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Detection of swine-origin influenza A (H1N1) viruses using a localized surface plasmon coupled fluorescence fiber-optic biosensor

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ABSTRACT

Swine-origin influenza A (H1N1) virus (S-OIV) was identified as a new reassortant strain of influenza A virus in April 2009 and led to an influenza pandemic. Accurate and timely diagnoses are crucial for the control of influenza disease. We developed a localized surface plasmon coupled fluorescence fiber-optic biosensor (LSPCF-FOB) which combines a sandwich immunoassay with the LSP technique using antibodies against the hemagglutinin (HA) proteins of S-OIVs. The detection limit of the LSPCF-FOB for recombinant S-OIV H1 protein detection was estimated at 13.9 pg/mL, which is 10^3-fold better than that of conventional capture ELISA when using the same capture antibodies. For clinical S-OIV isolates measurement, meanwhile, the detection limit of the LSPCF-FOB platform was calculated to be 8.25×10^4 copies/mL, compared with 2.06×10^6 copies/mL using conventional capture ELISA. Furthermore, in comparison with the influenza A/B rapid test, the detection limit of the LSPCF-FOB for S-OIV was almost 50-fold in PBS solution and 25-fold lower in mimic solution, which used nasal mucosa from healthy donors as the diluent. The findings of this study therefore indicate that the high detection sensitivity and specificity of the LSPCF-FOB make it a potentially effective diagnostic tool for clinical S-OIV infection and this technique has the potential to be applied to the development of other clinical microbe detection platforms.

1. Introduction

Influenza A viruses can cause epidemics and sometimes trigger pandemics. Changes in influenza virus antigenicity result from antigenic drift and antigenic shift. Such antigenic changes create new viral variants that may not be recognized by pre-existing antibodies (Garten et al., 2009). Any newly emerging influenza A virus subtypes pose a threat to human health and could potentially lead to significant morbidity and mortality (Lu et al., 2009). The receptors for influenza A viruses are α-2,3 and α-2,6 sialic acid (SA) linked glycosylated oligosaccharides which are dominantly expressed on respiratory cells of avian and human species respectively. Therefore, the receptor specificity for hemagglutinin (HA) is considered an important determinant of the host range of influenza A viruses. Pigs, which express both receptors on their respiratory tract cells, have been called "mixing vessels" for generating new influenza viruses (Smith et al., 2009) as they are naturally susceptible to infection by both avian and human influenza A viruses (Naffakh and van der Werf, 2009). Previous studies indicated that pigs are frequently involved in interspecific transmission events and recent reports indicated that H1N1, H3N2 and H1N2 influenza A viruses are endemic in swine populations worldwide (Naffakh and van der Werf, 2009; Brown, 2000; Van Reeth, 2007). In April 2009, a novel swine-origin influenza A (H1N1) virus (S-OIV) was isolated from humans in Mexico and the United States (Garten et al., 2009; Fraser et al., 2009). Recent studies have shown that the infectivity of the S-OIV is more efficient and fatal than seasonal influenza viruses (Lau et al., 2009; Munster et al., 2009; Maines et al., 2009). S-OIV was identified as a reassortant strain

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with six gene segments (PB2, PB1, PA, HA, NP and NS) from triple-reassortant influenza A viruses circulating in North American pigs. Two other gene segments (NA and M) were isolated from Eurasian swine influenza viruses. This S-OIV (serotype H1N1) is responsible for the first influenza pandemic of the 21st century. Up until 4 July 2010, the world health organization (WHO) reported that there were more than 214 countries and overseas territories or communities with reported laboratory-confirmed cases of this S-OIV (including at least 18,311 deaths). In addition, a variety of unusual clinical symptoms have been associated with this virus compared with common seasonal influenza viruses, such as hospitalization because of severe pneumonia and respiratory failure (Munster et al., 2009). Notably, reports have indicated that the S-OIV is naturally resistant to amantadine and rimantidine, but is still susceptible to oseltamivir and zanamivir (van der Vries et al., 2010; Rungrotmongkol et al., 2009). Therefore, a rapid and accurate diagnostic method is needed to confirm S-OIV in suspected patients. Currently, the standard procedure for influenza A virus detection and classification entails conventional virus isolation in embryonated eggs or MDCK cells, followed by HA and neuraminidase (NA) subtyping using serological or reverse transcription-polymerase chain reaction (RT-PCR) methods (Beigel et al., 2005). The currently available antigen detection methods are based on the influenza viral nucleoprotein that is conserved in all influenza A viruses, examples include the FLU OIA TEST (Biostar), FLU A (BD Bioscience) and FLU A/B (Formosa Biomedical Tech) detection assays (He et al., 2007). When emergency control and containment of these types of S-OIVs is required, these types of rapid tests which provide results within 15 min, are widely used in clinics and hospitals.

