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Performance of six influenza rapid tests in detecting human influenza in clinical specimens

Aeron C. Hurt [a,b,*], Robert Alexander [c], Jan Hibbert [c], Nicola Deed [a], Ian G. Barr [a,b]

[a] *World Health Organisation Collaborating Centre for Reference and Research on Influenza, 45 Poplar Road, Parkville, Victoria 3052, Australia*
[b] *Monash University, School of Applied Sciences, Churchill, Victoria 3842, Australia*
[c] *Royal Children's Hospital, Department of Microbiology and Virology, Flemington Road, Parkville, Victoria 3052, Australia*

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Abstract

Background: The rapid diagnosis of influenza can alter the management of a patient's illness, resulting in reduced antibiotic usage, correct use of influenza antivirals and reduced length of stay in hospital emergency departments. The rapid tests have also been used to detect outbreaks in institutions and may play a role in pandemic influenza control.
Objectives: To test six different rapid influenza tests, in a head-to-head comparison for the detection of seasonal influenza types A and B, compared to laboratory-based tests.
Study design: One hundred and seventy-seven clinical specimens taken from mostly paediatric patients between June and October 2006 were tested using six influenza diagnostic tests and three laboratory-based techniques (immunofluorescence, cell culture and real-time RT-PCR).
Results and conclusion: Compared with cell culture, five of the rapid tests (Binax Now Influenza A&B, Directigen EZ Flu A + B, Denka Seiken Quick Ex-Flu, Fujirebio Espline Influenza A&B-N, and Quidel QuickVue Influenza A + B Test) demonstrated a similar influenza A sensitivity of between 67–71% and a specificity of 99–100%, however one rapid test (Rockeby Influenza A Antigen Test) had a significantly lower influenza A sensitivity of only 10% (specificity was 100%). For the five kits that detected influenza B antigen, sensitivity was considerably lower than that seen for influenza A (sensitivity for all the kits was 30%), although the number of specimens containing influenza B viruses was low.

Keywords: Influenza; Rapid test; Rapid kit; Diagnostic

1. Introduction

Influenza is a respiratory infection that can result in significant morbidity and mortality, particularly in the young, elderly or immunosuppressed (Simonsen, 1999). However, rapid diagnosis of influenza infection can facilitate the use of appropriate treatment, both improving the patients clinical outcome and significantly reducing hospital costs (Bonner et al., 2003). A large number of rapid influenza detection tests are now available that are simple to use, quick (generate a result in 15 min) and can be performed outside the laboratory. In general, previous studies have reported the tests to have a good specificity (99–100%), however different studies have reported significantly different sensitivity values even when evaluating the same test (Storch, 2003). For example the reported influenza A sensitivity for the Becton Dickinson Directigen Flu A + B Test ranges from 44% (Cazacu et al., 2004) to 96% (Chan et al., 2002). While study-to-study variability in sensitivity with the same test can be explained by the differences in specimen type, influenza type/subtype circulating, patient age and the 'gold standard' test used for comparison, these differences highlight the difficulties in comparing results of individual rapid tests when they are

* Corresponding author at: World Health Organisation Collaborating Centre for Reference and Research on Influenza, 45 Poplar Road, Parkville, Victoria 3052, Australia. Tel.: +61 3 9389 1392.
E-mail address: aeron.hurt@influenzacentre.org (A.C. Hurt).

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evaluated in different studies. To date there are few reports of evaluations that have included more than three rapid tests within the same study (Rodriguez et al., 2002). In this study, however, we have conducted a head-to-head comparison of six influenza rapid tests for their ability to detect influenza A and B on the same clinical specimens and compared these results with laboratory detection methods for influenza.

2. Methods

2.1. Clinical specimens

One hundred and seventy-seven respiratory samples (150 nasopharyngeal aspirates; 8 nasal swabs; 8 bronchioalveolar lavages; 6 sputum samples; 5 throat swabs) were obtained from patients with influenza-like illness at the Royal Children's Hospital, Melbourne, Australia between June and October 2006. Patients were aged from 4 days to 64 years old (with 78% of patients being five years old or less), 59% of patients were male and 41% female.

2.2. Rapid influenza tests

Six influenza rapid tests—Binax Now Influenza A&B (Binax; Portland, USA), BD Directigen EZ Flu A + B (BD EZ; Sparks, MD, USA), Denka Seiken Quick Ex-Flu (Denka; Japan), Fujirebio Espline Influenza A&B-N (Fujirebio; Japan), Rockeby Influenza A Antigen Test (Rockeby; Singapore) and Quidel QuickVue Influenza A + B Test (Quidel; San Diego, CA, USA), were evaluated for their ability to detect influenza antigen by carefully following each of the manufacturer's own protocols. The tests were performed on undiluted original specimens as soon as possible after sample collection, but no longer than 24 h after collection. All of the kits detect and differentiate between influenza A and B, except for the Rockeby test which detects only influenza A.

