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Comparison of the Simplexa FluA/B & RSV Direct Assay and Laboratory-Developed Real-Time PCR Assays for Detection of Respiratory Virus

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The results of the Focus Simplexa FluA/B & RSV Direct assay were compared to those of laboratory-developed reverse transcription PCR tests for 498 nasopharyngeal swabs. Concordance rates were 96.6% (476/493; κ = 0.91), 97.6% (481/493; κ = 0.47), and 99.2% (488/492; κ = 0.94) for influenza A, influenza B, and respiratory syncytial virus, respectively.

Traditionally, rapid point-of-care detection of influenza A virus (FluA) and influenza B virus (FluB) and respiratory syncytial viruses (RSV) has been performed using antigen immunoassays, which have low sensitivity (1, 2, 3). In comparison, nucleic acid amplification tests (NAAT) have improved sensitivity but increased time to result (4). Recently, several NAAT that provide results in <2 h and require little hands-on time have become commercially available. The U.S. Food and Drug Administration (FDA)-cleared Simplexa Flu A/B & RSV Direct assay (Focus Diagnostics, Cypress, CA) is a real-time reverse transcription (RT)-PCR system that detects FluA, FluB, and RSV in about 1 h from nasopharyngeal swabs that do not require nucleic acid extraction. The assay consists of the Simplexa Flu A/B & RSV Direct reagents, a 3 M Integrated Cycler, and direct amplification discs.

We compared the results of the Simplexa Direct assay to those of well-characterized laboratory-developed real-time RT-PCR tests (LDTs) for 498 prospectively collected nasopharyngeal swabs at the University of Washington Molecular Virology Laboratory during the 2013 peak influenza season in Seattle, WA (2 January to 9 February). The median patient age was 48 years (range, 16 days to 96 years); 90% of the subjects were ≥21 years of age. Samples with discordant Simplexa Direct and LDT results and a subset of samples with concordant negative results were tested by the Prodesse ProFlu+ assay (Hologic Gen-Probe, San Diego, CA). The Simplexa Direct and Prodesse assays were performed according to the manufacturers' protocols. For Simplexa Direct, 50 μl of reaction mix from a single-use tube was added to the reagent well on one wedge of an 8-wedge amplification disc, 50 μl of sample was added to the sample well, the wedge was covered, the disc was placed on the cycler, and the run was started. Each run could accommodate up to eight samples. Results for FluA, FluB, RSV, and an internal amplification control were provided as positive or negative and as PCR cycle threshold (C_T) values. Samples with uninterpretable amplification plots and viral target-negative samples that were negative for the internal amplification control were reported as invalid. RNA extraction and LDTs were carried out as previously described (5, 6).

The reproducibility of Simplexa Direct was assessed by calculating the coefficients of variation (CVs) of C_T values obtained for a panel of 8 clinical nasopharyngeal swabs (two negative samples and one sample positive for high and low concentrations of FluA, FluB, and RSV). Samples were divided into aliquots and tested eight times on different days by different technicians. The sensitivity of Simplexa Direct was compared to that of LDTs by testing 10-fold serial dilutions of positive clinical samples by both assays.

TABLE 1 Comparison of Simplexa and LDTs for detection of FluA, FluB, and RSV in 498 nasopharyngeal swab samples

LDT result	No. of samples with indicated Simplexa result			Total no. of samples
	Positive	Negative	Invalid	
FluA				
Positive	108	13	0	121
Negative	4	368	5	377
Total	112	381	5	498
FluB				
Positive	7	3	1	11
Negative	9	474	4	487
Total	16	477	5	498
RSV				
Positive	35	3	2	40
Negative	1	453	3	457
Total	36	456	5	497

FluA was detected in 121 samples by LDTs and 112 by Simplexa Direct; FluB was detected in 11 samples by LDTs and 16 by Simplexa Direct; RSV was detected in 40 samples by LDTs and 36 by Simplexa Direct (Table 1). The overall concordance rates for the two methods (excluding invalid results) were 96.6% (476/493; κ = 0.91), 97.6% (481/493; κ = 0.47), and 99.2% (488/492; κ = 0.94) for FluA, FluB, and RSV, respectively. Positive percentages of agreement were 86.4%, 36.8%, and 89.7% for FluA, FluB, and RSV, respectively.

