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Implementation of a rapid influenza A/B and RSV direct molecular assay improves emergency department oseltamivir use in paediatric patients

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Abstract

Purpose. Influenza A virus (FluA), influenza B virus (FluB) and respiratory syncytial virus (RSV) illnesses increase hospitalizations during seasonal epidemics.

Methodology. To determine the utility of the Simplexa FluA/B & RSV Direct Assay (Direct Flu/RSV) and its impact on oseltamivir use, we offered this assay to emergency department (ED) patients with influenza-like illness.

Results. Utilization of the Direct Flu/RSV provided a turnaround time (TAT) of 2 hours. Compared to the flu season prior to implementation of the Direct Flu/RSV, clinicians were more likely to prescribe 5 days of oseltamivir therapy for Direct Flu/RSV-positive patients in comparison to those with a negative test.

Conclusions. Use of Direct Flu/RSV provides results rapidly, which leads to more appropriate use of oseltamivir. The ease of use of this assay and quick TAT allows for prompt decision-making, which is essential for patient care and effective disease control during the influenza season.

INTRODUCTION

Respiratory viruses, such as influenza virus (flu) and respiratory syncytial virus (RSV), have a major impact on the healthcare system. Even though influenza virus and RSV can affect both adults and children, infections with these viruses are particularly dangerous for young children, the elderly, pregnant women and those who are immunocompromised [1–3]. Treatment with oseltamivir is available and is highly recommended for children <2 years of age, as the risk of complications due to influenza infection is highest for them [4]. However, anyone who is diagnosed with influenza should be started on therapy as soon as possible, especially those who have underlying co-morbidities, require hospitalization or have severe disease [4]. Due to this large, highly susceptible population, the 'flu season' often results in increased hospitalizations and mortality rates [4]. These seasonal epidemics, which peak during the winter months, place excess strain on hospital emergency departments (EDs) nationwide. While the sudden increase in patients presenting to the ED is clearly problematic, other factors, such as bed availability for admitted patients, proper isolation precautions for infection control purposes, lengthy turnaround times (TATs) for diagnostic testing, and timely and appropriate treatment, are all important issues faced by hospitals.

In many laboratories, the current method for the detection of respiratory viruses is real-time PCR [5]. This method is more sensitive and specific than both the rapid antigen point-of-care assays and viral culture [6]. However, most of these real-time PCR methods are manual and require multiple steps to complete. As a result, this time-consuming assay delays test results that are needed to inform prompt management decisions in the ED. DiaSorin Molecular's (formerly Focus Diagnostic's) Simplexa FluA/B & RSV Direct Assay (Direct Flu/RSV; Cypress, CA, USA) is a direct sample-to-answer rapid, qualitative RT-PCR assay that detects influenza A (FluA) and influenza B (FluB) viruses and RSV.

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Keywords: influenza virus; Simplexa Flu/RSV; emergency department; oseltamivir.

Abbreviations: AdV, adenovirus; CLIA, Clinical Laboratory Improvement Amendments; Direct Flu/RSV, Simplexa Flu A/B & RSV Direct Assay; ED, emergency department; Flu, influenza virus; FluA, influenza A virus; FluB, influenza B virus; hMPV, human metapneumovirus; ILI, influenza-like illness; PIV1-3, parainfluenza 1-3; Rhino, rhinovirus; RSV, respiratory syncytial virus; TAT, turnaround time.

The clinical laboratory can help alleviate pressures encountered in the ED by utilizing a molecular assay, such as the Direct Flu/RSV, to help diagnose these infections quickly. The aim of this study is to evaluate the performance of the Direct Flu/RSV assay for paediatric patients presenting to the ED and determine its influence on treatment decisions made in the ED.

METHODS

Patient samples post-implementation

Specimens were collected from patients presenting to the ED who were suspected of an influenza-like illness from November 2014 through March 2015. The majority of samples received were nasopharyngeal aspirates; however, nasopharyngeal swabs were also collected. A total of 1483 ED samples were tested over this 5-month period. Of note, there was low circulation of FluB during this evaluation period.

