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Evaluation of multiplex assay platforms for detection of influenza hemagglutinin subtype specific antibody responses

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ABSTRACT

Influenza hemagglutination inhibition (HI) and virus microneutralization assays (MN) are widely used for seroprevalence studies. However, these assays have limited field portability and are difficult to fully automate for high throughput laboratory testing. To address these issues, three multiplex influenza subtype-specific antibody detection assays were developed using recombinant hemagglutinin antigens in combination with Chembio, Luminex®, and ForteBio® platforms. Assay sensitivity, specificity, and subtype cross-reactivity were evaluated using a panel of well characterized human sera. Compared to the traditional HI, assay sensitivity ranged from 87% to 92% and assay specificity in sera collected from unexposed persons ranged from 65% to 100% across the platforms. High assay specificity (86–100%) for A(H5N1) rHA was achieved for sera from exposed or unexposed to hetorosubtype influenza HAs. In contrast, assay specificity for A(H1N1)pdm09 rHA using sera collected from A/Vietnam/1204/2004 (H5N1) vaccinees in 2008 was low (22–30%) in all platforms. Although cross-reactivity against rHA subtype proteins was observed in each assay platform, the correct subtype specific responses were identified 78%–94% of the time when paired samples were available for analysis. These results show that high throughput and portable multiplex assays that incorporate rHA can be used to identify influenza subtype specific infections.

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1. Introduction

Influenza serology tests such as the hemagglutination inhibition (HI) and virus microneutralization (MN) assays are used for retrospective diagnosis and assessment of immune status. Both tests detect antibodies that inhibit the interaction of influenza hemagglutinin (HA) with receptors on red blood cells or cultured cells. The HA glycoprotein, originally named for its capability to agglutinate erythrocytes (Hirst, 1941), is a homotrimer with each monomer consisting of an ectodomain, transmembrane domain, and cytoplasmic tail (Verhoeyen et al., 1980). The precursor form of HA, HA0, is expressed as a single chain that is cleaved by host proteases to generate the native form of the protein. Native HA is comprised of HA1 and HA2 chains linked by a disulfide bond (Steinhauer, 1999).

Antibodies to acute viral infections appear in the blood as early as 10–14 days after infection (Katz et al., 2011; Miller et al., 2008) and can be maintained for decades after infection (Crotty et al., 2003). The continual antigenic drift and occasional antigenic shift of influenza viruses create complex antibody profiles in individuals over a life time of repeated exposure to multiple influenza viruses and vaccines, impeding the resolution and interpretation of influenza subtype specific immunity.

The HI and MN assays are the most reliable and commonly used tests for serologic surveys and seroepidemiologic investigations (Katz et al., 2011; Laurie et al., 2013). Serosurveys evaluate a population's immune status to emerging influenza viruses. This is an important public health surveillance tool which can be used to identify susceptible populations and asymptomatic infections to determine virus transmission rates, and provide more accurate esti-

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mates of disease severity (Broberg et al., 2011; Laurie et al., 2013), which in turn can aid the risk assessment of viruses for their pandemic or epidemic potential. Public health serosurveillance studies often involve the collection of thousands of specimens, highlight the need for high-throughput platforms.

Whereas the HI assay can be performed with inactivated viruses provided by reference laboratories (such as, via https://www.internationalreagentresource.org/), the MN assay requires infectious viruses. Virus propagation needed for both assays may require heightened biological safety levels for emerging influenza viruses and limit assay portability. The development of sensitive and specific serologic assays that do not use whole virus, but do use non-infectious recombinant proteins, offers the potential for improved assay standardization and inter-laboratory reproducibility. At the same time, this change would also make the serology assays more suitable for remote locations, biological safety level 2 laboratories, and high-throughput applications.

In their current form, the HI and MN require technical expertise, limiting their use to experienced laboratories with access to virus. Reagent preparation, liquid handling, and data collection are time consuming components of testing. Alternative serologic assays, such as enzyme-linked immunosorbent assay (ELISA), have been attempted to improve the functionality of serologic assays. Nonetheless, for influenza A virus, ELISA has lacked subtype specificity (Burlington et al., 1985) and multiplexing capabilities (Watson et al., 2009). The multiplex protein microarray was developed to investigate antibody profiling by Koopmans and colleagues, the bivariate model of microarray using recombinant hemagglutinins (rHAs) from A(H1N1)pdm09 and A/1918(H1N1) influenza viruses was used to achieve high sensitivity and specificity (Boni et al., 2013; Freidl et al., 2014, 2016; Huijskens et al., 2013; te Beest et al., 2014). More recently, Wang et al. developed mPLEX-Flu assay in which MagPix platform using full length HAs from multiple strains of influenza A and B viruses, investigated antibody profile and antigenic profile of HA (Wang et al., 2015). Our previous study indicated that assay sensitivity and specificity for one novel subtype HA maybe lower in persons exposed to other novel subtype HA than those unexposed to any novel subtype HA (Li et al., 2014) Despite these recent advances, assay sensitivity and specificity, using a panel of well-characterized sera from recently exposed and unexposed persons, has not been fully investigated in this context.

