Detection of Influenza A and B Viruses With the Sofia Analyzer

A Novel, Rapid Immunofluorescence-Based In Vitro Diagnostic Device

Kent Lewandrowski, MD,1 John Tamerius, PhD,2 Marilyn Menegus, PhD,3 Paul D. Olivo, MD, PhD,2 Ron Lollar,2 and Elizabeth Lee-Lewandrowski, PhD1

Key Words: Influenza virus; Rapid diagnostic test

DOI: 10.1309/AJCP7ZTLJCP3LLMA

Abstract

This report describes the clinical evaluation of a novel fluorescent immunoassay (FIA), Sofia Influenza A+B FIA (Quidel, San Diego, CA), for the rapid detection and differentiation of influenza A and B viruses. A total of 2,047 subjects provided nasal swabs and nasopharyngeal swabs or aspirates. The overall sensitivity and specificity for influenza A virus vs virus culture were 94% and 95%, respectively, and for influenza B virus were 89% and 96%, respectively. Fourteen hundred and sixty-one specimens were available for testing with reverse transcriptase–polymerase chain reaction (RT-PCR). The sensitivity of the Sofia Influenza A+B FIA for detecting influenza A and B viruses compared with the RT-PCR method was 78% and 86%, respectively. A high percentage of the positive specimens had low cycle threshold values, and almost all of these were positive with the Sofia test. This high level of sensitivity demonstrates that the Sofia influenza A+B FIA could improve the usefulness of rapid influenza virus testing.

The accurate detection of influenza virus in respiratory tract specimens is an important component in the treatment of patients with influenza by health care providers and the control of influenza epidemics by public health officials. Many types of in vitro diagnostic tests are offered by laboratories to aid in the diagnosis of an influenza virus infection.1 Traditional culture methods are reasonably accurate but labor intensive and slow. Direct fluorescent antigen (DFA) methods are more rapid but also require a skilled technologist. Molecular diagnostic tests based on reverse transcriptase–polymerase chain reaction (RT-PCR) methods are highly sensitive but are generally expensive, often require highly skilled laboratory personnel, and have a rapid turnaround time of 2 hours or more.2 Rapid influenza antigen detection tests (RIDTs), such as lateral flow assays, are simple to perform and provide results in minutes but have demonstrated lower than desired sensitivity in many cases.3

The sensitivity problem of RIDTs was reported to be more pronounced with the 2009 H1N1 pandemic strain.4-6 Many studies and 2 recent meta-analyses reported a wide range of sensitivities for various RIDTs used in different clinical circumstances.3,7,8 Nevertheless, RIDTs remain a useful clinical tool, especially because the diagnosis of influenza cannot be made on the basis of clinical criteria alone.9 RIDTs have been shown to improve decision making in the emergency department.10 In particular, studies have also shown that RIDTs improve patient care by reducing the number of radiographs, unnecessary antibiotic usage, and emergency department length of stay.11,12 It also has been demonstrated that combining clinical findings with rapid tests can improve appropriate use of antiviral therapy.13 Effective and appropriate antiviral therapy depends on
obtaining accurate results in a timely manner because anti-
viral therapy is most effective within 48 hours of onset of
symptoms.14-16 One report emphasized the importance of
RIDTs for travelers with influenza-like illness (ILI), and the
value of RIDTs has been shown to be particularly high in
resource-limited settings.17-19 There is a need, therefore, for
a rapid influenza test that has greater sensitivity and does
not rely on subjective interpretation.

Recently a novel fluorescent immunoassay (FIA) (Sofia
Influenza A+B FIA, Quidel, San Diego, CA) was developed
to detect and differentiate influenza A and B in patient speci-
mens. The Sofia Influenza A+B FIA is a commercially avail-
able in vitro diagnostic device that detects viral nucleoprotein
antigens. Although capable of detecting a broad range of
influenza A virus subtypes, it was not designed to specifically
identify the virus subtype. The test is a fluorescence-based
lateral flow immunoassay in which results are analyzed objec-
tively by a compact instrument (Sofia Analyzer) rather than
visually. Instead of antibody-tagged colored microparticles,
the Sofia technology uses a unique polystyrene microbead
that has been impregnated with a chelate of europium. The
europium compound yields a very efficient conversion of UV
energy from 365 nm to a wavelength of 618 nm. This large
Stokes shift avoids problems associated with many fluores-
cent compounds that may be present in the test samples. The
use of europium-based immunofluorescence also provides for
high analytic sensitivity.

In this report we describe a multicenter prospective
clinical trial in which the Sofia Influenza A+B FIA test was
compared with viral culture and RT-PCR for detecting influ-
enza A and B viruses in respiratory specimens from patients
with ILI.