Laboratory detection of respiratory viruses

For rapid detection of FluA, FluB and RSV, the FDA-cleared Simplexa FluA/B & RSV Direct Assay (Direct Flu/RSV) was utilized after initial verification according to Clinical Laboratory Improvement Amendments (CLIA) of 1988 regulations and performed as previously described [7]. Both nasopharyngeal aspirates and nasopharyngeal swabs are FDA-cleared sample types for use on the Direct Flu/RSV assay. FluA and FluB were detected by PCR using the conserved region of the matrix gene, while RSV was detected by the M gene [8]. Extraction of unprocessed samples and amplification were performed in one protocol according to the Simplexa FluA/B and RSV package insert [8]. Samples were batched and run approximately every hour, alternating between two instruments, and the test was only offered during the laboratory hours of operation (7:30 am–11:00 pm). The results were reported via the laboratory information system, which was the same for the pre- and post-implementation periods.

If the Direct Flu/RSV was negative, the sample was forwarded to a laboratory-developed respiratory viral panel (RVP), which has been our standard assay method for respiratory viral testing prior to acquiring the Direct Flu/RSV instrument and during the non-flu season [9]. The RVP is a qualitative real-time PCR that detects FluA, FluB, RSV, rhinovirus (rhino), human metapneumovirus (hMPV), adenovirus (AdV) and parainfluenza 1–3 (PIV1-3). The targets used for the detection of these agents are described in Pierce *et al* [9]. The extraction, real-time PCR, primer/probes and data analysis were performed following the protocol published by Pierce *et al.* [9]. Those samples that were negative by the Direct Flu/RSV but positive by the RVP were not tested by a third assay. Direct Flu/RSV-positive samples were not tested by the RVP, given that the published sensitivity and specificity for all three targets was high and our in-house verification supported this performance [7].

Collection of patient information for treatment outcomes

The beginning of 'flu season' is defined at our hospital when $\geq$10 % of patient samples tested for flu are positive. The effect of Direct Flu/RSV implementation on oseltamivir use was evaluated by analysing patients seen in the ED who exhibited an influenza-like illness and had a test performed with results being obtained. For treatment outcome analysis, only those patients who tested positive for flu were included in the test 'positive' group, while those who were positive for RSV were included in the test 'negative' group. The slight difference in the time periods is due to the natural fluctuation of flu season, which varies annually. One dose of oseltamivir is defined as one administration of the medication (e.g. not a full course); a full course is defined as administration of oseltamivir twice a day for 5 days. The definition of influenza-like illness (ILI) was defined by influenza surveillance ICD-9 codes, as validated through epidemiological surveillance criteria [10, 11]. The data were extracted through the Department of Biomedical and Health Informatics at the Children's Hospital of Philadelphia. Patient information, including chart review of documented phone calls, was collected and stored in a secure database, RedCap. Patients for whom a respiratory test had been ordered but for whom no results are available were excluded. The Institutional Review Board of the Children's Hospital of Philadelphia approved this study.

Statistical analysis

The Mann–Whitney U test was used to determine statistical significance for the duration of oseltamivir treatment between patients having either a positive or negative test during the pre- and post-implementation period. This test was used in order to compare two independent groups, and because oseltamivir usage was distributed non-parametrically.

RESULTS

Direct Flu/RSV TAT

The performance of the Direct Flu/RSV assay was assessed during the 2014–2015 flu season (November–March). The TAT was calculated as the time from when the sample was received in the laboratory to the time that the results were reported. As expected, the Direct Flu/RSV provided a quicker TAT of approximately 2 h as compared to an average of 6.7 h (range 3–30 h) for the RVP. This is an approximately threefold difference in TAT between the two assays.