A fiber-optic biosensor, based on the optical property of localized surface plasmon coupled fluorescence (LSPCF) combined with a sandwich immunoassay, has been proposed by our group as a tool to study protein–protein interactions. Experimentally, the detection limit of the LSPCF fiber-optic biosensor (LSPCF-FOB) has been demonstrated to be as low as 1 pg/mL when detecting mouse immunoglobulin G (IgG) interacting with anti-mouse IgG (Hsieh et al., 2007). In addition, the mechanism of fluorescence enhancements of fiber-optic biosensor with metallic nanoparticles has been studied using scattering theory (Ng and Liu, 2009). Our previous reports also demonstrated that the LSPCF-FOB is able to measure alpha-fetoprotein in human serum (Chang et al., 2009). In addition, the LSPCF-FOB has been applied to enhance the detection sensitivity of severe acute respiratory syndrome (SARS) coronavirus nucleocapsid protein in diluted serum to a lower limit of 0.1 pg/mL (Huang et al., 2009). The LSPCF-FOB offers many useful features including ease of operation and disposability. In addition, based on the ultra high detection sensitivity and quantitative ability, this biosensor can be used for the early diagnosis of diseases in clinics.

In this study, we used S-OIV antibodies to develop a LSPCF-FOB assay to allow and enhance clinical detection of S-OIV. Our results indicated that the detection limit of LSPCF-FOB for recombinant S-OIV HA protein detection was at 13.9 pg/mL, which is 103 lower than that observed with conventional capture ELISA. For the detection of clinical S-OIV isolates, the LSPCF-FOB was able to detect 8.25×10^4 copies/mL of S-OIVs, which is 25-fold higher than the detection sensitivity demonstrated by conventional capture ELISA assays. Compared with the influenza A/B rapid test, the detection limit of the LSPCF-FOB for S-OIV was almost 50-fold higher in phosphate buffer saline (PBS) solution and 25-fold higher in mimic solution, which used nasal mucosa from healthy donors as the diluent. We propose that the high sensitivity and specificity of the LSPCF-FOB make it a potentially useful tool for clinical S-OIV diagnosis.

2. Experimental procedures

2.1. Glycosylated recombinant HA protein preparation

The glycosylated recombinant HA (rHA) proteins were prepared using a baculovirus cell based system. Briefly, HA proteins from the A/Vietnam/1203/2004 H5N1 strain were prepared by the cotransfection of baculovirus transfer vector with BaculoGold-linearized baculovirus DNA (BD Biosciences, Bedford, MA, USA) into *Spodoptera frugiperda* (Sf9) cells (Invitrogen, Carlsbad, CA, USA) using the BaculoGold transfection solution set (BD Biosciences), and were subsequently amplified in the same cells. HA was recovered from the cell supernatant by metal affinity chromatography using Ni Sepharose high-performance resin (GE Healthcare, Piscataway, NJ, USA). Fractions containing HA were combined and subjected to ion-exchange chromatography using a MonoQ HR10/10 column (GE Healthcare). HA oligomers, trimers and monomers were separated by gel filtration chromatography using a Hi-Load 16/60 Superdex 200-pg column (GE Healthcare), then confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting using a mouse monoclonal anti-His antibody or a mouse monoclonal anti-HA antibody.