Materials and Methods

Clinical Sites

This study was conducted at 16 distinct sites in the
United States and involved a range of operator skill levels
(Table 1). All procedures were in accord with the ethical
standards of the institution in which the experiments were
performed, including obtaining approval from a local institu-
tional review board (IRB) or a third-party IRB.

Clinical Study Subjects

Inclusion Criteria

Patients enrolled in the study had 1 or more of the
following signs and symptoms: fever (temperature ≥38°C
either at the time of visit or onset within the previous 2
days), nasal congestion, rhinorrhea, sore throat, cough, head-
ache, myalgia, or malaise.

All enrolled subjects or a parent or guardian gave their
informed consent before enrollment.

Exclusion Criteria

Patients who were either treated with anti-influenza
antivirals or vaccinated with a live-attenuated influenza virus
nasal vaccine within the previous 7 days were excluded from
the study.

Specimen Collection

Nasal swabs (NS), nasopharyngeal (NP) swabs, and NP
aspirates (NPA) were collected using standard procedures.

Viral Culture

All specimens were transported on ice (not frozen),
to the laboratory for culture within 48 hours of specimen
collection. Influenza virus detection in cell cultures was
performed either at the laboratory of Diagnostic Hybrids Inc
(DHI, Athens, OH), a Quidel Company, or at the 2 clinical
sites (University of Rochester and BSR Laboratories) that
had virus and cell culture expertise. Influenza virus was
identified in infected, cultured cells using a DFA method.
Protocols for each laboratory are described below.

DHI Viral Cell Culture/DFA Staining Method

R-Mix Too Cluster Plates (DHI) were inoculated with
a 0.2-mL aliquot of the specimen and incubated at 35°C to
37°C, 5% CO₂ for 40 to 48 hours. The inoculated cells were
recovered from tissue culture and tested for influenza A
and B with DFA staining using D3 Duet DFA Influenza A/
Respiratory Virus ID Kit (DHI) for identifying influenza A
and the D3 Ultra DFA Respiratory Virus ID Kit (DHI) for
identifying influenza B.

| Table 1 |

<table>
<thead>
<tr>
<th>Location</th>
<th>Type of Practice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Austin, TX</td>
<td>Pediatric clinic</td>
</tr>
<tr>
<td>Chicago, IL</td>
<td>Emergency medicine</td>
</tr>
<tr>
<td>Norristown, PA</td>
<td>Family medical clinic</td>
</tr>
<tr>
<td>Miami, FL</td>
<td>Family medical clinic</td>
</tr>
<tr>
<td>Naperville, IL</td>
<td>Family medical clinic</td>
</tr>
<tr>
<td>Northglenn, CO</td>
<td>Family medical clinic</td>
</tr>
<tr>
<td>Far Rockaway, NY</td>
<td>Family medical clinic</td>
</tr>
<tr>
<td>Rochester, NY</td>
<td>Pediatric clinic</td>
</tr>
<tr>
<td>Kansas City, MO</td>
<td>Pediatric medical clinic</td>
</tr>
<tr>
<td>San Diego/Carlsbad, CA</td>
<td>Family medical clinic</td>
</tr>
<tr>
<td>Tampa, FL</td>
<td>Family medical clinic</td>
</tr>
<tr>
<td>Florence, KY</td>
<td>Family medical clinic</td>
</tr>
<tr>
<td>Panorama City, CA</td>
<td>Family medical clinic</td>
</tr>
<tr>
<td>Mt. Sterling, KY</td>
<td>Family medical clinic</td>
</tr>
<tr>
<td>Akron, OH</td>
<td>Family medical clinic</td>
</tr>
<tr>
<td>Milwaukee, WI</td>
<td>Emergency medicine</td>
</tr>
</tbody>
</table>
University of Rochester Viral Cell Culture/DFA Staining Method

Specimens were transported daily from Twelve Corners Pediatrics, Rochester, NY, to the nearby virus laboratory at the University of Rochester Medical Center, Rochester, NY. Madin Darby Canine Kidney cell culture tubes (DHI) were inoculated with a 0.26-mL aliquot of the specimen and incubated at 34°C. The inoculated cells were recovered from tissue culture on day 7 and tested for influenza A and B with the Light Diagnostics Influenza A and B DFA Kit (EMD Millipore, Billerica, MA).

BSR Laboratory (Belton, Texas) Viral Cell Culture/DFA Staining Method

R-Mix Too Shell Vials (DHI) were inoculated with a 0.25- to 0.4-mL aliquot of the specimen and incubated at 35°C to 37°C (non-CO2) for 48 to 72 hours. The inoculated cells were recovered from tissue culture and tested for influenza A and B using D3 FastPoint L-DFA Influenza A/Influenza B (DHI).