2.3. Laboratory-based techniques

Samples were processed for routine viral diagnosis in the virology laboratory at the Royal Children's Hospital, Melbourne, using a direct immunofluorescence assay (DIF) and a rapid enhanced tissue culture combined with immunofluorescence assay (RETCIF) (Alexander et al., 2001), utilising specific monoclonal antibodies to identify a range of respiratory viruses. Samples were inoculated into three different cell lines (MDCK, LLC-MK2 and Hep G cells) between 2 and 24 h post-sample collection (majority of samples inoculated between 2 and 6 h following sample collection). An aliquot of 200 μl of the undiluted clinical specimen was stored at −80 °C for RNA extraction and subsequent real-time RT-PCR analysis at the WHO Collaborating Centre for Influenza, Melbourne, Australia. RNA extraction was performed using a MagnaPure extraction system (Roche) in accordance with manufacturer's instructions. Real-time

Table 1
Comparison of the influenza A and B sensitivity, specificity, positive predictive value (PPV) and negative predictive values (NPV) for the six different rapid tests to RETCIF

Rapid test	Influenza A				Influenza B			
	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Binax Now Influenza A&B	36/49 (**73**)	127/128 (**99**)	36/37 (**97**)	127/140 (**91**)	3/10 (**30**)	167/167 (**100**)	3/3 (**100**)	167/174 (**96**)
BD Directigen EZ Flu A + B	34/49 (**69**)	128/128 (**100**)	34/34 (**100**)	128/143 (**90**)	3/10 (**30**)	167/167 (**100**)	3/3 (**100**)	167/174 (**96**)
Denka Seiken Quick Ex-Flu	35/49 (**71**)	128/128 (**100**)	35/35 (**100**)	128/142 (**90**)	3/10 (**30**)	167/167 (**100**)	3/3 (**100**)	167/174 (**96**)
Fujirebio Espline Influenza A&B-N	33/49 (**67**)	128/128 (**100**)	33/33 (**100**)	128/144 (**89**)	3/10 (**30**)	167/167 (**100**)	3/3 (**100**)	167/174 (**96**)
Rockeby Influenza A Antigen Test	5/49 (**10**)	128/128 (**100**)	5/5 (**100**)	128/172 (**74**)	Test detects influenza A antigen only			
Quidel Quickvue Influenza A + B Test	33/49 (**67**)	128/128 (**100**)	33/33 (**100**)	128/144 (**89**)	3/10 (**30**)	167/167 (**100**)	3/3 (**100**)	167/174 (**96**)

RT-PCR detection was performed using Taqman One-Step RT-PCR Master Mix Reagents (Applied Biosystems, USA) and utilising separate influenza A primers and probe as described previously (Heine et al., 2007) and influenza B primers (Poddar, 2002) with SYBR green (Qiagen QuantiTect SYBR green RT-PCR kit) using an Applied Biosystems 7500 Real Time PCR-System.

3. Results

Of the177 clinical specimens obtained, 49 were found to be positive for influenza A antigen by RETCIF; the laboratory based technique which has been used in this evaluation as the 'gold standard' for the purposes of rapid test comparisons. An additional specimen was determined to be influenza A positive by real-time RT-PCR (i.e. 50 influenza A positive specimens), while one less specimen was found to be influenza A positive by DIF (48 influenza A positive specimens). Only ten specimens in the study were found to be influenza B positive by RETCIF, while nine and six of these were detected as influenza B by RT-PCR and DIF, respectively. From the remaining influenza negative specimens, four were found to be positive for adenovirus, one positive for cytomegalovirus, one parainfluenza virus type 1, four parainfluenza virus type 3, 44 for respiratory syncytial virus (RSV), and 2 with mixed infections containing both adenovirus and RSV.

Five of the six rapid tests evaluated (Binax, BD EZ, Denka, Fujirebio and Quidel) detected a similar number of influenza A positive specimens (33–36/49), resulting in sensitivities ranging from 67% to 73% (Table 1). However, the Rockeby test, detected only 5/49 influenza A positive specimens, giving a sensitivity of 10% (Table 1). Of the ten influenza B specimens detected by RETCIF, three were detected by each of the five rapid tests (Rockeby test does not detect influenza B) (Table 1) resulting in a sensitivity of 30% for all the rapid tests, a value significantly lower than the influenza A sensitivity. The specificity of all of the rapid tests was excellent (99–100% for both influenza A and B), with the Binax test the only assay to give a single false influenza A positive result, for a sample that was found to contain RSV by RETCIF (Table 1).

