to currently available gold-standard detection methods [enzyme-linked immunosorbent assay (ELISA) and reverse transcription polymerase chain reaction (RT-PCR)], SPR showed a similar sensitivity but greater efficiency and simplicity in terms of infection detection. Out of 26 samples collected from patients with dengue in Indonesia, SPR was able to correctly identify all 16 positively infected individuals at a lower concentration and a shorter period of time compared to ELISA and RT-PCR. This study revealed that SPR is a promising tool for DENV detection and potentially other diseases as well.

1. Introduction

Dengue is an acute febrile disease that is caused by the transmission of dengue virus (DENV), which depends on the vector mosquito *Aedes* sp. (Kalayanarooj, 2011). This virus has four serotypes (DENV-1, DENV-2, DENV-3 and DENV-4) that can cause dengue fever (DF), dengue haemorrhagic fever and dengue shock syndrome (DSS) (Bäck and Lundkvist, 2013). This disease causes up to 10,000 mortalities annually, with 100 million infections across 125 countries (Stanaway et al., 2016; Messina et al., 2014). Although many novel therapeutics are still under development, its intensity of transmission has increased because of the lack of commercial anti-virals (Paranavitane et al., 2014). Although dengue is mostly found in tropical countries, the dramatic increase in temperature due to global warming may lead to its spread into sub-tropical regions, longer dengue transmission seasons, faster viral amplification rate, longer vector survival and higher mosquito biting rates (Kraemer et al., 2015; Messina et al., 2019; Gubler, 2011; Murray et al., 2013). Currently, half the world's population live in areas suitable for dengue infection (Bhatt et al., 2013; Brady et al., 2012). Recently, it has been reported that Indonesia has the highest infection rate in Southeast Asia and the

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second-highest infection rate in the world after Brazil (Wahyono et al., 2017). Moreover, by 2080, over 6.1 billion people are predicted to be at risk for DENV transmission (Messina et al., 2019). Therefore, advances in its treatment and detection are needed to diminish the risks.

DENV, a positive single-stranded RNA virus, encodes three structural proteins that are part of the mature virus particle [capsid (C), envelope (E) and precursor membrane (PrM)], as well as seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) that are expressed specifically in infected cells (Chuang et al., 2016). Non-structural protein 1 (NS1), a well-known co-factor for the RNA replication of this virus, is detected in blood one day after the onset of fever until day 9 (Alcon et al., 2002; Muller and Young, 2013). NS1 has been known to have a cross-reactive, auto-antibody mechanism, as it has the capability for molecular mimicry with human coagulator molecules (Lin et al., 2011). NS1 can induce the release of cytokines and directly takes part in vascular leakage by binding with TLR4 and endothelial glycocalyx (Modhiran et al., 2015; Glasner et al., 2017). These phenomena, vascular leakage and endothelial dysfunction, occur because of endothelial cell apoptosis, which is induced by cross-reactive antibodies (Lin et al., 2003). At a further stage, both phenomena may cause platelet function disruption, fibrin formation interruption, endothelial cell damage, coagulation defects and macrophage activation (Lin et al., 2011; Young et al., 2000).

The diagnosis of DENV infections occurs via viral RNA detection or NS1 antigen detection at the early stages of infection, or through IgM or IgG antibody detection in the latter stages of infection (after day 7), as the clinical symptoms of infected patients are similar to those of many other diseases (Paranavitane et al., 2014; Parikh et al., 2012). The methods of detection of DENV infections include enzyme-linked immunosorbent assay (ELISA), lateral flow and reverse transcription polymerase chain reaction (RT-PCR) (Lai et al., 2019), with ELISA and lateral flow being the most commonly used ones (Peeling et al., 2010; Muller et al., 2017). However, antibody detection often yields unreliable results in cases in which the patients have been infected previously with DENV, and NS1 has a low detection sensitivity with currently available commercial kits. In general, ELISA requires an intensive laboratory setup, as well as trained personnel, and is time-consuming, whereas lateral flow has a lower specificity but is simpler (Lai et al., 2019). On the other hand, RT-PCR is superior in terms of its capacity to identify serotypes and is highly sensitive. However, as with ELISA, RT-PCR requires advanced technology, along with adequate skill (Ahmed and Broor, 2014).

