Accurate PCR Detection of Influenza A/B and Respiratory Syncytial Viruses by Use of Cepheid Xpert Flu+RSV Xpress Assay in Point-of-Care Settings: Comparison to Prodesse ProFlu+

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ABSTRACT The Xpert Flu+RSV Xpress Assay is a fast, automated *in vitro* diagnostic test for qualitative detection and differentiation of influenza A and B viruses and respiratory syncytial virus (RSV) performed on the Cepheid GeneXpert Xpress System. The objective of this study was to establish performance characteristics of the Xpert Flu+RSV Xpress Assay compared to those of the Prodesse ProFlu+ real-time reverse transcription-PCR (RT-PCR) assay (ProFlu+) for the detection of influenza A and B viruses as well as RSV in a Clinical Laboratory Improvement Amendments (CLIA)-waived (CW) setting. Overall, the assay, using fresh and frozen nasopharyngeal (NP) swabs, demonstrated high concordance with results of the ProFlu+ assay in the combined CW and non-CW settings with positive percent agreements (PPA) (100%, 100%, and 97.1%) and negative percent agreements (NPA) (95.2%, 99.5%, and 99.6%) for influenza A and B viruses and RSV, respectively. In conclusion, this multicenter study using the Cepheid Xpert Flu+RSV Xpress Assay demonstrated high sensitivities and specificities for influenza A and B viruses and RSV in ~60 min for use at the point-of-care in the CW setting.

KEYWORDS CLIA-waived, DNA polymerase, influenza, respiratory syncytial virus

Viral respiratory infections are leading causes of morbidity and mortality worldwide in young children, pregnant women, older adults, and patients with comorbidities (1–3). Influenza virus and respiratory syncytial virus (RSV) remain common and burdensome infections, especially at the extremes of age (4, 5). Rapid detection of these infections enables timely and accurate management and potentially reduces transmission (6). Point-of-care (POC) molecular tests have been shown to decrease overall time to receipt of test results, testing costs, isolation times for hospitalized patients, and duration of hospitalization (7–9). Improvements in turnaround times afforded by POC tests for viral respiratory infections may also result in fewer unnecessary diagnostic imaging studies and antibiotic treatments (1). Standards of care across various medical specialties reflect widespread integration of molecular diagnostic testing. Although widely used, current rapid antigen

diagnostic lateral flow assays have variable, suboptimal test characteristics, as found by the Centers for Disease Control and Prevention (10). Newer point-of-care molecular assays can provide more accurate and timely results (11–13).

The Xpert Flu+RSV Xpress Assay is a fast, automated *in vitro* molecular diagnostic test for qualitative detection and differentiation of influenza A and B (influenza A/B) viruses and RSV performed on the Cepheid GeneXpert Xpress System. The Xpert Flu+RSV Xpress Assay was designed for nasopharyngeal (NP) swab specimen testing by nonlaboratory staff in a Clinical Laboratory Improvement Amendments (CLIA)-waived (CW) setting. The instrument is highly automated and includes a touch-screen laptop and step-by-step instructional videos for untrained users. The self-contained cartridges are disposable, single-use devices containing all required reagents. Each module of the instrument contains a syringe drive for dispensing fluids within the cartridge, a sonicator for lysing cells, and a thermocycler for reverse transcription-PCR (RT-PCR). The objective of this study was to establish performance characteristics of the Xpert Flu+RSV Xpress Assay compared to those of the FDA-cleared commercially available Prodesse ProFlu+ real-time RT-PCR assay (ProFlu+) (Hologic, San Diego, CA, USA) for the detection of influenza A and B viruses and RSV in a CLIA-waived setting.

