Multi-center Evaluation of the Cepheid Xpert® Xpress SARS-CoV-2/Flu/RSV Test

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Abstract

With the approach of respiratory virus season in the Northern Hemisphere, clinical microbiology and public health laboratories will need rapid diagnostic assays to distinguish SARS-CoV-2 from influenza and respiratory syncytial virus (RSV) infections for diagnosis and surveillance. In this study, the clinical performance of the Xpert® Xpress SARS-CoV-2/Flu/RSV test (Cepheid, Sunnyvale, CA, USA) for nasopharyngeal swab specimens was evaluated in four centers: Johns Hopkins Medical Microbiology Laboratory, Northwell Health Laboratories, NYC Public Health Laboratory, and Los Angeles County/University of Southern California (LAC+USC) Medical Center. A total of 319 nasopharyngeal swab specimens, positive for SARS-CoV-2 (n = 75), influenza A (n = 65), influenza B (n = 50), RSV (n = 38), or negative (n = 91) by the standard of care nucleic acid amplification tests at each site were tested using the Cepheid panel test. The overall positive percent agreement for the SARS-CoV-2 target was 98.7% (n=74/75) and the negative agreement was 100% (n= 91) with all other analytes showing 100% total agreement (n= 153). Standard of care tests to which the Cepheid panel was compared included the Cepheid Xpert Xpress SARS-CoV-2, Cepheid Xpert Xpress Flu/RSV, the GenMark ePlex respiratory panel, the BioFire Respiratory panels 2.1 and v1.7, the DiaSorin Simplexa COVID-19 Direct, and the Hologic Panther Fusion SARS-CoV-2 assays. The Xpert Xpress SARS-CoV-2/Flu/RSV test showed high sensitivity and accuracy for all analytes included in the test. This test will provide a valuable clinical diagnostic and public health solution for detecting and differentiating SARS-CoV-2, influenza A and B, and RSV infections during the current respiratory virus season.

Keywords

Cepheid, SARS-CoV-2, Influenza, Flu, RSV, 4Plex

Introduction
The potential concurrent circulation of SARS-CoV-2, influenza viruses, and respiratory syncytial virus (RSV) may prove to be a challenge for healthcare providers and clinical microbiology and public health laboratories. The ability to differentiate the diseases caused by these viruses is essential for patient management, infection control, as well as public health surveillance and response. These viruses can cause infections that present with very similar symptoms making clinical differentiation between them very difficult (1). Clinical microbiology and public health laboratories are likely to face pressure to offer parallel testing for these viruses, optimally using rapid assays with simultaneous detection of SARS-CoV-2, influenza A/B, and RSV. Critically, positivity for one target does not rule-out infection with another respiratory virus, with co-infection with SARS-CoV-2 and influenza as well as other respiratory viruses reported (2-7). Therefore, an optimal diagnostic algorithm for testing patients with influenza-like disease is a multiplex assay that combines the four targets to simultaneously test for SARS-CoV-2, influenza A, influenza B, and RSV.

The Xpert Xpress SARS-CoV-2/Flu/RSV test (Cepheid, Sunnyvale, CA, USA) is a multiplexed rapid real-time reverse transcriptase PCR (rRT-PCR) that can detect and differentiate SARS-CoV-2, influenza A, influenza B, and RSV. This test is the first and only test thus far that detects all four viruses in a single quadriplex panel to receive Emergency Use Authorization (EUA) from the US Food and Drug Administration (FDA). The test is a closed test that integrates specimen extraction, reverse transcription, amplification, and target detection with minimal hands on time and approximately 36 minutes time to results. In this study, we describe the performance of the test in four different laboratories.

Materials and Methods
The Xpert® Xpress SARS-CoV-2/Flu/RSV test. The test received FDA-EUA for viral RNA detection in upper respiratory tract specimens that include nasopharyngeal or nasal swabs and nasal wash/aspirates. The test cartridge is run on the GeneXpert® Dx, GeneXpert Xpress, or the GeneXpert Infinity systems, and result interpretation is performed by the instrument software.

