Short communication

Comparison of conventional lateral-flow assays and a new fluorescent immunoassay to detect influenza viruses

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A B S T R A C T

Sofia, a novel, fluorescent lateral-flow immunoassay was compared with two conventional colorimetric assays, Quickvue Influenza A+B and Directigen FLU A+B, to identify influenza viral antigen from patient nasopharyngeal specimens. A total of 118 frozen original influenza-positive specimens and 57 prospective specimens were examined. Using rt-PCR as a referee assay, sensitivity values (%) for influenza A/B of 80.0/74.8, 73.3/59.3 and 73.3/40.7 were obtained using the Sofia, Quickvue and Directigen assays, respectively. All assays demonstrated reduced sensitivity for influenza B as compared with influenza A virus. With respect to the Sofía assay, the sensitivity of influenza B for the Directigen assay was significantly diminished. False positive results were not observed in the Sofia and Directigen assays. The Quickvue assay produced 3 false-positive results (2 influenza A and 1 influenza B) resulting in a specificity (%) of 96 and 98 for influenza A and B, respectively. Cross-reactivity to other respiratory viruses was not observed among immunoassays. A sensitivity rank (highest to lowest) of rt-PCR > culture > Sofía > Quickvue > Directigen was established using dilutions of influenza A and B. Sofia provides enhanced diagnostic sensitivity and objective result interpretation over conventional colorimetric immunoassays.

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1. Introduction

Diagnostic immunoassays rely on specific antigen–antibody binding for accurate results. Although variations in format exist, lateral flow immunoassays generally use a hydrophobic reaction membrane strip containing bound, anti-target antibodies. Specimen, treated with reagents to produce target antigen-conjugates, is allowed to migrate along the membrane, producing a colored line if the antigen-conjugate is captured by the bound antibodies. Result interpretation is subjective and dependent on the strength of the color signal.

Advantages demonstrated by lateral flow assays have made them a cornerstone for influenza virus detection. Rapid result time permits point-of-care testing to occur in emergency departments and physician office settings on a 24 h, 7-day-a-week basis. Thus, optimal health care with respect to the administration of antiviral agents or the institution of infection control measures, including patient isolation, can occur readily. Immunoassays are also relatively inexpensive, require little or no equipment, little technical training, and produce results in about 15 min.

Despite these advantages, the sensitivity of rapid influenza immunoassays is lacking with respect to that obtainable using culture or molecular assays. This was exemplified during the recent influenza A/2009 H1N1 pandemic. Reports of low, unacceptable sensitivity values, ranging from 18 to 70% depending on which referee assay was chosen for comparison, tarnished the perception of immunoassay worth as diagnostic tools (CDC, 2009; Ginocchio et al., 2009; Leonardi et al., 2010; Leonardi, 2011).

A novel lateral-flow immunoassay, Sofia (Quidel; San Diego, CA, USA) has recently received CLIA-waived status for diagnostic testing. Instead of subjective, colorimetric results, this assay employs immunofluorescent technology to detect influenza nucleoprotein, producing objective data. Sofia was compared with two other lateral-flow immunoassays, Quickvue Influenza A+B (Quidel) and Directigen FLU A+B (Becton Dickinson, Sparks, MD, USA), to identify influenza viruses from original nasopharyngeal specimens. The Directigen and Quickvue immunoassays have been utilized widely for diagnostic testing and research evaluations (Chartrand et al., 2012). The latter assay has also been CLIA-waived for diagnostic use.

2. Materials and methods

2.1. Specimens

A total of 118 original patient nasopharyngeal swab specimens, identified previously as influenza-positive by immunoassay,
culture and/or rt-PCR were used to evaluate immunoassays. Specimens were stored at either −20 or −70 °C and were obtained during the respiratory virus season from 2006 to 2011. In addition, 57 nasopharyngeal specimens, obtained during the current 2011–2012 influenza season, were evaluated prospectively. These specimens were stored at 2–8 °C prior to testing which was performed within 72 h post-collection. Determination of patient age (children 12 and under vs. adults) and influenza A subtype were also made on study specimens.

Aliquots of consensus-positive influenza A and B virus (NYS-DOH, proficiency testing event # 121, sample nos. 1312 & 1314) were used to prepare sample dilutions which were tested subsequently by immunoassay, shell vial culture and rt-PCR methods. A rank order of sensitivity was established. Different dilution series were used for the influenza A and B samples because of the high titer of the influenza A specimen. Dilutions were chosen and tabulated when an immunoassay failed to identify virus. At each dilution, a sample was tested by each immunoassay once. When a negative immunoassay result occurred at a given dilution, a second sample was tested to confirm the result.

