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Evaluation study of a portable impedance biosensor for detection of avian influenza virus

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A B S T R A C T

Current methods for detection of avian influenza virus (AIV) based on virus culture and RT-PCR are well established, but they are either time consuming or require specialized laboratory facilities and highly trained technicians. A simple, rapid, robust, and reliable test, suitable for use in the field or at the patient's bedside, is urgently needed. In this study, the performance of a newly developed portable impedance biosensor was evaluated by comparison with real-time reverse transcriptase PCR (rRT-PCR) and virus culture for detection of AIV in tracheal and cloacal swab samples collected from experimentally H5N2 AIV infected chickens. The impedance biosensor system was based on a combination of magnetic nanobeads, which were coated with AIV subtype-specific antibody for capture (separation and concentration) of a target virus, and a microfluidic chip with an interdigitated array microelectrode for transfer and detection of target virus, and impedance measurement of the bio-nanobeads and AI virus complexes in a buffer solution. A comparison of results obtained from 59 swab samples using virus culture, impedance biosensor and rRT-PCR methods showed that the impedance biosensor technique was comparable in sensitivity and specificity to rRT-PCR. Detection time for the impedance biosensor is less than 1 h.

1. Introduction

Avian influenza viruses (AIV), belonging to the family *Orthomyxoviridae*, have caused global concerns due to the potential pandemic threat for public health and enormous economic losses. Rapid detection and identification of AIV is crucial to controlling the outbreaks. Currently, different diagnostic techniques for AIV detection are available, such as virus culture, ELISA, rapid influenza diagnostic test kits, RT-PCR, and microarray technology "Flu Chips" (Stamboulian et al., 2000; Boon et al., 2001; Poehling et al., 2002; Montalto, 2003; Ng et al., 2006; Xie et al., 2006; Townsend et al., 2006; Mehlmann et al., 2006; Liu et al., 2006; He et al., 2007; Takekawa et al., 2010). Among them, virus culture, the "gold standard" of detection has the highest sensitivity and reliability, but remains a very time-consuming procedure. Rapid influenza diagnostic tests, including monoclonal antibody (MAb)-based antigen capture kits (Lu, 2003) and PCR/rRT-PCR molecular assays (Atmar et al., 1996; Lee et al., 1997; Spackman et al., 2003; Lee and Suarez, 2004) have been developed but have drawbacks that include poor specificity, low sensitivity, or complex procedures that require a relatively sophisticated laboratory and a highly trained technician. In a report from a WHO working group (WHO, 2006), it was pointed out that "A simple, rapid, robust and reliable test, suitable for use in the field or at the patient's bedside, is urgently needed".

Considerable effort has been directed towards the development of simple biosensors for the detection of AIV. Examples include non-labeling technologies such as surface plasmon resonance (Schofield and Dimmock, 1996; Kortt et al., 1999; Critchley and Dimmock, 2004) and quartz-crystal microbalance (Sato et al., 1996, 1999; Hewa et al., 2009), that both show great potential in the area of sensor research but also have had limited success in specificity. An interferometric biosensor based on antibody–antigen interaction was reported for detection of purified AI H7 and H8 in buffer solution (Xu et al., 2007). An Australian team developed an ion channel switch biosensor for AIV detection (Oh et al., 2008). Compared to surface plasmon resonance, quartz-crystal microbalance and interferometric biosensors, electrochemical biosensors appear

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very promising because they require minimal instrumentation and are readily integrated with microelectronics (Pavlovic et al., 2008). Impedance biosensors are more sensitive than electrochemical biosensors that employ amperometry, where the signal depends critically on the relative proximity of the active site and the electrode surface (Wang et al., 2006).