MATERIALS AND METHODS

During the 2014-2015 respiratory virus season (September 2014 to March 2015), 12 sites from across the United States participated in the study. All users were considered CW users throughout the study, and they did not receive any trainer instruction or peer tutoring prior to the beginning of or during the study. CW users reviewed only the written product instructions provided by Cepheid (quick reference guide [QRG] and package insert) and were required to teach themselves how to install the instruments, use the instruments, and run an assay. After the study, 2 of the 12 study sites were determined to be non-CW sites because testing had been performed in moderately complex laboratories instead of CLIA-waived settings. Samples were tested identically by users, regardless of setting. Patients with respiratory infection symptoms were recruited from a spectrum of settings, including emergency departments (EDs), outpatient and urgent care clinics, and hospital inpatient services. The study was approved by central Institutional Review Boards (IRB) and local IRBs, depending on the particular site.

NP swab specimens in universal transport medium (UTM; Copan Diagnostics) were prospectively collected from consenting/assenting participants during the 2014-2015 respiratory virus season. Patients of all ages were eligible to participate in the study with appropriate consent and/or assent. Enrolled patient ages ranged from younger than 5 years of age to older than 60 years of age. In addition, deidentified, preselected frozen residual NP swab specimens supplemented the overall sample size to achieve adequate numbers of positive samples for each of the viral targets. Inclusion criteria were signs and symptoms of respiratory infection. Fresh NP swab specimens in UTM were stored at 2 to 8°C until testing, which was required to be completed within 24 h of collection using the Xpert Flu+RSV Xpress Assay. Specimens were stored at 2 to 8°C at the site until all testing was completed. Subsequently, specimens were shipped to a centralized lab and stored at 2 to 8°C until testing by the ProFlu+ Assay, as the reference test method, within 48 h of collection. Preselected frozen specimens in UTM were thawed and stored at 2 to 8°C until testing with the Xpert Flu+RSV Xpress Assay, which was required to be completed within 24 h of thawing. Specimens were stored at 2 to 8°C until all testing was complete. For the preselected frozen specimens, inclusion criteria were the following: collection of specimens from patients with signs and symptoms of respiratory infection, storage at −70°C or below, less than one freeze-thaw cycle, and presence of sufficient excess specimen for testing. Patients or samples previously included in the study were excluded to avoid duplicate testing.

All Xpert Flu+RSV Xpress Assay testing and processing were performed within 24 h of prospective specimen collection, utilizing three simple steps, as follows: (i) transfer the liquid sample to the cartridge via the pipette supplied with the test kit, (ii) start the test, and (iii) read the result. Summary and detailed test results, available in ~60 min, were the following: positive or negative for influenza A and/or B virus and/or RSV, no result/repeat test, or instrument error. The test was repeated one time if no result/repeat test or instrument error was obtained on the initial test. If this recurred on the repeat test, the result was recorded as a test failure. External controls (one negative and one positive for flu A/B virus and one positive for RSV) were tested daily prior to testing patient specimens on any given day that patient specimens were tested, by each new operator prior to testing any patient specimens, and whenever a new lot of Xpert Flu+RSV Xpress Assay reagents was received. The external-control results were verified as complete and valid prior to testing any patient specimens. The results obtained from the Xpert Flu+RSV Xpress Assay were compared to those obtained by the ProFlu+ assay, which was determined to be the appropriate reference standard based on discussions between the FDA and Cepheid. Discordant results between the two assays were resolved by bidirectional sequencing using primers other than those in the Xpert Flu+RSV Xpress Assay by an external vendor. Data were summarized in tables of overall CW and non-CW sites and categorized by fresh versus frozen specimens. Test characteristics were determined with associated 95% confidence intervals (CIs), including true positive (TP), true negative (TN), false positive (FP), false negative (FN), positive percent agreement (PPA), negative percent agreement (NPA), and accuracy.