2.2. Conventional capture ELISA for rHA protein detection

Immunoplates (PerkinElmer, Shelton, CT, USA) were first coated with 100 μL of 10 μg/mL rabbit anti-S-OIV H1 polyclonal antibody (ProSci, Poway, CA, USA) (a-H1, the capture antibody) at room temperature overnight. After blocking with 5% nonfat milk in PBST (PBS solution containing 0.05% Tween 20) for 1 h, 100 μL of serially diluted rHA protein in PBS were added to each well and incubated at 37 °C for 2 h. The plates were then washed five times with PBST and 100 μL of sheep anti-S-OIV H1 polyclonal antibody (5 μg/mL) was added, followed by a 2-h incubation at 37 °C. Diluted peroxidase-conjugated anti-sheep antibody was then added and incubated at 37 °C for 1 h. After three washes, the plates were incubated with 200 μL of substrate (0.015% o-phenylenediamine dihydrochloride (Cat No. P4664, Sigma–Aldrich Corp. MO, USA) for another 30 min at 37 °C. Reactions were stopped by the addition of 3 N HCl and the absorbance was measured with a spectrophotometer at 492 nm. A blank control in the absence of rHA protein was included for normalization of the absorbance readings. Each reaction was performed in duplicate.

2.3. Determination of the S-OIV virus titers using hemagglutination and real-time RT-PCR

The clinical isolate F1338 was cultured with MDCK cells. The viral supernatant was collected and filtered through a 0.45 μm filter, then the virus titer was measured using a hemagglutination assay and real-time RT-PCR. For the hemagglutination assay, 2-fold diluted viruses were incubated separately in 96-well plates (50 μL/well) with 50 μL of 0.75% guinea pig red blood cells at 37 °C for 2 h. Hemagglutination was then observed and recorded. For real-time RT-PCR, the standard procedures described by the centers for disease control (CDC) protocol for real-time RT-PCR for S-OIVs were followed. Briefly, viral RNA was extracted using a QIAGEN Viral Amp kit (QIAGEN, Hilden, Germany), then quantitative real-time RT-PCR was performed on the isolated RNA using an ABI one-step RT-PCR kit (P/N 4309169) (Applied Biosystems, CA, USA) and an ABI 7000 real-time PCR thermocycler. For S-OIV RNA detection, H1-specific primers and probes were used for RT-PCR amplification (SW-H1 forward: 5′-GTGCTATAAACACCAGCCTYCCA-3′; SW-H1 reverse: 5′-CGGGATATTCCTTAATCCTGTRGC-3′; SW-H1 probe: FAM-5′-CAGAATA TACAXTCCRGTCACAATTGGARAA-3′, where XT = BHQ1

dT). Amplification conditions for the H5 primers were 48 °C for 30 min and 95 °C for 10 min, followed by 45 cycles at 95 °C for 15 s and 60 °C for 1 min. H1N1 quantities were calculated by interpolation from a standard curve generated by the parallel running of serial dilutions of known quantities of the H1 (HA) segments of cloned plasmids.

2.4. Conventional capture ELISA for detection of clinical S-OIV isolates

a-H1-coated (1 μg/well) immunoplates, prepared as described above, were used for the detection of clinical S-OIV isolates. Briefly, S-OIV culture supernatants were collected and the virus titers were measured using a hemagglutination assay and real-time RT-PCR. Serial dilutions of the S-OIV in PBS solution (100 μL/well) were added to plates and incubated at 37 °C for 2 h. The plates were then washed five times with PBST and 100 μL of sheep anti-H1 polyclonal antibody (5 μg/mL) was added incubated for 2 h at 37 °C. Diluted peroxidase-conjugated anti-sheep antibody was then added and incubated at 37 °C for 1 h. After washing, the plates were incubated with 200 μL of substrate (0.015% o-phenylenediamine dihydrochloride, Sigma–Aldrich) for another 30 min at 37 °C. Reactions were stopped by the addition of 3 N HCl and absorbances were measured with a spectrophotometer at 492 nm. Alternatively, to mimic the real situation in a clinic, S-OIVs were also diluted with human nasal mucosa and subjected to the detection procedure described above. A blank control in the absence of clinical S-OIV was included for normalization of the absorbance readings. Each reaction was performed in duplicate.

2.5. Flu A/B rapid test for the detection of clinical S-OIV isolates

In the conventional rapid influenza diagnostic test (RIDT), Flu A/B rapid test (MeDiPro, Lot. MS19911, Formosa Biomedical, Taiwan) was compared with the sensitivity of conventional capture ELISA and the LSPCF-FOB. The Flu A/B rapid test was carried out according to the manufacture's protocol. Briefly, serially diluted S-OIVs in PBS solution or nasal mucosa (50 μL/tube) were incubated in tubes with extraction solution (containing lysis solution and reactive antibody-conjugated HRP). The strip coated with anti-nucleoprotein monoclonal antibody, was inserted into each tube and incubated at room temperature for 15 min. Reactive bands corresponding to the control, Flu A or Flu B were observed.