Microfluidic platforms in biosensor technology have numerous advantages such as process integration (sample pretreatment including preconcentration, separation and detection), portability, handling of small sample volumes, and high-throughput analysis (Wang et al., 2003; Liu et al., 2004, 2006). The successful integration of these laboratory procedures on a single microchip device has been demonstrated (de Mello and Beard, 2003; Wang et al., 2003; Liu et al., 2004, 2006). Impedance measurement/detection is a powerful detection method, ideal for easy integration into multi-array or microprocessor-controlled diagnostic tools and incorporation into miniaturized analytical systems. Impedance measurement/detection can be used for 'in field' measurements in combination with a portable measuring unit, and can also provide real-time in situ monitoring (Guan et al., 2004; Bolis et al., 2006). Interdigitated array microelectrodes have advantages in impedance measurement. For example, interdigitated array microelectrodes can maximize the impedance change at the surface, decrease the detection time, minimize interfering effects of non-target analytes in the solution, and have high signal/noise ratio (Thomas et al., 2004; Radke and Alocilja, 2005). An interdigitated array microelectrode and impedance measurement platform is potentially ideal for real time use in the field, especially when it is combined with a portable measuring unit, and an impedance measurement/detection system.

In our previous studies, extensive research was conducted to develop impedance biosensors for rapid detection of foodborne bacterial pathogens (Ruan et al., 2002; Yang et al., 2004a, 2004b; Wang et al., 2008). Since the size of an AI virus particle is 80–120 nm in diameter, whereas a foodborne bacterium, such as a rod-shaped cell of *Escherichia coli* O157:H7, is about 1–1.5 μm long and 0.5 μm wide, there are many differences in biological components, physical structure, binding sites, etc. between AI virus and bacteria cells. We attempted to explore the new application of the impedance biosensor for AIV detection, and the concept was proved with detection of killed purified AIV H5N1 (Wang et al., 2009). However, the reported biosensor system employed commercial impedance analyzer and commercial gold interdigitated array microelectrode, and did not design and integrate the components into miniaturized potable device, which could not do in-field detection of AIV in swab samples. In addition, no magnetic nanobeads based bioseparation was used for sample pretreatment, which is critical for real sample detection and in-field applications. Therefore, the purpose of the present study was to compare the performance of a newly developed portable impedance biosensor that is based on the combination of magnetic nanobeads bioseparation, microfluidic platform with interdigitated array microelectrode, and impedance measurement, with rRT-PCR for detection of AIV in tracheal and cloacal swab samples collected from experimentally H5N2 infected chickens.

2. Materials and methods

2.1. H5N2 virus preparation

Reference strain of low pathogenic AIV H5N2 (H5N2/ck/PA/87) maintained at Wiley Lab at Penn State University was propagated in specific-pathogen-free (SPF) embryonating chicken eggs. The fresh prepared H5N2 virus in chorioallantoic fluid (CAF), which was titered as $1 \times 10^{6.2}$ ELD_{50}/ml (ELD_{50}: 50% Egg Lethal Dose) and a HA titer 1:128/50 μl, was aliquoted 5 ml/tube and saved at −80 °C freezer for inoculation of experimental chickens and positive control virus in various test methods in this study.

2.2. Experimental chickens and virus inoculation

White leghorn specific pathogen free (SPF) chickens hatched from SPF fertile eggs were raised in cages in room-1 at Wiley biocontainment facility (WBCF) at Penn State University, and were used for AIV inoculation at 11-weeks of age. Two days prior to inoculation, 16 SPF chickens were transferred into two negative pressure isolators in room-3 at the WBCF, 8 chickens per isolator (maximum capacity). After 2 days in adaption of new environment, the 16 experimental chickens were each inoculated with a 2:3 (v/v) diluted H5N2 virus (4 ml virus was mixed with 6 ml viral transport media), 1.0 ml/bird (4×10^5 ELD_{50}/bird) via nasal, ocular and oral routes of administration. All inoculated birds were wing-banded for their individual identifications. SPF chicken remained in room-1 were served as negative controls. All research facilities including WBCF using experimental animals at Penn State University have met or exceeded AAALAC international standards and have been awarded full accreditation.

