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Evaluation of the Cepheid Xpert Flu Assay for Rapid Identification and Differentiation of Influenza A, Influenza A 2009 H1N1, and Influenza B Viruses

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The Xpert Flu Assay cartridge is a next-generation nucleic acid amplification system that provides multiplexed PCR detection of the influenza A, influenza A 2009 H1N1, and influenza B viruses in approximately 70 min with minimal hands-on time. Six laboratories participated in a clinical trial comparing the results of the new Cepheid Xpert Flu Assay to those of culture or real-time PCR with archived and prospectively collected nasal aspirate-wash (NA-W) specimens and nasopharyngeal (NP) swabs from children and adults. Discrepant results were resolved by DNA sequence analysis. After discrepant-result analysis, the sensitivities of the Xpert Flu Assay for prospective NP specimens compared to those of culture were 97.5%, 100%, and 93.8%, respectively. We conclude that the Xpert Flu Assay is an accurate and rapid method that is suitable for on-demand testing for influenza viral infection.

lower respiratory tract viral infections, including those due to influenza virus, are among the most common infectious diseases in humans, and they are associated with significant morbidity and mortality. These infections are a major economic burden in terms of both health care costs and lost income and productivity of workers (14, 15). Hospitalization of the very young or very old usually occurs, with increased admission rates for those individuals with underlying medical conditions or comorbidities (5). Antiviral treatment for influenza is available and effective if the disease is identified early in the course of illness, so rapid and accurate laboratory diagnosis is particularly important in both inpatient and outpatient settings (2, 6, 9). Some studies show that even treatment within the first 4 days of illness can have a positive impact on viral clearance and the disease’s course (10). In addition, knowing the etiologic agent of these infections can result in significant improvement of patient management by permitting the judicious use of antiviral agents in an era where antiviral resistance is continuing to increase. The introduction of highly accurate and rapid molecular assays for respiratory virus detection has prompted many laboratories to discontinue traditional culture or rapid, antigen-based techniques for influenza virus testing (3, 4, 11–13, 16, 18, 25). During the 2009 H1N1 influenza A virus pandemic, subtyping of influenza virus strains became important for monitoring the spread of the outbreak and managing high-risk patients. The emergence of this pandemic strain prompted several manufacturers to develop laboratory test systems to detect and subtype influenza viruses. Studies have shown the superior sensitivity of molecular diagnostic assays compared to that of other assays for the detection of respiratory viruses (8, 23, 24). Accurate assays with a short turnaround time (TAT) would promote appropriate antiviral therapy, potentially reduce antibiotic use, and initiate proper clinical management of patients in both inpatient and outpatient settings.

To date, five commercial molecular assays have received FDA clearance for the detection of viral respiratory pathogens, including the xTAG Respiratory Viral Panel (Luminex Corp., Austin, TX), Prodesse ProFlu+ (Gen-Probe Inc., San Diego CA), FilmArray (Idaho Technology Inc., Salt Lake City, UT), Liat Influenza A/B (Iquum, Marlborough, MA), and the Cepheid Xpert Flu Assay (Cepheid, Sunnyvale, CA). Commercial assays range from those that detect only influenza A and B viruses to those that detect as many as 15 viral pathogens (18–20, 22). The identification of other viral respiratory pathogens can still be important in the inpatient setting, since knowledge of the etiologic agent can be a factor relevant to the overall clinical management of a patient.

From a workflow perspective, conventional molecular assays in the United States not only require a licensed skilled technologist to run test batches but may require up to 8 h or more to complete. Complexity and a longer hands-on time for an assay are typically associated with a higher cost per test, especially when low numbers of specimens are tested. As the respiratory virus season wanes, maintaining higher-complexity assays may be challenging in the
day-to-day operation of a clinical laboratory due to low volume. Some institutions may choose to develop population-specific testing algorithms, for example, by testing only inpatients using assays that cover a broader range of virus types. Currently, the only Clinical Laboratory Improvement Amendments (CLIA)-waived laboratory tests for influenza virus are point-of-care antigen detection assays that typically lack sensitivity and have variable specificity (1, 8). The American Society for Microbiology does not recommend rapid antigen tests for primary screening for influenza virus because of their suboptimal positive predictive value (PPV) and low sensitivity. (Algorithm available at www.asm.org). In addition, the Centers for Disease Control and Prevention also warn physicians of the limited sensitivity of rapid antigen assays and that a negative result should be interpreted with caution (1a). However, the desire for short TAT and accuracy still exists. High-complexity molecular methods, despite using PCR, do not necessarily satisfy the need for a short TAT.