The test detects targets in the SARS-CoV-2 genes E and N2 similar to the Xpert Xpress SARS-CoV-2 test (8), but in contrast to the latter, the targets are combined in the same optical detection channel and hence does not provide separate results for each of the two targets.

Details of the analytical evaluation of the assay including the analytical sensitivity are available in the most updated assay’s package insert (https://www.fda.gov/media/142437/download).

Research use only (RUO) cartridges were distributed to four different sites to compare the performance of the Xpert Xpress SARS-CoV-2/Flu/RSV test to the standard-of-care (SOC) SARS-CoV-2, influenza A, influenza B, and RSV tests. Each site tested archived specimens with the SOC assay in use at the site as the comparators.

Specimens and standard of care testing

Table 1 lists the participating sites and their standard of care test methods. For all sites, specimens were collected from both symptomatic and asymptomatic patients of all age groups. Of note, no co-infections with SARS-CoV-2 and influenza A or B, or RSV were noted in the tested cohort at all sites.

Northwell Health Laboratories: Nasopharyngeal swab specimens (n = 76) were initially collected in 3 mL universal transport medium (UTM- various manufacturers). The Panther Fusion SARS-CoV-2 assay was used as the SOC test for the detection of SARS-CoV-2 (https://www.fda.gov/media/136156/download) and the GenMark ePlex Respiratory Pathogen Panel was the SOC for influenza A, influenza B, and RSV targets (https://www.accessdata.fda.gov/cdrh_docs/pdf16/K163636.pdf). Residual specimens were immediately aliquoted and frozen at -80°C, remaining frozen until this study was performed.
Samples were thawed and immediately tested on the Xpert Xpress SARS-CoV-2/Flu/RSV test. In addition, a side by side testing at the same time with the BioFire Respiratory Panel 2.1 was performed to collect comparative data for the two assays as well. Notably, 20 specimens were positive for other targets on the BioFire panel that included adenovirus (n = 1), Chlamydia pneumoniae (n = 1), endemic coronavirus (n = 10), enterovirus/ rhinovirus (n = 1), human metapneumovirus (n = 1), Mycoplasma pneumoniae (n = 1), and human parainfluenza virus (n = 5). Those 20 were considered negatives for SARS-CoV-2, influenza A, influenza B, and RSV.

NYC Public Health Laboratory, Department of Health and Mental Hygiene: Nasopharyngeal swab specimens (n = 80) collected in viral transport medium (VTM) were initially tested by the standard of care CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel for influenza A and influenza B or Xpert Xpress SARS-CoV-2 test for SARS-CoV-2 (https://www.fda.gov/media/136314/download). Negative nasopharyngeal swab specimens were preliminarily identified by a negative result with the Xpert Xpress SARS-CoV-2 test and were confirmed negative for Influenza A/B and RSV by the BioFire FilmArray Respiratory Panel v1.7 prior to testing by the Xpert Xpress SARS-CoV-2/Flu/RSV test. Specimens were stored at -70°C until Xpert Xpress SARS-CoV-2/Flu/RSV analysis.

The Johns Hopkins Hospital Laboratory: Nasopharyngeal swab specimens (n = 75) were collected in VTM from 45 adults and 30 pediatric patients (< 18 years). SOC testing was performed by the Xpert Xpress SARS-CoV-2 test, the Xpert Xpress Flu/RSV test, and the GenMark ePlex Respiratory Pathogen Panel assay. Residual specimens were aliquoted and frozen at -70°C until tested by the Xpert Xpress SARS-CoV-2/Flu/RSV test for the current study.

LAC+USC Medical Center, University of Southern California. Nasopharyngeal swab specimens (n = 88) were collected in VTM or UTM (various manufacturers). Initial standard of care testing...
was performed using the Xpert Xpress SARS-CoV-2 test, Xpert Xpress Flu/RSV test and the Simplexa COVID-19 Direct test (DiaSorin, n = 6 specimens). Left-over specimens were stored at -70°C until the time of testing with the Xpert Xpress SARS-CoV-2/Flu/RSV panel test. In addition to the qualitative result of RNA detected or RNA not detected, correlation of the cycle threshold values was made, where available, between assays.