TABLE 1 Demographics of enrolled subjects

Parameter	Value for the parameter (no. of patients [% of total])	
	Overall[a]	CW[b]
Gender		
Female	1,406 (57.7)	1,192 (57.3)
Male	1,028 (42.2)	887 (42.6)
No response	1 (0.04)	1 (<0.0)
Total	2,435	2,079
Age group		
≤5 yr	422 (17.3)	374 (18.0)
6–21 yr	421 (17.3)	375 (18.0)
22–59 yr	1,302 (53.5)	1,094 (52.6)
≥60 yr	288 (11.8)	237 (11.4)
Unknown	2 (0.1)	NA[c]
Total	2,435	2,080
Healthcare setting category		
ED	1,120 (46.0)	793 (38.1)
Urgent care/walk-in clinic	688 (28.3)	688 (33.1)
Outpatient	565 (23.2)	550 (26.4)
Inpatient	62 (2.5)	49 (2.4)
Total	2,435	2,080

[a]CLIA-waived sites and non-CLIA-waived sites combined.
[b]CLIA-waived sites only.
[c]NA, not applicable.

RESULTS

Overall. A total of 2,553 patients and samples were included in the study, with 2,288 fresh prospective and 265 frozen specimens. Of the above, 2,435 specimens were retained for analysis; 2,176 fresh and 259 frozen specimens met study criteria and had reportable results for both the Xpert Flu+RSV Xpress Assay and the ProFlu+ assay. Patients included 1,406 female (57.7%) and 1,028 male (42.2%) patients, 422 (17.3%) of ≤5 years of age, 421 (17.3%) of 6 to 21 years of age, 1,302 (53.5%), of 22 to 59 years of age, 288 (11.8%) of ≥60 years of age, and 2 (0.1%) of unknown age. The patients were enrolled from the following locations: 1,120 patients (46%) from the ED, 688 patients (28.3%) from the urgent care/walk-in clinic, 565 (23.2%) outpatients, and 62 (2.5%) inpatients. The majority of specimens tested (95.0%, 2,337/2,461) were successfully completed on the first attempt by the Xpert Flu+RSV Xpress Assay. A total of 115 of the 124 specimens were retested, of which 98 yielded valid results after a single retest. There were 17 specimens with invalid results on retest which were excluded from the analyses. Overall, 1,743 (71.6%) of the results were negative, and 692 (28.4%) were positive, as follows: 346 (14.2%) for influenza A virus, 172 (7.1%) for influenza B virus, 162 (6.7%) for RSV, 9 (0.4%) for influenza A virus and RSV, and 3 (0.1%) for influenza B virus and RSV. In aggregate, compared to results with the ProFlu+ assay, the Xpert Flu+RSV Xpress Assay demonstrated high PPA, NPA, and accuracy values for the detection of influenza A and influenza B viruses and RSV (Table 1). For the total group, there were 111 discordant results that were resolved by bidirectional sequencing. Following discordant resolution, the PPAs for influenza A virus, influenza B virus, and RSV were 100% (95% CI, 98.89 to 100%), 100% (95% CI, 97.82 to 100%), and 97.73% (95% CI, 94.30 to 99.11%), respectively; the NPAs were 99.38% (95% CI, 98.94 to 99.64%), 99.87% (95% CI, 99.61 to 99.95), and 99.91% (95% CI, 99.68 to 99.98%), respectively.

CLIA-waived setting. As described above, following completion of the study, two sites were determined to have testing performed in a non-CW setting; therefore, the subset of 10 CW site results are presented, as follows. A total of 2,080 of the NP swab specimens (1,871 fresh and 209 frozen) met study criteria and were tested at CW sites using the Xpert Flu+RSV Xpress Assay. Patient demographics were the following: 1,192 (57.3%) female patients, 887 (42.6%) male patients, 374 (18.0%) of ≤5 years of age, 375 (18.0%) of 6 to 21 of years, 1,094 (52.6%) of 22 to 59 years of age, and 237 (11.4%) of

TABLE 2 Xpert Flu+RSV Xpress Assay performance versus ProFlu+ on NP swab specimens for CW sites and non-CW sites combined

