JCM
Journals.ASM.org

Evaluation of the Xpert Flu Rapid PCR Assay in High-Risk Emergency Department Patients

Andrea F. Dugas,[a] Alexandra Valsamakis,[b] Charlotte A. Gaydos,[a,c] Michael Forman,[b] Justin Hardick,[c] Pranav Kidambi,[a]* Sharmeen Amin,[a]* Alisha Gupta,[a]* Richard E. Rothman[a,c]

Department of Emergency Medicine, Johns Hopkins University, Baltimore, Maryland, USA[a]; Department of Pathology, Johns Hopkins University, Baltimore, Maryland, USA[b]; Department of Medicine, Division of Infectious Disease, Johns Hopkins University, Baltimore, Maryland, USA[c]

We prospectively evaluated the performance of Cepheid's GeneXpert Xpert Flu assay in a target population of 281 adults presenting to the emergency department with an acute respiratory illness who met Centers for Disease Control and Prevention (CDC) criteria for recommended antiviral treatment. Compared with the Prodesse ProFlu+ assay, Xpert Flu had an overall sensitivity of 95.3% and specificity of 99.2%.

The majority of patients seeking care for influenza and other respiratory viruses present to episodic outpatient care settings, such as emergency departments (EDs) or other urgent or primary care settings, where rapid diagnosis and treatment are critical (1). Due to nonspecific symptoms, a provider's clinical diagnosis of influenza has low sensitivity, leading providers to rely on diagnostic testing for an accurate influenza diagnosis (2). Most commercially available real-time PCR (rt-PCR) tests are typically run in batches and require separate nucleic acid extraction, which significantly delays the results. The current antigen detection tests are rapid but have poor-to-moderate sensitivities, ranging from 10% to 70% (3). Rapid random-access PCR-based influenza tests, such as the GeneXpert Xpert Flu assay (Cepheid, Sunnyvale, CA, USA), may have clinical utility in filling this diagnostic gap, since it has a reported time to result of approximately 80 min and has a significantly higher sensitivity than rapid antigen detection tests (4).

Prior to integration into routine clinical use, the clinical performance of Xpert Flu in the target population requires evaluation. Although clinicians in the outpatient episodic care setting may test an array of patients, accurate and rapid influenza testing with Xpert Flu would be most important in patients for whom the test result would impact clinical management, namely, those who meet the Centers for Disease Control and Prevention (CDC) criteria for antiviral therapy and are at risk for potential influenza-related complications. Several of these conditions, such as advanced age and pneumonia, have been associated with the decreased sensitivity of rapid antigen-based testing, highlighting the importance of evaluating Xpert Flu in this population (5). In order to fully translate rapid PCR-based testing into clinical practice, we prospectively evaluated the sensitivity and specificity of Xpert Flu in adult ED patients with an acute undifferentiated respiratory illness who met CDC criteria for recommended antiviral treatment.

Adult ED patients with an undifferentiated acute respiratory illness who met CDC criteria for recommended influenza antiviral treatment at an urban university-affiliated tertiary-care ED were prospectively enrolled between December 2012 and March 2013. After written consent was obtained, as approved by the Johns Hopkins University institutional review board (IRB), a nasopharyngeal swab was collected from each patient and placed in 3 ml of viral transport medium (MicroTest M4RT; Remel, Lenexa, KS, USA). All the samples were aliquoted, stored at −70°C until completion of the study, and tested after a single freeze-thaw cycle. All

TABLE 1 Subject characteristics

Characteristic	Subject data[a]
Total no. of subjects	281
Age (yr)[b]	50 (38–58)
Gender (male)	119 (42)
Race	
African American	228 (81)
White	44 (15)
Other	9 (3.2)
CDC guidelines for antiviral treatment met	
Hospital admission	123 (44)
Complications/pneumonia	19 (6.8)
Age ≥ 65 yr	39 (14)
Chronic disease	
Pulmonary	176 (63)
Cardiovascular	64 (23)
Renal	32 (11)
Hematologic	23 (8.2)
Metabolic	70 (25)
Neurologic	25 (8.9)
Immunosuppression	72 (26)
Pregnancy	1 (0.4)
Morbid obesity	25 (8.9)
Nursing home residence	7 (2.5)
Native American	0 (0)

[a] Data are no. (%) unless otherwise indicated.
[b] Age is presented as the median (interquartile range).