**Ethical concerns.** The study protocol was reviewed and approved by the institutional review boards at the study testing site or conducted as a quality improvement project consistent with institutional policies.

**Statistical analysis.** Positive and negative percent agreement for the Xpert Xpress SARS-CoV-2/Flu/RSV test were calculated using two-by-two tables. Overall accuracy, positive percent agreement, negative percent agreement, and 95% confidence intervals were calculated using the MedCalc Statistical Software version 19.2.6 (MedCalc Software Ltd, Ostend, Belgium). Linear regression analysis was performed using GraphPad Prism for comparing cycle thresholds (Ct values) of different assays.

**Results**

**Agreement of the Xpert Xpress SARS-CoV-2/Flu/RSV test with comparator standard of care methods.**

A total of 166 nasopharyngeal swab specimens initially tested by the SOC assays for SARS-CoV-2 were tested by the Xpert Xpress SARS-CoV-2/Flu/RSV test. The Xpert Xpress SARS-CoV-2/Flu/RSV test missed only one positive (initially positive with the Xpert Xpress SARS-CoV-2 with Ct values of E = 41.1 and N2 = 43.3). The overall agreement was 99.3% (n= 165/166), with positive agreement of 98.7% (n= 74/75) (95% CI of 91.02 - 99.8) and negative agreement of 100% (n= 91/91) (Table 2). Notably, Two Xpert Xpress SARS-CoV-2 positive specimens had single Ct values of N2 = 41.5 or N2 = 39.2 and negative E target (Figure 1A) The Xpert Xpress
SARS-CoV-2/Flu/RSV test showed excellent agreement for cycle thresholds (Ct) values with both the Xpert Xpress SARS-CoV-2 [E gene, slope = 1.027 ± 0.02, Y-intercept -0.59 ± 0.6 (R² = 0.97)] and N2 gene, slope 1.01 ± 0.02, Y-intercept 1.9 ± 0.7 (R² = 0.97)] and Panther Fusion SARS-CoV-2 tests [(ORF1ab gene, slope 1.03 ± 0.04, Y-intercept 0.6 ± 0.9, R² = 0.97)] (Figure 1A and B).

Agreement of the Xpert Xpress SARS-CoV-2/Flu/RSV test with the SOC influenza A/ influenza B/ RSV tests at the four sites was 100%. A total of 65 influenza A, 50 influenza B, and 38 RSV positive NPS specimens, as well as 50 negative specimens (notably, the negatives were a subset of the 91 SARS-CoV-2 negative samples (Table 2) that were confirmed as negatives for influenza A, influenza B, and RSV before the study) were tested and compared to the SOC tests (Table 3). Performance was comparable to the Xpert Xpress Flu/ RSV test, BioFire Respiratory Panel 2.1 (RP2.1), GenMark ePlex Respiratory Pathogen Panel, and the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel. Notably the Xpert Xpress SARS-CoV-2/Flu/RSV test showed good correlation at the Ct values level with the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel for both influenza A [Cepheid channel A1: slope 0.8 ± 0.1, Y-intercept 2.7 ± 2.8 (R² = 0.75)]; [Cepheid channel A2: slope 0.8 ± 0.1, Y-intercept 4.7 ± 2.4 (R² = 0.79)] and influenza B [Slope 0.8 ± 0.1, Y-intercept 2.4 ± 2.8 (R² = 0.76)] (Figure 1C and D).

Discussion

The SARS-CoV-2 pandemic will impact algorithms for influenza-like-illness (ILI) testing. In a typical influenza season, molecular testing for influenza is recommended if the patient is likely to be hospitalized or if a conclusive influenza diagnosis could impact the patient’s management (9). Testing for additional respiratory viral pathogens that can cause influenza-like illness, such as RSV, human parainfluenza viruses, enteroviruses/rhinoviruses, and human metapneumoviruses among others may be warranted in pediatric, elderly, and immunocompromised patients (10). Offering SARS-CoV-2 testing along with influenza testing...
for symptomatic patients will be essential with the start of the 2020-2021 influenza season in the Northern hemisphere driven by the widespread community prevalence of SARS-CoV-2 and the very similar clinical presentations. Differentiation of COVID-19 (Coronavirus disease caused by SARS-CoV-2) from influenza and other respiratory diseases in hospitalized and non-hospitalized patients will also be critical for public health surveillance and response to the ongoing pandemic.

