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Comparison of Xpert Flu rapid nucleic acid testing with rapid antigen testing for the diagnosis of influenza A and B

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ABSTRACT

Influenza infections are associated with thousands of hospital admissions and deaths each year. Rapid detection of influenza is important for prompt initiation of antiviral therapy and appropriate patient triage. In this study the Cepheid Xpert Flu assay was compared with two rapid antigen tests, BinaxNOW Influenza A & B and BD Directigen EZ Flu A + B, as well as direct fluorescent antibody testing for the rapid detection of influenza A and B. Using real-time, hydrolysis probe-based, reverse transcriptase PCR as the reference method, influenza A sensitivity was 97.3% for Xpert Flu, 95.9% for direct fluorescent antibody testing, 62.2% for BinaxNOW, and 71.6% for BD Directigen. Influenza B sensitivity was 100% for Xpert Flu and direct fluorescent antibody testing, 54.5% for BinaxNOW, and 48.5% for BD Directigen. Specificity for influenza A was 100% for Xpert Flu, BinaxNOW, and BD Directigen, and 99.2% for direct fluorescent antibody testing. All methods demonstrated 100% specificity for influenza B. These findings support the use of the Xpert Flu assay in settings requiring urgent diagnosis of influenza A and B.

1. Introduction

Seasonal influenza infections are associated with greater than 200,000 hospital admissions and greater than 30,000 deaths each year in the United States (Thompson et al., 2006; Clark and Lynch, 2011). Rapid detection of influenza in clinical samples is critical in both ambulatory and inpatient settings to allow prompt treatment with antiviral agents, to reduce the risk of further transmission through proper implementation of infection control practices, and to limit the inappropriate use of antibiotics (Clark and Lynch, 2011). The rapid rule out of influenza may also help to avoid unnecessary hospital admission and patient isolation, as well as to reduce the inappropriate use of antiviral drugs.

Numerous commercial rapid antigen assays are available for the detection of influenza virus and inform these decisions about therapy and patient triage. These rapid tests require minimal technical expertise and have the short turnaround times necessary for clinical decision-making. The rapid antigen tests used most commonly include the BinaxNOW Influenza A & B Test and the BD Directigen EZ Flu A + B Test. The performance characteristics of these tests for the detection of influenza A 2009 H1N1 were studied extensively following the 2009 influenza pandemic. BinaxNOW demonstrated sensitivities from 11.1% to 60.3% and specificities from 93.6% to 100.0% when reverse transcriptase PCR (RT-PCR) assays were used as the reference method (CDC, 2009; Ginocchio et al., 2009; Vasoo et al., 2009; Fuenzalida et al., 2010; Liao et al., 2011). BD Directigen showed similar sensitivity and specificity for 2009 H1N1, 49.0–71.9% and 97.0–100.0%, respectively (Vasoo et al., 2009; Karre et al., 2010; Cheng et al., 2011). These rapid antigen tests exhibit comparable performance for the detection of influenza A (H3N2) and influenza B (Hurt et al., 2007; CDC, 2009).

Given the relatively poor sensitivity of rapid antigen tests, negative rapid antigen results may require follow up with more sensitive testing (CDC, 2010). Alternatively, the rapid test of choice for influenza diagnosis in some centers is direct fluorescent antibody testing, which can be performed in 1–4 h and can be more sensitive than rapid antigen tests (Pollock et al., 2009). However, direct fluorescent antibody testing requires a high level of technical expertise, is difficult to adapt to the high throughput required for pandemic testing, and remains less sensitive than real-time RT-PCR (rRT-PCR) (Ginocchio et al., 2009; Pollock et al., 2009).

Most available commercial and lab-developed influenza rRT-PCR tests are of very high complexity, requiring experienced and highly skilled staff, as well as specialized molecular diagnostic laboratory facilities to perform. Perhaps more importantly, these tests are batched, thereby prolonging turn-around-time and reducing

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clinical utility. The ideal influenza diagnostic would therefore combine the sensitivity and specificity of rRT-PCR with the rapidity and simplicity of the rapid antigen test.