2.3. Sample collection and processing

Tracheal and cloacal swabs were collected at day 2 of post inoculation (PI) and continued daily for 7 consecutive days. At time of swab collection, 2 tracheal swabs and 2 cloacal swabs obtained from the same two birds were pooled for 1 tracheal swab sample and 1 cloacal swab sample, respectively, the two tracheal or cloacal swabs were placed into a sterile tube containing 3 ml VTM. To process swabs for testing, all swabs were vortexed vigorously for 15–20 s and then squeezed to remove as much of the organic material as possible. The squeezed dry-cotton swabs were discarded in a biohazard waste bag for autoclave, the liquid swab samples were kept at 4 °C refrigerator to allow large particles and organic materials participating to the bottom of the test tube. The samples were then ready to be tested by the impedance biosensor and rRT-PCR tests. For virus isolation in (ECE), 0.9 ml supernatant of each swab pool was treated with 0.1 ml multiple antibiotics mix at room temperature for 40 min to remove possible microbial contaminations. All processed swab samples were kept at 4 °C refrigerator if test is done within 48 h, otherwise at −80 °C freezer.

2.4. Preparation of monoclonal antibody and magnetic nanobeads

2.4.1. Biotin labeling of monoclonal avian influenza H5 antibody

The monoclonal avian influenza H5 antibody (2.2 mg/ml) obtained from the PSU Animal Diagnostic Laboratory was labeled with biotin using the EZ-Link Sulfo-NHS-Biotinylation Kit (Pierce, Rockford, IL) according to the supplied instruction. The level of biotin incorporation was measured to be 4–5 mole biotin per mole antibody by using the HABA assay.

2.4.2. Magnetic nanobead/antibody conjugation

The 150 nm diameter streptavidin-coated magnetic beads (R&D System, Minneapolis, MN) were first conjugated to the biotin-labeled monoclonal antibody described previously. Briefly, 25 μl of streptavidin-coated beads were washed by adding 250 μl of PBS to the beads in a microcentrifuge tube and vortexing 5–10 s. A magnet developed in our group (Lin et al., 2010) was then applied to the sample for 5 min to attract the nanobeads and remove the waste solution with a pipette. 100 μl of PBS and 50 μl of biotin-labeled antibody were added to the nanobeads and mixed for

30 min at 15 RPM on an orbital rotator to allow the streptavidin-coated nanobeads and biotin-labeled antibody to bind and form a nanobead + antibody complex. After mixing, the magnet was applied to the sample for 5 min to attract the beads + antibody complex, and remove the waste solution. The beads + antibody complexes were washed again with 150 μl of PBS, the magnet applied for 5 min, and the waste solution removed.

2.4.3. Sample preparation for impedance biosensor tests

Sample preparation for the impedance biosensor tests started by adding 200 μl of the previously collected swab sample solution to the beads + antibody complex. The sample tube was then vortexed 5–10 s and allowed to incubate at 37 °C for 30 min. This incubation time allowed any AI H5N2 virus present in the swab sample to bind to the monoclonal avian influenza H5 antibodies that were coupled to the nanobeads. After incubation the magnet was applied to the sample for 5 min to separate the beads + antibody + virus complex from the waste solution. The sample complex was then washed with 200 μl of the measuring solution (KPL Washing Solution diluted 1:200,000, KPL, Gaithersburg, MD) used for the impedance biosensor. The magnet was applied to the sample complex for 5 min, and after removing the waste, was finally resuspended in 150 μl of measuring solution. After this procedure the samples were ready for the impedance biosensor tests, or stored at 4 °C up to 72 h for the impedance test.