In response to the COVID-19 global pandemic, a growing number of molecular diagnostic assays have become commercially available; assays differ in detection targets as well as analytical sensitivity, and most demonstrate high specificity (11-29). Variables that contribute to assay performance include the type of specimen examined, the specimen collection in relation to the course of illness, as well as adequacy of specimen collection (30-34). The Xpert Xpress SARS-CoV-2 test received FDA EUA on March 20, 2020 and was the first assay authorized for use in CLIA-waived settings. The use of this sensitive test in the laboratory and point-of-care setting (8, 28, 35, 36), has reduced health care workers exposure risk in emergency rooms due to the short turnaround time (37).

The laboratory diagnosis of influenza during the 2020–2021 flu season is of particular importance due to the circulation of SARS-CoV-2, overlapping clinical presentations of both influenza and COVID-19, as well as distinct infection control and public health ramifications of the two viruses in the setting of the global COVID-19 pandemic. Testing all patients presenting with influenza-like illness (ILI)/ COVID-19-like illness (CLI) for relevant circulating respiratory viruses will be critical to the diagnosis and appropriate care of the patient, to identify co-infections, and to initiate appropriate public health interventions and collect accurate surveillance data. In addition, RSV detection is particularly valuable due to the seasonal overlap with influenza and similar symptoms in some patient populations including pediatrics and immunocompromised patients. The Cepheid Xpress Flu/RSV test is one of a few rapid
molecular assays available for influenza and RSV diagnosis. The test, since its implementation in the microbiology laboratories or in CLIA waived settings, showed high sensitivity and specificity for detection of influenza A, B, and RSV and positive clinical impact associated with the rapid availability of the results (38-42).

A limited number of commercial molecular assays, as of the writing of this manuscript in addition to the Cepheid Xpert Xpress SARS-CoV-2/Flu/RSV test have combined SARS-CoV-2 testing with other viruses such as influenza or RSV. These tests include small panels developed by the CDC (Influenza SARS-CoV-2 (Flu SC2) Multiplex Assay), and Roche Molecular Systems (Cobas SARS-CoV-2 & Influenza A/B) for the Cobas and Liat systems. In addition to these small panels, extended panels from BioFire Diagnostics, LLC (BioFire Respiratory Panel 2.1 (RP2.1) and RP2.1-EZ), Qiagen (QIAstat-Dx Respiratory SARS-CoV-2 Panel), and GenMark (ePlex Respiratory Pathogen Panel 2 (RP2)) are available. The Cepheid Xpert Xpress SARS-CoV-2/Flu/RSV test is currently the only commercially available quadriplex panel that combines detection of SARS-CoV-2, influenza A and influenza B viruses, and RSV. The test has a fast time to results and is available for CLIA-waived and high throughput format platforms. Our study showed that the Xpert Xpress SARS-CoV-2/Flu/RSV test has high sensitivity and accuracy for the four assay targets. As a result, implementing multiplex SARS-CoV-2, Influenza A and B, and RSV test is projected to have a positive impact during the respiratory virus season of 2020-2021 associated with the upfront testing of SARS-CoV-2, influenza A and B, and RSV and the rapid turn-around time.

Acknowledgements

The authors would like to acknowledge the staff in the participating laboratories without whom the study would not have been possible.
Cepheid provided the Xpert® Xpress SARS-CoV-2/Flu/RSV test cartridges.

References


Figure legends and tables

Figure 1. Correlation of the Cepheid Xpert® Xpress SARS-CoV-2/Flu/RSV test cycle threshold (Ct) values with the standard of care tests Ct values for (A and B) SARS-CoV-2, (C) influenza A, and (D) influenza B. Notably, The Cepheid Xpert Xpress SARS-CoV-2/Flu/RSV test detects SARS-CoV-2 genes E and N2 similar to the Xpert Xpress SARS-CoV-2 test, but in contrast the two targets are not differentiated (panels A and B).
Table 1. Specimens and standard of care testing for each study site.