Cepheid has developed a moderately complex integrated nucleic acid amplification system that automates sample preparation and real-time PCR and detects the influenza A, influenza A 2009 H1N1, and influenza B viruses. This new Xpert Flu Assay is similar to the previous influenza A 2009 H1N1 virus test from Cepheid, which was released for emergency use only during the 2009 pandemic (7, 21). The newer assay provides enhanced detection of influenza A virus, separately identifies the pandemic 2009 H1N1 strain, and detects influenza B virus. The assay can be used in a routine clinical microbiology laboratory or nearer to a patient, which might have an impact on patient management. In this study, we compared the performance of the Cepheid Xpert Flu Assay to that of culture or an alternate PCR method in a clinical trial using both prospective and archived respiratory samples.

MATERIALS AND METHODS

Study population and sample collection. The prospective arm of this study was conducted at five health care institutions across the United States and Australia during 13 May 2010 through 29 October 2010. In the archived arm of the study, specimens were collected from three institutions from 23 February 2007 through 19 March 2010. One site collected only NP swabs, and the other two sites collected NA-W specimens. Eligible patients included children and adults with suspected influenza virus infection from whom NA-W specimens or NP swab specimens were collected according to the institutions’ standard practices. Table 1 shows the age distribution of the study population. Only residuals of specimens submitted for such testing were used; therefore, informed consent was waived by the institutional review board (IRB) at each site. Duplicate specimens from the same patient were excluded.

For the fresh, prospectively collected specimens, after the establishment of patient eligibility and following the standard testing performed at each institution, a portion of the specimen was used for Xpert Flu Assay testing. Inclusion criteria for fresh prospective specimens included those NA-W or NP specimens for which there was sufficient excess material for testing by the Xpert Flu Assay, culture (if not previously conducted as part of the standard-of-care testing), and DNA sequence analysis. Fresh prospective specimens that were excluded were those specimens from subjects enrolled previously in the study.

Inclusion criteria for archived specimens included those NA-W or NP specimens that had been stored at −70°C. Sufficient excess specimen had to be available for Xpert testing, Xpert retesting, DNA sequence analysis, and Prodesse ProFlu+ testing (if viral culture was not performed at the time of sample collection). Specimens requiring DNA sequence analysis were shipped to a single reference laboratory for testing.

Investigational sites. Prospective samples were collected at three Australian and two U.S. sites. Archived specimens were obtained from three U.S. sites. The enrollment sites were North Hollywood, CA; Charlotteville, VA; Westmead, Australia; Neulands, Australia; Randwick Australia; and Aurora, CO. The study was performed with the approval or waiver of the IRB at each investigational site.

Molecular detection. The Xpert Flu Assay was performed at each participating institution as instructed by the manufacturer. Briefly, for NA-W specimens, 600 μl of sample was placed into a 3-ml UTM (universal transport medium) tube. The sample was inverted three times, 300 μl was added to the Xpert cartridge, and binding reagent 1 was added. For nasal swab specimens, 300 μl of the UTM containing the swab was added to the cartridge and then binding reagent 1 was added. For each day of study testing, three positive controls (one for influenza A virus, one for influenza A 2009 H1N1 virus, and one for influenza B virus) and one negative control provided by the sponsor were tested with the Xpert Flu Assay prior to the testing of patient samples. Any Xpert Flu Assay not yielding results on the first attempt was repeated once using new reagents and a new cartridge according to the manufacturer’s instructions. If a result was not obtained upon retesting, the results were reported as indeterminate.