2.5. Impedance biosensor tests

2.5.1. Checking and cleaning interdigitated array microelectrode chips

The interdigitated microelectrode chips fabricated in the High Density Electronics Center at the University of Arkansas consists of 25 pairs of electrode fingers that are each 10 μm × 250 μm. These electrodes sit in a microfluidic channel that had a depth of 35 μm. After the chips were received, they were observed under 5× magnification to check for irregular features (e.g. damaged electrodes or foreign objects on the electrodes). Using a 1 ml syringe and a single channel syringe pump, the chips were then rinsed with Milli-Q water for 10 min (flow rate = 5 ml/h) to ensure that any residues or foreign materials remaining on the electrodes from the fabrication process were removed. Following this, the stability of chips was checked by measuring their impedance. Using the syringe pump, the measuring solution was injected through the microfluidic channel for 2 min at a flow rate of 1 ml/h, after which there was a 2 min waiting period before the impedance value was recorded. This waiting period was to ensure that there was no fluid movement in the microfluidic channel, which might affect the impedance reading. This was repeated three times to make sure the chip was stable. Finally, it was rinsed with Milli-Q water for 5 min (flow rate = 1 ml/h).

2.5.2. Impedance biosensor tests

Following the step-by-step introduction shown on the screen of the biosensor device, negative controls, swab samples, or washing solutions were injected into the biosensor system. The impedance values were automatically recorded and the result of AIV negative or positive samples was displayed.

2.6. Real-time RT-PCR (rRT-PCR) test

Real-time RT-PCR (rRT-PCR) protocol for AIV detection was from USDA National Veterinary Services Laboratories (NVSL) (Document # AVPRO1510.04). The rRT-PCR test was conducted at Wiley lab at Pennsylvania State University. Extraction of RNA from any virus present in the swab samples was accomplished using Qiagen RNeasy kit (Qiagen, Valencia, CA) following the instructions, and Qiagen OneStep RT-PCR Kit (Qiagen, Valencia, CA) was used for the rRT-PCR reaction. The rRT-PCR test was performed on Applied Biosystems 7300 Real-Time PCR System (Applied Biosystems, Foster City, CA). All swab samples were tested by both rRT-PCR and the impedance biosensor for result comparisons between the two assays.

2.7. Dot-ELISA test

Dot-ELISA was used in this study to test specific reactions of AIV Mabs to their specific subtypes. The Dot-ELISA procedures were described in previously published article (Lu, 2003) and same procedures were used in this study.

2.8. Statistic analysis

The means and standard deviations were calculated based on three replicates. *t*-Tests were used to obtain the *P*-values using Excel software.

3. Results

3.1. Evaluation of anti-H5 monoclonal antibody with Dot-ELISA

To ensure the quality of the antibody being used in the impedance biosensor testing described above, it was necessary to perform some preliminary tests. Dot-ELISA analysis (Lu, 2003) was employed to check the immuno-interaction between antibody and antigen, and also the cross-reaction with other AIV subtypes. Both H5 subtypes (H5N1, H5N2, H5N3, H5N9) and other AIV subtypes (H7N2, H9N2, H4N2) were tested. Newcastle disease virus (NDV), an RNA virus that causes influenza-like symptoms in birds and chickens, was also tested to check cross-reactivity. The results clearly showed that there was no binding between the selected antibody, NDV, and other AIV subtypes (H7N2, H9N2, H4N2), indicating good specificity of the selected antibody. Only AIV H5 subtypes appeared as visible dots on the strips. Among the four different H5 subtypes (H5N1, H5N2, H5N3, H5N9), the selected antibody showed the best binding affinity to the target H5N2 virus, displaying the largest dot size and the highest intensity.

3.2. Impedance biosensor system

An earlier version of the impedance biosensor system designed and developed in our group has been described previously (Li et al., 2006; Lin et al., 2010). The present version of the impedance biosensor contains a magnetic separator, an impedance detector (a microfluidic chip with an included interdigitated array microelectrode), and a laptop with data acquisition software. The impedance biosensor system was based on a combination of magnetic bio-nanobead separation/concentration procedure and a microfluidic chip with interdigitated array microelectrode for impedance measurement. Briefly, a poultry swab sample (typically a tracheal or cloacal swab that is obtained using a cotton-tipped applicator) is dipped into a buffer solution in which magnetic nanobeads are added. Magnetic nanobeads are pre-coated with an anti-H5 monoclonal antibody that specifically binds only to the H5 subtype of AI virus. After mixing a sample with the bio-nanobeads, a magnetic field is applied to retain the nanobead–H5 AI virus complex and the rest of the sample is then washed away. This procedure results in the separation and concentration of target AI virus into a small volume of buffer solution. Following the bio-nanobead pretreatment, the resulting nanobead–H5 AI virus complex solution is then injected into the impedance biosensor. The microfluidic chip is designed and fabricated as a flow-through device to deliver the target virus to an embedded interdigitated array microelectrode for