Analysis of the data from patients less than 5 years old ($n = 137$) was found to significantly increase the sensitivity of the rapid test kits (Table 2). For example the sensitivity of the Binax test increased from 73% (based on data from all aged patients) to 91% when calculated on specimens obtained from patients under 5 years of age. Other kits also increased between 17% and 19% in sensitivity if only samples from children less than 5 years old were considered (with the exception of the Rockeby test which increased only 2% in sensitivity). Sensitivity values did not differ significantly between the less than 5-year old cohort and the less than 2-year old cohort.

4. Discussion

Comparisons between the sensitivities of rapid tests evaluated in different studies are inherently difficult given the variability in study designs (patient age, sample type and comparators). As such, studies like this present one, which compared six different rapid influenza tests head-to-head on clinical samples, are of significant value to physicians, general practitioners and others in choosing which influenza rapid test to use. Apart from the Rockeby test, the other five tests demonstrated very similar influenza A sensitivity, which increased significantly when analysing only specimens from children under five years of age. Higher sensitivities have been reported previously in samples from young children compared to adults (Alexander et al., 2005; Cazacu et al., 2004; Ruest et al., 2003), presumably as a consequence of higher levels and longer duration of viral shedding in the younger age group (Frank et al., 1981). In addition, previous reports have also found significantly higher sensitivities when analysing nasopharyngeal aspirates (85% of the specimens tested in this study), compared with other specimen types such as throat swabs (Smit et al., 2007). Probably as a consequence of testing mainly nasopharyngeal aspirates from paediatric patients, influenza A sensitivity values from this study were higher than previously reported for the Binax (59% in (Smit et al., 2007)) and BD EZ (41% in (Weinberg and Walker, 2005)) tests. In contrast, the sensitivity of the Fujirebio test has previously been reported to be higher than determined in this study (93% in one study (Mitamura et al., 2004) and 100% in another (Hara et al., 2005)). The authors are unaware of any previously published evaluations of either the Rockeby test, the Quidel QuickVue Influenza A + B Test (the version that differentiates between influenza A and B)

Table 2
Sensitivity of rapid test kits based on data selected from different patient age ranges

Rapid test	Influenza A sensitivity (%); age range			
	0–2 years	0–5 years	0–15 years	All ages
Binax Now Influenza A&B	23/27 (**89**)	30/33 (**91**)	34/40 (**85**)	36/49 (**73**)
BD Directigen EZ Flu A + B	23/27 (**89**)	29/33 (**88**)	32/40 (**80**)	34/49 (**69**)
Denka Seiken Quick Ex-Flu	23/27 (**89**)	29/33 (**88**)	33/40 (**83**)	35/49 (**71**)
Fujirebio Espline Influenza A&B-N	22/27 (**85**)	28/33 (**85**)	31/40 (**78**)	33/49 (**67**)
Rockeby Influenza A Antigen Test	4/27 (**15**)	4/33 (**12**)	5/40 (**13**)	5/49 (**10**)
Quidel QuickVue Influenza A + B Test	23/27 (**89**)	28/33 (**85**)	31/40 (**78**)	33/49 (**67**)

or the Denka Seiken Quick Ex-Flu Test. In the study by (Weinberg and Walker, 2005), the BD EZ kit had a significantly lower sensitivity than the previous Becton Dickinson rapid test, the BD Directigen Flu A + B. However, comparing the influenza A sensitivity in patients under 15 years of age of the BD EZ test from this current study (80%) and the BD Directigen Flu A + B sensitivity from our previous study (81%) (Alexander et al., 2005), which used the same laboratory test comparator and similar specimen types, it appears that the two kits have a very similar sensitivity and specificity.

Unfortunately insufficient influenza B viruses were collected during the study period to gain statistically significant data on influenza B sensitivity. However, the limited results suggested that the tests performed poorer compared to influenza type A, a finding that should be investigated further, particularly given that the majority of studies performed previously have found only small differences between influenza A and B sensitivities (Cazacu et al., 2004; Dunn et al., 2003; Landry et al., 2004; Ruest et al., 2003).

This study demonstrates that the influenza rapid tests evaluated have a high specificity with a high positive predictive value (PPV), but lower sensitivities and negative predictive values (NPV) compared to real-time RT-PCR, DIF and RETCIF (which all gave very similar results for both influenza A and B detection). Five of the six rapid tests included in the study performed similarly, however the Rockeby test was found to be inferior, with a significantly lower influenza A sensitivity compared to the other tests in this evaluation. The use of these tests is likely to increase in the future, both for seasonal influenza and in the event of a pandemic situation. While there is little data yet available on the performance of these kits on humans infected with avian influenza, a recent study found that six different rapid tests (including Binax, Quidel and BD EZ) detected cell culture grown H5N1 virus as efficiently as conventional H3N2 or H1N1 isolates (Chan et al., 2007), however these levels may be higher than is present in H5N1 human clinical samples.