In order to overcome the limitations of the current methods in detecting DENV, NS1 can be considered as a promising marker of viral infection, because it can be observed at the onset of illness and its levels are associated with the severity of disease. Essentially, NS1 antigen detection refers to the binding affinity between the NS1 antigen protein and the anti-NS1 antibody protein (Paranavitane et al., 2014; Libraty et al., 2002). Antibodies have been used widely to detect NS1 in plasma samples using ELISA, but their interaction with the antigen has not yet been described precisely. Therefore, surface plasmon resonance (SPR) was used, as it is the most reliable biosensor for measuring molecular interactions (Patching, 2014). SPR is a sensitive, specific, label-free technique that can measure binding affinities in real time on the basis of kinetic constants and thermodynamic parameters (Kumbhat et al., 2010). Furthermore, this method has successfully identified the kinetics of biological molecules, including protein binding, antibody–ligand binding, various foods, lipid raft formation and drugs (Fu et al., 2019; Zhang et al., 2019; Visentin et al., 2018; Bahri et al., 2019; Ryu et al., 2019; Mowla et al., 2018).

Previously, we showed that SPR can be utilised for detecting kinetic constants and thermodynamic models of NS1 and anti-NS1 (unpublished). In this study, we develop a new diagnostic tool for the detection of dengue virus from patient plasma. Despite commercially available kits that use lateral flow technology and the potential for ELISA or RT-PCR as a gold standard for detection, these approaches all suffer from issues related to sensitivity, efficiency, and/or require skilled personnel, which limit their value to rapidly detect virus infection. In purpose to delay treatment and impacts the rate of spread of infection, hence, SPR is used to detect the NS1, which is a cofactor for the RNA replication of dengue virus. The NS1 antigen protein was used as an analyte that targeted anti-NS1 antibody, with their interaction resulting in a change of refractive index. By comparing to the available gold-standard detection methods, SPR showed similar sensitivity, but greater efficiency and simplicity in infection detection. Therefore, this study revealed that SPR is a promising tool for DENV detection and potentially other diseases as well.

2. Materials and methods

2.1. Non-structural protein 1 (NS1) and Anti-NS1

The anti-NS1 monoclonal antibody was a type 2 DENV (HM 388) that was more than 90% pure (EastCoast Bio, Inc., North Berwick, ME, USA) and could recognize protein NS1 antigen in four DENV serotypes based on preliminary research that utilized ELISA method and samples from four serotypes. The plasma samples used in this study were collected from stored biological material from 26 patients that belonged to the Health Research and Development Agency under the Ministry of Health of the Republic of Indonesia and was taken in 2015–2016 with a total of 26 samples of acute patients on first day of the arrival at the hospital. The confirmation of a positive DENV infection was performed using RT-PCR Lanciotti and NS1 ELISA detection kit Focus Diagnostics Dengue Virus IgG Capture DxSelect™ USA. Ethical approval to conduct this research was granted by the National Institute of Health Research and Development, Ministry of Health, Indonesia (KE.01.05/EC/407/2012).

2.2. Antibody anti – NS1 immobilisation

After a CMD3 (carboxyl methildextrand, 3D; Xantec Bioanalytic, Bionavis Ltd., Tampere region, Finland) sensor chip was installed on the SPR instrument (MP-SPR or Multi-Parametric Surface Plasmon resonance Navi™ 220A NAALI, Bionavis Ltd, Finland), both channels were rinsed with a mixed solution of 2 M NaCl and 0.01 M NaOH for 7 min. Subsequently, the chip surface was activated with a solution of 0.05 M NHS and 0.2 M EDC, which was injected into both channels for 7 min. Immobilisation of NS1 began by mixing NS1 with a 5 mM (pH 5) MES buffer solution at an NS/MES ratio of 1:100, which was then injected into Channel 1 for 7 min. This step was repeated three times. The reference channel, Channel 2, was injected without NS1. The vacant parts of the sensor that were not immobilized with antibody anti NS1 were blocked using 1% BSA solution dissolved in 500 uL of acetic acid solution (pH 4.2) for 5 min and post wait for 1 min. A 1 M solution of ethanolamine (pH 8; Sigma-Aldrich, St. Louis, MO, USA) was used to deactivate both channels for 5 min, and 50 mM NaOH was used to rinse both channels after deactivation for 1 min twice. All injection processes were performed at a flow rate of 30 μL/min except for immobilization process that was carried out at flow rate of 20uL/min.