2.6. Preparation of the optic fiber and setup of the biosensor

A plastic multimode optical fiber (Mitsubishi Rayon Co., LTD., Tokyo, Japan) of 1 mm in diameter was used in this experiment. The fibers were stripped off the cladding by immersion in ethyl acetate, then the stripped portion was washed with 2-propanol to clean the stripped surface. Chemical adsorption was carried out using covalent binding forces to immobilize the capture antibody onto the surface of the stripped fiber. The protocol for chemical adsorption has been described previously (Hsieh et al., 2007). The modified stripped portion of each optical fiber was first coated with 300 μL of 1 μg/mL a-H1 (the capture antibody) at 4 °C overnight. After blocking with 10 mg/mL bovine serum albumin-PBS solution for 1 h, 300 μL of serially diluted rHA proteins in PBS solution, serially diluted UV-irradiated S-OIVs in PBS solution and 500-fold diluted UV-irradiated S-OIVs in mimic solution, were added into each reaction chamber and incubated at room temperature for 2 h. The schemes for the sandwich complex and the fluorescence probe are shown in Supplement Fig. S1.

2.7. Preparation of the LSPCF probe

The LSPCF probe is composed of fluorophores which are labeled with detection antibodies, connecting to protein A and adsorbed to colloidal GNP (Au–PA, ψ = 25 nm, Aurion, Wageningen, Netherlands), as shown in Supplement Fig. S1. The detection antibody was a-H1 antibody fluorescently labeled with Lightning-Link Atto633 (Innova Biosciences, Cambridge, UK) using a commercial labeling kit. To produce the fluorescence probe, 1 μg/mL of fluorophore-labeled detection antibody was mixed with Au–PA at a concentration of 1.86×10^{10} particles/mL and incubated for 10 h at 4 °C in the dark.

2.8. Measurement using the LSPCF-FOB

A 632.8 nm He–Ni laser was used as the excitation light source and a microscope objective (20×, NA = 0.45) was used to achieve the best coupling efficiency. The fluorophore (Atto633), of which the central wavelength of the emission spectrum was 657 nm, was excited by the enhanced localized electromagnetic field close to the GNP surface during measurement. A band pass filter (central wavelength = 660 nm) was introduced into this setup to allow fluorescence detection via a photomultiplier tube. Experimentally, a lock-in amplifier was used to increase the signal to noise ratio.

3. Results

3.1. Detection of rHA proteins and clinical S-OIV isolates use conventional capture ELISA

HA glycoproteins consist of the major parts of influenza viral envelope, which contain the antigenic regions and are usually used to be subtype identification. Glycosylated HA proteins of the S-OIV were selected as detected targets for diagnosis of S-OIV infection. The recombinant glycosylated HA (rHA) proteins were produced using the baculovirus cell based system. The trimer or monomer forms (HA0 was composed of HA1 and HA2 domains) of the rHA proteins were confirmed by SDS-PAGE and western blot analysis (Supplement Fig. S2). To develop convenient and accurate assays for S-OIV detection, we first set up a conventional capture ELISA using polyclonal antibodies (a-H1) against S-OIV HA proteins. We used the rHA protein as the antigen for sensitivity evaluation in this assay. The results shown in Fig. 1 demonstrate a linear relationship between the O.D. 492 nm and the concentrations of rHA protein over the range 12.5–800 ng/mL; the correlation coefficient (R^2) is 0.9916 and the error bar indicates one standard deviation in the

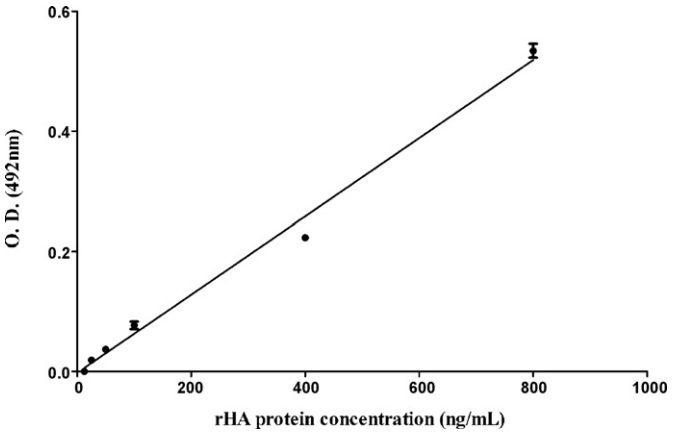

Fig. 1. The linear relationship between the O.D. 492 nm and rHA protein concentrations over the range 12.5–800 ng/mL, the correlation coefficient (R^2) is 0.9916 and the error bar indicates one standard deviation in the measurement.

