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Performance Characteristics of Xpert Flu/RSV XC Assay

Elena B. Popowitch,[a] Melissa B. Miller[a,b]

UNC Health Care, Clinical Microbiology-Immunology Laboratory,[a] and UNC School of Medicine, Department of Pathology and Laboratory Medicine,[b] Chapel Hill, North Carolina, USA

The Xpert Flu/RSV XC assay was compared to laboratory-developed tests (LDTs) ($n = 207$) and the Xpert Flu assay ($n = 147$) using archived nasopharyngeal swabs. The percentages of positive agreements with LDTs were 97.8% for influenza A, 97.2% for influenza B, and 89.3% for RSV. The sensitivity of influenza detection was improved with the Xpert Flu/RSV XC assay compared to the Xpert Flu assay.

Influenza and respiratory syncytial virus (RSV) are significant causes of morbidity and mortality in the United States. On average, between 1997 and 2009, >19,000 deaths were attributable to influenza and >11,000 were attributable to RSV (1) annually. The low sensitivity of rapid antigen tests and the delayed time to result of viral cultures 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 (2, 3). The Xpert Flu/RSV XC assay (Cepheid, Sunnyvale, CA) is a newly FDA-cleared rapid, random-access molecular test capable of detecting and differentiating influenza A, influenza B, and RSV viruses from nasal wash fluid samples/aspirates and nasopharyngeal (NP) swabs. The new Xpert assay allows extraction, amplification, and detection to take place within a single-use disposable cartridge, with results available in 63 min. In addition to expanding the detection to include RSV, the Xpert Flu/RSV XC assay includes new and additional primer sets to provide redundant coverage for the influenza viruses and to minimize the impact of genetic drift. Workflow was also improved by placing all wet reagents inside the cartridge, and the extraction was optimized. Given these improvements, we sought to ascertain the performance characteristics of the Xpert Flu/RSV XC assay, especially compared to the previous Xpert Flu assay.

We conducted a verification study using archived NP swabs collected between September 2013 and January 2015 that were chosen by convenience sampling to enrich for positive samples. Nasopharyngeal specimens were collected using the Becton Dickinson universal viral transport system (Sparks, MD) from patients exhibiting symptoms of an upper respiratory infection and submitted to UNC Health Care for testing. Clinical results had been obtained via the Xpert Flu assay, the Nanosphere Verigene respiratory virus plus (Northbrook, IL), or the GenMark repiratory viral panel (RVP) (Carlsbad, CA). Sample remnants were stored at −70°C prior to study initiation. The reference method was defined as our laboratory-developed tests (LDTs) for influenza A/B and RSV, which were performed as previously described (4, 5) using published primer and probe sequences (6, 7). Influenza A- or B-positive samples were also tested with the Xpert Flu cartridge if they were not already tested as part of standard diagnostic testing. The Xpert Flu/RSV XC and Xpert Flu assays were performed according to the manufacturer's instructions with one exception: instead of inverting the sample tube 5 times, it was vortexed for 5 s.

Of the 207 NP swabs tested, 172 were positive by both the Xpert Flu/RSV XC test and the LDT: 36 influenza A 2009 H1N1, 14 influenza A H3, 39 untyped influenza A, 35 influenza B, 17 RSV A, and 31 RSV B. Four swabs were positive for both influenza A and RSV. An additional sample was dually positive only by the LDT; the Xpert Flu/RSV XC assay was positive for influenza A but did not detect the RSV. Two swabs were positive by the Xpert Flu/RSV XC test alone (influenza B, cycle threshold [C_T], 35.0; RSV, C_T, 35.6), and 7 were positive by the LDT alone (2 influenza A, C_T, 34.1, 36.6; 5 RSV, C_T range, 36.2 to 38.3). Of the 9 discordant results, 8 were initially tested with the GenMark RVP assay; each was positive for the analyte in question, indicating that the Xpert-only positive results may be true positives. Thirty-one swabs were negative by both tests, including samples established by the GenMark assay to be positive for adenovirus ($n = 2$); human metapneumovirus ($n = 1$); rhinovirus ($n = 5$); coronaviruses 229E ($n = 1$), HKU1 ($n = 3$), NL63 ($n = 1$), and OC43 ($n = 3$); and parainfluenza viruses 1 to 4 (PIV 1 to 3, $n = 1$ each; PIV 4, $n = 2$). Table 1 shows the performance of the Xpert Flu/RSV XC test compared with our LDTs. The mean LDT C_T value of concordantly positive samples was 25.5 (95% confidence interval [CI], 24.7, 26.4; range, 15.11 to 39.57), while the mean LDT C_T of the 7 samples missed by the Xpert Flu/RSV XC test was 36.5 (95% CI, 35.3, 37.7; range, 34.06 to 38.32), indicating less strongly positive samples. This difference was statistically significant ($P < 0.0001$, Student's t test).