The Prodesse ProFlu+ assay was run with only a portion of the archived specimens (Table 2). Two sites performed the ProFlu+ assay. One site used the bioMérieux EasyMag and followed the procedure according to the manufacturer’s instructions. The other site used the Qiagen EZ1 with the Virus 2.0 protocol using a 200-μl input and a 60-μl elution volume. The Prodesse samples were subjected to the same freeze-thaw cycle as the Xpert Flu Assay. Controls for the ProFlu+ assay were run according to the manufacturer’s package insert. During the study, one site observed inhibition rates of approximately 4% (NA-W) and 0% (NP) using the ProFlu+ assay.

Viral culture. Viral culture was performed at four of the testing sites. Viral culture was performed with a subset of the archived specimens (Table 3) and all of the prospective specimens (Tables 4 and 5). Three sites employed Madin-Darby canine kidney cell shell vial cultures with incubation for 2 to 7 days, depending on the site, followed by immunofluorescence assay utilizing reagents from Light Diagnostics (Millipore), Billerica, MA; Boots-Celltech Diagnostics, Plainview, NY; or Chemicon International (Millipore), Billerica, MA, and different staining protocols. One site used R-Mix Too Ready Cells for rapid culture of influenza virus (Diagnostic Hybrids, Athens, OH). The R-Mix assay was performed as directed by the manufacturer. Collection in UTM or viral transport medium was used at those sites that collected NP swabs.

DNA sequence analysis. All specimens positive for influenza A virus by culture or the ProFlu+ assay were sequenced to identify the 2009 H1N1 strain. All influenza B virus discrepant samples were also sequenced. Sequences for analysis were generated with primers that flank the genomic sequences targeted by the Xpert Flu Assay. Specimens were received at Cepheid on dry ice (frozen). Samples were thawed, and RNA extractions were performed using the Viral RNA Mini Isolation kit (Qiagen catalog no. 50906). cDNA was generated from purified RNA using the One Step reverse transcription–PCR Mini Isolation kit (Qiagen catalog no. 210212) with target-specific primers on a PTC200 DNA PCR thermal cycler (48-well dual module; MJ Research). The amplicon was analyzed for purity and concentration using an Agilent Bioanalyzer (catalog no. G2938C). The cDNA was aliquoted into 96-

<table>
<thead>
<tr>
<th>Table 1 Age distribution of the patients in this study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient age (yr)</td>
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<tr>
<td>≤5</td>
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<tr>
<td>6–21</td>
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<tr>
<td>22–59</td>
</tr>
<tr>
<td>≥60</td>
</tr>
<tr>
<td>Unknown</td>
</tr>
</tbody>
</table>
well microtiter plates and shipped to the vendor for sequencing. The cDNA amplicons were used as templates for bidirectional sequencing using Applied Biosystems BigDye Terminator Cycle Sequencing Kit. A positive-control sequencing reaction (pGEM) was included in each batch of sequencing runs. Sequencing reaction products were analyzed on ABI3730xl automated DNA analyzers. The fluorescence data were automatically analyzed using Sequence Analysis software (Applied Biosystems). Sequencher software (Gene Codes) was used to assemble the sequence data generated for each PCR product.

Acceptable sequence reads that were used to construct the consensus sequences met the following criteria: they (i) contained a minimum of 100 contiguous bases and (ii) had an average base-specific quality value or Phred quality score of ≥20. The consensus sequences were generated from base reads, which provided 4X sequence coverage. The identity of the consensus was determined by comparison against the viral database through the National Center for Biotechnology Information using the BLASTALL program. In order to be considered a match to a sequence within the database, the E value had to be <10e-20 for the specific target. DNA sequencing results were reported as either matching (H1N1) or not matching (not H1N1) for 2009 H1N1 sequences.

**Statistical analysis.** The sample size used for this study was calculated using the binomial distribution with the lower confidence interval (CI) as the acceptance criteria. For the DNA sequence analysis of influenza A virus-positive culture isolates, two-sided CIs were used to be conservative for the acceptance criterion. Two of two 2009 H1N1 strain positive by sequencing.