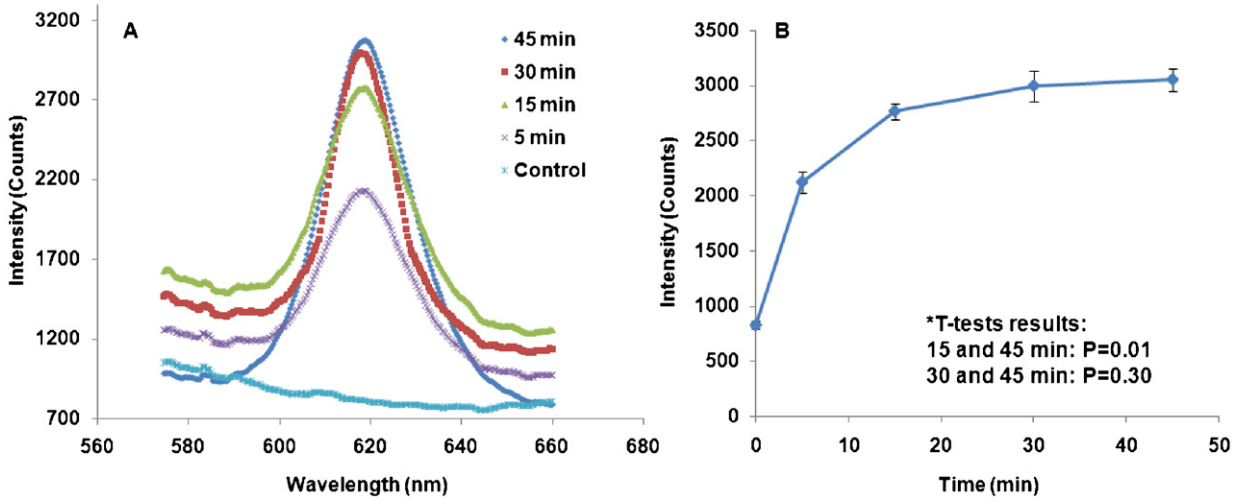

Fig. 1. (A) Typical fluorescence emission spectra and (B) the fluorescence intensity as a function of the incubation time for antibody–antigen (target AIV) binding.

impedance measurement. Changes in the impedance of the antibody coated nanobead–virus complex is measured and correlated to the presence of target AIV in the poultry swab sample.

3.3. Optimization of incubation time

Experiments were conducted to optimize the incubation time of the antibody–antigen complex (5, 15, 30, and 45 min). Because of their long-term photostability and high quantum yield, quantum dots (QDs) were selected as the fluorescent markers to label the target AIV for fluorescence measurement.

The results of these studies on optimization of the incubation period are shown in Fig. 1. The titer of target AIV used for the test was $1 \times 10^{3.2}$ ELD_{50}/ml. Fig. 1(A) shows the typical fluorescence emission spectra that were obtained. The control (PBS), which was subjected to the same treatment as the sample, gave no emission peak. The spectrum of the sample had a maximum emission at 620 nm, which was identical to the characteristic emission peak of the QDs being used in this study. This indicates the successful capture of the QDs-labeled target AIV to the antibody coated nanobeads. The fluorescence intensity as a function of the incubation time for antibody–antigen (target AIV) binding is presented in Fig. 1(B). The peak intensity increases with increasing incubation time from 5 to 45 min, and the relative steady state was obtained when the incubation time was equal to or greater than 30 min. Based on the *t*-tests results, the *P*-value between 15 min and 45 min was calculated to be 0.01, showing the peak intensity was significantly different between the two time points. However, the *P*-value between 30 min and 45 min was 0.30, indicating no significant difference after extending the incubation time to 45 min. Hence, an incubation time of 30 min was applied to the subsequent experiments.