Table 1
Detection of clinical S-OIV isolates using conventional capture ELISA. "+" represents positive results; "–" represents negative results.

Target	S-OIV (H1N1)						
Dilution folds	Dilute with PBS 5×	10×	20×	40×	80×	160×	320×
Copies/mL	1.65×10^7	8.25×10^6	4.13×10^6	2.06×10^6	10.3×10^6	5.16×10^5	2.58×10^5
Results	+	+	+	+	–	–	–

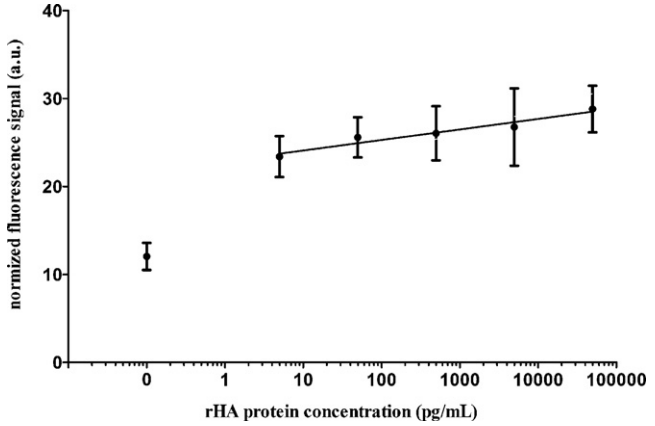

Fig. 2. The linear relationship between the normalized fluorescence signals and rHA protein concentrations over the range 5–50 ng/mL, the correlation coefficient (R^2) is 0.9375 and the error bar indicates one standard deviation in each measurement.

measurement. According to international union of pure and applied chemistry (IUPAC) definition (Thomsen et al., 2003), the evaluated detection limit and analytical sensitivity of the conventional capture ELISA in this experiment are calculated to be 12.2 ng/mL and 17.7 ng/mL, respectively. For the detection of clinical S-OIV isolates, UV-irradiated S-OIV culture supernatants (the original S-OIVs were detected by RT-PCR at ~8.25×10^7 RNA copies/mL; data not shown) were diluted from 5 to 320-fold in PBS solution and measured by conventional capture ELISA. This assay was able to detect around 2.06×10^6 copies/mL corresponding to 40-fold dilution of the original viruses, as shown in Table 1.

3.2. Detection of rHA proteins and clinical S-OIV isolates use the LSPCF-FOB

To develop a highly sensitive assay for S-OIV detection, we established a LSPCF-FOB platform and analyzed its sensitivity using rHA proteins and S-OIV isolates. Different dilutions of rHA proteins in PBS solution (i.e., 0 pg/mL, 5 pg/mL, 50 pg/mL, 500 pg/mL, 5 ng/mL, and 50 ng/mL) were injected into the reaction chamber to interact with captured antibodies (a-H1), and this interaction was measured by the LSPCF probes. The results shown in Fig. 2 demonstrated a linear relationship between the normalized fluorescence signals and rHA protein concentrations over the range 5–50 ng/mL; the correlation coefficient (R^2) is 0.9375 and the error bar indicates one standard deviation in each measurement. We think the huge error bar of each measurement is caused by the injured core when the fiber was stripped off the cladding by immersion in ethyl acetate. The fluorescence signal was calculated by subtracting the background level from the steady fluorescence intensity in each measurement in which the fluorescence intensity was obtained as the measuring fluorescence normalized by laser beam intensity to reduce the perturbation of laser intensity. After that, the fluorescence signal was normalized by the background level in order to decrease the effect of misalignment in the system. According to IUPAC definition, the detection limit and analytical sensitivity of LSPCF-FOB in the experiment are estimated at 13.9 pg/mL and 3.7 pg/mL, respectively. The serial dilutions (from 10^{-1} to 10^{-5}) of each of the UV-irradiated S-OIVs in PBS solution were injected into the reaction chamber to interact with the immobilized a-H1 and form an <a-H1/S-OIV> complex on the modified stripped portion of the fiber surface. The detection limit was around a 1000-fold dilution of the original S-OIV culture supernatant (8.25×10^4 copies/mL) in PBS solution as shown in Table 2. Notably, the normalized fluorescence signal of the 10-fold diluted UV-irradiated avian influenza A (H5N1) virus culture supernatant was showed the same signal level with the 10-fold diluted blank medium which is the control group. To mimic *in vivo* isolation of influenza virus from the upper human respiratory tract, the S-OIV isolate was diluted in nasal mucosa from healthy donors (called mimic solution). A 500-fold dilution of S-OIV culture supernatant (1.65×10^5 copies/mL) in mimic solution was successful detected by the LSPCF-FOB compared with a 10-fold diluted influenza A (H5N1) virus culture supernatant, which corresponded to the medium only control as shown in Table 2.