The GeneXpert System from Cepheid is a cartridge-based, random access platform for performing nucleic acid extraction, PCR amplification, and real-time detection of products without intermediate sample-handling steps. Influenza testing on this platform was first introduced under Emergency Use Authorization during the 2009 pandemic for detection of influenza A and 2009 H1N1. In a comparison of the Xpert Flu A Emergency Use Authorization test with the Centers for Disease Control and Prevention (CDC) rRT-PCR assay, the GeneXpert demonstrated 91.0% sensitivity for influenza A (Miller et al., 2010). Another study showed the GeneXpert was 91.2% sensitive for detection of influenza A compared with the Luminex xTag Respiratory Virus Panel and 92.1% sensitive for detection of 2009 H1N1 compared with the Focus Diagnostics Influenza A/H1N1 (2009) rRT-PCR assay (Sambol et al., 2010). A 2nd generation Xpert Flu cartridge was developed subsequently and detects influenza A and B, with a call-out for 2009 H1N1. This 2nd generation cartridge showed 93.0% overall sensitivity for detection of influenza A and B in one study and 100.0% and 80.8% sensitivity for influenza A and B, respectively, in another, when compared with laboratory developed rRT-PCR assays (Popowitch et al., 2011; Salez et al., 2012). In contrast, another comparison of the 2nd generation cartridge with a laboratory developed rRT-PCR assay revealed sensitivities of 78.8% for influenza A and 76.5% for influenza B (Li et al., 2012). Finally, Xpert Flu demonstrated 98.1% and 93.8% sensitivity for influenza A and B, respectively, when Gen-Probe ProFlu+ rRT-PCR was used as reference (Novak-Weekley et al., 2012).

Though Xpert Flu provides an alternative to conventional rapid influenza testing, there is limited data directly comparing the performance of Xpert Flu with other rapid testing methodologies. In this study, we compared the Xpert Flu assay with BinaxNOW Influenza A & B, BD Directigen EZ Flu A + B, and direct fluorescent antibody testing, using the CDC Influenza rRT-PCR assays as the reference methods to determine test performance characteristics and suitability for settings requiring rapid diagnosis of influenza.

2. Materials and methods

2.1. Sample selection

Archival, frozen, nasopharyngeal (NP) samples from the Stanford University Medical Center Clinical Virology Laboratory collected between 2009 and 2012 were reviewed for inclusion in this retrospective study. Inclusion criteria included: adequate NP collection as assessed by direct viral exam and sufficient residual volume to perform GeneXpert Flu (Cepheid, Sunnyvale, CA), BinaxNOW Influenza A & B (Inverness Medical, Princeton, NJ), BD Directigen EZ Flu A + B (BD, Franklin Lakes, NJ), and CDC rRT-PCR for influenza A and influenza B. Two hundred specimens met these criteria and were de-identified and randomized. One hundred sixteen specimens were from adult patients and 84 specimens were from pediatric patients (<18 years). Four patients had two specimens tested. In each case, the second specimen was obtained from a unique collection, and three were collected more than 3 weeks after the first. Institutional Review Board approval was waived for this study.

2.2. Sample collection and direct fluorescent antibody testing

Fresh nasopharyngeal (NP) samples were collected using flocked swabs (Copan Diagnostics, Corona, CA) and placed into M4RT viral transport media (Remel, Lenexa, KS). Specimens were processed by centrifuging the transport medium, washing the cells with phosphate buffered saline, and then spotting the well-mixed sediment onto 8-well Teflon masked slides. The cells were then fixed with acetone and stained with a respiratory virus direct fluorescent antibody panel (Diagnostic Hybrids, Athens, OH) that includes antibodies for the detection of influenza A, influenza B, RSV, hMPV, adenovirus, and parainfluenza virus 1, 2, and 3. Samples were considered positive if one or more intact cells exhibited specific fluorescence. A minimum sampling of 15 columnar epithelial cells per well was required for a negative result. Samples with insufficient cells were not evaluated. All direct fluorescent antibody slides were read by at least two clinical laboratory scientists, which is standard protocol. Direct fluorescent antibody testing was performed within 48 h of specimen collection. Specimens included in the study were composed of direct fluorescent antibody confirmed cases of influenza A (72), influenza B (33), respiratory syncytial virus (11) [RSV], human metapneumovirus (9) [hMPV], adenovirus (9), parainfluenza 1 (1), parainfluenza 2 (1), parainfluenza 3 (7), and specimens negative for these viruses (57).

2.3. GeneXpert flu and rapid antigen testing

A single positive control for each test method was assayed daily. For the BinaxNOW Influenza A & B and BD Directigen EZ Flu A + B tests, the supplied controls were tested as per manufacturer's instructions at the start of each testing day. For the Cepheid Xpert Flu assay, cultured reference influenza A 2009 H1N1 (A/California/04/2009) and influenza B (B/Maryland/1/59) viruses were utilized. All external controls were acceptable each testing day.