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TABLE 2 LDT, Simplexa, and Prodesse results for 37 samples with discordant or invalid LDT/Simplexa results

LDT result(s) (C_T value)	Simplexa result(s) (C_T value)	Prodesse result (C_T value)
Negative	FluA (38.6)	Negative
Negative	FluA (39.0)	Negative
Negative	FluA (39.4)	Negative
RSV (28.6)	FluA (37.8), RSV (26.4)	RSV (18.1)
FluA (35.5)	Negative	Negative
FluA (36.9)	Negative	Negative
FluA (37.0)	Negative	Negative
FluA (37.0)	Negative	Negative
FluA (37.9)	Negative	Negative
FluA (38.0)	Negative	Negative
FluA (38.3)	Negative	Negative
FluA (38.9)	Negative	Negative
FluA (39.1)	Negative	Negative
FluA (39.3)	Negative	Negative
FluA (39.3)	Negative	Negative
FluA (39.6)	Negative	Negative
FluA (38.1), RSV (28.9)	Negative	RSV (23.0)
FluA (18.6)	FluA (20.6), FluB (29.1)	FluA (15.7)
FluA (22.9)	FluA (25.0), FluB (33.5)	FluA (17.5)
FluA (20.4)	FluA (20.6), FluB (35.7)	FluA (18.0)
FluA (23.4)	FluA (24.1), FluB (35.9)	FluA (19.6)
FluA (23.7)	FluA (25.2), FluB (32.8)	FluA (20.1)
FluA (23.8)	FluA (24.2), FluB (31.7)	FluA (21.0)
FluA (24.3)	FluA (27.2), FluB (35.5)	FluA (21.5)
FluA (26.0)	FluA (27.2), FluB (36.8)	FluA (22.1)
FluA (29.1)	FluA (20.1), FluB (32.7)	FluA (24.0)
FluB (35.7)	Negative	Negative
FluB (38.3)	Negative	Negative
FluB (38.8), RSV (25.7)	Invalid	RSV (15.1)
FluB (33.5)	Negative	FluB (28.4)
RSV (26.7)	Invalid	RSV (17.6)
RSV (38.0)	Negative	RSV (26.4)
RSV (37.3)	Negative	RSV (28.4)
Negative	RSV (35.9)	RSV (26.8)
Negative	Invalid	Negative
Negative	Invalid	Negative
Negative	Invalid	Invalid

Simplexa Direct, LDT, and Prodesse results for 37 samples with discrepant Simplexa Direct and LDT results are shown in Table 2. Of these, 27 had Simplexa Direct or LDT C_T values > 35, indicating viral copy numbers near the assay limits of detection. The low FluB kappa value and positive percent agreement were due to nine Simplexa Direct-positive/LDT-negative samples that were likely false-positive results, which may have been caused by a malfunctioning Integrated Cycler. All nine were FluB negative by the Prodesse assay and FluA positive by all three methods. Six of 22 LDT-positive/Simplexa Direct-negative samples were positive by the Prodesse assay, while 1 of 14 Simplexa Direct-positive/LDT-negative samples was positive by Prodesse. Forty concordant negative samples were negative by Prodesse. Eleven samples (2.8%) required repeat testing due to invalid results by Simplexa Direct; five were invalid after repeat testing. All samples had valid results by LDTs, which identified two positive samples among the five with final invalid Simplexa Direct results (Table 2).

The reproducibility of the Simplexa Direct assay was good, with CVs of C_T values ranging from 1.7% to 3.2% for each of the viral targets at low concentrations (mean C_T range, 30.3 to 34.5) and high concentrations (mean C_T range, 23.8 to 28.8). FluA and RSV detection results in serially diluted specimens were similar for the Simplexa Direct and LDT assays. However, Simplexa Direct was 10-fold less sensitive than the LDTs for detection of FluB.