### RESULTS

A total of 1,506 specimens were evaluated for this trial, including 700 prospective specimens and 806 archived specimens (Fig. 1). Of the 700 prospective specimens, 9 were ineligible and 52 were excluded from analysis, leaving a total of 639. Of the 806 archived specimens, 39 were ineligible and 19 were excluded, leaving a total of 748. Gender was not appreciably different among the groups, although the patient gender for 104 archived specimens was unknown (Table 1). The age distribution in both groups is shown in Table 1. The Xpert Flu Assay results were compared to the viral culture results for prospectively collected and archived clinical samples and the ProFlu+ assay results for archived specimens. Sensitivity and specificity data are shown in Tables 2 to 5 for the comparisons evaluated.

Table 2 summarizes the results of testing archived specimens by the Xpert Flu Assay and ProFlu+. The PPV for influenza A 2009 H1N1 virus was 96.7% due to one Xpert Flu Assay-positive, significant results for comparison of the Xpert Flu Assay to the reference test. Statistical analyses were performed using Fisher’s exact test. Fisher’s exact tests showed homogeneity of the Xpert Flu Assay results across the study sites, three test lots, four age categories, and both genders. Therefore, data were pooled across these groups for calculations of assay performance characteristics. Performance characteristics were calculated for the Xpert Flu Assay relative to viral culture or ProFlu+ if viral culture was not performed at the time of sample collection.

### TABLE 3 Comparison of Xpert Flu Assay with culture on archived specimens

<table>
<thead>
<tr>
<th>Sample type and isolate</th>
<th>No. of specimensa</th>
<th>% Sensitivity (95% CI)</th>
<th>% Specificity (95% CI)</th>
<th>% PPV (95% CI)</th>
<th>% NPV (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA-W</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Influenza A virus</td>
<td>150</td>
<td>79.7 (79.2–99.2)</td>
<td>99.9 (95.4–100)</td>
<td>98.4 (83.3–99.9)</td>
<td>98.3 (94.1–99.8)</td>
</tr>
<tr>
<td>Influenza B virus</td>
<td>150</td>
<td>96.7 (96.6–100)</td>
<td>100 (99.8–100)</td>
<td>100 (97.7–100)</td>
<td>99.6 (97.9–100)</td>
</tr>
</tbody>
</table>

a C' / X', culture positive/negative and sequencing of 2009 H1N1 strain target; X' / X', Xpert Flu Assay positive/negative.

b Two of three negative by sequencing; one of three influenza B virus positive.

c Influenza B virus positive by sequencing.

d Two of two negative by sequencing.
TABLE 4 Comparison of Xpert Flu Assay with culture on prospective specimens

<table>
<thead>
<tr>
<th>Sample type and isolate</th>
<th>No. of specimens</th>
<th>C+ X+</th>
<th>C− X+</th>
<th>C+ X−</th>
<th>C− X−</th>
<th>% Sensitivity (95% CI)</th>
<th>% Specificity (95% CI)</th>
<th>% PPV (95% CI)</th>
<th>% NPV (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA-W</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Influenza A virus</td>
<td>342</td>
<td>6</td>
<td>3b</td>
<td>1</td>
<td>332</td>
<td>85.7 (42.1–99.6)</td>
<td>99.1 (97.4–99.8)</td>
<td>66.7 (29.9–92.5)</td>
<td>99.7 (98.3–100.0)</td>
</tr>
<tr>
<td>Influenza A virus</td>
<td>342</td>
<td>4</td>
<td>4c</td>
<td>0</td>
<td>334</td>
<td>100 (39.8–100)</td>
<td>98.8 (97.0–99.7)</td>
<td>50.0 (15.7–84.3)</td>
<td>100 (98.9–100.0)</td>
</tr>
<tr>
<td>2009 H1N1 strain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Influenza B virus</td>
<td>342</td>
<td>7</td>
<td>2d</td>
<td>0</td>
<td>333</td>
<td>100 (65.2–100)</td>
<td>99.4 (98.1–99.9)</td>
<td>77.8 (45.0–95.9)</td>
<td>100 (99.1–100.0)</td>
</tr>
<tr>
<td>NP swab</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Influenza A virus</td>
<td>297</td>
<td>7</td>
<td>5e</td>
<td>0</td>
<td>285</td>
<td>100 (59.0–100)</td>
<td>98.3 (93.3–99.0)</td>
<td>58.3 (27.7–84.8)</td>
<td>100 (98.7–100.0)</td>
</tr>
<tr>
<td>Influenza A virus</td>
<td>297</td>
<td>5</td>
<td>3f</td>
<td>0</td>
<td>289</td>
<td>100 (47.8–100)</td>
<td>99.0 (97.0–99.8)</td>
<td>62.5 (24.3–91.5)</td>
<td>100 (98.7–100.0)</td>
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<tr>
<td>2009 H1N1 strain</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Influenza B virus</td>
<td>297</td>
<td>7</td>
<td>1g</td>
<td>1h</td>
<td>288</td>
<td>87.5 (47.3–99.7)</td>
<td>99.7 (98.1–100)</td>
<td>87.5 (47.3–99.7)</td>
<td>99.7 (98.1–100.0)</td>
</tr>
</tbody>
</table>