Specimen type (n)[a]	Target	No. of results by type[b]				PPA (% [95% CI])	NPA (% [95% CI])	Accuracy (% [95% CI])
		TP	FP	TN	FN			
Fresh (2,176)	Influenza A virus	250	101	1,825	0	100 (98.5–100)	94.8 (93.7–95.7)	95.4 (94.4–96.2)
	Influenza B virus	63	10	2,103	0	100 (94.3–100)	99.5 (99.1–99.8)	99.5 (99.2–99.8)
	RSV	125	8	2,039	4	96.9 (92.3–99.1)	99.6 (99.2–99.8)	99.4 (99.0–99.7)
Preselected frozen (259)	Influenza A virus	0	4	255	0	NA[c]	98.5 (96.1–99.6)	98.5 (96.1–99.6)
	Influenza B virus	100	2	157	0	100 (96.4–100)	98.7 (95.5–99.8)	99.2 (97.2–99.9)
	RSV	40	1	217	1	97.6 (87.1–99.9)	99.5 (97.5–100)	99.2 (97.2–99.9)
Fresh and preselected frozen combined (2,435)	Influenza A virus	250	105	2,080	0	100 (98.5–100)	95.2 (94.2–96.1)	95.7 (94.8–96.5)
	Influenza B virus	163	12	2,260	0	100 (97.8–100)	99.5 (99.1–99.7)	99.5 (99.1–99.7)
	RSV	165	9	2,256	5	97.1 (93.3–99.0)	99.6 (99.2–99.8)	99.4 (99.0–99.7)

[a]Fresh, specimen prospectively collected from consented/assented subjects; preselected frozen, specimen deidentified, frozen, and known to be positive or negative for a specific analyte. n, number of specimens.
[b]TP, true positive; FP, false positive; TN, true negative; FN, false negative.
[c]NA, not applicable.

≥60 years of age. The patients were enrolled from the following locations: 793 (38.1%) from the ED, 550 (26.4%) outpatients, 688 (33.1%) from an urgent care/walk-in clinic, and 49 (2.4%) inpatients. Overall, 1,492 (71.7%) of the results were negative, and 588 (28.3%) were positive, as follows: 295 (14.2%) for influenza A virus, 144 (6.9%) for influenza B virus, 138 (6.6%) for RSV, 9 (0.4%) for influenza A virus and RSV, and 2 (0.1%) for influenza B virus and RSV. Compared to results with the ProFlu+ assay, the Xpert Flu+RSV Xpress Assay demonstrated high PPA, NPA, and accuracy values for detection of influenza A and influenza B viruses and RSV for both fresh and frozen samples in a CW setting (Tables 2 and 3). For the CW group, there were 86 discordant results that were resolved by bidirectional sequencing. Following discordant resolution, the PPAs for influenza A and influenza B viruses and RSV were found to be 100% (95% CI, 98.7 to 100%), 100% (95% CI, 97.38 to 99.95%), and 98.01% (95% CI, 94.32 to 99.32%), respectively. NPAs were 99.27% (95% CI, 98.76 to 99.57%), 99.85% (95% CI, 99.55 to 99.95%), and 99.95% (95% CI, 99.71 to 99.99%), respectively.

DISCUSSION

This multicenter study evaluated the Cepheid Xpert Flu+RSV Xpress Assay to detect influenza A and B viruses as well as RSV in CW settings in the United States. Overall, results of the assay were highly concordant with those of the ProFlu+ assay. This finding is highlighted by the high PPAs and NPAs for influenza A and B viruses and RSV on fresh prospective NP swabs in a CW setting by nonlaboratory users.

Prior studies have evaluated similar point-of-care molecular assays for influenza A

TABLE 3 Performance of Xpert Flu+RSV Xpress versus ProFlu+ on NP swab specimens for CLIA-waived sites only