Received 13 August 2014 **Returned for modification** 2 September 2014
Accepted 17 September 2014
Published ahead of print 24 September 2014
Editor: Y.-W. Tang
Address correspondence to Andrea F. Dugas, adugas1@jhmi.edu.
* Present address: Pranav Kidambi, Mount Auburn Hospital, Cambridge, Massachusetts, USA; Sharmeen Amin, Sind Medical College, Dow University of Health Sciences, Karachi, Pakistan; Alisha Gupta, Shubham Hospital, Rohini, Delhi, India

doi:10.1128/JCM.02343-14

TABLE 2 Test characteristics of Xpert Flu in comparison to those of Prodesse ProFlu+

Variable	Comparative Xpert Flu Data for[a]:		
	All influenza strains ($n = 43$)	Influenza A ($n = 28$)	Influenza B ($n = 15$)
Influenza prevalence (%)	15 (11–20)	10 (6.7–14.1)	5.3 (3.2–8.7)
Test characteristic (%)			
Sensitivity	95.3 (84.2–99.4)	96.4 (81.7–99.9)	93.3 (68.1–99.8)
Specificity	99.2 (97.0–99.9)	99.6 (97.8–100)	99.6 (97.9–100)
Likelihood ratio			
Positive	113 (28.5–452)	244 (35–1,727)	248 (35–1,764)
Negative	0.05 (0.01–0.18)	0.04 (0.01–0.25)	0.07 (0.01–0.45)
Predictive value (%)			
Positive	95.3 (84.2–99.4)	96.4 (81.7–99.9)	93.3 (68.1–99.8)
Negative	99.2 (97.0–99.9)	99.6 (97.8–100)	99.6 (97.9–100)

[a] The 95% confidence intervals are listed in parentheses.

testing was performed in a blinded fashion. Testing with ProFlu+ (Hologic Gen-Probe, Inc., San Diego, CA, USA) and Xpert Flu (Cepheid, Sunnyvale, CA, USA) was performed according to the manufacturers' instructions, with the exception of samples that had indeterminate Xpert Flu results (6, 7). Samples with indeterminate Xpert Flu results were not retested due to volume constraints; these samples were omitted from the final analysis.

For the primary analysis, Prodesse ProFlu+ was considered the comparative standard. A similar subgroup analysis was performed to evaluate patients who had the highest-acuity illness (i.e., those admitted to the hospital). Data were analyzed utilizing basic descriptive statistics and Stata statistical software, release 11 (Stata Corp, College Station, TX).

Of the 303 subjects enrolled, 281 had sufficient data to be included in the final analysis. Of the 22 excluded subjects, 1 subject did not meet full inclusion criteria, 11 did not have ProFlu+ testing, 5 did not have Xpert Flu testing, and 5 had Xpert Flu tests that resulted in an error code. Among the 281 subjects included in the final analysis, 126 (44%) were admitted to the hospital. Additional details regarding the included subjects and the criteria for CDC-recommended antiviral treatment are listed in Table 1.

