Accuracy of Rapid Influenza Detection Test in Diagnosis of Influenza *A* and *B* Viruses in Children Less Than 59 Months Old

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Abstract

Influenza burden among children is underestimated. Rapid influenza diagnostic tests (RIDTs) may be helpful in the early diagnosis of the disease, but their results should be interpreted cautiously. The aim of our study was to estimate the accuracy of the rapid influenza detection test BD Directigen™ EZ Flu A+B (Becton, Dickinson and Company, Sparks, MD) used among children with influenza-like illness (ILI) consulted in the ambulatory care clinics. A total number of 150 patients were enrolled into the study. The inclusion criteria were: age of the child less than 59 months, presentation of ILI according to CDC definition (fever >37.8 °C, cough, and/or sore throat in the absence of another known cause of illness), and duration of symptoms shorter than 96 h. In all patients two nasal and one pharyngeal swab were obtained and tested by RIDT, RT-PCR, and real time RT-PCR. For or influenza A(H1N1)pdm09, virus sensitivity of RIDT was 62.2 % (95 %CI 53.4–66.5 %), specificity 97.1 % (95 %CI 93.4–99 %), positive predictive value (PPV) 90.3 % (95 %CI 77.5–96.5 %), and negative predictive value (NPV) 85.7 % (95 % CI 82.4–87.3 %). For influenza B, virus sensitivity was 36.8 % (95 %CI 23.3–41.1 %), specificity 99.2 % (95 %CI 97.3–99.9 %), PPV 87.5 % (95 %CI 55.4–97.7 %), and NPV 91.5 % (95 % CI 89.7–92.1 %). We conclude that the RIDT immunoassay is a specific, but moderately

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sensitive, method in the diagnosis of influenza type A and is of low sensitivity in the diagnosis of influenza B infections in infants and children.

Keywords
Children • Diagnosis • Influenza • Pharyngeal swabs • Rapid influenza diagnostic test • Virus

1 Introduction

Influenza is an acute viral disease which burden among children is underestimated (Principi et al. 2004). Depending on age, incidence rates of influenza may be 1.5–3.0 times higher than those for adults and are estimated to be between 10 and 40 % each year (Long et al. 1997). Influenza in the pediatric population leads to a significant increase in primary care visits, emergency department visits and hospitalizations due to complications (Neuzil et al. 2000).

The diagnosis of influenza is based on clinical symptoms and results of additional laboratory tests confirming presumptive diagnosis, including rapid influenza detection tests (RIDTs), real time polymerase chain reaction (RT-PCR), direct immunofluorescence assay (DIA), or viral culture. There are no typical symptoms for influenza, but the disease may be suspected in the presence of: the acute onset, cough, and fever >38.5 °C (Landry 2011; Friedman and Attia 2004). The presumptive diagnosis requires confirmation and the viral culture has been considered the gold standard for influenza diagnosis, but the delay in obtaining results makes it impractical for clinical-decision making. The polymerase chain reaction is more sensitive than standard viral culture, but is not widely available and expensive (Landry 2011). As an alternative, RIDTs are relatively inexpensive and can provide timely information for clinical diagnosis (Uyeki 2003). The positive aspects of a timely diagnosis of influenza include the opportunity to provide antiviral therapy, allowing implementation of measures to limit the spreading of the disease, avoiding unnecessary antibiotic therapy and ambulatory and hospital testing and costs (Angoulvant et al. 2011; Jennings et al. 2009; Esposito et al. 2003). However, physicians must be aware that the accuracy of interpretation of rapid-test results depends on many factors including: clinical presentation of the disease, duration of symptoms, age of the patient, sample type, prevalence of influenza in the community, test characteristics, even previous vaccination against influenza using life-attenuated vaccine (Poehling et al. 2011).

The aim of our study was to estimate the accuracy of the rapid influenza detection test BD Directigen™ EZ Flu A+B used among children younger than 59 months with symptoms of influenza-like illness (ILI) consulted in the ambulatory care clinics.

2 Methods

The children in whom the accuracy of the BD Directigen™ EZ Flu A+B test was assessed also were used as subjects in the accompanying paper in which we described the clinical outcomes of influenza caused by viruses type A and B (Nitsch-Osuch et al. 2013). The assessment of the rapid influenza test efficacy, perceived as a single unrelated ramification of the study and a disconnected technical and research issue, was analyzed separately and presented herein. The study protocol was approved by a Local Ethics Committee and informed consent was obtained from the children's parents.

The study was conducted in the double autumn and winter seasons from 2009 to 2011.