a: C+, culture positive/negative; sequencing of 2009 H1N1 strain target; X+, Xpert Flu Assay positive/negative.
b: Three of three novel H1N1 strain positive by sequencing.
c: Three of four novel H1N1 strain positive by sequencing; one of four influenza A virus positive.
d: Two of two influenza B virus positive by sequencing.
e: Three of five 2009 H1N1 strain positive by sequencing; two of five influenza A virus positive.
f: Two of three 2009 H1N1 strain positive by sequencing; one of three influenza A virus positive.
g: Influenza B virus positive by sequencing.
h: Influenza A virus (not influenza B virus) positive by sequencing.

ProFlu+ -negative strain for which confirmatory tests were not available. All other Xpert Flu Assay performance measures for NP swabs or NA-W specimens (sensitivity, specificity, PPV, and negative predictive value [NPV]) exceeded 98%. Only 3 of 425 specimens were Xpert Flu Assay positive and ProFlu+ negative. Sequencing data were available for only one NA-W specimen. This specimen was influenza A virus positive and identified as 2009 H1N1 by the GeneXpert assay. The strain could not be confirmed by sequencing. Four specimens were Xpert Flu Assay negative and ProFlu+ assay positive for influenza A virus. One specimen was negative for influenza virus by sequence analysis (false-positive ProFlu+ assay result), whereas two were 2009 H1N1 positive. Two specimens were unavailable for sequencing. Of the NP samples, only 16 were influenza B virus positive; contributing to the sensitivity of 93.8% based on one specimen that was ProFlu+ assay positive and Xpert Flu Assay negative but unavailable for sequencing.

Table 3 summarizes the results of the testing the archived specimens that were identified as containing 2009 H1N1 by the Xpert Flu Assay and culture positive/negative, sequencing of 2009 H1N1 strain target and sequencing of specimens that disagree with Xpert results; X−, Xpert Flu Assay negative.

Table 4 summarizes the results of testing of prospectively collected specimens by the Xpert Flu Assay and culture. There were 18 specimens (9 NA-W and 9 NP swabs) that were Xpert Flu Assay positive but culture negative. Sequence analysis indicated that 16 samples contained influenza A virus RNA; however, two specimens that were identified as containing 2009 H1N1 by the Xpert Flu Assay did not contain sequences compatible with 2009 H1N1.

TABLE 5 Comparison of Xpert Flu Assay with culture on prospective specimens, corrected after discrepant-result analysis by sequencing