3.4. Determination of the threshold of the impedance biosensor device

Determination of the device threshold plays a very important role, and it could be a key factor to minimize the false positive and false negative ratio during detection. Therefore, it is necessary to conduct tests to set up a reasonable and practical threshold for the most recent version of the AI impedance biosensor. To determine the threshold, a series of 10-fold dilutions of the purified target virus were prepared and then detected using the impedance biosensor. The results of these studies are presented in Fig. 2(A). Experiments were conducted with a wide range of virus titers spanning from $1 \times 10^{0.2}$ ELD_{50}/ml to $1 \times 10^{5.2}$ ELD_{50}/ml, with a negative control also being employed (PBS alone). The signals obtained at virus titer lower than $1 \times 10^{1.2}$ ELD_{50}/ml were poorly discriminated because their signal did not exceed 3 times the standard deviation (0.96 kΩ) from the control measurement. The detection limit of this biosensor system was determined based on signal/noise (maximum standard deviation) ratio ≥ 3, which was equal to 6 kΩ.

Table 1
Results obtained for rRT-PCR and impedance biosensor in comparison with virus culture.

Method	Swab	No. of specimens				Sensitivity[e] (%)	Specificity[f] (%)	PPV[g] (%)	NPV[h] (%)
		TP[a]	TN[b]	FP[c]	FN[d]				
rRT-PCR	Tracheal	21	14	0	0	100	100	100	100
	Cloacal	9	9	4	2	81	69	69	81
Biosensor	Tracheal	21	9	5	0	100	64	81	100
	Cloacal	6	13	0	5	55	100	100	72

A total of 59 swab samples (35 tracheal and 24 cloacal) were detected by rRT-PCR and the portable impedance biosensor. Virus culture was employed as the reference method (gold standard).

[a] TP, true positives. Samples identified as positive by virus culture were considered TP.
[b] TN, true negatives. Samples identified as negative by virus culture were considered TN.
[c] FP, false positives. Samples identified as positive by rRT-PCR or impedance biosensor, but negative by virus culture were considered FP.
[d] FN, false negatives. Samples identified as negative by rRT-PCR or impedance biosensor, but positive by virus culture were considered FN.
[e] Sensitivity = number of TP specimens/(number of TP + number of FN specimens).
[f] Specificity = number of TN specimens/(number of TN specimens + number of FP specimens).
[g] PPV (positive predictive value) = TP/(TP + FP).
[h] NPV (negative predictive value) = TN/(TN + FN).