2.3. SPR analysis

The detection of NS1 antigen was performed in two sequential stages. The first stage was to find out the response signal generated

when bonding occurred between antibodies anti NS1 that were immobilized on sensor surface with NS1 antigen as the analyte at concentration of 800 nM, 80 nM, 8 nM, 0.8 nM, dan 0.08 nM. This serial dilution was then used as a standard curve to figure out the concentration of NS1 antigen in clinical plasma. The analyte used in this stage was standardized NS1 antigen (Abcam) that was dissolved into blood plasma that has been confirmed as a negative DENV using Dengue NS1 ELISA or RT-PCR method.

The second stage involved the detection of NS1 antigens in clinical patient plasma samples. The detection of the NS1 antigen protein inside the plasma sample started by adding 5 μL of the sample to 495 μL of Hepes-buffered saline Tween (HBST) running buffer (pH 7.4; BioNavis Ltd., Tampere, Finland). The solution was then homogenised using a sonicator before flowing at a rate of 30 μL/min, with a 3 min pre-injection followed by injection and waiting for 10 min. In order to disrupt the bonds between the ligand and the analyte, a 10 mM glycine solution (pH 2; Sigma-Aldrich) was used, and this procedure was repeated twice. Contaminants were washed by flowing 500 μL of 0.05 M NaOH (Sigma-Aldrich) at a rate of 30 μL/min, 1 min injection and 1 min wait. The data obtained were extracted using the SPR Navi™ DataViewer software, whereas the sensitivity, specificity and cut-off point values were analysed using bivariate statistics in addition to the interval score from Wilson's method.

2.4. ELISA analysis

ELISA was performed according to the kit instructions for DENV detection through IgG and IgM antibody detection and NS1 detection (Focus Diagnostics Dengue Virus IgG Capture DxSelect™, Focus Diagnostics Dengue Virus IgM Capture DxSelect™ and Focus Diagnostics Dengue NS1 Antigen DxSelect™; DiaSorin Molecular LLC, Cypress, CA, USA).

2.4.1. IgG/IgM antibody ELISA analysis.

The ELISA well plate of each of the diagnosis kits was covered by inactivated original DENV types 1–4. 100 μL of the plasma sample diluted at 1:100 was added to each well, and the plates were incubated at room temperature (RT) for 1 h to cause an interaction between the anti-DENV antibody in the plasma sample and the DENV antigen. The antigen suspension was then discarded, and the wells were filled with 300 μL of wash buffer in an ELISA washing machine. The plates were then incubated for 5 min and washed three times. Subsequently, 100 μL of horseradish-peroxidase-conju gated anti-human IgG/IgM was added to the wells and incubated for 30 min at RT. This procedure was repeated three times, followed by the addition of 100 μL of enzyme substrate and the chromogen available in the kit and incubation for 10 min at RT until the colour changed. The reaction was stopped, and colour changes were quantified spectrophotometrically on the basis of the optical density (OD) at 450 nm (Bio-Rad Model 550; Bio-Rad Laboratories, Hercules, CA, USA). The positive and negative controls were available in the kit. Positive reactivity was determined if the OD value was greater than or equal to 1. However, if the OD value was between 1.1 and 0.9, the procedure was repeated.