Detection of Influenza Virus Using Sofia Influenza A+B FIA

The Sofia analyzer contains a microprocessor-controlled optics unit that scans the length of the nitrocellulose test strip, thereby exposing the strip to UV light from a UV light-emitting diode. A positive result for either analyte is determined by the detection of a fluorescent signal at levels above a signal threshold set on scanning a “negative-control” line. This process is controlled by a specific algorithm embedded in the Sofia analyzer. The test strip is processed in the Sofia analyzer, which automatically scans the test strip, collects and analyzes the fluorescence data, and then calculates and reports the result in about 1 minute. There is no visual signal, and the laboratory technician must use the Sofia analyzer to obtain a test result. These features eliminate the subjectivity involved in visually read lateral flow assays. The test result can be printed directly from the instrument and/or transmitted to a laboratory information system.

The Sofia Influenza A+B FIA testing was performed in accordance with the manufacturer’s recommendations. Study sites were instructed to follow the Sofia Influenza A+B FIA package insert, and the Sofia analyzer was used in compliance with the manufacturer’s user manual (Quidel). After the sample was prepared and added to the cassette, it was incubated for 15 minutes, either on the bench top or in the Sofia analyzer, before being analyzed.

External Positive and Negative Controls

Positive and negative external controls were provided with the Sofia FIA kit. These noninfectious swab specimens were tested as instructed in the package insert. Positive and negative external controls were run at each site on each day of use of the Sofia Influenza A+B FIA during the clinical study. The negative control contains a lysate of group C Streptococcus. The positive control contains recombinant influenza nucleoprotein and uniformly gives a positive signal if the analyzer is functioning properly.

Detection of Influenza Virus RNA With RT-PCR

Influenza A and B virus RNA was extracted from the specimens using the EasyMag (bioMérieux, Lyon, France). Viral RNA was detected with RT-PCR with the Quidel Molecular Influenza A+B kit (Quidel) and the ABI 7500 thermocycler instrument (Life Technologies, Carlsbad, CA).

Results

Clinical Performance

A total of 2,152 subjects from 16 sites (Table 1) were enrolled in this study. Because of technical problems, deviation from the protocol, or lack of culture results, 105 specimens were excluded from the study. Data from 2,047 subjects were included in the analyses. Six hundred sixty-five subjects provided an NS; 733 provided an NP swab, and 649 provided an NPA/wash specimen. Table 2 shows the gender and age distribution of the 2,047 subjects enrolled in the study.

Comparison of Sofia Influenza A+B FIA With Virus Culture

Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for Sofia Influenza A+B FIA compared with culture were calculated for each virus and each sample type Table 3 and Table 4. The overall sensitivity of the Sofia Influenza A+B FIA for detecting influenza A virus vs culture was 94% (292/310). The overall specificity was 95% (1,650/1,737). The sensitivity for each specimen type was 90%, 97%, and 99% for NS, NP swabs, or NPA, respectively (Table 3). The specificity ranged from 95% to 96% across all sample types.
The overall sensitivity of the Sofia Influenza A+B FIA for detecting influenza B virus compared with culture was 90% (247/274). The overall specificity was 96% (1,707/1,771). The sensitivity for each type of specimen was 89%, 90%, and 88% when testing NS, NP swabs, or NPA, respectively (Table 4). The specificity ranged from 96% to 97% for all sample types.

### Comparison of Sofia Influenza A+B FIA With RT-PCR

The overall sensitivity of the Sofia Influenza A+B FIA for detecting influenza A virus compared with RT-PCR was 78% (260/333; confidence interval, 73.6-82.5) **Table 5.** The overall specificity was 100%. The sensitivity for each specimen type was 89%, 90%, and 88% when testing NS, NP swabs, or NPA, respectively (Table 4). The specificity ranged from 96% to 97% for all sample types.

The overall sensitivity of the Sofia Influenza A+B FIA for detecting influenza B virus in comparison with RT-PCR was 86% (211/245) (Table 5). The overall specificity was 98.4% (1,197/1,216). The sensitivity of the Sofia Influenza A+B FIA compared with PCR for each type of specimen was 96%, 87% and 88% when testing NS, NP swabs, or NPA, respectively (data not shown). The NPV and PPV were 97.2% and 92.7%, respectively.

