Journal of Clinical Virology 55 (2012) 329–333

Contents lists available at SciVerse ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Early detection of influenza A and B infection in infants and children using conventional and fluorescence-based rapid testing

Barbara Rath[a,*], Franziska Tief[a], Patrick Obermeier[a], Ewelina Tuerk[a], Katharina Karsch[a], Susann Muehlhans[a], Eleni Adamou[a], Susanne Duwe[b], Brunhilde Schweiger[b]

[a] Department of Paediatrics, Division of Pneumonology-Immunology, Charité University Medical Centre, Augustenburger Platz 1, Berlin, Germany
[b] Division of Influenza/Respiratory Viruses, National Reference Centre for Influenza, Robert Koch Institute, Nordufer 20, 13507 Berlin, Germany

ARTICLE INFO

Article history:
Received 31 July 2012
Accepted 3 August 2012

Keywords:
Influenza
RSV
Rapid testing
Infants
Children
POC

ABSTRACT

Background: The appropriate management of infants and children with influenza depends on the accurate and timely diagnosis, ideally at the point of care (POC).
Objectives: To evaluate the use of simultaneous RSV/influenza rapid testing with QuickVue™ test strips as well as (the use of) novel, fluorescence-based, rapid influenza antigen testing (SOFIA™) in infants and children with influenza-like illness (ILI).
Study design: The Study was conducted in a real-time surveillance program at the Charité Department of Pediatrics in collaboration with the National Reference Centre for Influenza at the Robert Koch Institute (RKI) in Berlin, Germany (Charité Influenza-Like Disease = ChILD Cohort).
Results: During the 2010/2011 influenza season, 395 infants and children were simultaneously tested using QuickVue™ FluA&B and RSV10 rapid tests at POC compared to independent RT-PCR. Sensitivities were 62.7/67.8% for Influenza/RSV overall, but highest in infants <1 year with 76.0/76.2%. The evaluation of the fluorescence-based rapid test SOFIA™ with frozen laboratory samples (derived from the 2008/2009 and 2010/2011 national surveillance) yielded sensitivities of 97.7/86.7/86.7/81.7% for influenza A(H1N1)pdm09/A(H3N2)/B-Victoria/B-Yamagata in samples with CT values <34, and 80.2/79.8/67.5/62.5% for all CT values combined. The same method used at POC with 649 consecutive ChILD patients in 2011–2012 yielded sensitivity/specificity/PPV/NPV values of 78.9/99.7/96.6/97.3%. Again, sensitivities were highest in infants (85.7%) and small children <2 years (88%).
Conclusions: Fluorescence-based rapid antigen testing provides a highly sensitive and specific tool for POC diagnostics of acute influenza in the paediatric age group, especially in infants and small children <2 years, when viral loads are at their peak and treatment decisions are imminent.

1. Background

Accurate and timely diagnosis of influenza is crucial for effective infection control in a busy in-hospital and emergency room setting and may help to reduce the exposure to unnecessary antibiotics and diagnostic procedures.[1] Children are also effective transmitters of influenza[2,3] necessitating targeted infection control measures to minimise the risk of nosocomial and household transmission. Current guidelines recommend the antiviral treatment of influenza infection in children with underlying conditions.[4] In this sensitive age group, timely diagnosis and treatment within the first 48 h after disease onset is key.[5]

The positive predictive value of a "clinical influenza diagnosis" in the paediatric age group remains below 40%.[6] A recent study in Scotland confirmed how difficult it can be to distinguish between influenza and other respiratory pathogens on clinical grounds alone. In particular, infections with rhinovirus and parainfluenza virus are commonly mistaken for influenza.[7] In infants, influenza may be difficult to distinguish from infections with respiratory syncytial virus (RSV), especially in times of simultaneous epidemic peaks during the winter months.[8]

It is therefore crucial to have highly sensitive and specific diagnostic tools available. Conventional rapid tests for influenza show a wide range of sensitivities when different methodologies are compared, but even the same test performed at different centres may yield very different sensitivities.[9] Second generation POC tests have been developed offering standardized readings of test results.[10] There is a clear need for standardisation with respect to sampling procedures and case management practices.[11] Until influenza testing and treatment guidelines are widely recognized and used, clinicians may continue to test and treat influenza with inconsistency.[11]

* Corresponding author. Tel.: +49 30 450 666664; fax: +49 30 450 566931.
E-mail address: Barbara.Rath@gmail.com (B. Rath).

1386-6532/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jcv.2012.08.002

In this study, we evaluated different rapid diagnostic approaches in the context of a real-time surveillance and quality management program at the Charité University Department of Pediatrics in collaboration with the National Reference Centre (NRC) for Influenza at the Robert Koch Institute in Berlin, Germany (Charité Influenza Like Disease = ChILD Cohort).[12] We report the simultaneous evaluation of lateral flow influenza and RSV rapid antigen detection assays using conventional, visually interpreted test strips. We then evaluated a novel fluorescence-based rapid test with automated readings in the laboratory as well as at the bedside.

2. Objectives

The objectives of the POC evaluation study in the ChILD cohort are as follows:

- To evaluate the performance of a conventional and an innovative fluorescence-based rapid antigen detection assay in the real-life setting.
- To measure the performance of both rapid tests in detection of influenza viruses typically circulating in Germany and Central Europe, compared to RT-PCR based on national surveillance data.
- To study the sensitivity of the POC assays in infants compared to the overall paediatric age group.