<table>
<thead>
<tr>
<th>Study Site</th>
<th>Specimen Source</th>
<th>Number of specimens tested</th>
<th>Positive for SARS-CoV-2</th>
<th>Positive for influenza A</th>
<th>Positive for influenza B</th>
<th>Positive for RSV</th>
<th>Negatives</th>
</tr>
</thead>
<tbody>
<tr>
<td>------------------------</td>
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<td>-----------------------------</td>
<td>--------------------------</td>
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</tr>
</tbody>
</table>

NPS, nasopharyngeal swabs; UTM, Universal Transport Medium; VTM, Viral Transport Medium. * Specimens were also tested by the BioFire RP 2.1. ** 20 were positives for targets other than SARS-CoV-2, influenza A, influenza B, and RSV. # Specimens were also tested by the GenMark ePlex respiratory pathogen panel. ## 6 were also tested by DiaSorin COVID-19 direct assay.
Table 2. Agreement of the Xpert® Xpress SARS-CoV-2/Flu/RSV test with the comparator standard of care SARS-CoV-2 test.

<table>
<thead>
<tr>
<th></th>
<th>Pos/Pos</th>
<th>Pos/Neg</th>
<th>Neg/Pos</th>
<th>Neg/Neg</th>
<th>PPA (95% CI)</th>
<th>NPA (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All methods</td>
<td>74</td>
<td>0</td>
<td>1</td>
<td>91</td>
<td>98.7%</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(91.02 - 99.8)</td>
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</tr>
<tr>
<td>XpertXpress SARS-CoV-2</td>
<td>58</td>
<td>0</td>
<td>1</td>
<td>41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Panther Fusion SARS-CoV-2 assay</td>
<td>16</td>
<td>N/A</td>
<td>0</td>
<td>N/A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BioFire Respiratory Panel RP 2.1</td>
<td>N/A</td>
<td>0</td>
<td>N/A</td>
<td>30</td>
<td></td>
<td></td>
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<tr>
<td>BioFire Respiratory</td>
<td>N/A</td>
<td>0</td>
<td>N/A</td>
<td>20</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Cepheid Xpert Xpress SARS-CoV-2/Flu/RSV test result/comparator test result (SARS-CoV-2)
Panel RP v1.7 Pos, positive; Neg, negative; PPA, positive percent agreement; NPA, negative percent agreement; CI, confidence interval. N/A, Not Applicable.
Table 3. Agreement of the Xpert® Xpress SARS-CoV-2/Flu/RSV test with the comparator standard-of-care influenza A, influenza B, and RSV tests.

<table>
<thead>
<tr>
<th></th>
<th>Cepheid Xpert Xpress SARS-CoV-2/Flu/RSV test result/ comparator test result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pos/Pos</td>
</tr>
<tr>
<td><strong>Influenza A</strong></td>
<td></td>
</tr>
<tr>
<td>All methods</td>
<td>65</td>
</tr>
<tr>
<td>BioFire Respiratory Panel 2.1 (RP2.1)</td>
<td>10</td>
</tr>
<tr>
<td>Xpert® Xpress Flu/RSV</td>
<td>31</td>
</tr>
<tr>
<td>GenMark ePlex Respiratory Pathogen Panel</td>
<td>4</td>
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<tr>
<td>CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel</td>
<td>20</td>
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<tr>
<td>BioFire Respiratory Panel RP v1.7</td>
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<tr>
<td><strong>Influenza B</strong></td>
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<td>All methods</td>
<td>50</td>
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<tr>
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<tr>
<td>Test Description</td>
<td>Pos</td>
</tr>
<tr>
<td>-------------------------------------------------------</td>
<td>-----</td>
</tr>
<tr>
<td>GenMark ePlex Respiratory Pathogen Panel</td>
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<tr>
<td>CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel</td>
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<tr>
<td>BioFire Respiratory Panel RP v1.7</td>
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<tr>
<td>RSV</td>
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<tr>
<td>All methods</td>
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<tr>
<td>XpertXpress Flu/RSV</td>
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<td>BioFire Respiratory Panel RP v1.7</td>
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</table>

Pos, positive; Neg, negative. N/A, Not Applicable.