

Received: 2012.03.06
Accepted: 2012.07.14
Published: 2012.10.22

Accuracy of rapid influenza diagnostic test and immunofluorescence assay compared to real time RT-PCR in children with influenza A(H1N1)pdm09 infection*

Wiarygodność szybkiego testu diagnostycznego i metody immunofluorescencji bezpośredniej w odniesieniu do real-time RT-PCR u dzieci z grypą wywołaną wirusem typu A (H1N1)pdm09

Authors' Contribution:

- A** Study Design
- B** Data Collection
- C** Statistical Analysis
- D** Data Interpretation
- E** Manuscript Preparation
- F** Literature Search
- G** Funds Collection

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Summary

Introduction:

The influenza burden among children is underestimated. The aim of our study was to estimate the accuracy of the rapid influenza detection test (RIDT) BD Directigen™ EZ Flu A+B® and direct immunofluorescence assay (DFA) used among children with influenza-like illness (ILI) consulted in the ambulatory care clinic.

Material/Methods:

A total of 150 patients were enrolled in the study. Inclusion criteria were: age less than 59 months, presentation of ILI according to the CDC (Centers for Disease Control and Prevention) definition (fever >37.8°C, cough and/or sore throat in the absence of another known cause of illness), duration of symptoms shorter than 96 hours. Two nasal swabs and one pharyngeal swab were obtained from patients and tested by RIDT, DFA and real time RT-PCR as the reference method.

Results:

For influenza A (H1N1)pdm09 virus sensitivity of RIDT was 62.2% (95% CI 46.5–76.2%), specificity 97.1% (95% CI 91.8–99.4%), PPV 90.3% (95% CI 74.3–98%), NPV 85.7% (95% CI 78.1–91.5%), for DFA sensitivity was 60% (95% CI 51.9–63.2%), specificity 96% (95% CI 88.7–98.8%), PPV 93.1% (95% CI 80.5–98%), NPV 72.7% (95% CI 67.2–74.9%). Analysis of logistic regression revealed that the chance of receiving a true positive result of RIDT was twice as high when the test was conducted during the first 48 hours of symptoms (OR 0.40 vs OR 0.22).

Conclusions:

The accuracy of RIDT is comparable with DFA and both methods are very specific but moderately sensitive in diagnosis of influenza in young children. Both methods may be recommended for screening for influenza among children.

Key words:

influenza • rapid influenza diagnostic test • direct immunofluorescence assay • children

* Source of support: scientific grant from the Department of Family Medicine, Warsaw Medical University, 1MH/2010.



Streszczenie

Wprowadzenie: Grypa stanowi u dzieci istotny, ale niedoceniany i nieoszacowany problem kliniczny i epidemiologiczny. Celem pracy było porównanie wiarygodności szybkiego testu diagnostycznego w kierunku grypy BD Directigen EZ Flu A+B[®] z metodą immunofluorescencji bezpośredniej w odniesieniu do metody real time RT-PCR u dzieci zgłaszających się do ambulatorium z objawami grypopodobnymi.

Materiał/Metody: Do badania włączono 150 dzieci, kryteriami włączenia do badania były: wiek poniżej 59 miesięcy życia, występowanie objawów grypopodobnych, zgodnych z definicją CDC: gorączka $\geq 37,8^{\circ}\text{C}$, kaszel, ból i/lub zaczerwienienie gardła, utrzymywanie się objawów ≤ 96 godzin. U pacjentów pobrano wymaz z nosa i z gardła i poddano badaniu z użyciem RIDT i/lub DFA, a wyniki zwerfikowano metodą real time RT-PCR.

Wyniki: Metoda RIDT charakteryzowała się w odniesieniu do wykrywania zakażeń wywołanych wirusem grypy A (H1N1)pdm09: czułością 62,2% (95% CI 46,5–76,2%), swoistością 97,1% (95% CI 91,8–99,4%), PPV 90,3% (95% CI 74,3–98%), NPV 85,5% (95% CI 78,1–91,5%), podczas gdy metoda DFA charakteryzowała się: czułością 60% (95% CI 51,9–63,2%), swoistością 96% (95% CI 88,7–98,8%), PPV 93,1% (95% CI 80,5–98%), NPV 72,7% (95% CI 67,2–74,9%). Analiza regresji logistycznej wykazała, że szansa na uzyskanie prawdziwie dodatniego wyniku RIDT była dwukrotnie wyższa, gdy badanie wykonywano w ciągu 48 godzin od pojawienia się objawów chorobowych (OR 0,40 vs OR 0,22).