3. Study design

3.1. Patient population and sampling method

The evaluation was carried out in the context of the IRB-approved influenza quality management (QM) program at the Charité Department of Paediatrics in collaboration with the NRC for Influenza at the Robert Koch Institute (Charité Influenza-Like Disease = ChILD Cohort).[12–14] Paediatric inpatients at Charité and children (age 0–18) presenting to the paediatric emergency rooms were screened regularly based on predefined ILI criteria. The screening and the rapid testing of nasopharyngeal samples were performed in real-time by a specifically trained independent quality management team.

3.2. Rapid testing using QuickVue™ Influenza A + B and QuickVue™ RSV 10 (Quidel Inc.)

Nasopharyngeal swabs/aspirates underwent immediate and simultaneous rapid testing using the QuickVue™ RSV10 and QuickVue™ Influenza A + B (Quidel Inc.) dipstick immunoassays according to standard protocol [See product package inserts: QV RSV10 #1179500 (10/10) and QV Influenza A + B #1063809 (10/10)]. Briefly, undiluted naspharyngeal aspirate or a NaCl suspension from nasopharyngeal swabs were mixed with the extraction buffer. Both RSV10 and influenza A&B test strips were dipped into the solution back-to-back. The result was read manually at 10 min.

3.3. Rapid testing using the new fluorescence-based SOFIA™ Influenza A + B test (Quidel Inc.)

Pilot study 2011: Patient specimens tested positive by real-time PCR during the seasons 2008/2009 and 2010/2011 were stored frozen and selected according to their PCR threshold (CT) values. The majority of samples were obtained from the nationwide community-based influenza sentinel. Influenza reference viruses obtained from the virus collection of the NRC for Influenza were propagated in embryonated hens' eggs and the EID_{50} determined.

Table 1
Sensitivity, specificity, PPV, NPV, positive and negative DLR for influenza and RSV in ChILD patients <1 year and >1 year of age using QuickVue™ rapid tests at point of care.

	All		<1 year		>1 year	
	FLU	RSV	FLU	RSV	FLU	RSV
Sensitivity	62.7	67.8	76.0	76.2	58.4	47.1
Specificity	98.0	98.5	97.8	97.5	98.1	99.1
PPV	91.4	88.9	86.4	91.4	93.8	80.0
NPV	88.3	94.6	95.8	92.2	82.5	95.9
Positive DLR	30.6	35.2	30.0	45.6	31.0	50.4
Negative DLR	00.4	00.2	00.4	00.3	00.2	00.5

PPV: positive predictive value; NPV: negative predictive value; DLR: diagnostic likelihood ratio.

ChILD cohort: During the 2011/2012 flu season, the same procedure was performed at bedside in nasopharyngeal samples obtained from ChILD patients as during 2011/2012.

The SOFIA™ Influenza A + B test kit (Quidel Inc.) was used that employs a lateral-flow immunofluorescence technique interpreted with the SOFIA™ Analyzer (Quidel Inc.) to detect influenza virus nucleoprotein. Using this test allows for the differential detection of influenza A and B antigens [See product package insert: Sofia Flu #1170000DE0 (09/11)]. After a sample extraction step using 260 μl of the specimen an aliquot was pipetted onto the test cassette and incubated for 15 min. The cassette was then inserted into the SOFIA™ Analyzer where the test strip was scanned, the fluorescent signals analyzed using method-specific algorithms, and the result reported within 1 min.

3.4. PCR detection

Nasopharyngeal swabs from children belonging to the ChILD cohort during 2010/2011 and 2011/2012 were hand-delivered to the NRC for Influenza. After arrival specimens were resuspended and adjusted to 4 ml with sterile minimal essential medium with HEPES (Gibco BRL, Eggenstein, Germany) and 100 U/ml penicillin/streptomycin (GIBCO), aliquoted and stored at −70 °C.

RNA was extracted from 300 μl of the sample using the MagAttract Viral RNA 48 Kit (Qiagen) and eluted in 80 μl elution buffer. Synthesis of cDNA and real-time PCR for detection of A(H1N1)pdm09 and A(H3N2) viruses were carried out as recently described.[15] Identification and differentiation of influenza B viruses were performed according to Biere et al.[16] For detection of RSV, cDNA was analysed by a generic RSV TaqMan PCR located in the RSV N-protein gene as described by Reiche et al.[17] All reactions were carried out using the Light Cycler® 480 real-time PCR system (Roche Deutschland Holding GmbH, Germany), the ABI PRISM® 7500 Sequence Detection System (Applied Biosystems, Germany) or the Mx3005 real-time PCR thermal cycler (Stratagene).

4. Results

4.1. Simultaneous rapid testing, using QuickVue™ Influenza A + B and QuickVue™ RSV10 dipstick immunoassays during the 2012/11 season

A total number of 395 consecutive ChILD cohort subjects (mean age 2.7 years, median age 1.3 years, SD 3.5, 56% male) were tested at the point of care during the peak of the 2010/2011 flu season (January 26 through April 31, 2011). The sensitivities, specificities, PPV, NPV and positive and negative diagnostic likelihood ratio (DLR) are displayed in Table 1. Differentiation between influenza A and B was correct in all instances (data not shown).