Influenza virus detection

Of the 1483 samples tested, 617 samples were negative for FluA, FluB and RSV by both the Direct Flu/RSV and RVP assay (41.6 %). Three hundred and forty-eight (23 %) were positive for Flu A by the Direct Flu/RSV. Seven samples (7/348, 2 %) were negative by the Direct Flu/RSV but positive by the RVP, resulting in a high sensitivity of 98 % and a low false-negative rate of 2 %. Overall, 5 of the 1483 samples (0.3 %) were positive for FluB. All of the samples that were

positive for FluB were detected by the Direct Flu/RSV. Two samples were excluded from the analysis because one was reproducibly invalid and the other produced an error on the Direct Flu/RSV platform, suggesting that the specimen quality or matrix was incompatible with the Direct Flu/RSV. There was one co-infection observed with FluB and RSV, which were both positive by the Direct Flu/RSV assay.

RSV detection

Of the 1483 samples tested, 519 (35 %) were positive for RSV. Of the 519 positive samples, the Direct Flu/RSV detected 496, having a sensitivity of 96 %. Twenty-six samples were negative by the Direct Flu/RSV, resulting in a 5 % false-negative rate. Interestingly, of the false-negatives, 20 (77 %) were only positive for RSV A (data not shown). Notably, one patient was positive for RSV and FluA. The Direct Flu/RSV assay correctly detected RSV but did not identify FluA.

Treatment outcomes

An overview of the patient demographics is shown in Table 1. For the 2012–2014 pre-implementation period, the overall positivity rate by the RVP was 29.9, 19.9 and 29.2 % for FluA, FluB and RSV, respectively (Table 2). The rate of FluA detected by the Direct Flu/RSV assay was similar during the 2014–2015 post-implementation (23 %), but there was a significantly lower positivity rate for FluB (0.3 %) and a higher rate for RSV (35 %) (Table 2). This likely represents seasonal variations in the circulation of these viruses, as opposed to differences in detection by the two assays.

To determine the impact of the Direct Flu/RSV on treatment decisions, we analysed the differences in oseltamivir usage pre- and post-implementation of the Direct Flu/RSV assay. Use of oseltamivir was defined as receipt of at least one dose of oseltamivir. A reduction in overall oseltamivir use and duration was seen in the post-implementation period, where only 31.0 % of the patients analysed received oseltamivir (compared to 61.5 %), with an average duration of 3.24 days (compared to 4.10 days) (Table 3).

During the pre-implementation phase, of the patients that were truly negative for FluA/B, 48.7 % were still given at least one dose of oseltamivir (Table 3). Additionally, while patients with a positive result during the pre-implementation phase had a longer duration of prescribed oseltamivir (4.23 days) compared to patients who were negative (3.75 days), this difference was not statistically significant (P=0.99; Table 3). Analysis of the post-implementation period revealed a significantly lower percentage (14.3 %, P<0.001) of negative patients receiving antiviral therapy compared to the pre-implementation period (Table 3). Furthermore, the duration of oseltamivir was significantly longer for positive patients (mean=4.42 days) compared to negative patients (mean=1.18 days, P<0.001) (Table 3).

These results were also stratified by admission status. During the pre-implementation period, for those who were admitted, there was a slight difference in the duration of oseltamivir between those who tested negative and those who tested positive, 2.67 days versus 3.39 days, but this was not statistically significant (P=0.2281, Table 4). However, there was a significant difference in the duration of oseltamivir use between those who tested negative and those who tested positive in those who were discharged, 3.80 days versus 4.92 days (P<0.001, Table 4). Conversely, the difference in the duration of oseltamivir treatment for the post-implementation period was more pronounced in patients who were discharged, and the mean duration of oseltamivir for those who tested negative was much shorter than for those