3.3. Detection of clinical S-OIV isolates use conventional RIDT

The conventional RIDT, Flu A/B rapid test, is currently used for the clinical diagnosis of influenza A and B virus infection. We used this assay to detect the clinical S-OIV isolates diluted in PBS. As shown in Table 3, the 20-fold dilution (4.13×10^6 copies/mL), or less diluted samples, of the original viruses showed a reactive band. The detection limit of the Flu A/B rapid test was also a 20-fold dilution (4.13×10^6 copies/mL) of the original S-OIVs in mimic solution.

Table 2
Detection of clinical S-OIV isolates using LSPCF-FOB. "+" represents positive results; "–" represents negative results.

Target	S-OIV (H1N1)				
Dilution folds	Dilute with PBS 10×	100×	1000×	5000×	10000×
Copies/mL	8.25×10^6	8.25×10^5	8.25×10^4	1.65×10^4	8.25×10^3
Results	+	+	+	–	–

Target	Medium	H5N1	H5N1	S-OIV (H1N1)
Dilution folds	Dilute with PBS 10×	10×	Dilute with mimic solution 10×	500×
Copies/mL	none	none	none	1.65×10^5
Results	–	–	–	+

Table 3
Detection of clinical S-OIV isolates using conventional RIDT. "+" represents positive results; "−" represents negative results.

Target	S-OIV (H1N1)							
Dilution folds	Dilute with PBS							
	1×	5×	10×	15×	20×	30×	40×	100×
Copies/mL	8.25×10^7	1.65×10^7	8.25×10^6	5.50×10^6	4.13×10^6	2.75×10^6	2.06×10^6	8.25×10^5
Results	+	+	+	+	+	−	−	−

Target	S-OIV (H1N1)				
Dilution folds	Dilute with mimic solution				
	4×	20×	40×	80×	120×
Copies/mL	2.06×10^7	4.13×10^6	2.06×10^6	1.03×10^6	6.88×10^5
Results	+	+	−	−	−

Table 4
Comparison of different assays for S-OIV detection.

Detection assay	Target protein	Time cost	Sensitivity (copies/mL)
Rapid test	Nucleoprotein	~15 min	4.13×10^6
Conventional capture EIA	Hemagglutinin	>2 h	2.06×10^6
LSPCF-FOB	Hemagglutinin	~2 h	8.25×10^4
RT-PCR	X	6 h	10^2–10^3

4. Discussion

In this study, the rHA protein was successfully detected at a concentration of 25 ng/mL in PBS solution using conventional capture ELISA. To lower the detection limit and improve the analytical sensitivity of capture ELISA, an LSPCF-FOB platform was used. Our results demonstrated that the LSPCF-FOB is capable of detecting the concentration of rHA protein at pg/mL level in PBS solution and the detection limit was calculated at 13.9 pg/mL based on IUPAC definition. Therefore, the LSPCF-FOB improves the detection limit 10^3-fold better compared with conventional capture ELISA using the same capture antibody. A comparison of different assays for S-OIV detection is shown in Table 4. At present, there are two assays recommended for use in the diagnosis of S-OIV in clinical samples. The first option is a molecular diagnostic assay based on nucleic acid detection, such as real-time RT-PCR or RT-PCR. Real-time RT-PCR is the best choice for clinical diagnosis of S-OIV in respiratory specimens and can further differentiate between S-OIVs and seasonal influenza A viruses by using specific primers and probes. The sensitivity of real-time RT-PCR is over 97% and its detection limit is around 10^2–10^3 copies/mL (Bose et al., 2009; He et al., 2009; van der Vries et al., 2010; Guo et al., 2009; Chan et al., 2009; Wenzel et al., 2009; Yang et al., 2009). However, both assays are complex and cannot be readily performed in primary health care settings. Although the findings of this study indicate that the LSPCF-FOB is not as sensitive as real-time RT-PCR or RT-PCR, it can improve the antigen detection limit and diagnose a certain pathogen within a few hours. Whereas, the assay time using the RT-PCR system means that confirmation of S-OIV is often reported too late to be of clinical or epidemiological relevance (Chan et al., 2009; Cunha et al., 2009).