Of the 281 subjects, 43 (15%) were positive for influenza by ProFlu+; 28 were positive for influenza A, and 15 were positive for influenza B. Compared to ProFlu+, Xpert Flu had sensitivities of 95.3% (95% confidence interval [CI], 84.2% to 99.4%) overall, 96.4% (95% CI, 81.7% to 99.9%) for influenza A, and 93.3% (95% CI, 68.1% to 99.8%) for influenza B (Table 2). Although Xpert Flu also detects 2009 H1N1, no sample was positive for influenza A, 2009 H1N1; the main circulating strain during the 2012-2013 season was H3N2. Restricting the comparison to the patients with the highest-acuity illness (i.e., requiring hospital admission), Xpert Flu had 100% sensitivity and specificity for influenza A (sensitivity, 100% [95% CI, 76% to 100%]; specificity, 100% [95% CI, 97% to 100%]) and for influenza B (sensitivity, 100% [95% CI, 57% to 100%]; specificity, 100% [9% CI, 97% to 100%]).

This is the first time that the Xpert Flu rapid diagnostic test has been evaluated in a high-acuity ED population, where undifferentiated patients are evaluated and treated. In this high-acuity target population, Xpert Flu had high overall sensitivity and specificity compared those of ProFlu+, similar to what has been reported by several previous studies performed in more general patient populations (4, 8–13). From a clinical viewpoint, diagnosing influenza and initiating antiviral treatment in the admitted population is most critical, as antivirals have shown substantial benefit, including a reduction in mortality rates, in this population (14–16). One previous study demonstrated poor performance of Xpert Flu among hospitalized patients; however, our study showed excellent performance among the subpopulation of admitted patients, with 100% sensitivity and specificity (17).

This evaluation of Xpert Flu was performed in a single inner-city ED and did not include otherwise healthy patients or children, thus potentially reducing the generalizability to all patients in various geographic locations. Additionally, we did not evaluate the sensitivities of H1N1 strains. However, when prospectively evaluated in a population of undifferentiated ED patients who already had or were at increased risk for influenza-related complications, Xpert Flu demonstrated high sensitivity and specificity. With demonstrated high levels of sensitivity and specificity in a clinical ED population, and a rapid turnaround time of 80 min, Xpert Flu has significant potential to aid clinicians who work in episodic care settings, such as EDs or urgent care centers, where rapid influenza diagnosis and management can be challenging.

ACKNOWLEDGMENTS

This work was supported by the Johns Hopkins Clinical Research Scholars Program (5KL2RR025006) and by Cepheid (Sunnyvale, CA).

REFERENCES

1. **Linder JA, Bates DW, Platt R.** 2005. Antivirals and antibiotics for influenza in the United States, 1995–2002. Pharmacoepidemiol. Drug Saf. **14:** 531–536. http://dx.doi.org/10.1002/pds.1067.
2. **Stein J, Louie J, Flanders S, Maselli J, Hacker JK, Drew WL, Gonzales R.** 2005. Performance characteristics of clinical diagnosis, a clinical decision rule, and a rapid influenza test in the detection of influenza infection in a community sample of adults. Ann. Emerg. Med. **46:**412–419. http://dx.doi.org/10.1016/j.annemergmed.2005.05.020.
3. **Centers for Disease Control and Prevention.** 2009. Evaluation of rapid influenza diagnostic tests for detection of novel influenza A (H1N1) virus—United States, 2009. MMWR Morb. Mortal. Wkly. Rep. **58:**826–829. http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5830a2.htm.
4. **Sambol AR, Iwena PC, Pieretti M, Basuc S, Levic MH, Gilonsked DK, Mosesd DK, Marolae LJ, Ramamoorthye P.** 2010. Validation of the Cepheid Xpert Flu A real time RT-PCR detection panel for emergency use authorization. J. Clin. Virol. **48:**234–238. http://dx.doi.org/10.1016/j.jcv.2010.06.001.