The Simplexa Direct assay performed well compared with our established LDTs, demonstrating no significant differences in detection of FluA, FluB, and RSV in clinical specimens. Samples with discordant results were most likely due to low viral loads near the assay limits of detection. Our study was limited by the fact that samples were tested sequentially by Simplexa Direct, LDTs, and Prodesse, with sample freeze/thaw cycles between each test. This may have caused an underestimation of assay concordance due to loss of viral RNA in subsequent tests. A higher-than-expected proportion of samples (2.8%) had invalid Simplexa Direct results, including two that were positive for RSV by LDTs and Prodesse. All samples tested were nasopharyngeal samples collected in Universal Transport Medium, which is the FDA-approved sample type.

This study was the first comparison of LDTs to the newly developed Simplexa FluA/B & RSV Direct assay that provides results directly from specimens in about 1 h. A study that compared the Nanosphere Verigene RV+ assay to the Simplexa Flu A/B & RSV assay, which requires nucleic acid extraction and conventional real-time PCR, reported Simplexa sensitivities for FluA, FluB, and RSV of 82.8%, 76.2%, and 94.6%, respectively (7). A study comparing LDT to the same Simplexa Flu A/B & RSV assay with a modified protocol that eliminated nucleic acid extraction but used the conventional real-time PCR format reported overall sensitivities of 95.1% and 99.6 (8).

Compared to LDTs, the Simplexa Flu A/B and RSV Direct assays showed good performance. Advantages over LDTs include rapid speed and ease of use. Simplexa Direct will provide accurate results to patients more quickly than LDTs, allowing more-rapid antiviral treatment and patient management.

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REFERENCES

1. **Liao RS, Tomalty LL, Majury A, Zoutman DE.** 2009. Comparison of viral isolation and multiplex real-time reverse transcription-PCR for confirmation of respiratory syncytial virus and influenza virus detection by antigen immunoassays. J. Clin. Microbiol. **47:**527–532.
2. **Drexler JF, Helmer A, Kirberg H, Reber U, Panning M, Müller M, Höfling K, Matz B, Drosten C, Eis-Hübinger AM.** 2009. Poor clinical sensitivity of rapid antigen test for influenza A pandemic (H1N1) 2009 virus. Emerg. Infect. Dis. **15:**1662–1664.
3. **Hurt AC, Alexander R, Hibbert J, Deed N, Barr IG.** 2007. Performance of six influenza rapid tests in detecting human influenza in clinical specimens. J. Clin. Virol. **39:**132–135.
4. **Chartrand C, Leeflang MM, Minion J, Brewer T, Pai M.** 2012. Accuracy of rapid influenza diagnostic tests: a meta-analysis. Ann. Intern. Med. **156:**500–511.
5. **Kuypers J, Wright N, Morrow R.** 2004. Evaluation of quantitative and type-specific real-time RT-PCR assays for detection of respiratory syn-

cytial virus in respiratory specimens from children. J. Clin. Virol. **31:** 123–129.
6. **Munro SB, Kuypers J, Jerome KR.** 2013. Comparison of a multiplex real-time PCR assay with a multiplex Luminex assay for influenza virus detection. J. Clin. Microbiol. **51:**1124–1129.
7. **Alby K, Popowitch EB, Miller MB.** 2013. Comparative evaluation of the Nanosphere Verigene RV+ assay and the Simplexa Flu A/B & RSV kit for detection of influenza and respiratory syncytial viruses. J. Clin. Microbiol. **51:**352–353.
8. **Hindiyeh M, Kolet L, Meningher T, Weil M, Mendelson E, Mandelboim M.** 8 May 2013. Evaluation of Simplexa Flu A/B & RSV for direct detection of influenza viruses (A and B) and respiratory syncytial virus in patient clinical samples. J. Clin. Microbiol. [Epub ahead of print.] doi:10.1128/JCM.00286-13.