Comparison of the Xpert Flu/RSV XC and Xpress Flu/RSV Assays

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ABSTRACT Molecular diagnostics for influenza and respiratory syncytial virus (RSV) have become commonplace, and various tests and systems have been cleared by the FDA for use in the United States. We performed a retrospective study to compare the Cepheid Xpress Flu/RSV assay with the Xpert Flu/RSV XC assay, using laboratory-developed tests (LDTs) as the reference method. The Xpress assay was 100% accurate compared to LDTs, whereas the Xpert Flu/RSV XC assay was 96.0% accurate. The Xpress test was determined to be faster and more sensitive than the XC assay.

KEYWORDS Cepheid, Xpert, influenza, molecular diagnostics, respiratory syncytial virus

Influenza and respiratory syncytial virus (RSV) are significant causes of morbidity and mortality in the United States. The Centers for Disease Control and Prevention estimates that influenza results in up to 60.8 million illnesses, 710,000 hospitalizations, and 56,000 deaths annually (1). RSV infections trigger >2 million outpatient visits and 57,000 hospitalizations for children under the age of 5 years annually (2), and it is estimated that 11,000 to 17,000 adults die of RSV infection each year (3). The low sensitivity of rapid antigen tests and the delayed time to result of viral culture, combined with the improved sensitivity afforded by molecular methods, have led to an increase in FDA-cleared tests and systems designed to detect these viruses from nasopharyngeal (NP) specimens. The Xpert Flu/RSV XC and Xpert Xpress Flu/RSV assays (Cepheid, Sunnyvale, CA) are rapid, random-access molecular tests capable of detecting and differentiating influenza A, influenza B, and RSV viruses from nasal wash fluid samples/aspirates and NP swabs. Neither of the assays subtypes influenza A or differentiates RSV A from RSV B. The Xpert platform allows for extraction, amplification, and detection to take place within a single-use disposable cartridge. The Xpress version of the test became available in 2017. In addition to reduced time to result (32 versus 63 min), the Xpress test targets multiple segments of RNA for each influenza virus to increase sensitivity and specificity. We previously reported the Xpert Flu/RSV XC test to have positive percent agreements (PPAs) of 97.8% for influenza A, 97.2% for influenza B, and 89.3% for RSV relative to our laboratory-developed tests (LDTs) (4). We aimed to determine whether the Xpress test offers improved PPA.

MATERIALS AND METHODS

Study specimens. The nasopharyngeal (NP) swabs tested (n = 201) were collected from 197 symptomatic patients. Patients were seen as inpatients (n = 69), in outpatient clinics (n = 66), and in the emergency department (n = 66) and were categorized into the following age groups: 0 to 1 (n = 32), 2 to 10 (n = 39), 11 to 18 (n = 9), 19 to 64 (n = 81), 65 to 74 (n = 19) and ≥75 (n = 17) years. Specimens were collected using the Becton Dickinson universal viral transport system (Sparks, MD) from patients exhibiting symptoms of a respiratory infection and were submitted to University of North Carolina (UNC) Health Care for testing between November 2015 and February 2017. Most of the swabs were collected between January and April 2016 (n = 61) and between December 2016 and February 2017 (n = 138),

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TABLE 1 Xpert Flu/RSV and Xpress assays versus LDT, all analytes

Assay and result	LDT result (no. of samples) (n = 200)	
	+	–
Flu/RSV XC		
+	142[a]	0
–	8[b]	50
Xpress		
+	150	0
–	0	50

[a]Influenza A 2009 H1N1 (n = 29), influenza A H3 (n = 27), influenza B (n = 30), RSV A (n = 27), RSV B (n = 28), RSV A+B (n = 1).
[b]Influenza A 2009 H1N1 (n = 1), influenza A H3 (n = 3), RSV A (n = 3), RSV B (n = 1).

representing two influenza seasons. Clinical results had been obtained via the Xpert Flu/RSV XC assay or the GenMark respiratory viral panel (RVP) assay (Carlsbad, CA). Positive specimens were selected to evenly characterize the target menu: negative specimens to encompass the entire period defined by the positives. Sample remnants were stored at −70°C prior to study initiation.

Specimen testing. The reference method was defined as our LDTs, for which 200 μl of specimen was extracted using the total nucleic acid isolation kit on MagNAPure (Roche Applied Science, Indianapolis, IN), with amplification and detection performed on ABI 7500 FAST (Life Technologies, Carlsbad, CA). The influenza A/B virus and RSV A/B LDTs used primer and probe sequences previously described for influenza A and influenza B viruses (5) and separately for RSV A and RSV B (6). The GenMark RVP was used to subtype influenza A-positive samples as necessary. The Xpert assays were performed according to the manufacturer's instructions with one exception: the sample tube was vortexed for 5 s rather than inverted 5 times to reduce the risk of contamination and per our standard practice.

