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# Direct Detection of Influenza A and B Viruses in Less Than 20 Minutes Using a Commercially Available Rapid PCR Assay

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**We compared an FDA-cleared rapid (<20 min) PCR assay (Cobas Liat; Roche Diagnostics) to our routine influenza A and B real-time PCR assay (Simplexa Flu A/B & RSV Direct; Focus Diagnostics) using respiratory swabs ($n = 197$). The Cobas Liat influenza A and B assays demonstrated sensitivities of 99.2% (123/124) and 100% (23/23), respectively, while showing a specificity of 100% for each target.**

Influenza viruses are a significant cause of morbidity and mortality worldwide. Although a diagnosis of influenza can often be made on clinical grounds, laboratory testing may be needed to guide antimicrobial therapy, determine isolation precautions, and provide epidemiologic data. Historically, the laboratory diagnosis of influenza has been accomplished using cell culture, rapid antigen testing, and, more recently, real-time PCR. Cell culture techniques have good specificity for influenza A and B, but these methods are limited by prolonged turnaround time. Rapid antigen tests provide results in <20 min, but numerous studies have demonstrated that they may suffer from low sensitivity (1–3). Over the past decade, real-time PCR has become the test of choice for the laboratory diagnosis of influenza A and B due to its high sensitivity and specificity and reduced turnaround time compared to conventional cell culture (4–6). However, the majority of real-time PCR assays are performed in moderate- to high-complexity laboratories due to their requirement for preanalytic nucleic acid extraction, testing on a moderate-complexity platform, and subsequent interpretation by a trained laboratory technologist. Although many real-time PCR assays for influenza A and B provide results in as little as 1 to 4 h, the sample must be transported to a testing laboratory, thereby limiting the ability of health care providers to make rapid patient management decisions based on the results of the laboratory test.

Recently, the Food and Drug Administration (FDA) cleared a rapid PCR assay (Cobas Liat Influenza A/B; Roche Diagnostics, Indianapolis, IN) for the detection and differentiation of influenza A and B viruses. The Cobas Liat (lab in a tube) assay is cleared for testing nasopharyngeal (NP) swabs in viral transport media (VTM) and was labeled as moderately complex at the time of this evaluation. However, a submission for Clinical Laboratory Improvement Amendments (CLIA)-waived status is pending (Roche Diagnostics, personal communication). The Cobas Liat platform is a closed, sample-to-answer system that utilizes real-time PCR to detect influenza viruses A and B in approximately 20 min. In this study, we compared the performance of the Cobas Liat to that of our routine real-time PCR assay (Simplexa Flu A/B & RSV Direct; Focus Diagnostics, Cypress, CA) using clinical swab samples ($n = 197$) placed in 3 ml of VTM. Specimens (NP swabs [$n = 123$], throat swabs [$n = 41$], and nasal swabs [$n = 33$]) were submitted for routine testing by the Simplexa Direct assay on the Integrated Cycler (Focus Diagnostics) according to the manufacturer's recommendations. This process includes pipetting 50 μl of VTM and reaction mix (Focus Diagnostics) directly into the supplied Direct Amplification Disc (Focus Diagnostics). Testing by the Focus Simplexa assay requires ~60 min.

Among the 197 samples included in this study, 121 (61.4%) were archived specimens initially tested by the Focus Simplexa during December 2014 and subsequently stored at −20°C. All archived samples were selected based on an initial positive result for influenza A ($n = 119$) or influenza B ($n = 2$) by the Focus Simplexa assay; however, samples were not selected based on the strength of positivity. The remaining 76 (38.6%) samples were prospectively collected between February and March 2015, tested by the Focus Simplexa assay, and then stored at 4°C for ≤48 h prior to analysis by the Cobas Liat. The prospective samples were consecutive and not selected based on the results of initial testing. Among both the archived and prospective samples, specimens testing positive by the Focus Simplexa showed an average crossing point ($C_p$) value of 29.7 (range, 20.4 to 39.9). Testing by the Cobas Liat PCR system was performed according to the manufacturer's FDA-cleared package insert, which calls for 200 μl of VTM to be pipetted into the supplied assay tube and tested on the Cobas Liat Analyzer (Roche Diagnostics). The Cobas Liat assay tube includes all the reagents required for nucleic acid extraction and real-time PCR amplification and detection. The Cobas Liat Analyzer compresses the assay tube to selectively release reagents from each tube segment, moving the sample from one segment to another and controlling the reaction conditions at different temperatures. The result interpretation is automated by the Liat Analyzer, and a report of detected or not detected for influenza A or B is shown on the screen of the Liat Analyzer. Samples showing discrepant results (i.e., positive by the Focus Simplexa and negative by the Roche Cobas Liat) were retested by the Focus assay. Archived samples that were initially positive by the Focus assay but negative

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**TABLE 1** Comparison of the Roche Cobas Liat and Focus Simplexa Direct for detection of influenza A

| Cobas Liat influenza A | Focus Simplexa influenza A | | Sensitivity (%) (95% CI) | Specificity (%) (95% CI) |
|---|---|---|---|---|
| | No. positive | No. negative | | |
| Positive | 123 | 0 | 99.2 (95.1–99.9) | 100 (94.0–100) |
| Negative | 1 | 73 | | |

**TABLE 2** Comparison of the Roche Cobas Liat and Focus Simplexa Direct for detection of influenza B

| Cobas Liat influenza B | Focus Simplexa influenza B | | Sensitivity (%) (95% CI) | Specificity (%) (95% CI) |
|---|---|---|---|---|
| | No. positive | No. negative | | |
| Positive | 23 | 0 | 100 (83.1–100) | 100 (97.4–100) |
| Negative | 0 | 174 | | |

by repeat testing were considered negative during data analysis. Statistical calculations were performed using GraphPad software (GraphPad Software, Inc., La Jolla, CA).

Following the testing of 197 clinical swab samples, the results of the Cobas Liat were compared to those of our routine method (Focus Simplexa Flu A/B & RSV Direct), which was considered the reference standard for this evaluation. The Roche Cobas Liat assays demonstrated a sensitivity of 99.2% (123/124) (95% confidence interval [CI], 95.1% to 99.9%) for influenza A and 100% (23/23) (95% CI, 83.1% to 100%) for influenza B. One sample (nasal swab) was positive by the Focus Simplexa but negative by the Cobas Liat influenza A assay. This sample was repeat tested by the Focus Simplexa and was confirmed to be positive for influenza A, with a $C_p$ value of 35.9 (out of 40 cycles total). Interestingly, 14 other samples showed a $C_p$ value higher than 35.9 by the Focus Simplexa, and all of these were positive by the Cobas Liat. The specificity of the Cobas Liat influenza A and influenza B assays was 100% (Tables 1 and 2). No samples yielded an invalid result by either the Focus or Cobas Liat assays.

In this study, we compared a rapid (<20 min) PCR assay to our routine real-time PCR for the detection of influenza A and B. To our knowledge, this is the first report describing the performance of the Roche Cobas Liat platform, which performed comparably to the Focus influenza A and B Direct assays with an overall agreement of 99.5% (196/197). The Cobas Liat platform requires <5 min of hands-on time and may help bridge the gap between rapid antigen testing and standard real-time PCR. The implementation of rapid (<20 min) PCR assays, such as the Roche Cobas Liat, into hospital clinical labs, emergency departments, urgent care sites, or primary care offices may allow for a diagnosis to be made while the patient is being seen and, therefore, assist in patient management decisions.

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