2.4.2. NS1 ELISA analysis

The wells were covered with anti-NS1 antibodies. Each 100 μL aliquot of the sample mixture comprised 50 μL of a diluent containing the secondary antibody and 50 μL of the sample (or control). The plates were incubated at RT for 1 h to cause an interaction between the anti-NS1 antibody and the NS1 antigen protein, which also interacted with the secondary antibody in the sample diluent. The antigen suspension was discarded, and the wells were filled with 300 μL of wash buffer in an ELISA washing machine. The plates were incubated for 5 min and washed six times, and 100 μL of the conjugate was added to the wells and incubated for 30 min at RT. This was then followed by six washes with 300 mL of wash buffer, with 5 min inoculation between each wash. A 100 μL aliquot of the TMB liquid substrate available in the kit was added, and the plates were incubated for 20 min at RT until the colour changed. The reaction was stopped, and the availability of the NS1 antigen was confirmed spectrophotometrically on the basis of the OD at 450 nm (Bio-Rad Model 550; Bio-Rad Laboratories). The positive and negative controls used were available in the kit, and the results were determined as above.

2.5. RT-PCR analysis

The methods described here followed those described by Lanciotti, Calisher, Gubler, Chang and Vorndam (1992). First, an RNA DENV target extracted from blood plasma of DENV suspected patients (QIAamp Viral RNA Mini Kit, Germany) was converted into a DNA copy (cDNA) with reverse transcriptase. The RT-PCR mixture reagent (5 μL of 5X master mix, 2.5 μL of 10 μM Den Gr F primer, 2.5 μL of 10 μM Den Gr R primer, 1 μL of 10 μM dNTP, 10 pmol of enzyme and 8 μL of nuclease-free water) was mixed under laminar flow before being placed in a biosafety cabinet to allow the addition of 5 μL of the RNA sample, positive control and negative control. The mixture was then placed in a ProFlex™ PCR System machine (Thermo Fisher Scientific, Waltham, MA, USA) for reverse transcription under the following conditions: 30 min at 50 °C, 2 min at 94 °C, 15 s at 94 °C, 30 s at 60 °C, 31 s at 72 °C and 10 min at 72 °C, followed by a hold at 4 °C. The product was then dissolved in nuclease-free water at a ratio of 1 : 50 and then inserted into the PCR machine once more with a different reagent (12.5 μL of 2× GoTaq Green Master Mix, 1.5 μL of 10 μM Den Gr F primer, 1.5 μL of 10 μM R-TS1, 1.5 μL of 10 μM R-TS2, 1.5 μL of 10 μM R-TS3 and 1.5 μL of 10 μM R-TS4), followed by the addition of 5 μL of the dissolved PCR product for the last run (2 min at 94 °C, 1 min at 94 °C, 1 min at 65 °C, 29 s at 72 °C and 10 min at 72 °C, with an infinite hold at 4 °C).

2.6. Lateral flow assay analysis

The lateral flow assay analysis was conducted according to the instructions on the kit. A 100 μL aliquot of plasma was applied at one end of the strip on the sample pad. The sample then migrated through the conjugate release pad, which contained anti-NS1 bound to the protein target, and continued to migrate along the strip to the detection zone. The results were represented by lines appearing with various intensities, which were visible within 15 min. The read-out was assessed by the eye: two lines (control line + detection line) indicated positive results and a single line (without the detection line) represented negative results.

2.7. Management and data analysis

The obtained SPR data from serial dilutions of NS1 antigen which was dissolved in DENV negative plasma and clinical samples were extracted using SPR NaviTM Data Viewer software. The result of extracted data from serial dilutions were subsequently analysed to acquire affinity value using SPR NaviTM Trace DrawerTM (TD) software whilst the results of extracted data from clinical sample were further analysed to gain a cut-off point by SPPS analysis. The estimated NS1 antigen concentration values in clinical samples were obtained based on serial dilution curves and sensorgram graph for each clinical sample were analysed using the SPR NaviTM Trace DrawerTM (TD) software.