Wnioski: Wiarygodność RIDT i DFA u dzieci jest porównywalna, czułość obu testów jest umiarkowana, a swoistość bardzo wysoka. Oba testy mogą być używane jako metody skriningowe w diagnostyce grypy u dzieci z objawami grypopodobnymi.

Słowa kluczowe: grypa • szybkie test diagnostyczny • immunofluorescencja bezpośrednia • dzieci

Full-text PDF: <http://www.phmd.pl/fulltxt.php?ICID=1015040>

Word count: 2490

Tables: 3

Figures: –

References: 33

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Abbreviations: **DFA** – direct immunofluorescence assay; **LR–** – negative likelihood ratio; **LR+** – positive likelihood ratio; **NPV** – negative predictive value; **PPV** – positive predictive value; **real time RT-PCR** – real time reverse transcriptase polymerase chain reaction; **RIDT** – rapid influenza diagnostic test.

BACKGROUND

Influenza virus is a highly contagious agent that affects individuals of all ages and plays an important worldwide role by causing epidemics and pandemics. Since the signs and symptoms of influenza are similar to other respiratory viral infections, the differential diagnosis of influenza may be difficult, even impossible, when based solely on clinical symptoms. Laboratory diagnosis of influenza is critical not only for its treatment and surveillance, but also for its prevention and control [27,32].

Laboratory testing for influenza has historically been of questionable value for the management of patients with influenza, because of limited test sensitivity, long turnaround times, and a lack of effective therapy [21,24]. The development of more rapid and accurate tests for the detection of influenza now enables the laboratory to provide a prompt, definitive diagnosis, which allows clinicians

to initiate modern antiviral therapy within 48 hours from the disease onset, limit the injudicious use of antibacterial agents, implement appropriate infection control measures, decrease the duration of hospital isolation, reduce ancillary testing, and decrease health costs [9,10,11,13,17,19,25,26].

Laboratory diagnosis of influenza can be accomplished by detection of the virus or the patient's immune response to the virus. Diagnostic approaches for identification of the virus include viral isolation, detection of viral antigens by immunospecific assays (immunofluorescence microscopy), rapid influenza detection test (RIDT – based on optical immunoassay or immunoenzymatic assay), and detection of viral nucleic acid by use of amplification testing (RT-PCR, real time RT-PCR). Antibody detection is usually accomplished by virus neutralization (virus NT) and hemagglutinin inhibition (HI) tests conducted to monitor seroconversion to a specific virus strain or to determine the immune status, for example, after vaccination [21].

Diagnosis of influenza by immunoenzymatic assay has led to the development of easy-to-use, self-contained diagnostic kits that can provide results within 15 minutes after specimen collection. The World Health Organization has issued recommendations for the use of such kits [33]. In contrast to traditional laboratory methods, such as virus isolation from cell culture or real-time PCR, RIDTs are widely used in a routine clinical setting. However, RIDTs may have low to moderate sensitivity and their results should be interpreted very carefully by practitioners [9,11,33].

Another rapid detection method is direct immunofluorescence assay (DFA), which can generate results within 1–4 hours but requires specialized and experienced staff and specialized equipment. Generally, the sensitivity of DFA is higher than that of the rapid antigen test. Sensitivity of DFA is also higher among pediatric patients, especially those admitted with high fever and spreading high amounts of viruses [3,14,16,19,23].

The aim of our study was to estimate the accuracy of two rapid influenza diagnostic methods, RIDT and DFA, compared to real time RT-PCR in children younger than 59 months with acute symptoms of respiratory tract infection consulted in an ambulatory care setting. We chose this age group of patients because influenza is an epidemiological, clinical and economic problem for these patients, their caregivers and health care services. Depending on age, attack rates of influenza may be 1.5–3 times higher than for adults and are estimated to be between 10 and 40% each year [28]. Influenza in the pediatric population leads to a significant increase in primary care visits, emergency department visits and hospitalizations due to complications [28].

According to our current knowledge, our study is the first one in Poland exploring this problem.