Other common respiratory viruses (respiratory syncytial virus, adenovirus, parainfluenza types 1–3 and human metapneumovirus) were also tested by the immunoassays using original patient specimens. Presence of these viruses was confirmed using shell-vial culture and immunofluorescent antibody methods (D³Ultra DFA respiratory virus and D³ DFA Metapneumovirus ID assays; DHI, Athens, OH, USA).

2.2. Immunoassays

All immunoassays were performed according to manufacturer’s procedures. Specimens were tested simultaneously by all immunoassays. Comparison of immunoassays with respect to ease of use, result reporting, result time, and equipment needs was evaluated.

2.3. Resolution of discrepant immunoassay results by rt-PCR

A molecular rt-PCR assay (ProFLU+, GenProbe/Prodesse; Waukesha, WI, USA) served as the “gold standard” confirmatory method to decipher discordant or negative results in specimens tested among the immunoassays. Shell vial culture (R-mix; Diagnostic Hybrids, Athens, OH, USA) was also inoculated (100 µL) if the remnant specimen volume allowed, so that comparative sensitivity of the immunoassays and culture could be evaluated with respect to gold standard rt-PCR. Cultures were harvested at 48 h post-inoculation and examined using fluorescent antibodies (DHI).

2.4. Statistics

95% confidence intervals were computed for assay sensitivity values using a modified method of Wald (Agresti and Coull, 1998).

3. Results

3.1. Specimens

In 118 influenza-positive specimens (92 type A and 26 type B), 3 specimens negative by all immunoassays and rt-PCR were excluded from the study. Of the remaining 115 retrospective specimens, 20 were negative among all immunoassays (16 type A and 4 type B) but were confirmed as positive using rt-PCR. Virus was identified in 5 of the 57 prospective specimens (4 influenza A and 1 influenza B). One prospective specimen was negative by all immunoassays but confirmed as A/H3-like using rt-PCR.

When retrospective and prospective specimens are separately analyzed, similar sensitivity/specificity values (%) for influenza A and B of approximately 80/100 in were obtained for the Sofia assay. Quickvue and Directigen assay sensitivity for influenza A and B in the retrospective samples were also essentially the same as when all samples were combined. However, influenza A sensitivity values (%) in the Quickvue and Directigen assays were respectively reduced in the prospective samples, producing values of 60 (3 of 5 positive) and 40 (2 of 5 positive samples). All assays produced 100% sensitivity for influenza type B because all correctly identified the one positive prospective sample.

Specimens from children, 12 and under accounted for 84 of the total 175 (48%) samples studied. Of these, 58 of 84 (70.2%) were influenza influenza-positive (51 type A, 7 type B). The pandemic influenza A/2009/H1N1 subtype accounting for 38 of the 51 (74.5%) influenza A-positive specimens among children. Of the total 97 influenza A specimens studied, 48, 31 and 7 were identified as pandemic A/2009/H1N1, seasonal A/H3N2-like and seasonal B/H1N1-like agents, respectively. Subtype identity was not determined for 11 of the 97 influenza A specimens.

3.2. Immunoassay performance

Discrepant results (10 influenza A and 12 influenza B specimens) were produced among the immunoassays (Table 1). Using this data, sensitivity values (%) for influenza A/B of 80/74.8, 73.3/59.3 and 73.3/40.7 were obtained with the Sofia, Quickvue and Directigen assays, respectively. All immunoassays were more sensitive for influenza A rather than influenza B viruses. No false positive results were obtained for the Sofia and Directigen assays; the Quickvue assay exhibited 3 false-positive results, producing specificity (%) values of a 96.0/98.0 for influenza A/B, respectively.

Among 33 cases of discrepant or negative immunoassay results, enough specimen volume remained to permit inoculation of R-mix shell-vial cultures. With respect to rt-PCR, culture identified influenza in 27/33 samples (81.8% sensitivity). All immunoassays produced negative results when tested with other common respiratory viruses.

Dilutions of known positive-influenza A and B specimens, obtained from remnant proficiency testing samples, compared viral detection among immunoassay, culture and molecular assays (Table 2). A rank order for sensitivity was established. From high to low: rt-PCR > culture > Sofia > Quickvue > Directigen. The Directigen and Quickvue assays performed poorly in identifying influenza B samples, failing to identify antigen at dilutions of 1:30 and 1:75, respectively. Sofia produced positive influenza B results at a dilution of 1:800. Although the use of a single sample to presently test the various diagnostic assays at each dilution precludes the ability to calculate statistical significance, the results clearly demonstrate a trend toward increased sensitivity for the Sofia assay and warrants further, more extensive investigation.