3. Results

3.1. Fast detection of small amounts NS1 protein by SPR

Two phases generated in the whole detection process of SPR over time: association phase and dissociation phase (Fig. 1(a)). The association phase occurred when the interaction between the NS1 protein and the anti-NS1 antibody protein (as a ligand) started to occur as the sample flowed over the sensor's surface. This can be seen between 5 s and 600 s (injection phase). This phase induced an increase in the refractive index to almost 9×10^{-3} degrees of the maximum for the highest concentration, reaching a minimum at around 5×10^{-4} degrees for the lowest one. The dissociation phase occurred when the sample was no longer flowing through the sensor but the HBST buffer solution was, which led to some non-specific interactions occurring with the sensor or any residues of NS1 protein washed out with the buffer. This phase can be seen from 600 s to 1200 s, at which the signal declined dramatically to as much as 2×10^{-3} degrees maximum for an 800 nM sample. Thus, the overall time required to detect the NS1 protein by SPR is approximately 600 s.

There was a gradual increase of NS1 detection using SPR and ELISA (Fig. 1(b) and (c)), indicating that higher concentrations result in greater detection of the NS1 protein. Although both techniques are capable of identifying the NS1 protein in a sample, SPR appears to have a higher sensitivity, as it can detect NS1 at concentrations as low as 0.8 nM (Fig. 1(b)). However, ELISA was only able to detect NS1 at concentrations greater than 8 nM (Fig. 1(c)).

3.2. Detection of NS1 by SPR, lateral flow, ELISA and RT-PCR

ELISA and RT-PCR have both been used as the gold standard to declare that patients were confirmed positive for DENV. The ELISA NS-1 test and lateral NS-1 were performed to confirm the existence of NS-1 antigen in patients as research subjects. On the other hand, IgM and IgG detection were carried out to achieve data related to the type of infection in the subject. Therefore, the results of both methods were compared with those obtained by SPR. Out of the 26 samples of patient plasma, 16 were positively infected by DENV, whereas the rest were uninfected (Table 1). Based on the ELISA test result of IgM and IgG, subject was divided into primer infection and secondary infection as much as 3(18.75%) and 13(81.25%) respectively (Table 1). As another commercially available option for NS1 detection, we used lateral flow; however, this method detected only 15 positive cases rather than the 16 detected by ELISA and RT-PCR. Thus, NS1 appears to be the most reliable marker for detecting dengue infections.

In order to determine the effects of SPR in detecting dengue infections, the NS1 protein was identified in SPR with an immobilised anti-NS1 antibody. The results showed that all 16 positive samples were able to change the sensorgrams' signal by a minimum of 0.00113°, whereas the normal samples were more than 0.00075° less. This indicated that dengue-infected samples generate sensorgrams signals with more than 0.001°. While SPSS analysis obtained sensorgrams signal cut off value of 0.0008. The sensitivity and specificity value of SPR method to detect NS-1 antigen in clinical sample compared to the gold standard of ELISA and RT-PCR were 100% (80.64–100%; 95% CI) and 100% (72.5–100%; 95% CI) respectively (Table 2). Besides detecting dengue infections, the concentration of NS1 can also be predicted using SPR-based methods. Table 1 shows that dengue-infected samples can be detected at a minimum NS1 concentration of 0.301 nM, with a signal of 0.00113°. These results demonstrate that SPR is a highly sensitive biosensor for dengue infections, with a precise detection ability that is comparable to that of ELISA and RT-PCR and better than that of lateral flow.

3.3. SPR offers rapid NS1 detection with less lab work

Fig. 2 compares the total time required to identify NS1 among the procedures used, showing that RT-PCR takes the longest (6.5 h). In contrast, lateral flow consists of only two main steps and requires only 12 min. On the other hand, ELISA requires up to 4.3 h to finish its three main operations in the procedure,

