A comparison of a rapid test for influenza with laboratory-based diagnosis in a paediatric population

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Abstract

The rapid and accurate detection of influenza A and B in a hospital setting is useful to confirm infection, exclude other diseases and assist in the management of patient illness including the possible use of specific antiviral therapy. We evaluated the use of the Directigen Flu A+B in a paediatric hospital laboratory in comparison with the established diagnostic tests direct immunofluorescence, viral culture and reverse transcriptase-polymerase chain reaction. A total of 193 respiratory specimens were examined and the Directigen test detected positive samples with an 80.8 per cent sensitivity and a specificity of 100 per cent. This study confirms other paediatric studies which have found the Directigen Flu A+B to be less sensitive than traditional laboratory tests but nevertheless to have a potential role in patient management especially when a positive result is obtained. *Commun Dis Intell* 2005;29:272–276.

Keywords: influenza, rapid tests, Directigen, point of care, diagnostic

Introduction

Influenza is a major cause of respiratory disease outbreaks in the winter months and while it is usually a self-limiting disease in healthy individuals, it can cause severe illness and mortality in the elderly, immunosuppressed and very young patients.[1,2] In children, influenza has been associated with increased outpatient visits, hospital admissions and antibiotic usage.[3,4] However, rapid diagnosis of influenza has been shown to significantly alter the management of the patient's illness, resulting in a reduction in diagnostic tests performed, reduced antibiotic use, increased antiviral use and reduced length of stay in a hospital emergency department.[5]

A number of laboratory tests are used for the diagnosis of influenza but most require highly skilled laboratory staff and equipment, and are often too time consuming to be useful in determining timely treatment options. Recently however, a number of rapid tests for influenza have become available which are simple and can be performed outside the laboratory without specialised equipment or extensive training.[6,7] The major limitations in using these tests have been the lack of sensitivity and specificity when compared to standard laboratory tests. Their performance has also been shown to be variable depending on the age of the study group and the type of sample being tested. The highest sensitivity with rapid test kits has been reported in studies from young children using nasopharyngeal aspirates (NPA) or swabs as the respiratory sample and even when testing is limited to these types of samples, some variation has been reported.[6–13] We undertook the current study to evaluate the Directigen Flu A+B rapid test using mainly NPA samples from children in comparison with three laboratory diagnostic tests used for influenza diagnosis, direct immunofluorescence (DIF), rapid enhanced tissue culture combined with immunofluorescence (RETCIF) and a multiplex reverse transcriptase polymerase chain reaction (RT–PCR) for the differential detection of influenza A and B viral genes.

Materials and methods

Respiratory samples were obtained from patients with acute respiratory symptoms attending the Royal Children's Hospital, Melbourne, between August and the beginning of October 2003. Samples were processed for routine viral diagnosis in the virology laboratory using DIF and RETCIF as previously described.[14] Samples were tested using the Directigen Flu A+B rapid test (Becton Dickinson and Co., Maryland, USA) as per the manufacturer's recommendations. A test was performed using internal kit positive and

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negative controls on each new kit. Specimens for the rapid test were prepared by aliquoting 200 µl of NPA (or bronchoalveolar lavage) into a tube and adding eight drops of extraction buffer (Reagent E) according to the manufacturer's instructions. Swabs were extracted in 16 drops of extraction buffer. The preparation and testing procedure takes approximately 10–15 minutes to perform depending on the number of samples tested and the result is read by eye. An aliquot (300–500 µl) of the original specimen was stored at –70° C for the RT–PCR assay. A non-nested, in-house multiplex RT–PCR assay was used for the detection of influenza type A and influenza B. Briefly, primers used to detect the influenza A matrix gene (amplicon size 322 bp) and the influenza B NS gene (amplicon size 109 bp) were modified from Poddar[15] (primer sequences available on request). Viral RNA was extracted from 140 µl of clinical sample using the QIAamp® Viral RNA Mini Kit (QIAGEN, Australia) in accordance with the manufacturer's instructions. RT–PCR was carried out using the Titan One Tube RT–PCR System (Roche, Australia) according to the manufacturer's recommendations using 5 µl of the extracted RNA per reaction with an PTC–200TM thermocycler (MJ Research, Waltham, MA). PCR product was analysed by gel electrophoresis using 10 µl of amplified product which was run on a 2.5 per cent agarose gel containing ethidium bromide with control influenza A and B samples.