The sensitivity of Sofia Influenza A+B FIA compared with RT-PCR for detecting influenza A and B viruses was dependent on the level of RNA in the sample as assessed by the PCR cycle threshold value **Figure II.** Seventy-seven (77%) percent of the patients who were PCR positive for influenza A had cycle threshold values less than or equal to 30, and 96% of these were detected as positive on the Sofia Influenza A+B FIA. Similarly, about 78% of the patients who were PCR positive for influenza B had cycle threshold values less than or equal to 30, and 99% of these tested positive on the Sofia assay.

### Discussion

In this study, the Sofia Influenza A+B FIA, performed by hospital and physician office personnel using NS, NP swabs, or NPA specimens, yielded good sensitivity and specificity

### Tables

- **Table 3: Sofia FIA Performance vs Influenza A Culture**
  - **Table 4: Sofia FIA Performance vs Influenza B Culture**
  - **Table 5: Performance of Sofia FIA for Influenza A and B Viruses Compared With RT-PCR**
Figure 1: Sensitivity of the Sofia fluorescent immunoassay for influenza A and B viruses compared with reverse transcriptase–polymerase chain reaction (RT-PCR) as a function of cycle threshold (Ct) values. Bars show the cumulative percentage of 929 nasal and nasopharyngeal swab samples that were positive with Sofia when the RT-PCR results gave the indicated Ct values (Sofia positives/RT-PCR positives). Triangles indicate the cumulative percentage of RT-PCR positive samples (number positive/total positive × 100). A, Influenza A virus. B, Influenza B virus.

in comparison with cell culture and RT-PCR. These performance results, combined with some of the practical advantages of this technology, demonstrate that the Sofia Influenza A+B FIA could be significantly useful in many health care delivery settings. This test has all the advantages of traditional RIDTs but offers an objective readout. In addition, the sensitivity seen in this study is higher than all reports on RIDTs.

Although cell culture was traditionally considered the gold standard for sensitivity assessments, recent molecular methods have been shown to be the most sensitive for detecting these viruses. One problem with using RT-PCR as a comparator is that the clinical significance of low levels of viral RNA remains controversial. The ability to detect viral RNA continues after symptoms have waned and certainly after antiviral therapy has been effective. In the present prospective study, most of the samples yielded high levels of viral RNA for which the sensitivity of the Sofia FIA was almost 90%. This suggests that the sensitivity is highest in circumstances in which the results are clinically most useful to support effective antiviral therapy.

This study focused primarily on children with ILI and included a comparatively low percentage of elderly patients. Further studies are needed to demonstrate the clinical performance of this test in older patients, especially because RIDTs have been reported to perform less effectively in hospitalized, elderly patients. Also, although analytical studies showed excellent performance for detecting numerous influenza A virus subtypes, viral subtyping was not performed in this study.

The treatment of patients with ILI has been shown to be significantly benefited by the availability of a rapid result of an influenza virus detection test. The ideal RIDT should be rapid, sensitive, specific, simple to perform, economically feasible, and, perhaps most importantly, provide actionable results for the physician. When used within 48 hours of onset of symptoms, the Sofia FIA will yield highly accurate, objective results within 15 minutes. Indeed, of the patients with PCR-confirmed influenza A virus infection in this clinical study, 80% had relatively high viral loads (cycle threshold values less than 30); of these, 96% tested positive with Sofia. The Sofia assay system invites more clinical studies to firmly establish the benefits it can bring to patient care, hospital bed management, and reduction of nosocomial spread of influenza infections. The Sofia FIA’s clinical performance in comparison with culture and PCR was remarkably good and assures the delivery of actionable results to physicians within minutes of their patients’ visits. RIDTs generally have had very good specificity, which provides clinicians the confidence to act appropriately when a positive result is obtained. In this study, the higher sensitivity achieved with Sofia and the high NPV demonstrates that Sofia can reduce reflex testing of negative samples and reduce the number of cases managed as presumptive influenza while clinicians wait for the results of confirmatory testing. Additional clinical studies are needed to more fully delineate the contribution that this test could make to improved treatment of patients with ILI in various health care settings.

From the 1Department of Pathology, Massachusetts General Hospital, Harvard Medical School, Boston, MA; 2Quidel Corp, San Diego, CA; and 3Department of Clinical Virology, University of Rochester Medical Center, Rochester, NY.
This study was supported by funding from Quidel Corp.
Address reprint requests to Dr Lewandrowski: Dept of Pathology, Massachusetts General Hospital, Boston, MA; Elewandrowski@partners.org.

Drs Lewandrowski and Lee-Lewandrowski have received research funds and consulting fees from Quidel Corp. Drs Olivo and Tamerius and Mr Lollar are employees of Quidel Corp.

Acknowledgments: We thank the health care providers at the trial sites, including the physicians, nurses, technologists, and laboratory supervisors.

References