In comparing these immunoassays from a laboratory-use perspective, the Quickvue assay was simplest to perform, required less procedural steps, offered the fastest result time and did not require any operational equipment. However, result interpretation for Quickvue was cumbersome as the technician sometimes labored to visualize a pink result line imposed on a pink membrane background. Although the background of the Directigen cassette membrane was less pink in color, visualization of faintly-pink positive results were also sometimes difficult to interpret. The Directigen assay did not require equipment in its operation and produced results in approximately 15 min.

The Sofia assay required a fluorescent analyzer to perform the assay. It also had the most procedural steps in assay operation. Despite these limitations, Sofia demonstrated enhanced sensitivity over its competitors, especially with respect to influenza B
viruses. In addition, Sofia allowed objective results to be obtained, permitted the potential integration into a laboratory information system, could store 500 results in the analyzer memory, provided a hard-copy result and could be operated in either a read now or walk-away testing mode.

4. Discussion

Although the role of lateral flow immunoassays for rapid influenza testing was well established, the reliability of these methods came into question during the 2009 influenza A/H1N1 pandemic. This outbreak necessitated an accurate diagnosis of influenza. Further, the ability to distinguish seasonal and pandemic influenza A/H1N1 subtypes was necessary because of the differing antiviral drug sensitivity profiles of these viruses. Lateral-flow immunoassays could not perform either function effectively.

In subsequent influenza seasons, enzyme immunoassays have been utilized in a limited capacity. Adequate assay specificity permits its use as a first screening tool to rule in disease. Meta-analysis of 159 studies comparing rapid influenza immunoassays reported a pooled specificity (%) of 98.2, supporting this role (Chartrand et al., 2012). However, highly heterogeneous sensitivity values (95% CI of 57.9% to 66.6%) clearly suggest that negative results cannot stand alone and require confirmation by DFA, cell culture or molecular assay.

Direct fluorescent-antibody (DFA) immunoassays demonstrate enhanced sensitivity over their enzymatic counterparts with one investigation reporting similar performance characteristics between DFA and PCR in identifying pandemic influenza (Pollack et al., 2009). An influenza A/2009 H1N1-specific fluorescent immunoassay was also developed, permitting laboratories lacking molecular diagnostic capability the ability to definitively identify this new pathogen (Leonardi, 2010). Unlike enzyme immunoassays, DFA requires extensive technical training, equipment, and greater assay turn-around times. These pitfalls would thus preclude DFA testing in any point-of-care or emergency room setting.

A decrease in sensitivity for influenza B as compared to influenza type A was demonstrated among all immunoassays. This finding parallels the meta-analysis study which reported pooled sensitivity values (%) of 64.6/52.2 for influenza A/B, respectively (Chartrand et al., 2012). A plausible explanation for this finding may rest with the comparative “virulence” of the influenza types. Influenza A leads to more severe disease and higher rates of hospitalization and death than infection with type B (Fiore et al., 2011). Differences

Table 1
Performance characteristics of 3 direct antigen detection assays using rt-PCR as a referee assay to evaluate discrepant and negative immunoassay results.

<table>
<thead>
<tr>
<th>ITEM</th>
<th>Influenza type A specimens</th>
<th>Influenza type B specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sofia</td>
<td>Quickvue</td>
</tr>
<tr>
<td>TP</td>
<td>72</td>
<td>66</td>
</tr>
<tr>
<td>FN</td>
<td>18</td>
<td>24</td>
</tr>
<tr>
<td>TN</td>
<td>51</td>
<td>49</td>
</tr>
<tr>
<td>FP</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>SENSITIVITY (%)</td>
<td>80.0</td>
<td>C1 (95%)</td>
</tr>
<tr>
<td></td>
<td>73.3</td>
<td>70.5-87.0</td>
</tr>
<tr>
<td>TN</td>
<td>73.3</td>
<td>73.3</td>
</tr>
<tr>
<td>NPV (%)</td>
<td>159</td>
<td>100</td>
</tr>
</tbody>
</table>

FP: false positive; FN: false negative; TN: true negative; TP: true positive. Sensitivity was calculated as TP/TP+FN x 100%. Specificity was calculated as TN/TN+FP x 100%. PPV: positive predictive value, calculated as TP/TP+FP; NPV: negative predictive value, calculated as TN/TN+FN. CI: 95% confidence interval.

4 ProFlu+ and ProFAST+ assays (GenProbe-Prodesse; Waukesha, WI).

5 In 33 cases of discrepant or negative results among the antigen detection assays, enough specimen remained for inoculation of R-mix shell vials. Viral antigen was culture-confirmed in 27 of 33 cases (81.8% sensitivity).