TABLE 1 Comparison of the Xpert Flu/RSV XC to laboratory-developed real-time PCR using nasopharyngeal swabs ($n = 207$)

	LDT	
Xpert Flu/RSV XC	+	−
+	172[a]	2[b]
−	7[c]	31

[a] Concordant positives: 89 influenza A, 35 influenza B, and 48 RSV positive samples.
[b] Two false-positive samples: 1 influenza B and 1 RSV.
[c] Seven false-negative results: 2 influenza A, 2 RSV A, and 3 RSV B positive samples.

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Address correspondence to Melissa B. Miller, Melissa.Miller@unchealth.unc.edu.

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TABLE 2 A subset of the sample bank was used to compare Xpert Flu and Xpert Flu/RSV XC to LDT for influenza detection ($n = 147$)

Comparative test	LDT +	LDT −
Xpert Flu		
+	108[a]	0
−	18[b]	21
Xpert Flu/RSV XC		
+	124[c]	1[d]
−	2[e]	20

[a] Samples positive by both the Xpert Flu test and the LDT included 34 influenza A/H1N1, 4 influenza A/H3, 39 influenza A not typed, and 31 influenza B.
[b] Of those missed by the Xpert Flu test, 4 were influenza A/H1N1-, 10 were influenza A/H3-, and 4 were influenza B-positive specimens.
[c] More true-positive samples were detected by the Xpert Flu/RSV XC assay, including 36 influenza A/H1N1-positive, 14 influenza A/H3-positive, 39 influenza A not typed, and 35 influenza B-positive samples.
[d] There was 1 influenza B false positive.
[e] Both Xpert Flu/RSV XC false negatives were influenza A/H1N1-positive samples. One was collected from an adult 6 days after symptom onset, the other from an adult with no noted indications of a respiratory infection.

When the new Xpert Flu/RSV XC cartridge was compared to that of the Xpert Flu assay for the detection of influenza viruses ($n = 147$) (Table 2), it was found to be slightly simpler (1 set-up step eliminated), slightly faster (63 versus 75 min), and significantly more sensitive ($P = 0.0002$, Fisher's exact test). When the C_T values of the LDT influenza-positive samples were analyzed, there was a significant difference between those that were positive by both the Xpert Flu and Flu/RSV XC cartridges and those that were positive by the Flu/RSV XC cartridge alone (23.7 versus 34.3; $P < 0.0001$). The difference between LDT-positive/Xpert Flu/RSV XC-positive/Xpert Flu-negative (Xpert Flu false negative) and LDT-positive/Xpert Flu/RSV XC-negative/Xpert Flu-negative samples (false negative by both Xpert tests) was not significant (C_T 34.3 versus 35.3; $P = 0.57$). Notably, of 10 samples collected between October 2014 and January 2015 that were identified as falsely negative by Xpert Flu for influenza A/H3 (i.e., positive by GenMark RVP, negative by Xpert Flu), all were positive by the Xpert Flu/RSV XC assay.

The overall accuracy of the Xpert Flu/RSV XC assay compared to the LDT was 95.8% (204/213), with 99.1% (211/213), 99.5% (212/213), and 97.2% (207/213) accuracy for influenza A, influenza B, and RSV, respectively. The sensitivity/specificity was 97.8%/100% for influenza A, 100%/99.4% for influenza B, and 90.6%/99.4% for RSV. The precision of Xpert Flu/RSV XC results was evaluated for influenza A/H1N1, influenza A/H3, influenza B, and RSV using CLSI EP-15 guidelines (8). Daily precision was 0.90 to 2.3 C_T, depending on the viral target, while overall precision was 0.81 to 2.1 C_T (data not shown). Although the LDT was more sensitive, particularly for RSV, it is a batched test with a turnaround time of 8 to 24 h. The Xpert Flu/RSV XC test provides a result in 63 min. The assay is classified as moderately complex; setup is simple, taking ~2 min, and can be done by nonmolecular personnel. It does not subtype influenza A or differentiate RSV A from RSV B, but the sensitivity is significantly improved over the existing Xpert Flu assay.

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