MATERIAL AND METHODS

A total of 150 patients (70 boys and 80 girls) aged 0-59 months (mean age 34.3 months, SD 14.3 months) were included in the study. All patients came from ambulatory care settings and met symptoms of influenza-like illness (ILI) according to the CDC definition (fever >38.7°C plus cough and/or sore throat in the absence of another known cause of illness) lasting less than 4 days (96 hours). Exclusion criteria for the study were age older than 59 months, duration of symptoms longer than 96 hours, current antibiotic therapy or antiviral therapy.

The observation was performed in two consecutive influenza seasons: 2009/2010 and 2010/2011. Nasopharyngeal swabs were taken from all patients by previously trained staff using special artificial sticks (ending with viscose). The specimens were tested for influenza with both the rapid influenza diagnostic test (RIDT) BD Directigen™ EZ Flu A+B® (Becton, Dickinson and Company, Sparks, MD) (150 patients) and direct immunofluorescence assay IMAGENTM Influenza Virus A and B (Oxoid) (95 patients), strictly according to the manufacturer’s instructions. Results of RIDT were available during 15 minutes in the office. DIA and real time RT-PCR were performed by qualified personnel at the National Influenza Center in Warsaw (Poland). Isolation of viral RNA was conducted with Maxwell® 16 Viral Total Nucleic Acid Purification

Table 1. Formulae used for calculation of accuracy of RIDT and DFA

Value	Calculation formula
Sensitivity	No. of true positives/no of true positives and false negatives
Specificity	No. of true negatives/ no of true negatives and false positives
Positive predictive value	No. of true positives/ no of true positives plus no. of false positives
Negative predictive value	No. of true negatives/ no of true negatives plus no. of false negatives
Positive likelihood ratio	Sensitivity/ 1 minus specificity
Negative likelihood ratio	1 minus sensitivity/ specificity

Kit (Promesa Corp., USA). One-step RT-PCR was conducted with Transcriptor One-step RT-PCR Kit (Roche Diagnostics, Switzerland). Real time RT-PCR was conducted with Invitrogen SuperScript™ III Platinum® One-Step Quantitative RT-PCR System (Invitrogen, USA) using the CDC Realtime RTPCR (rRTPCR) Protocol for Detection and Characterization of Swine Influenza (version 2009) (http://www.who.int/csr/resources/publications/swineflu/CDCrealtimeRTPCRprotocol_20090428.pdf).

The sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, negative likelihood ratio of rapid influenza detection test and direct immunofluorescent assay compared to real time reverse transcriptase polymerase chain reaction (real time RT-PCR) were calculated, by using standard formulae (table 1) and the medical statistical calculator available at www.medcal3000.com. 95% confidence intervals were calculated. The analysis of logistic regression was used to describe the impact of duration of symptoms on the sensitivity of RIDT (OR and 95% CI were calculated). The statistical program CRAN-R was used. The approval of the Local Ethical Committee of Warsaw Medical University was obtained for the study (no. KB 165/2011).

RESULTS

For RIDT there were obtained 28 true positive, 102 true negative, 3 false positive and 17 false negative results. For DFA there were obtained 27 true positive, 48 true negative, 2 false positive and 18 false negative results.

The accuracy of RIDT and DFA compared to real time RT-PCR is presented in table 2.

The positive and negative predictive values were calculated for estimated prevalence of influenza at the level of 20%.

The chance of obtaining a true positive result of RIDT or DFA was twice as high when the duration of symptoms was shorter than 48 hours and, generally, the chance of obtaining a true positive result of RIDT decreased with longer duration of symptoms (table 3).



Table 2. Comparison of the accuracy of rapid influenza detection test (RIDT) and direct immunofluorescent assay (DFA)

	RIDT [%]	DIF [%]
Sensitivity	62.2 95 CI 46.5–76.2%	60 95 CI 51.9–63.2
Specificity	97.1 95 CI 91.8–99.4	96 95 CI 88.7–98.8
Positive predictive value	90.3 95 CI 74.3–98	93.1 95 CI 80.5–98
Negative predictive value	85.7 95 CI 78.1–91.5	72.7 95 CI 67.2–74.9
Positive likelihood ratio	21.778 95 CI 6.977–67.979	15 95 CI 4.594–55.566
Negative likelihood ratio	0.389 95 CI 0.267–0.567	0.417 95 CI 0.372–0.542
Kappa score	0.652 95 CI 0.509–0.794	0.57 95 CI 0.413–0.632

Discussion

Our results indicate a comparable and moderate accuracy of both rapid influenza diagnostic methods, RIDT and DFA, in the detection of infections caused by A (H1N1) pdm09 virus in a homogeneous group of children aged 0–59 months consulted in primary care settings with symptoms of influenza-like illness.