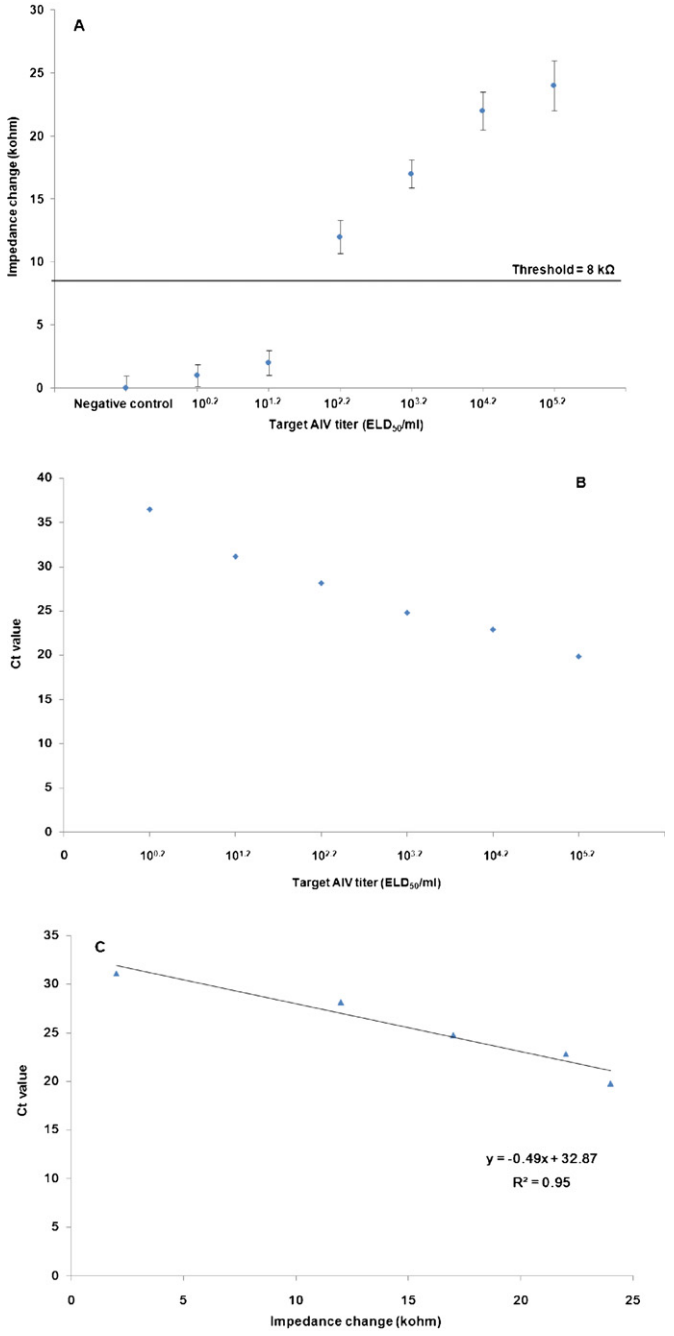

Fig. 2. (A) Relationship between AIV titer and the observed impedance change; (B) real-time RT-PCR for detection of 10-fold serial dilutions of H5N2. The Ct (cycle threshold) value is defined as the number of cycles required for the fluorescent signal to cross the threshold (i.e. exceeds background level); and (C) correlation between the reading of impedance change (kΩ) from impedance biosensor and reading of Ct value from rRT-PCR.

The threshold of the biosensor for the positive detection is set as: biosensor detection limit (6 kΩ) + maximum standard deviation (2 kΩ) = 8 kΩ. Hence, any detection signal greater than 8 kΩ is considered as a positive sample and any signal lower than 8 kΩ is considered a negative sample. The experimental detection limit of the biosensor for detection of the purified AIV H5N2 was observed to be $1 \times 10^{2.2}$ ELD_{50}/ml. As a comparison, rRT-PCR was also used for the detection of 10-fold serial dilutions of H5N2 (Fig. 2(B)). A linear relationship was found between the readings from impedance change (kΩ) measured by impedance biosensor and Ct value tested by rRT-PCR (Fig. 2(C)) in the virus titer range from $1 \times 10^{1.2}$ ELD_{50}/ml to $1 \times 10^{5.2}$ ELD_{50}/ml, and a correlation equation was described as: $y = 0.49x + 32.87$ ($R^2 = 0.95$), where y is the reading of Ct value from rRT-PCR and x is the reading of impedance change (kΩ) from impedance biosensor. In the rRT-PCR assay, a positive reaction is detected by accumulation of a fluorescent signal. The Ct (cycle threshold) value is defined as the number of cycles required for the fluorescent signal to cross the threshold (i.e. exceeds background level). Ct value is inversely proportional to the amount of target nucleic acid in the sample.