A total of 150 children (47 % boys and 53 % girls) younger than 5 years (72 % children older than 24 months, 5 % younger than 12 months, 23 % children aged 12–24 months) were enrolled into the study. All patients were consulted at the ambulatory care clinics in Warsaw, Poland by general practitioners or pediatricians and presented symptoms of influenza-like illness shorter than 4 days. In 50 % of the patients, symptoms lasted longer than 48 h, in 9 % shorter than 24 h, in 41 % between 24 and 48 h. Fever >37.8 °C was reported in all patients, cough and/or sore throat was present in 93 % cases.

Biological material (nasal and pharyngeal swabs) taken from the patients were tested with a RIDT (BD Directigen™ EZ Flu A+B; Becton, Dickinson & Company, Sparks, MD). The isolation of viral RNA was conducted with Maxwell® 16 Viral Total Nucleic Acid Purification Kit (Promesa Corp., USA) and one-step RT-PCR was carried out with a Transcriptor RT-PCR Kit (Roche Diagnostics, Switzerland). Samples positive for influenza type A virus in RT-PCR were tested with a real time RT-PCR (Invitrogen SuperScript™ III Platinum® One-Step Quantitative RT-PCR System; Invitrogen, USA).

The Directigen EZ Flu A+B test was described by manufacturer as a chromatographic assay to qualitatively detect influenza A and B viral antigens in samples processed from respiratory specimens. When specimens were processed and added to the test device, influenza A or B viral antigens bound to anti-influenza conjugated antibodies to visualize particles in the corresponding A and B test strips. A positive result for influenza A was visualized as a reddish purple line at the test 'T' and control 'C' positions in the Directigen EZ Flu A read window. A positive result for influenza B was visualized as a reddish purple line at the test 'T' and control 'C' positions in the Directigen EZ Flu B read window.

Sensitivity, specificity, positive and negative predictive values (PPV and NPV), positive and negative likelihood ratio (LH+ and LH−), and kappa score of RIDT compared to RT-PCR were separately calculated for influenza type A and influenza type B virus. 95 % confidence intervals were calculated for all values. The statistical analyses were performed using SPP (ver. 15.0 for Windows, Chicago, IL).

3 Results

According to RT-PCR results the total number of 64 cases of influenza was diagnosed (incidence rate 40 %): 19 cases of influenza caused by virus type B and 45 cases of influenza caused by type A virus. *Real time* – RT-PCR revealed that all cases of influenza A were caused by subtype A(H1N1)pdm09. The accuracy of the RIDT was calculated for influenza type A (H1N1)pdm09, influenza type B. For influenza type A(H1N1)pdm09 infection the following results were obtained: 28 true positive, 102 true negative, 3 false positive, and 17 false negative. For influenza type B infection there were obtained: 7 true positive, 130 true negative, 1 false positive, and 12 false negative results. Values of sensitivity, specificity, PPV, NPV, LH+, LH− and kappa score of the rapid influenza detection test BD Directigen™ EZ Flu A+B compared to RT-PCR are presented in the Tables 11.1 and 11.2.

Table 11.1 Accuracy of rapid influenza detection test BD Directigen™ EZ Flu A+B compared to RT-PCR results for influenza A(H1N1)pdm09 virus

	Value	95 % CI
Sensitivity	62.2 %	53.4–66.5 %
Specificity	97.1 %	93.4–99 %
PPV	90.3 %	77.5–96.5 %
NPV	85.7 %	82.4–87.3 %
LH+	21.8	8.0–64.9
LH−	0.39	0.34–0.50
Kappa score	0.65	0.51–0.72

PPV positive predictive value, *NPV* negative predictive value, *LH+* positive likelihood ratio, *LH−* negative likelihood ratio

Table 11.2 Accuracy of rapid influenza detection test BD Directigen™ EZ Flu A+B compared to RT-PCR results for influenza B virus

	Value	95 % CI
Sensitivity	36.8 %	23.3–41.1 %
Specificity	99.2 %	97.3–99.9 %
PPV	87.5 %	55.4–97.7 %
NPV	91.5 %	89.7–92.1 %
LH+	48.3	8.6–296.9
LH−	0.66	0.59–0.79
Kappa score	0.48	0.27–0.55

PPV positive predictive value, *NPV* negative predictive value, *LH+* positive likelihood ratio, *LH−* negative likelihood ratio

4 Discussion

The present study shows that rapid influenza detection test BD Directigen™ EZ Flu A+B has moderate sensitivity (62.2 %) in detection of influenza A(H1N1)pdm09 virus and low sensitivity in detection of influenza B infection (36.8 %) among children with influenza-like illness younger than 59 months consulted in ambulatory care settings. The specificity of the test was high for both influenza A(H1N1)pdm09 virus (97.1 %) and influenza type B virus (99.2 %). Positive predictive values were also high and ranged from 87.5 % for influenza type B virus to 90.3 % for influenza type A(H1N1) pdm09 virus. Negative predictive values ranged from 85.7 % for influenza A(H1N1)pdm09 virus to 91.5 % for influenza B.