RIDT is another diagnostic option. RIDTs are based on immunochromatographic lateral flow tests which use monoclonal antibodies directed against the nucleoprotein of influenza virus, and can be performed in around 15 min with minimal training (Taylor et al., 2009). However, although most RIDTs can be used for distinguishing between influenza A and influenza B viruses, few can further classify influenza A subtypes. Furthermore, many studies on RIDTs report a detection limit of 10^6–10^7 copies/mL for seasonal influenza viruses and a low sensitivity (less than 50%) for S-OIV detection (Faix et al., 2009; Chan et al., 2009; Vasoo et al., 2009; Balish et al., 2009; Cunha et al., 2009; Sabetta et al., 2009; Drexler et al., 2009). Experimentally, in comparison with the conventional RIDT and Flu A/B rapid test, the LSPCF-FOB platform developed in this study increased the detection sensitivity at least 50-fold for S-OIV diagnosis in PBS solution and 25-fold in mimic solution. The low detection limit of LSPCF-FOB is based on several properties. Firstly, the LSPs excite and enhance fluorescence with high efficiency near the gold nanoparticle (GNP) surface. Secondly, the fluorescence is amplified because more than 120 fluorophores are presented on each LSPCF probe and are excited simultaneously. Thirdly, on the fluorescence probe, the protein A which is immobilized on the GNP surface, acts not only as a linker by connecting to the secondary antibody labeled fluorophore but also as a spacer that effectively avoids metal-induced quenching effects (Hsieh et al., 2007). Fourthly, the fluorescence signal is usually detected at the distal end of the optical fiber in a conventional setup. In such situations, the coaxial propagation of the pumping laser beam induces a strong background signal, which results in an apparent lower detection limit. In contrast, the LSPCF-FOB detects the fluorescence signals beside the reaction region. This results in a significant increase in the efficiency of fluorescence collection compared with the conventional setup (Chang et al., 1996). Finally, the sandwich immunoassay configuration in the LSPCF-FOB exhibits higher specificity than other types of immunoassays, such as direct labeling and single-capture (Kingsmore, 2006). We therefore propose that the LSPCF-FOB is an effective alternative to conventional methods for the clinical diagnosis of S-OIV. The LSPCF-FOB also has the potential to be developed into a high-throughput screening or detection assay for different subtypes of influenza A virus.

5. Conclusions

In this study, we set up a novel H1N1 diagnostic platform, LSPCF-FOB, using capture and detection polyclonal antibodies against HA proteins of S-OIV. Experimentally, a linear response between the normalized fluorescence signal and the concentration of rHA protein was obtained in the range of 5–50 ng/mL. In addition, the detection limit and analytical sensitivity of LSPCF-FOB in the experiment were calculated at 13.9 pg/mL and 3.7 pg/mL, respectively. Compared with conventional capture ELISA, the detection limit of the LSPCF-FOB for rHA protein detection is 10^3-fold better by using the same capture antibodies. Furthermore, compared with conventional RIDTs, the LSPCF-FOB platform enhanced the detection sensitivity of S-OIV diagnosis up to 50-fold in PBS solution and 25-fold in mimic solution. Our experimental results revealed that the LSPCF-FOB offers higher detection sensitivity and specificity for S-OIVs, using PBS or mimic solution as a diluent, compared with conventional capture ELISA or RIDTs. We propose that the LSPCF-

FOB has the potential to be developed into a rapid and accurate diagnostic platform for S-OIV detection in the near future.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2010.08.060.

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