3.5. Detection of AIV H5N2 from infected chickens

The results for detection of AIV in tracheal and cloacal swab samples collected from experimentally infected chickens are summarized in Table 1. The latest version of the impedance biosensor was compared with rRT-PCR in terms of sensitivity, specificity, PPV (positive predictive value) and NPV (negative predictive value) using the virus culture results as the gold standard assay. A total of 59 swab samples, which included 35 tracheal swabs and 24 cloacal swabs were tested. For tracheal swab samples, both the impedance biosensor and rRT-PCR were able to detect the target AIVs with an excellent sensitivity and NPV of 100%, although the impedance biosensor method showed less specificity (64%) and PPV (81%). For cloacal swab samples, much better specificity and PPV of 100% were obtained by impedance biosensor in comparison with rRT-PCR, which had specificity and PPV of 69%. The observed sensitivity and NPV were 81% for rRT-PCR and 55%, 72% for the impedance biosensor, respectively. Considering the detection time was less than 1 h for the impedance biosensor and the user-friendly setup in the field, the developed impedance biosensor method has great potential as an alternative for surveillance of epidemic outbreaks caused by AIV.

4. Discussion

This study was conducted in light of a continuing need for rapid sensitive methods for the identification and detection of avian influenza viruses for use in the field or at the patient's bedside. We evaluated the performance of a newly developed portable impedance biosensor system by comparing it to rRT-PCR for detection of AIV in the swab samples collected from experimentally infected chickens. Although based on a small sample size, the present study covered the optimization of incubation time, the evaluation of the used antibody, and the use of both purified virus sample and swab samples, which provided a rigorous test of the performance of the impedance biosensor device.

The sensitivity and specificity of a biosensor is greatly dependent on the antibodies being used in the biosensor. The combination of monoclonal antibodies that were raised against unique epitopes on the AI virus Hemagglutinin (HA) and Neuraminidase (NA) antigens is a logical approach for highly specific detection of AIV while simultaneously minimizing interference from other AIV subtypes that could be present in a field sample. Therefore, in our study, the monoclonal anti-H5 antibody was selected as a capture antibody to selectively capture the target AI virus.

Analysis indicated that the portable impedance biosensor had identical sensitivity (100%) and less specificity (64%/100%) for virus detection in tracheal swabs, and better specificity (100%/69%) and less sensitivity (55%/81%) for virus detection in cloacal swabs, when compared to rRT-PCR. This is probably due to the different detection principle with the rRT-PCR method. The impedance biosensor is based on an immunoassay, and rRT-PCR depends on the use of primers and probes. For the reported rapid immunoassays, such as point-of-case (POC) tests (Drexler et al., 2009; Vasoo et al., 2009; Faix et al., 2009) and the direct fluorescent antibody (DFA) staining

(Ginocchio et al., 2009; Ganzenmueller et al., 2010), the sensitivity for influenza A H1N1 detection ranged from 11% to 67%. In contrast to this, a quartz crystal microbalance immunosensor (Hewa et al., 2009) showed a slightly higher sensitivity of 76% for influenza type A detection without the use of nanoparticles for signal amplification, and 81% with nanoparticle amplification. However, this study was only capable of type-specific viral detection and could not be used for the influenza virus subtype identification. Thus, the modified impedance biosensor presented in the current study offers a number of advantages: (1) the impedance biosensor assay is portable and has great potential for conducting tests on-site or in-field, dramatically reducing the turnaround time for AIV detection; (2) easy operation through step-by-step instructions displayed on the screen of the device makes it user-friendly for field operators with minimal laboratory-based knowledge; (3) it is a time-efficient technology with a total detection time of 30–60 min; (4) the interdigitated array microelectrode based microfluidic biochip can be continuously used until a positive sample is obtained; and (5) the present study demonstrated that the sensitivity and specificity of the impedance biosensor was comparable to rRT-PCR when it was applied to virus detection from swab samples. However, like most current AIV detection methods (immunoassays and molecular methods), the impedance biosensor has a limitation on not being able to differentiate between live and dead virus.

In summary, this study demonstrated that the most recent version of the portable impedance biosensor is suitable for the purpose of rapid screening of AIV from poultry swab samples (tracheal and cloacal). It has great potential to serve as a rapid and sensitive diagnostic and epidemiological tool for detection of AIV in the field. Furthermore, it is entirely possible that this device could also be used for rapid and sensitive detection of samples obtained from human subjects.

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