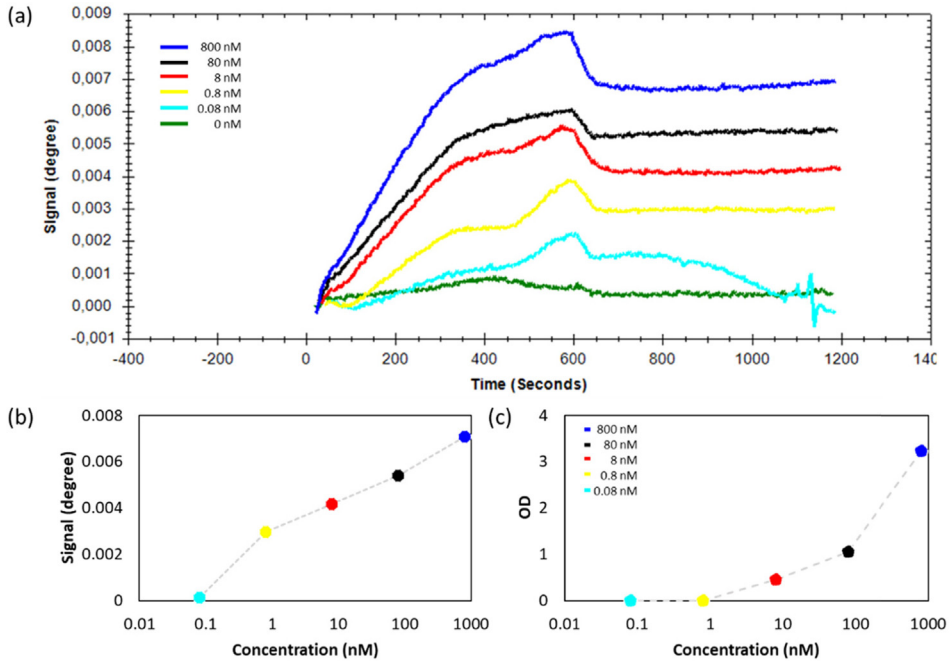

Fig. 1. Time and concentration analysis for detecting NS1 using SPR. (a) Sensorgram analysis using SPR can detect NS1 in just 600 s. (b) Detection of various NS1 concentrations by SPR analysis. (c) Detection of various NS1 concentrations by ELISA.

Table 1
Comparison of NS1 detection among RT-PCR, ELISA, lateral flow and SPR.

Sample number	Test sample	RT-PCR	ELISA			Lateral flow	SPR		
			IgM	IgG	NS1		Conc. (nM)	Signal (degrees)	Result
1	Positive	+	+	–	+	+	3.134	0.00252	+
2	Positive	+	–	+	+	+	0.333	0.00125	+
3	Positive	+	–	+	+	+	0.313	0.00115	+
4	Positive	+	–	–	+	+	0.301	0.00113	+
5	Positive	+	–	+	+	+	3.061	0.00234	+
6	Positive	+	+	+	+	–	0.884	0.00173	+
7	Positive	+	–	+	+	+	2.312	0.00229	+
8	Positive	+	–	+	+	+	5.182	0.00314	+
9	Positive	+	–	+	+	+	4.353	0.00297	+
10	Positive	+	–	+	+	+	4.352	0.00273	+
11	Positive	+	+	+	+	+	3.381	0.00264	+
12	Positive	+	+	+	+	+	7.124	0.00398	+
13	Positive	+	+	+	+	+	2.217	0.00194	+
14	Positive	+	–	+	+	+	0.543	0.00136	+
15	Positive	+	+	–	+	+	1.823	0.00188	+
16	Positive	+	–	+	+	+	0.342	0.00127	+
17	Normal	–	–	–	–	–	0.005	0.00042	–
18	Normal	–	–	–	–	–	0.034	0.00075	–
19	Normal	–	–	+	–	–	0.005	0.00044	–
20	Normal	–	–	+	–	–	0.027	0.00074	–
21	Normal	–	–	–	–	–	0.004	0.00040	–
22	Normal	–	–	+	–	–	0.006	0.00047	–
23	Normal	–	–	–	–	–	0.003	0.00033	–
24	Normal	–	–	+	–	–	0.007	0.00059	–
25	Normal	–	–	+	–	–	0.007	0.00057	–
26	Normal	–	–	+	–	–	0.003	0.00034	–

Table 2
The Comparison of Protein NS1 DENV Results between SPR Method with PCR and ELISA as the Gold Standard.