Results

In this study we analysed a clinical sample from each of 193 paediatric patients aged from nine days to 15 years (66% of samples were from patients aged two years or younger) of which 53.4 per cent were male and 46.6 per cent were female. The sample types consisted of 183 nasopharyngeal aspirates, four nasal swabs, three throat swabs and three bronchoalveolar lavages. Of the 193 specimens examined, 99 were considered positive for influenza by DIF. All influenza isolates were influenza A with no influenza B viruses and when sub-typed using DIF, all were A(H3) with no A(H1) viruses. A small number (less than 10%) of samples required re-testing after dilution as they gave invalid results initially but on dilution and re-testing gave valid results. The Directigen kit was easy to use and detected influenza A in 80 samples and no influenza B. When this was compared to the results obtained with DIF testing (Table 1) the Directigen kit showed a sensitivity of 80.8 per cent and a specificity of 100 per cent (Table 2). No false positives were obtained with the kit, giving a 100 per cent PPV (positive predictive value) but only an 83.2 per cent NPV (negative predictive value) when compared to DIF (Table 2). All of the samples that were positive by DIF also yielded positive isolates using cell culture in combination with IF using the RETCIF method, therefore comparisons with Directigen and RETCIF were identical to those made with Directigen and DIF (Tables 1 and 2). The sensitivity of the Directigen test compared to DIF and RETCIF was also analysed in three different age groupings. Children 0–2 years had an 87.5 per cent sensitivity, children five years and below had a sensitivity of 83.5 per cent and children 6–15 years had a sensitivity of 62.5 per cent.

Table 1. Detection of influenza A virus in clinical samples by DIF/RETCIF, multiplex RT–PCR* and Directigen Flu A+B

	DIF or RETCIF +	DIF or RETCIF –	RT–PCR+	RT–PCR–
Directigen +	80	0	78	2
Directigen –	19	94	16	95

* Note that two samples were unavailable for RT–PCR testing.

Table 2. Comparison of performances of the Directigen Flu A+B rapid test kit to DIF or RETCIF or multiplex RT–PCR, and comparison of the performance of multiplex RT–PCR to the DIF or the RETCIF assay

	Directigen Flu A+B compared to:		RT–PCR compared to:
	DIF or RETCIF	Multiplex RT–PCR	DIF or RETCIF
Sensitivity %	80.8	83.0	95.9
Specificity %	100	97.9	100
PPV %	100	97.5	100
NPV %	83.2	85.6	95.9
Accuracy %	90.2	90.6	97.9

A comparison of the Directigen test with an in-house non-nested multiplex RT–PCR assay yielded similar results to the use of DIF or RETCIF as the comparators with a sensitivity and specificity of 83 per cent and 97.9 per cent, respectively (Tables 1 and 2). When the RT–PCR assay was compared to the DIF or RETCIF results, the concordance was very good with discrepant results seen in only four of 191 samples, giving the RT–PCR assay a sensitivity of 95.9 per cent and specificity of 100 per cent with PPV and NPV of 100 per cent and 95.9 per cent respectively (Table 2). All four of these discrepant results were from samples that were positive by DIF or RETCIF and two were also positive by Directigen. Two of these samples (NPA's) yielded smeared PCR product on both initial testing and repeat testing which were considered inconclusive and scored as negative while the other two samples failed to produce detectable PCR product. Twenty-four non-influenza viruses were also detected using routine DIF and culture with IF [11 respiratory syncytial virus (RSV), five cytomegalovirus (CMV), five parainfluenza–3 (PI–3) and three dual infections – RSV+PI–3, RSV+CMV and RSV+herpes simplex virus 1]. The Directigen kit and the RT–PCR were negative for all these samples.