5. **Chan MC, Lee N, Ngai KL, Leung TF, Chan PK.** 2014. Clinical and virologic factors associated with reduced sensitivity of rapid influenza diagnostic tests in hospitalized elderly patients and young children. J. Clin. Microbiol. **52:**497–501. http://dx.doi.org/10.1128/JCM.02316-13.
6. **Hologic Gen-Probe, Inc.** 2013. Prodesse Proflu+ assay package insert. Hologic Gen-Probe Inc., San Diego, CA.
7. **Cepheid.** 2012. Xpert Flu assay package insert. Cepheid, Sunnyvale, CA.
8. **Salez N, de Lamballerie X, Zandotti C, Gazin C, Charrel RN.** 2013. Improved sensitivity of the novel Xpert flu test for detection of influenza B virus. J. Clin. Microbiol. **51:**4277–4278. http://dx.doi.org/10.1128/JCM.02125-13.
9. **Salez N, Ninove L, Thirion L, Gazin C, Zandotti C, de Lamballerie X, Charrel RN.** 2012. Evaluation of the Xpert Flu test and comparison with in-house real-time RT-PCR assays for detection of influenza virus from 2008 to 2011 in Marseille, France. Clin. Microbiol. Infect. **18:**E81–E83. http://dx.doi.org/10.1111/j.1469-0691.2012.03792.x.
10. **Popowitch EB, Rogers E, Miller MB.** 2011. Retrospective and prospective verification of the Cepheid Xpert influenza virus assay. J. Clin. Microbiol. **49:**3368–3369. http://dx.doi.org/10.1128/JCM.01162-11.
11. **Novak-Weekley SM, Marlowe EM, Poulter M, Dwyer D, Speers D, Rawlinson W, Baleriola C, Robinson CC.** 2012. Evaluation of the Cepheid Xpert Flu assay for rapid identification and differentiation of influenza A, influenza A 2009 H1N1, and influenza B viruses. J. Clin. Microbiol. **50:**1704–1710. http://dx.doi.org/10.1128/JCM.06520-11.
12. **Jenny SL, Hu Y, Overduin P, Meijer A.** 2010. Evaluation of the Xpert Flu A panel nucleic acid amplification-based point-of-care test for influenza A virus detection and pandemic H1 subtyping. J. Clin. Virol. **49:**85–89. http://dx.doi.org/10.1016/j.jcv.2010.07.005.
13. **DiMaio MA, Sahoo MK, Waggoner J, Pinsky BA.** 2012. Comparison of Xpert Flu rapid nucleic acid testing with rapid antigen testing for the diagnosis of influenza A and B. J. Virol. Methods **186:**137–140. http://dx.doi.org/10.1016/j.jviromet.2012.07.023.
14. **McGeer A, Green KA, Plevneshi A, Shigayeva A, Siddiqi N, Raboud J, Low DE.** 2007. Antiviral therapy and outcomes of influenza requiring hospitalization in Ontario, Canada. Clin. Infect. Dis. **45:**1568–1575. http://dx.doi.org/10.1086/523584.
15. **Muthuri SG, Myles PR, Venkatesan S, Leonardi-Bee J, Nguyen-Van-Tam JS.** 2013. Impact of neuraminidase inhibitor treatment on outcomes of public health importance during the 2009–10 influenza A(H1N1) pandemic: a systematic review and meta-analysis in hospitalized patients. J. Infect. Dis. **207:**553–563. http://dx.doi.org/10.1093/infdis/jis726.
16. **Hsu J, Santesso N, Mustafa R, Brozek J, Chen YL, Hopkins JP, Cheung A, Hovhannisyan G, Ivanova L, Flottorp SA, Saeterdal I, Wong AD, Tian J, Uyeki TM, Akl EA, Alonso-Coello P, Smaill F, Schünemann HJ.** 2012. Antivirals for treatment of influenza: a systematic review and meta-analysis of observational studies. Ann. Intern. Med. **156:**512–524. http://dx.doi.org/10.7326/0003-4819-156-7-201204030-00411.
17. **Pierro A, Gaibani P, Rossini G, Landini MP, Sambri V.** 2013. Clinical application of a molecular method based on real time RT-PCR for detection of influenza A(H1N1)v virus. New Microbiol. **36:**405–408.