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References

Alexander R, Hurt AC, Lamb D, Wong FY, Hampson AW, Barr IG. A comparison of a rapid test for influenza with laboratory-based diagnosis in a paediatric population. Commun Dis Intell 2005;29:272–6.

Alexander R, Lamb D, White D, Wentzel T, Politis S, Rijnsburger J, et al. 'RETCIF': a rapid, sensitive method for detection of viruses, applicable for large numbers of clinical samples. J Virol Methods 2001;97:77–85.

Bonner AB, Monroe KW, Talley LI, Klasner AE, Kimberlin DW. Impact of the rapid diagnosis of influenza on physician decision-making and patient management in the pediatric emergency department: results of a randomized, prospective, controlled trial. Pediatrics 2003;112:363–7.

Cazacu AC, Chung SE, Greer J, Demmler GJ. Comparison of the directigen flu A + B membrane enzyme immunoassay with viral culture for rapid detection of influenza A and B viruses in respiratory specimens. J Clin Microbiol 2004;42:3707–10.

Chan KH, Lam SY, Puthavathana P, Nguyen TD, Long HT, Pang CM, et al. Comparative analytical sensitivities of six rapid influenza A antigen detection test kits for detection of influenza A subtypes H1N1, H3N2 and H5N1. J Clin Virol 2007;38:169–71.

Chan KH, Maldeis N, Pope W, Yup A, Ozinskas A, Gill J, et al. Evaluation of the Directigen Flu A + B test for rapid diagnosis of influenza virus type A and B infections. J Clin Microbiol 2002;40:1675–80.

Dunn JJ, Gordon C, Kelley C, Carroll KC. Comparison of the Denka-Seiken INFLU A.B-Quick and BD Directigen Flu A + B Kits with direct fluorescent-antibody staining and shell vial culture methods for rapid detection of influenza viruses. J Clin Microbiol 2003;41:2180–3.

Frank AL, Taber LH, Wells CR, Wells JM, Glezen WP, Paredes A. Patterns of shedding of myxoviruses and paramyxoviruses in children. J Infect Dis 1981;144:433–41.

Hara M, Takao S, Fukuda S, Shimazu Y, Kuwayama M, Miyazaki K. Comparison of four rapid diagnostic kits using immunochromatography to detect influenza B viruses. Kansenshogaku Zasshi 2005;79:803–11.

Heine H, Trinidad L, Selleck P, Lowther S. Rapid detection of highly pathogenic avian influenza H5N1 virus by TaqMan reverse transcriptase-polymerase chain reaction. Avian Dis 2007; in press.

Landry ML, Cohen S, Ferguson D. Comparison of Binax NOW and Directigen for rapid detection of influenza A and B. J Clin Virol 2004;31:113–5.

Mitamura K, Yamazaki M, Ichikawa M, Kimura K, Kawakami C, Shimizu H, et al. Evaluation of an immunochromatography test using enzyme immunoassay for rapid detection of influenza A and B viruses. Kansenshogaku Zasshi 2004;78:597–603.

Poddar SK. Influenza virus types and subtypes detection by single step single tube multiplex reverse transcription-polymerase chain reaction (RT-PCR) and agarose gel electrophoresis. J Virol Methods 2002;99:63–70.

Rodriguez WJ, Schwartz RH, Thorne MM. Evaluation of diagnostic tests for influenza in a pediatric practice. Pediatr Infect Dis J 2002;21:193–6.

Ruest A, Michaud S, Deslandes S, Frost EH. Comparison of the Directigen Flu A + B Test, the QuickVue influenza test, and clinical case definition to viral culture and reverse transcription-PCR for rapid diagnosis of influenza virus infection. J Clin Microbiol 2003;41:3487–93.

Simonsen L. The global impact of influenza on morbidity and mortality. Vaccine 1999;17:S3–10.

Smit M, Beynon KA, Murdoch DR, Jennings LC. Comparison of the NOW Influenza A & B, NOW Flu A, NOW Flu B, and Directigen Flu A + B assays, and immunofluorescence with viral culture for the detection of influenza A and B viruses. Diagn Microbiol Infect Dis 2007;57:67–70.

Storch GA. Rapid diagnostic tests for influenza. Curr Opin Pediatr 2003;15:77–84.

Weinberg A, Walker ML. Evaluation of three immunoassay kits for rapid detection of influenza virus A and B. Clin Diagn Lab Immunol 2005;12:367–70.