The Comparison of Protein NS1 DENV Results		Gold standard (RT-PCR & ELISA) Method		
		Positive	Negative	Total
SPR Method	Positive	16	0	16
	Negative	0	10	10
	Total	16	10	26 (Ahmed and Broor, 2014; Alcon et al., 2002)

including kit preparation and addition of the primary and secondary antibodies. Our experiments showed that SPR required 0.95 h of operation time and 0.45 h after immobilisation.

4. Discussion

The clinical effects of DENV-infected patients are non-specific and similar to many other diseases, which might lead to a misdiagnosis (Parikh et al., 2012). Moreover, there are currently no available anti-viral drugs or specific therapies for treating this disease (Parkash and Shueb, 2015). Therefore, an early and accurate laboratory diagnostic test is urgently required for disease confirmation, patient triage and prevention of infection spread (Tricou et al., 2010). There are currently many available detection techniques that are used routinely for clinical assessment, namely, rapid detection tests (including NS1 antigen detection, detection of IgM and IgG) and detection of NS1 antigen, IgM/IgG antibody detection using ELISA and RT-PCR. However, these methods have several limitations; for example, they require a long time, they are laborious and require a high skill level, they cannot detect small amounts of sample or they have a poor sensitivity for detecting DENV infections.

NS1 proteins are very imperishable glycoprotein that possess epitope similarity with the other four DENV serotypes and makes NS1 protein from the other dengue serotypes are recognizable by antibody anti NS1 DENV. The similar NS1 epitope sequence between DENV serotypes are ^{25}VHTWTEQYKFQ^{35}; ^{112}KYSWKS/TWGKAK^{123}; ^{193}AVHADMGYWIES^{204}; ^{266}GPWHLGKLE^{274}; ^{294}RGPSLRTTT^{302} (Falconar and Young, 1991; Puttikhunt et al., 2011). A study by Chen et al. (2010) revealed the presence of specific common epitope for DENV which was ^{11}LRYSWKTWGKA^{121}.^{32}.

In this study, we assessed the usefulness and applicability of SPR for accurately detecting DENV infections in plasma samples originating from in-patients. The obtained data revealed that SPR is a competitive alternative to RT-PCR and ELISA, the current gold standards in testing for DENV infections. Moreover, this study revealed that SPR-based biosensors have an excellent sensitivity, with the ability to detect small amounts of NS1 protein in a sample (limit up to 0.300 nM), a level at which measuring samples with low concentrations is often difficult (Nguyen et al., 2015). SPR also showed a promising accuracy, as it has a higher refractive index for each concentration compared to the OD of ELISA. Principally, SPR is an optical biosensor that operates on the basis of the adsorption of the target analyte (NS1 antigen) to a selective bio-recognition element immobilised on a sensor surface (anti-NS1 antibody), which causes a change in the fluorescent signal that is measured by a detector (Parkash and Shueb, 2015). When the plasma sample containing NS1 comes into contact with the surface, an NS1 antigen–anti-NS1 antibody binding via affinity interaction occurs, which consequently induces an increase in the refractive index on the SPR sensor's surface. Changes in the refractive index are tracked by coupling of incident light with a propagating surface plasmon on a gold surface in real time. This light is proportional to the amount of NS1 antigen attached to the antibody in the unit. Therefore, SPR is able to compete with ELISA and RT-PCR in terms of sensitivity and specificity.