<table>
<thead>
<tr>
<th>Sample type and isolate</th>
<th>No. of specimens</th>
<th>Cs+ X+</th>
<th>Cs− X+</th>
<th>Cs+ X−</th>
<th>Cs− X−</th>
<th>% Sensitivity (95% CI)</th>
<th>% Specificity (95% CI)</th>
<th>% PPV (95% CI)</th>
<th>% NPV (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA-W</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Influenza A virus</td>
<td>342</td>
<td>9</td>
<td>0</td>
<td>1</td>
<td>332</td>
<td>90.0 (55.5–99.8)</td>
<td>100 (99.1–100.0)</td>
<td>100 (71.7–100.0)</td>
<td>99.7 (98.3–100.0)</td>
</tr>
<tr>
<td>Influenza A virus</td>
<td>342</td>
<td>7</td>
<td>1</td>
<td>0</td>
<td>334</td>
<td>100 (65.2–100.0)</td>
<td>99.7 (98.3–100.0)</td>
<td>87.5 (47.4–99.7)</td>
<td>100 (99.1–100.0)</td>
</tr>
<tr>
<td>novel H1N1 strain</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Influenza B virus</td>
<td>342</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>333</td>
<td>100 (71.7–100.0)</td>
<td>100 (99.1–100.0)</td>
<td>100 (71.7–100.0)</td>
<td>100 (99.1–100.0)</td>
</tr>
<tr>
<td>NP swab</td>
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<td></td>
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<td></td>
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<tr>
<td>Influenza A virus</td>
<td>297</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>285</td>
<td>100 (77.9–100.0)</td>
<td>100 (99.0–100.0)</td>
<td>100 (77.9–100.0)</td>
<td>100 (99.0–100.0)</td>
</tr>
<tr>
<td>Influenza A virus</td>
<td>297</td>
<td>7</td>
<td>1</td>
<td>0</td>
<td>289</td>
<td>100 (65.2–100.0)</td>
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<td>87.5 (47.4–99.7)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Influenza B virus</td>
<td>297</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>289</td>
<td>100 (68.8–100.0)</td>
<td>100 (99.0–100.0)</td>
<td>100 (68.8–100.0)</td>
<td>100 (99.0–100.0)</td>
</tr>
</tbody>
</table>

a: Cs+, culture positive/negative; sequencing of 2009 H1N1 strain target and sequencing of specimens that disagree with Xpert results; X−, Xpert Flu Assay negative/positive.
In addition, three specimens that were identified as influenza A virus positive by the Xpert assay were actually 2009 H1N1 positive by sequencing. There were two specimens that were Xpert Flu Assay negative and culture positive (Table 4). Of those specimens, one contained sequences consistent with influenza A virus even though it was identified as influenza B virus by culture. PPV in this data set was low but increased considerably after discrepant-result analysis was performed (Table 5).

One explanation of the slightly lower sensitivity (compared with that of a reference method) of fresh samples is the possibility that freezing of samples may enhance sensitivity by releasing viral nucleic acid from intracellular sites. The PPV for the H1N1 subtype was only 87.5% in this data set due to the lower number of influenza A virus-positive samples encountered in the prospective specimens during the clinical trial.

**DISCUSSION**

Early detection of circulating viruses can help hospitals prepare for each respiratory virus season. Subtyping of influenza viruses was an important focus for clinical laboratories during the pandemic because this information allowed laboratories to quickly assess the extent of the 2009 H1N1 virus’s prevalence in their community. There were also subtype-dependent treatment considerations, because the 2009 H1N1 virus is sensitive to oseltamivir, whereas seasonal H1N1 influenza A virus was resistant to the drug. In the 2010-2011 season, the 2009 H1N1 strain replaced the seasonal H1N1 influenza A virus strain, reducing the clinical need to separately identify the pandemic virus. Rapid detection of influenza virus allows health care providers to treat patients with oseltamivir, limit the use of unnecessary antimicrobial agents, assign respiratory isolation resources more efficiently, and potentially decrease lengths of stay in the hospital. If patients in the outpatient setting are diagnosed with influenza and treated in a timely fashion, this may decrease the morbidity and mortality, as well as the economic burden, due to this illness by reducing the spread of the disease. To have this impact, however, testing would need to be performed expeditiously so that patients could get their antiviral medications in time to impact the course of the disease.

Molecular assays can be competitive in terms of cost per test compared to culture-based techniques while providing an improved turnaround time and increased sensitivity over viral culture. Nevertheless, one study has shown that even with the implementation of molecular respiratory virus testing, hospital costs may not be impacted (17). More outcome studies are needed to fully understand the impact of molecular testing in the clinical setting. Favorable results for cost avoidance will help laboratories convince administrators of the value of molecular testing for respiratory viruses.