Table 2
Ability of diagnostic assays to identify influenza A and B viruses using dilutions of positive proficiency testing samples (New York State Department of Health-CLEP Program).

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Dilution</th>
<th>Antigen detection</th>
<th>Culture</th>
<th>rt-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influenza A</td>
<td>1:100</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>1:400</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>1:100</td>
<td>−</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Influenza B</td>
<td>1:10,000</td>
<td>−</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>1:250</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>1:30</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>1:75</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>1:100</td>
<td>+</td>
<td>−</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>1:800</td>
<td>+</td>
<td>−</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>1:1,280</td>
<td>−</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND: no data.

All assays were performed according to manufacturer’s recommended procedures.

Shell vial cultures (R-mix, DHI, Athens, OH) were inoculated with specimens (180 μl), harvested after 24 h and stained with appropriate monoclonal antibodies (DHI, Inc.).
Nucleic acids extracted using an EasyMag (Biomerieux, Inc.) and tested using ProFlu+ and ProFAST+rt-PCR assays (Gen Probe/Prodesse; Waukesha, WI).
in virulence also exist among individual subtypes of influenza A, where A/H3N2 demonstrates far greater disease severity than seasonal A/H1N1 infection (Chartrand et al., 2012). Greater disease severity usually means greater viral load and thus, greater observed sensitivity in studies that predominantly examine influenza A samples, particularly the A/H3N2-like subtype. Among influenza A specimens evaluated presently, 7, 31, and 48 were identified as seasonal H1-like, seasonal H3-like and 2009 H1N1-like subtypes, respectively.

Variations in assay sensitivity can also be influenced by the age of patients submitting specimens for study. Influenza immunoassays reportedly perform better in children than adults, presumably because higher viral loads and longer duration of shedding occur in the former (Fiore et al., 2011). Meta-analysis has reportedly demonstrated an approximate 13% increase in assay sensitivity when children were studied (Chartrand et al., 2012).

The Quickvue and Directigen assays produced modestly greater sensitivity values than obtained in the meta-analysis of pooled studies examining these products. In addition, influenza A/B sensitivity for Sofia was even higher than observed among its competitors. A study bias based on the selection of specimens tested may account for the increased sensitivity values observed presently. Factors including a high percentage of positive-influenza A to B specimens (ratio of approximately 4:1), the high percentage of children’s specimens studied (approximately 50%) and a paucity of seasonal H1N1 samples would all favor increased assay sensitivity.

A second source of bias may exist with respect to samples which were positive by all immunoassays but not confirmed presently using gold standard rt-PCR. Positive confirmation was not performed because the amount of sample left following immunoassay testing was often insufficient to permit such analysis. Further, the fact that these specimens were previously identified as positive using methods considered more sensitive than immunoassay (culture and/or rt-PCR) and were currently identified as positive by 3 immunoassays which detect differing antigens and or antigenic sites seem to make the potential for false positive results highly unlikely.

In head-to-head, same-specimen, simultaneous evaluation, the Sofia outperformed its competitors providing greater accuracy over traditional colorimetric immunoassays. Sofia also produced comparable sensitivity to culture, however, the frozen-retrospective patient specimens may have contained non-viable virus which would enhance antigen immunoassay detection but negatively bias culture results. In the dilution studies, culture demonstrated greater sensitivity over the Sofia assay.

Sofia may represent a next step in the evolution of immunoassays, combining lateral flow and fluorescent antibody detection formats. The choice of fluorescent rather than colorimetric detection is at least partially responsible for the improved sensitivity demonstrated by Sofia because the former is known to increase sensitivity while also widening the dynamic assay detection range over the latter (Gibbs, 2001). Even greater sensitivity in future assays may be achieved with the development of luminescent immunoassays, which presumably may amplify results similar to that seen in PCR.

With respect to sensitivity, the Sofia assay demonstrated a statistically significant increase in influenza B viruses over the Directigen assay. Although a trend toward higher sensitivity for influenza A was observed for Sofia, the results among assays were equivalent statistically. A larger clinical study may be needed to demonstrate any potential significance for influenza A viruses among these assays.

Features offered by Sofia, including production of objective results, operation in a run-now or walk-away mode, and having hard copy and memory stored results make it valuable to the diagnostic laboratory. The enhanced sensitivity demonstrated by the Sofia assay helps reestablish the value of these methods for rapid diagnostic testing, allowing meaningful patient management decisions to be made at the point-of-care.

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References