Specimen type (n)[a]	Target	No. of results by type[b]				PPA (% [95% CI])	NPA (% [95% CI])
		TP	FP	TN	FN		
Fresh (1,871)	Influenza A virus	215	85	1,571	0	100 (98.3–100)	94.9 (93.7–95.9)
	Influenza B virus	56	10	1,805	0	100 (93.6–100)	99.4 (99.0–99.7)
	RSV	112	6	1,750	3	97.4 (92.6–99.5)	99.7 (99.3–99.9)
Preselected frozen (209)	Influenza A virus	0	4	205	0	NA[c]	98.1 (95.2–99.5)
	Influenza B virus	78	2	129	0	100 (95.4–100)	98.5 (94.6–99.8)
	RSV	31	0	177	1	96.9 (83.8–99.9)	100 (97.9–100)
All NP swabs (2,080)	Influenza A virus	215	89	1,776	0	100 (98.2–100)	95.2 (94.2–96.1)
	Influenza B virus	134	12	1,934	0	100 (97.2–100)	99.4 (98.5–99.7)
	RSV	143	6	1,927	4	97.3 (93.2–98.9)	99.7 (99.3–99.9)

[a]Fresh, specimen prospectively collected from consented/assented subjects; preselected frozen, specimen deidentified, frozen, and known to be positive or negative for a specific analyte. n, number of specimens.
[b]TP, true positive; FP, false positive; TN, true negative; FN, false negative.
[c]NA, not applicable.

and B viruses (11, 13–18). Two of these reports of the Alere i Influenza A&B Assay (Scarborough, ME, USA) demonstrated rapid results with high sensitivity within 15 min and used nasal swabs, which are more comfortable for patients (11, 17). However, some of these studies found lower specificities (62.5% for influenza A virus and 53.6% for influenza B virus in one study and 85.6% for influenza A virus and 96.3% for influenza B virus in another study) than those found in the present study (11, 15). Therefore, these assays may require confirmatory testing, depending on the clinical scenario; also, an additional test would be needed to evaluate for RSV since that target is not included in the Alere i Influenza A&B Assay. Similar to results of this study, the Cobas Influenza A/B Assay (Roche Molecular Systems, Pleasanton, CA, USA) has reported high sensitivities (97.7% for influenza A virus and 98.6% for influenza B virus) as well as high specificities (99.2% for influenza A virus and 99.4% for influenza B virus) for a 20-min CW molecular assay; however, the assay also does not include RSV (13). In 2016, the Enigma MiniLab assay for influenza A/B viruses and RSV (Enigma Diagnostics, Porton Down, Salisbury, United Kingdom) was evaluated at a single-center study in the United Kingdom with nonlaboratory personnel trained to use the molecular assay (12). That platform provided results in 95 min with sensitivities of 81.8% for influenza A virus, 100% for influenza B virus, and 97.7% with the use of the reference standard, the Luminex xTAG Respiratory Virus Panel (RVP) Fast, version 2 (Luminex, Austin, TX, USA) (12). However, the Enigma test is not FDA cleared for CW testing, and specific training was required as described in the study (12, 19).

Our study had limitations. As with any test, there were indeterminate results in the present study; these were 0.7% overall and 1.1% in a CW setting. However, this rate compares favorably to a similar assay for influenza A/B viruses and RSV (Enigma) of 5.6% and to a CW influenza A/B virus test (Alere i) with a failure rate of 2 and 2.8% (11, 12). Additionally, if confirmatory testing is clinically indicated, then this will result in patient care or cohorting delays. As currently designed, the assay is limited to three targets. Future studies of these devices could be enhanced by performing all of the testing in a CW environment. Additionally, future results may potentially vary by viral season, given potentially shifting molecular targets as well as spectrum bias that may affect test performance based on the case mix of the patients. As tested, the platform had one module per system, which limited throughput. Having additional modules per unit would be an enhancement in a CW setting. During the study period, results were available in ~60 min. Future enhancements could include reduction of turnaround time to expedite decision making regarding disposition, cohorting, and potentially antiviral treatment, all of which impact busy acute care settings.

Conclusion. The Cepheid Xpert Flu+RSV Xpress Assay performed on the GeneXpert Xpress (GX-I) instrument was highly sensitive and specific compared with results obtained from the ProFlu+ assay. These data indicate that the assay is highly suitable and capable of facilitating reliable laboratory-quality PCR results within point-of-care environments. The assay is easy to use and requires no training. The results are fast, sensitive, and specific and can be used to inform real-time clinical decision making and improve surveillance.

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