The advantages of PCR over viral culture were readily demonstrated in this study, where the Xpert Flu Assay detected 18 additional (prospective) positive specimens containing influenza virus, all of which were confirmed by sequence analysis as containing influenza A or B virus. However, the Xpert Flu Assay identified two samples as 2009 H1N1 that could not be confirmed by sequence analysis. We speculate that this might be due to the greater efficiency of PCR used in the Xpert Flu Assay than PCR.
used prior to sequence analysis or to degradation of RNA in the samples before sequencing.

To date, only four other respiratory virus assays are FDA cleared in addition to the Cepheid Xpert Flu Assay. Analyte-specific reagents (ASRs) are also available from several manufacturers. Some laboratory-developed tests for respiratory viruses (i.e., home-brew assays) may cost less than FDA-cleared tests, but they do not have clinical trial data supporting their performance characteristics. Reimbursement for these assays is less likely than for FDA-cleared assays. In addition, adequate validation of lab-developed tests, even those using ASRs, becomes challenging, especially for laboratories that do not have access to sufficiently large numbers of samples appropriate for validation protocols. Another drawback of both ASRs and high-complexity molecular diagnostic testing in general is TAT; since testing is typically performed in batches, a possible 1-h analytical procedure often takes several days before a clinically actionable result is reported.

In summary, this clinical trial comparing the newly FDA-cleared Xpert Flu Assay to two current reference methods for influenza virus testing, the ProFlu+ assay and culture, found that performance of the Xpert Flu Assay was comparable to that of the molecular reference standard. Consistent with previous studies using molecular methods, we found a higher assay sensitivity of PCR than of viral culture in the prospective specimen set for which culture results were available. Although the PPV shown in Table 4 for prospective NA-W specimens compared to that of culture was low before resolution, bidirectional sequencing of the specimens indicated that the results of the Xpert Flu Assay were correct (Table 5). The assay is easy to use; all reagents are contained in the cartridge, and the assay requires only approximately 2 min of hands-on time. This allows deployment in facilities where licensed technologists may not be available or where technologists are unfamiliar with high-complexity PCR assays. Not only is the test relatively rapid (the time to a result is approximately 70 min), but the closed system helps mitigate the cross-contamination issues that can be problematic with other molecular-analysis-based assays. The format of the assay makes integration into a routine clinical laboratory reasonably simple.

A major advantage of rapid molecular testing with moderately complex assays, such as the Xpert Flu Assay, is that they can be deployed in rapid-response laboratories (STAT labs or core labs) in order to maximize the medical impact of the results. STAT labs, although open 24 h a day 7 days a week to provide rapid results in most hospitals, are not typically staffed by laboratory personnel with molecular diagnostic training, nor would such technologists have time to perform complex testing. The Xpert Flu Assay fits the profile of many tests currently being offered by STAT labs; it is relatively rapid (the time to a result is approximately 70 min), but the closed system helps mitigate the cross-contamination issues that can be problematic with other molecular-analysis-based assays. The format of the assay makes integration into a routine clinical laboratory reasonably simple.

The availability of FDA-cleared assays greatly enhances the ability of PCR technology to be deployed in a spectrum of laboratories, especially those that do not have the ability to develop their own assays or to use ASR-based assays. Administrators have to make many decisions when determining how molecular respiratory viral assays will be integrated into their laboratories and institutions. The analytical performance of the assay needs to be a primary consideration. In addition, cost, ease of use, and the available menu must be assessed before making any final decisions to move to a molecular assay. More FDA-cleared platforms allow laboratories expanded choices for the implementation of respiratory viral testing assays. For laboratories that do not have the skill set to maintain adequate cell culture techniques, a simple, accurate PCR assay may afford some institutions the ability to offer expanded testing services for respiratory viruses with the Xpert platform. The list price of the Xpert Flu Assay is approximately $50 per cartridge, although pricing would vary based on volume in the institution. The Xpert Flu Assay provides a rapid, reliable, and easily deployable way to test for influenza A and B viruses and identifies the presence of the novel 2009 H1N1 strain as well.

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