The archival, frozen samples were thawed at room temperature once and tested by the three rapid methods simultaneously. The individual performing the tests was blinded to the previous direct fluorescent antibody testing results. As rapid antigen testing results were available at 15 min, those results were collected prior to the availability of the GeneXpert results. The three test methods were as follows:

(1) BinaxNOW Influenza A & B testing was performed as per manufacturer's package insert by transferring 100 μL of viral transport media to the testing strip and interpreting results at 15 min. A calibrated pipette was used to transfer the media instead of the disposable pipettes supplied in the test kit.
(2) BD Directigen EZ Flu A + B testing was performed as per manufacturer's package insert with no deviations. Briefly, 300 μL of viral transport media was added to 4 drops of Reagent E, and vortexed. Three drops of this mixture were transferred to each of the Flu A and Flu B sample wells on the test device, and results were interpreted at 15 min.
(3) Xpert Flu testing was performed as per manufacturer's instructions. For each specimen, 300 μL of viral transport media was added to the sample opening of the test cartridge and the entire contents of an ampule of Binding Reagent 1 was added to the small opening. A calibrated pipette was used instead of the disposable pipette supplied by the manufacturer. The cartridge was then loaded into a GeneXpert module for testing. The GeneXpert software reported results after 77 min.

2.4. rRT-PCR

The CDC influenza rRT-PCR protocols for universal detection of influenza A and influenza B were modified for use with available reagents and instrumentation (WHO/CDC, 2009; Selvaraju and Selvarangan, 2010). Nucleic acids were extracted from 200 μL of specimen with the EZ1 Virus Mini Kit v. 2.0 on either the EZ1 or EZ1 Advanced XL workstations (Qiagen-USA, Valencia, CA) and eluted into a volume of 60 μL. Each 20 μL reaction on the

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Table 1
Summary of test performance for influenza A and B.

	Influenza A		Influenza B	
	Sensitivity[a]	Specificity	Sensitivity	Specificity
Xpert Flu	97.3% (89.6–99.5)[b]	100.0% (96.3–100.0)	100.0% (87.0–100.0)	100.0% (97.2–100.0)
Direct fluorescent antibody	95.9% (87.8–98.9)	99.2% (95.0–99.9)	100.0% (87.0–100.0)	100.0% (97.2–100.0)
BinaxNOW	62.2% (50.1–73.0)	100.0% (96.3–100.0)	54.5% (36.6–71.5)	100.0% (97.2–100.0)
BD Directigen	71.6% (59.8–81.2)	100.0% (96.3–100.0)	48.5% (31.2–66.1)	100.0% (97.2–100.0)

[a] Sensitivity and specificity are calculated using rRT-PCR as reference.
[b] The values in parentheses are the 95% confidence intervals.

Rotor-Gene Q (Qiagen-USA, Valencia, CA) contained 5 μL of extracted nucleic acids, 10 μL of 2× Superscript One-Step RT-PCR Reaction Mix, 0.5 μL SuperScript II Reverse Transcriptase/Platinum *Taq* DNA Polymerase (Invitrogen, Carlsbad, CA), and primers (Eurofins MWG Operon, Huntsville, AL) and probes (Biosearch Technologies, Novato, CA) at the concentrations shown in Supplementary Table. Each target was tested in an individual reaction. The reactions underwent reverse transcription at 50 °C for 30 min, reverse transcriptase inactivation and *Taq* hot-start at 95 °C for 2 min, and 45 cycles of 95 °C for 15 s and 55 °C for 45 s. Data were collected on the green channel. The threshold for each target was set at 0.03 normalized fluorescence units. Specimens with exponential amplification in the green channel and a crossing threshold ≤40 cycles were considered positive. Amplification of RNase P RNA confirmed adequate extraction and the absence of reaction inhibitors.

Influenza A positive extracted nucleic acids were subtyped for 2009 H1N1 using a lab-developed rRT-PCR assay (Trevino et al., 2011). Extracts negative for 2009 H1N1 by the lab-developed test were subtyped using Prodesse ProFAST+ reagents (Gen-Probe Prodesse, Waukesha, WI). Briefly, each 25 μL reaction on the Rotor-Gene Q contained 5 μL of extracted nucleic acids, 19.45 μL of ProFAST+ Supermix, 0.30 μL M-MLV Reverse Transcriptase, and 0.25 RNase Inhibitor. The reactions underwent reverse transcription at 42 °C for 30 min, reverse transcriptase inactivation and *Taq* hot-start at 95 °C for 10 min, 5 cycles of 95 °C for 30 s and 55 °C for 60 s, then 40 cycles of 95 °C for 10 s and 55 °C for 60 s. Data were collected on the green (H1), yellow (H3), and orange (2009 H1N1) channels. The threshold was set at 0.10 normalized fluorescence units for the green and orange channels, and 0.05 units for the yellow channel. Specimens with exponential amplification and a crossing threshold ≤40 cycles were considered positive.

3. Results

A total of 200 specimens were tested by Xpert Flu, direct fluorescent antibody, BinaxNOW Influenza A & B, and BD Directigen EZ Flu A + B. There were no BD Directigen internal quality control failures. Three Xpert Flu tests (1.5%; 3/200) and two BinaxNOW tests (1.0%; 2/200) failed internal quality control on initial testing but were each interpretable on the second attempt.