Table 1. Patient demographics

Patient demographics		Pre-implementation (2012–2013, 2013–2014) *n*=688	Post-implementation (2014–2015) *n*=1483
Mean age (years)		4.73	4.29
Median age and oseltamivir administered (years)		5.09	4.82
Mean age admitted (years)		5.15	3.96
Admittance		313/688 (41.5 %)	834/1483 (55.8 %)
Gender	Female	345 (50.2 %)	665 (44.8 %)
	Male	342 (49.9 %)	818 (55.2 %)
Race	Native American	0 (0 %)	1 (0.1 %)
	Asian	132 (4.7 %)	73 (4.9 %)
	African American	368 (53.5 %)	761 (51.3 %)
	Caucasian	199 (28.9 %)	413 (27.9 %)
	More than one race	13 (1.9 %)	46 (3.1 %)
	Other	75 (10.9 %)	187 (12.6 %)
	Unknown	1 (0.2 %)	2 (0.1 %)
Ethnicity	Hispanic/Latino	61 (8.1 %)	159 (0.7 %)
	Not Hispanic/Latino	627 (91.9 %)	1323 (89.2 %)
	Unknown	0 (0 %)	1 (0.1 %)

Table 2. Positivity rates for pre- and post-implementation. The overall rates of influenza A, B and RSV were calculated for the pre- and post-implementation periods

Target	Pre-implementation (2012–2013, 2013–2014) *n*=688		Post-implementation (2014–2015) *n*=1483	
	Number of positives (%)	Number of negatives (%)	Number of positives (%)	Number of negatives (%)
Influenza A	206 (29.9 %)	236 (34.3 %)	342 (23 %)	617 (42 %)
Influenza B	137 (19.9 %)		5 (0.3 %)	
RSV	125 (29.2 %)		519 (35 %)	

who tested positive, 1.12 versus 4.83 days (Table 4). For those who were admitted, there was also a difference in the duration of oseltamivir use between those who tested negative and those who tested positive, 2.10 days versus 3.63 (P=0.007, Table 4).

DISCUSSION

There was a clear difference in oseltamivir use pre- and post-implementation. Prior to the implementation of the Direct Flu/RSV test, a large number of patients who tested negative for respiratory pathogens still received oseltamivir for a prolonged period of time. Since the recommendations are to begin treatment as soon as possible, as any delay in treatment may put the patient at a higher risk for severe complications and greater disease impact, it is not surprising that oseltamivir usage was higher prior to the Direct Flu/RSV test, given that the RVP results took, on average, hours to obtain. Ideally, patients are sent home with an oseltamivir prescription, are called with the test results and are instructed to fill the oseltamivir prescription if the patient is positive for flu; however, this does not always occur. Given that the RVP assay requires on average 6.7 h to complete (3–30 h), patients were most likely discharged prior to the test results being obtained, causing overuse of oseltamivir in non-admitted patients in the pre-implementation period. Furthermore, for patients who were discharged, there was likely a delay in calling patients, which is not reflected in the data, for those who were admitted, as real-time results can be acted upon more quickly in the inpatient setting. While these data strongly suggest that changes in our testing protocols between pre- and post-implementation of the Direct Flu/RSV most likely resulted in the observed difference in oseltamivir usage, we cannot exclude other contributions, such as severity of disease due to the varying pathogenicity of circulating strains and the underlying co-morbidities of the patients.

Conversely, for 2014–2015, the Direct Flu/RSV test (unlike the RVP assay) was incorporated into an ED algorithm policy for the assessment of patients with an influenza-like illness (ILI). If a patient who presents with ILI is high-risk and requires either admission or treatment, the Direct Flu/RSV test was highly recommended to aid in care management and treatment. After implementation, more focused prescription and use of oseltamivir was observed. Since the Direct Flu/RSV takes only 2 h to complete, we hypothesize

Table 3. Oseltamivir usage during the pre- and post-implementation

Oseltamivir use and duration was determined for patients with negative and positive test results. The pre-implementation test was the RVP and the post-implementation test was the Direct Flu/RSV. A dose is defined as one administration of oseltamivir (e.g. one pill).