Discussion

The results in this study using the Directigen Flu A+B compared favourably with other studies in paediatric populations where influenza A was detected. Studies which also used DIF or viral culture as the 'gold standards' have reported sensitivities and specificities of 96 per cent and 99.6 per cent,[9] 86.6 per cent and 100 per cent,[10] 95 per cent and 88 per cent,[11] 43.8 per cent and 99.7 per cent,[12] 60 per cent and 100 per cent[13] respectively, for paediatric patient groups compared to our results of 80.8 per cent and 100 per cent. The studies by Cazacu *et al*[12] and Landry *et al*[13] obtained much lower sensitivity with the Directigen Flu A+B kit than in our study and other similar studies.[9,10,11] The reasons for this large difference are not apparent however some differences in the sample type were present with one study[12] using mainly nasal washes in their trial and the other[13] using a mixture of NP swabs and aspirates. In the test results contained in the BD Directigen Flu A+B kit booklet, when compared to virus isolation, NPA's gave the highest sensitivity followed by nasopharyngeal washes and nasopharyngeal swabs followed by throat swabs and lower nasal swabs. Also reported in the booklet is up to a 1,000-fold difference in the detection limits (as measured by $CEID_{50}$) for different viruses [A(H1N1), A(H3N2) and B], for example the 2 A(H3N2) viruses listed showed a 100-fold difference in detection limit. As the various studies were conducted at different times, different viruses may have been circulating in the studies that may be detected at varying levels with the Directigen rapid test. Influenza B has been reported to be detected at a similar[12] or lower level[9,10,13] than influenza A using the Directigen Flu A + B kit however as no B viruses were detected in our study this can not explain the discrepancy with some of these other studies. The proportion of younger children in each study may also affect the sensitivity of the Directigen Flu A+B kit. A consistent finding between studies has been the higher sensitivity of the kit when used on samples from children[16] compared to adults, especially in young patients (≤5 years[11]) or those aged under two years.[12] In our study the sensitivity increased to 87.5 per cent for samples from patients ≤2 years compared to an overall sensitivity of 80.6 per cent and decreased to 62.5 per cent with older children (6–15 years). As the majority of our samples were obtained from ≤2-year-old children, this may have contributed to the higher overall sensitivity compared to other studies.

The high PPV seen in our study (100% i.e. no false positives) and others (100%[10], 96%[9] and 90.5%[13]) with influenza A detection by the Directigen test, should give confidence to technicians and paediatricians that when they obtain a positive test, they can confidently confirm a clinical diagnosis or begin appropriate treatment, with the option of using a specific influenza antiviral drug immediately. A rapid positive result could reduce the need for further laboratory testing which would help offset the cost of the kit and performing the test. A previous study has shown rapid diagnosis of respiratory viral infections in children can result in significant reductions in hospital stays and antibiotic use as well as laboratory savings.[17] On the other hand a more cautious approach is warranted if a negative Directigen result is obtained, in view of the higher proportion of false negative results seen when using this test (NPV in our study was 83.2%, and in others was 99.6%,[9] 92.1%,[10] and 96.9%[13]). Previous studies using the Directigen kit have also noted that the test can produce a number of indeterminate or invalid tests initially Ruest *et al*[10] found that eight per cent of samples tested fell into this category and required diluting and re-testing. In our study we found less than 10 per cent of samples gave invalid results initially but on dilution and re-testing gave valid results.

When the rapid test was compared to a multiplex RT–PCR assay, the sensitivity and specificity was improved slightly due to the RT–PCR not detecting 4/97 of the DIF/RETCIF positive samples, two of which were positive by the rapid test. One possible explanation for this might have been the extra freeze thaw step these samples had prior to RT–PCR assay, which may have caused degradation of the viral RNA. Others have reported a lower sensitivity than our study when comparing the Directigen kit with a multiplex real-time PCR[18] however this is not surprising given the added sensitivity of real-time PCR.

The impact of influenza in children has been highlighted in recent studies especially in those under five years[19] and during the winters in Australia and in the United States of America (USA) in 2003–04, where a number of deaths were associated with influenza. Some 152 deaths were reported in children under 18 years in the USA[20] while three deaths were reported in one hospital in Sydney, Australia.[21] Hence methods that will rapidly and accurately diagnose influenza in children would be a useful addition to our current range of tests both in the laboratory and also in the wider community. During outbreaks, hospitals might even consider outpatient testing of children who present with respiratory illness to allow segregation of any who test positive to reduce nosocomial infections and reduce further testing.[5,22,23] In conclusion, the Directigen Influenza A+B is a relatively simple test that performed well when using samples that would be expected to contain the highest viral loads (NPA samples from a paediatric population) but still failed to detect influenza A in around 20 per cent of positive samples as detected by DIF or RETCIF or RT–PCR. Newer rapid tests for influenza which are now available, promise even better results than the current ones.[24]

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