Seventy four influenza A positive specimens were tested. The sensitivity was 97.3% (72/74) for Xpert Flu, 95.9% (71/74) for direct fluorescent antibody testing, 62.2% (46/74) for BinaxNOW, and 71.6% (53/74) for BD Directigen (Table 1). All specimens positive for influenza A by rapid antigen testing were detected by the Xpert Flu assay. Specificity for influenza A was 100% (126/126) for Xpert Flu, BinaxNOW, and BD Directigen, and 99.2% (125/126) for direct fluorescent antibody testing.

All influenza A positive specimens were subtyped. There were 56 2009 H1N1, 13 H3N2, and 5 previous, seasonal H1N1 viruses. Xpert Flu had a sensitivity of 96.4% for 2009 H1N1 (54/56) and 100.0% for H3N2 (13/13) and H1N1 (5/5). BinaxNOW detected 64.3% (36/56) of 2009 H1N1, 76.9% (10/13) of H3N2, and 00.0% (0/5) of H1N1 viruses. BD Directigen had a sensitivity of 75.0% (42/56) for 2009 H1N1, 76.9% (10/13) for H3N2, and 20.0% (1/5) for H1N1.

Of the 54 2009 H1N1 positive specimens detected by the Xpert Flu assay, all were subtyped accurately. None of the H3N2 or H1N1 viruses were subtyped incorrectly.

Thirty-three influenza B positive specimens were tested. Influenza B sensitivity was 100% (33/33) for Xpert Flu and direct fluorescent antibody testing, 54.5% (18/33) for BinaxNOW, and 48.5% (16/33) for BD Directigen (Table 1). All methods demonstrated 100% (167/167) specificity for influenza B.

4. Discussion

The accurate and prompt detection of influenza is crucial for patient triage and the timely initiation of antiviral therapy. In this study we compared the Cepheid Xpert Flu assay on the GeneXpert platform with direct fluorescent antibody testing and two rapid antigen tests, BinaxNOW Influenza A & B and BD Directigen EZ Flu A + B, using rRT-PCR as the reference method.

Sensitivity for influenza by direct fluorescent antibody testing in our clinical laboratory is comparable to the highest performing direct fluorescent antibody testing reported in the recent literature (Pollock et al., 2009). These results suggest that direct fluorescent antibody testing can be a reasonable respiratory virus testing option in experienced laboratories with low to moderate test volumes.

The rapid antigen tests demonstrated performance characteristics consistent with previous reports (Chartrand et al., 2012; Chu et al., 2012). These tests are simple to perform and continue to provide the most rapid results, which if positive, are of immediate clinical utility. However, our results confirm the low sensitivity of these assays and support use of more sensitive follow-up testing of rapid antigen negative specimens.

Xpert Flu, in contrast to the rapid antigen tests, showed sensitivities of 97.3% and 100.0% compared with rRT-PCR for influenza A and B, respectively. Previous Xpert Flu studies demonstrated similar assay performance (Miller et al., 2010; Sambol et al., 2010; Popowitch et al., 2011; Novak-Weekley et al., 2012; Salez et al., 2012). The two influenza A specimens not detected by Xpert Flu had late rRT-PCR crossing thresholds (39 cycles). Xpert Flu also provided accurate subtyping of 2009 H1N1. However, the clinical utility of this feature is unclear, since the co-circulating 2009 H1N1 and H3N2 viruses have the same susceptibility profiles to available anti-influenza drugs.

In laboratories seeking an alternative to rapid antigen testing, the Xpert Flu assay provides superior sensitivity in a rapid and simple format. This increased sensitivity should limit the need for reflexive testing of negative samples and therefore has the potential to reduce the cost associated with ruling out influenza infection. Future cost analyses are necessary to determine the financial impact of Xpert Flu compared with rapid antigen testing. However, given that Xpert Flu is less sensitive than conventional

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batched rRT-PCR assays, respiratory virus testing algorithms that include Xpert Flu may still require more sensitive methodologies to follow up negative results in patients at risk for severe disease, such as transplant recipients and others with immune compromise. Often, these patients are tested for a panel of respiratory viruses, so including Xpert Flu in the testing algorithm may help triage influenza positive patients and reduce additional respiratory virus panel testing. Future studies are necessary to determine the optimal use of Xpert Flu in respiratory virus testing algorithms for different patient populations.

5. Conclusions

In summary, this study demonstrates that Xpert Flu is more sensitive than two rapid antigen tests, BinaxNOW Influenza A & B and BD Directigen EZ Flu A + B. Importantly, Xpert Flu is as simple to use as the rapid antigen tests and generates results within 90 min, a turn-around-time sufficient to impact clinical decision-making. These findings support the use of the Xpert Flu assay in settings requiring the timely and accurate diagnosis of influenza.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jviromet.2012.07.023.

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