The 2009 A(H1N1) pandemic and A(H7N9) outbreaks highlight the public health need for rapid and reliable assays to detect influenza subtype-specific antibody responses (Boni et al., 2013; Broberg et al., 2011; Laurie et al., 2013). New technologies and assays are urgently needed to improve assay speed, portability, throughput, sensitivity, specificity, and reproducibility. To address these issues, we evaluated both rapid lateral flow and high throughput laboratory platforms in combination with a set of rHAs and a panel of well-characterized human serum specimens collected from persons exposed or unexposed to specific subtype influenza antigens. Results from these studies demonstrate the utility and caveats of using these platforms to assess influenza subtype-specific antibody responses following influenza virus infection, potentially when novel subtype influenza virus emerges.

2. Material and methods

2.1. Human sera

To evaluate test platforms, 21 paired acute (S1, 1–7 days post symptom onset) and convalescent (S2, 15–52 days post symptom onset) sera were collected from A(H1N1)pdm09 (pH1) virus-infected persons during the first wave of the 2009 A(H1N1) pandemic (April–July 2009) (Table 1). All patients showed seroconversion (a 4-fold or greater rise in antibody titer in convalescent compared with acute phase sera with convalescent sera titers ≥40) by MN and/or by HI (Table 1). The collection of these sera was a part of the CDC U.S. Public Health Emergency Response to the pandemic that did not require CDC Institutional Review Board (IRB) review. In addition, 15 paired pre- (S1, day 0) and post-vaccination (S2, day 28 or 56) sera were collected from U.S. residents enrolled in 2009 who received one dose of 15 μg pH1 monovalent, non-adjuvanted, split vaccine and 23 U.S. residents who received 2 doses of 90 μg A/Vietnam/1203/2004 (H5N1) (H5) monovalent, non-adjuvanted, split vaccine in 2008 (Table 1). S1 sera represent either pre-vaccination or acute sera, S2 sera represent either post-vaccination or convalescent sera (Tables 1 and 2). Subjects that received pH1 or H5 vaccines showed a 4-fold or greater rise in either turkey red blood cell (RBC) or horse RBC HI titer, respectively (Table 1). IRB approval was granted for the collection of H5N1 vaccination specimens by the Centers for Disease Control and Prevention (CDC; ClinicalTrials.gov identifier: NCT00417560) and for the pH1 vaccination sera by Emory University (IRB# 22371). Forty paired sera were collected from U.S. residents who received 2009–10 trivalent Northern Hemisphere inactivated vaccines and showed serum conversion to either A/Brisbane/59/2007 (H1N1) (sH1) and/or A/Brisbane/10/2007 (H3N2) (H3) (Table 1, data not shown). All serum panels were collected from healthy individuals aged 1–79 years of age (Table 1). The serum samples used in determination of sensitivity, specificity for unexposed or exposed to heterosubtypic influenza antigen were described in Table 2.

2.2. Chembio dual path platform

The Chembio Platform (Chembio Diagnostic Systems, NY) was used to construct a portable rapid influenza antibody test containing the full length ectodomain recombinant HAs with histidine tag from A/California/4/2009 (H1N1pdm09, pH1), cat# FR-180; A/Brisbane/59/2007 (H1N1, sH1), cat# FR-65; A/Brisbane/10/2007 (H3N2, H3), cat# FR-61; A/Vietnam/1203/2004 (H5N1, H5), cat# FR-39; A/shorebird/DE/68/2004 (H13N9, H13), cat# FR-73 were obtained through the International Reagent Resource (CDC, Atlanta, Georgia). Positive and negative control antigens included a recombinant protein A and H13 rHA, respectively. Each rHA and the protein A were sprayed onto a nitrocellulose strip mounted in a 95 mm × 50 mm plastic cassette (Fig. 1). The test utilized a filter pad on the vertical sample path to remove red blood cells when testing whole blood and a pad impregnated with dried colloidal gold conjugated protein A on the lateral buffer path to detect antibody bound to immobilized rHA antigens (Fig. 1). To perform the assay, 10 μl of serum or plasma was added to 250 μl of sample diluent and 55 μl of the diluted sample was then added to the sample port. After incubation at room temperature for 5 min, five drops of running buffer were added to the buffer port (Fig. 1). The colloidal gold-protein A/anti HA antibody/rHA or colloidal gold-protein A/human IgG/protein A was formed for testing antigen or protein A control, respectively. The test was read after 15 min of incubation at room temperature using a Chembio Rapid Influenza Immunity Test Reader. The threshold values were determined to achieve optimal sensitivity for post-vaccination or convalescent serum samples (S2) and specificity for serum samples collected from unexposed (S1) or exposed (S2) to heterosubtype influenza for all three platforms in this study (Tables 2 and 3). The threshold values for determining a positive result were established to achieve the combination of at least 87% (20/23) sensitivity for post-vaccination or convalescent serum samples (S2) and at least 65% (15/23) specificity for S1 sera from unexposed persons in Chembio DPP kits (Table 3).

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