	Pre-implementation (2012–2013, 2013–2014)		Post-implementation (2014–2015)	
Overall oseltamivir use	423/688 (61.5 %)		460/1483 (31.0 %)	
	Oseltamivir administered (≥1 dose)			
Influenza test result	No	Yes	No	Yes
Negative	177/345 (51.3 %)	168/345** (**48.7** %)	975/1137 (85.8 %)	162/1137** (**14.3** %)
Positive	88/343 (25.7 %)	257/343 (74.3 %)	58/347 (16.7 %)	292/347 (83.3 %)
Overall average oseltamivir duration (days)	4.10		3.24	
Influenza test result	Oseltamivir duration (days)		Oseltamivir duration (days)	
Negative	3.75		1.18	
Positive	4.23		4.42*	
P-value	0.99		<0.001	

*Statically significant by the Mann–Whitney U test.

**Statically significant difference by the Pearson chi-squared test, P<0.001.

Table 4. Oseltamivir use stratified by admission status

Oseltamivir use and duration were stratified by admission status and broken down by test result. The pre-implementation test was the RVP and the post-implementation test was the Direct Flu/RSV.

	Pre-implementation (2012–2013, 2013–2014)	Post-implementation (2014–2015)
Admitted patients	***n*=87**	***n*=163**
Influenza test result	Oseltamivir duration (days)	Oseltamivir duration (days)
Negative	2.67	1.12
Positive	3.39	4.83*
P-value	0.2281	<0.001
Discharged patients	***n*=168**	***n*=287**
Influenza test result	Oseltamivir duration (days)	Oseltamivir duration (days)
Negative	3.80	2.10
Positive	4.92	3.63*
P-value	<0.001	0.007

*Statically significant by the Mann–Whitney U test.

that ED physicians obtain results prior to patient discharge, which could result in more appropriate oseltamivir use. Also, for those who were discharged from the ED prior to knowledge of the test results, a more rapid test resulted in a quicker telephone notification, which then led to more appropriate use of oseltamivir. Additionally, more appropriate use of oseltamivir leads to decreased adverse side-effects from taking the drug unnecessarily. Of note, this reduction in oseltamivir usage was seen despite the Direct Flu/RSV test only being offered during the day (7:30 am–11:00 pm); based on this data, we might expect even more appropriate oseltamivir use when this test is offered 24/7. Additionally, as PCR technologies advance, we hypothesize that a larger impact may be observed with faster direct PCR methods (e.g. 20–30 min assays).

There were limitations to this study. Even though the pre-implementation period was evaluated over 2 years, there was still a large difference in the total number of patients included in the analysis (688 pre-implementation patients compared to 1483 post-implementation patients). This could be due to the incorporation of the Direct Flu/RSV assay into an official ED policy leading to more physicians utilizing this test for the diagnosis of flu. Indeed, during the pre-implementation period, the RVP was not included in the ED ILI algorithm and it is possible that many physicians based their diagnosis of flu on the patient's clinical presentation, forgoing testing. Additionally, patient co-morbidities were not included or assessed in the decision to administer oseltamivir. This analysis could provide useful information on oseltamivir use as some co-morbidities may have a significant impact on the treatment decision. However, stratification by admission provides some insight, as patients with severe co-morbidities are more likely to be admitted. Additional studies are needed to determine the impact of the Direct Flu/RSV on other outcomes, such as antibiotic usage/costs and overall hospital costs during flu season.

Taken together, we found that the high-quality performance of the Direct Flu/RSV rapid assay is useful to clinicians and has a positive impact in the ED by aiding in the decision to prescribe oseltamivir or not. The Direct Flu/RSV provides rapid and accurate results for the detection of influenza A and B viruses and RSV. The TAT for the Direct Flu/RSV was shorter than that for the laboratory-developed RVP. Therefore, offering this assay as a rapid test for the ED provides clinicians with prompt results that can be incorporated into the treatment decision-making process.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
The Institutional Review Board of the Children's Hospital of Philadelphia approved this study.

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