The values describing the accuracy of the rapid influenza detection test BD Directigen™ EZ Flu A+B calculated in our study were lower than those indicated by the manufacturer and calculated for viral culture as a gold standard. Our results concerning the accuracy of BD Directigen™ EZ Flu A+B test for detection of influenza A(H1N1)pdm09 are in agreement with the studies conducted by Karre et al. (2010) who found the sensitivity of this test of 48.7 %, specificity of 96.5 %, PPV of 88.6, and NPV of 77.3 %. Other studies also reported lower sensitivity of RIDTs in detection of influenza type B virus compared to influenza type A virus (Lucas et al. 2011). The present study indicates a good agreement between the two methods: RIDT and RT-PCR (kappa ranged from 0.48 for influenza type B to 0.65 for influenza type A virus) and this observation is consistent with the kappa 0.67 and 95%CI 0.56–0.76 in a study by Gordon et al. (2011).

This particular RIDT (BD Directigen™ EZ Flu A+B) has been evaluated by Blazguez et al. (2010) for detection of the novel influenza A (H1N1)2009 virus in children. The test showed good sensitivity (70.4 %), specificity (100 %), NPV (76.6 %), and PPV (100 %). Chan et al. (2002) found that the test detects a range of human and animal virus A subtypes, including H5N1 and H9N2 subtypes with high sensitivity (96 %), specificity (99.6 %), PPV (96 %), and NPV (99.6 %) for influenza A virus; the respective values for influenza B viruses were: 87.5, 96.8, 80, and 98 %.

The accuracy of different RIDTs was examined in other studies providing different, sometimes opposite results. Ganzenmueller et al. (2010) found that the Quidel QuickVue is not suitable for the diagnosis of infections caused by influenza A(H1N1) 2009 virus and DIA is a superior method. Stevenson and Loeffelholz (2010) also pointed to a poor accuracy of the Quidel Quick Vue test and Cheng et al. (2010) found the same in regard to the Rat Espline test. Hawkes et al. (2010) found sensitivity of RIDT BinaxNow influenza A and B kit (Inverness Medical, Montreal, Quebec, Canada) for detection of A(H1N1) 2009 infections higher in children than that reported among mixed adult-pediatric populations, but still remaining suboptimal. On the other side, a good diagnostic value of RIDT Quidel Quick Vue test has been reported by Lee et al. (2011) (sensitivity 70 %, specificity 97.5 %, PPV 97.4 %, NPV 71.2 %) and Louie et al. (2010) (sensitivity 66 %, specificity 84 %, PPV 84 %, NPV 64 %). Discrepancies in the results regarding RIDTs' accuracy above outlined may stem from the differences in sample types, age of patients studied, duration of symptoms, and viral spreading.

Despite moderate sensitivity for A(H1N1) pdm09 virus detection, the present results suggest that BD Directigen EZ Flu A+B® might be useful as a screening tool for the diagnosis of influenza

for children who are consulted in an outpatient setting due to symptoms of acute respiratory tract infection (Angoulvant et al. 2011). Potentially, the most important aspect of this rapid test is that it can provide timely, accurate and useful information for clinicians. The information can be provided in a real time when the diagnostics, isolation, and therapeutic questions need to be addressed and solved on the spot. However, clinicians must be aware of the limitations of RIDT and test results should be interpreted cautiously. During epidemic or pandemic seasons, false negative results occur more often than false positive results. The physician should consider forwarding respiratory specimens of patients with negative results for influenza obtained in RIDT testing to further RT-PCR, viral culture, or DIA verification, especially when community influenza activity is high and when the patient is at risk for a severe course of disease. For children at high risk for influenza-like illness during high-prevalence periods of influenza, empiric initiation of antiviral therapy should be considered even in case of negative RIDT results (Faix 2009).

One advantage of our study is a homogenous group of patients (children younger than 59 months), while most other studies dedicated to the evaluation of rapid influenza tests have been conducted in adult, mixed adult and pediatric patients, or among patients aged 0–18 years (Noyola and Demmler 2000). We chose young children for the study because the incidence of influenza, the risk of complications, and influenza-related hospitalizations are all high in this age-group (Principi et al. 2004; Neuzil et al. 2002). Another advantage seems to be that the study was conducted in an outpatient setting, while most other studies have been performed in hospitals or emergency units (Nitsch-Osuch et al. 2013; Poehling et al. 2011). A disadvantage, however, could be a relatively small number of patients enrolled, and a small number of positive results for influenza type B infection. Our findings are consistent with those reported in literature in that they confirm that the immunoassay is a specific, but not very sensitive method, in the diagnosis of influenza. Nevertheless, the rapid BD Directigen™ EZ Flu A+B test may be recommended for initial screening for influenza in primary care settings.

Conflicts of Interest The authors declare no conflicts of interest in relation to this article.

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