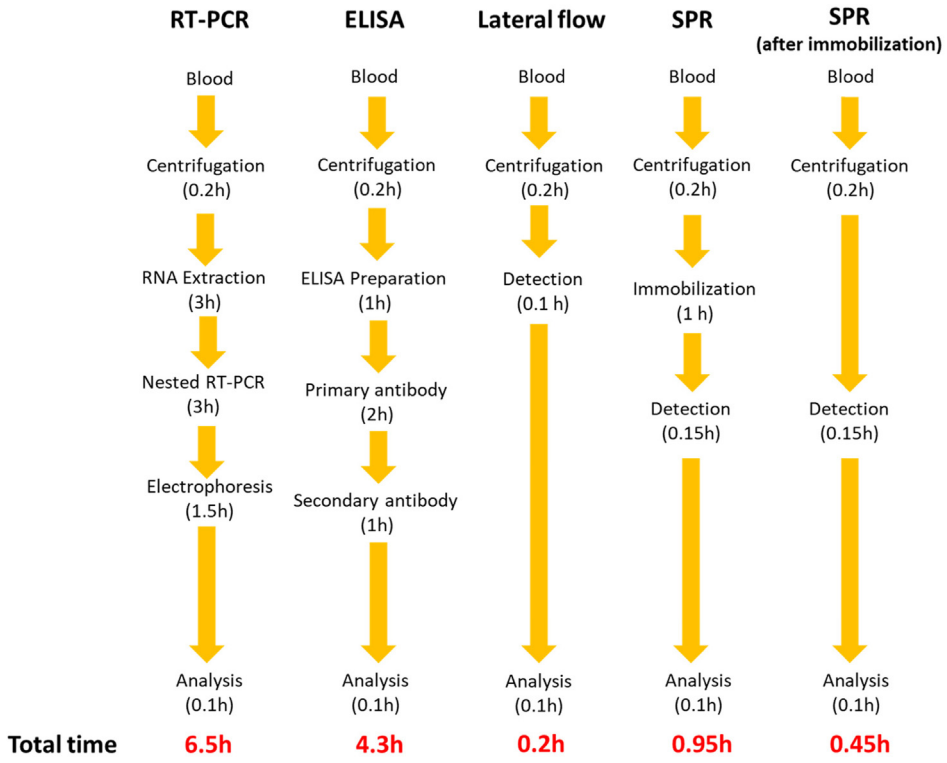

Fig. 2. Comparison of the time required for RT-PCR, ELISA, lateral flow, SPR and SPR after immobilisation.

The other advantages of SPR analysis are that it is time-efficient and does not require highly skilled personnel and is simpler than the other gold standards. SPR requires only immobilising anti-NS1 antibodies, which can be utilised several times in subsequent experiments. The number of times the immobilised anti-NS1 antibody can be used is unknown, but this should be tested in the future. Although lateral flow still requires a shorter time compared to the other procedures, its reliability in detecting NS1 is lower.

Although in this study there was no discrepancy between RT-PCR method and NS1 detection by SPR method, the possibility of SPR method generated false positive and false negative value still existed as a consequence of the NS1 protein detection result affected by the infection status of the patient, the geographical origin of the detected sample, and the reaction crosses with other members of the flaviviridae circulating in Indonesia (Aryati et al., 2013; Kosasih et al., 2013). Other issues arose from this study was related to the number of samples used which were considered to be small, hence it could not represent the geographical origin of the sample as well as the patient's infection status. There was also no sample that represented other members of flaviviridae, and consequently further research needs to be done in the future. The essence of SPR method was to ensure that the binding occurs between the target analytes and the ligand. The non – specific binding will lead to false negative or false positive. It can be minimized by blocking using BSA and diluting plasma samples.

Over the past decades, SPR has been shown to be one of the most powerful technologies for determining specificity, affinity and kinetic parameters during the binding of antigens and antibodies (Kim et al., 2006; Madeira et al., 2011; Unit, 2011). Consistent with these previous findings, SPR is a useful tool for the diagnosis of dengue NS1 owing to its sensitivity to small amounts of target, efficiency and minimum laboratory skills required. This method may be applied for detecting biomarkers of numerous diseases, including breast, ovarian and pancreatic cancer, as well as cardiac and neurological diseases (Nguyen et al., 2015).

5. Conclusions

In this study, it was found that the NS1 antigen of DENV can be detected using SPR. This method displays a high sensitivity and time efficiency and requires only standard-skilled personnel. Hence, this well-known biosensor can act as an alternative commercial assay to already existing tests for DENV detection. Further studies should be conducted with more samples in Indonesia and other tropical and sub-tropical regions to determine its reliability on a broader scale.

Conflict of interest

There is no conflict of interest.

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