Statistics. Statistical analyses were performed using GraphPad (La Jolla, CA). A P value of <0.05 was considered significant.

This study was approved by the University of North Carolina at Chapel Hill Institutional Review Board.

RESULTS

Of the 201 NP swabs tested, 142 were concordantly positive by the Xpert Flu/RSV XC assay, Xpress Flu/RSV assay, and LDT: 56 influenza A, 30 influenza B, and 56 RSV (27 A, 28 B, 1 A+B) (Table 1). Eight swabs tested positive by LDT and the Xpress assay but negative by the Flu/RSV XC assay (4 influenza A, 4 RSV A); 1 sample had an invalid result from two consecutive Xpress cartridges and was omitted from further analysis (Table 2). Fifty specimens were negative by both Cepheid cartridges and LDT, including samples found by the GenMark assay to be positive for rhinovirus (n = 4), coronavirus OC43 (n = 3), and 1 each of adenovirus, coronaviruses 229E and HKU1, parainfluenza 2, and parainfluenza 3.

As shown in Table 3, the mean LDT C_T value of the 56 concordant influenza A-positive swabs was 22.7 (95% confidence interval [CI], 21.2 to 24.2); for the 4 specimens that tested negative only by Xpert Flu/RSV XC assay, the mean was 35.8 (95% CI, 35.1 to 36.5). This difference was found to be significant (unpaired t test, $P < 0.0001$). Similarly, the mean LDT C_T value of the 57 concordant RSV-positive results was 22.39 (95% CI, 21.1 to 23.7); for the 4 specimens that tested negative

TABLE 2 Analysis of discrepant samples

Study ID	Test result with:				LDT C_T
	GenMark RVP	XC	Xpress	LDT	
1	RSV A	Negative	RSV	RSV A	33.01
5	Inf A,[a] H3	Negative	Inf A	Inf A	34.72
17	Inf A, H1N1	Negative	Inf A	Inf A	35.84
33	RSV A	Negative	RSV	RSV A	36.49
54	Inf A, H3	Negative	Inf A	Inf A	36.22
78	Inf A, H3	Negative	Inf A	Inf A	36.29
183	RSV B	Negative	RSV	RSV B	37.81
196	RSV A	Negative	RSV	RSV A	33.21

[a]Inf A, influenza A virus.

TABLE 3 Correlations between mean LDT C_Ts and positive results

Test status	LDT cycle threshold (mean [range; 95% confidence interval]) for:		
	Influenza A	Influenza B	RSV
XC+/Xpress+/LDT+	22.7 (14.2–35.4; 21.2–24.2)	24.8 (16.3–36.8; 22.9–26.7)	22.4 (15.4–34.6; 21.1–23.7)
XC–/Xpress+/LDT+	35.8 (34.7–36.3; 35.1–36.5)		35.1 (33.0–37.8; 32.8–37.5)

only by Xpert Flu/RSV XC assay, it was 35.1 (95% CI, 32.8 to 37.5). This difference was also found to be significant ($P < 0.0001$).

In this study, the PPA was 94.6% between the XC test and LDT versus 100% between the Xpress test and LDT. The percent negative agreements were both 100%. The Xpress test was 100% accurate compared to our reference LDTs.

DISCUSSION

The accuracy of the Xpert Flu/RSV XC assay compared to LDTs was 97.4%, 100%, and 98.7% for influenza A, influenza B, and RSV, respectively. The accuracy of the Xpert Xpress Flu/RSV assay was 100%. The clinical trial data in the package insert for the Xpress test indicate an accuracy of 98.6%. While this is slightly lower than the 100% accuracy we observed, the clinical trial included 2,065 NP swabs. A limitation of our study is the comparatively low number of samples tested. This limitation and the low discrepancy rate of 4% (8 out of 200) make it difficult to draw any conclusions regarding the performance of the assays in different populations or age groups.

A comparison of data from the package inserts for each test reveals consistently lower limits of detection for the Xpress test for each virus (mean 50% tissue culture infective dose [$TCID_{50}$]/ml, 0.65) than those for the Xpert XC assay (mean $TCID_{50}$/ml, 2.45). These data support our analysis of the C_T values in Table 3, which indicates that samples called positive by the Xpress but not the Xpert Flu/RSV XC assay have less virus present. Therefore, the expanded design of the Xpress test improved its analytical sensitivity.

The XC cartridge provides a result in 63 min, while the Xpress test provides a result in only 32 min. When testing for influenza or RSV only, a positive result may be reported in as little as 20 min (Xpress) or 40 min (XC). However, we have not assessed whether this decrease in time to result has a direct impact on patient care or clinic throughput.

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