Values estimated in our study, describing specificity and sensitivity of RIDT BD Directigen EZ Flu A+B[®], are lower than these indicated by the manufacturer and calculated for a viral culture as a gold standard [4]. This may be a result of different sample types taken in our study (pharyngeal and nasal swabs), while the manufacturer tested nasopharyngeal swabs or pharyngeal swabs. Our observation proves that the accuracy of RIDT should be evaluated in everyday practice because many factors may influence its results, including for example age of patients and the subtype of virus circulating in the population.

One of these possible factors influencing the sensitivity of RIDT and DFA is the duration of symptoms of ILI. In our study we found that the chance of obtaining a true positive result of RIDT was twice as low when the symptoms lasted longer than 48 hours and the logistic regression analysis indicated that longer duration of symptoms was negatively associated with RIDT performance. Our observation is consistent with the results obtained by Hawkes et al. [18]. Gordon et al. also observed that the sensitivity was significantly lower when the samples for RIDT were collected on the day of symptom onset compared to samples collected one or two days following symptom onset (41.7% vs 72.1%) [16]. Time-dependent sensitivity of a rapid antigen test in patients with A (H1N1)pdm09 influenza was also described by Lee et al. [22] – the most appropriate time frame for sample collection for the detection of influenza virus in RIDT was 24–96 hours (sensitivity 75–79.9%).

Table 3. Duration of symptoms as the predictor of true positive RIDT result

Duration of symptoms [hours]	OR	95% CI
>48	0.224	0.136–0.368
<48	0.402	0.269–0.603
<24	0.644	0.57–0.727
24–48	0.414	0.325–0.529
48–72	0.267	0.185–0.385
72–96	0.172	0.105–0.280

We found that the BD Directigen EZ A+B Flu test performed moderately well, with an intermediate sensitivity and a high specificity in comparison to the CDC real time RT-PCR assay for influenza A (H1N1)pdm09. Several studies have been published on the performance of the BD Directigen EZ A+B Flu test in detecting influenza A (H1N1) pdm09, with reported sensitivities ranging from 20.6% to 70.4% [1,5,6,10,18]. Our sensitivity estimate of 62% falls within the estimate of previously reported sensitivities and is very similar to the estimate of sensitivity of 70.4% from the Blazquez et al. study [5], but higher than reported by Karre et al. (48.7%) [20] and Vasoo et al. (46.7%) [32]. Possible reasons for the higher sensitivity of RIDT in our study may include the fact that we tested young children aged less than 59 months. It has been proven that the sensitivity of the RIDT increases with younger age of patients and it is believed to be a result of more intensive and longer lasting replication and shedding (transmission) of influenza virus in children compared to adults. Hawkes et al. found that the sensitivity of RIDT for detection of influenza A (H1N1)pdm09 virus was higher in a pediatric population than in a previously reported mixed adult patient population [18]. Gao et al. reported that the sensitivity of RIDT was higher among children younger than 2 years (85.7%) than patients aged 2–39 years (33.3%) [15]. A similar observation was reported by Landry et al. for seasonal influenza (the sensitivity of BD Directigen EZ Flu A+B – 75%) [21].

Another reason for higher sensitivity of RIDT reported in our study could be that we used fresh samples for testing while some researchers used frozen samples; studies using frozen specimens or specimens delayed in testing reported worse accuracy of RIDT [17,29].

The BD Directigen EZ Flu A+B[®] in our study revealed a moderate positive predictive value (68.6%), and high negative predictive value (94.1%). These results would be more typical for prevalence of influenza estimated as low (<10%) while we conducted the study during the autumn and winter season (between 1 November and 31 March) when the prevalence of the disease is expected to be higher, especially as the 2009/2010 season was a pandemic season with predominance of type (H1N1)pdm09 influenza virus. Moderate positive predictive value of the RIDT may be explained by the changing prevalence of influenza in different months, even different weeks of the season, as described by some authors [18,27].

Positive and negative likelihood ratios are independent values from the prevalence of the diseases and in our study we observed a high positive and low negative likelihood ratio (respectively), which is similar to data reported by other investigators: Poehling et al. found a positive and negative likelihood ratio of respectively 126 and 0.18 [27].

The second rapid method of diagnosis of influenza is a direct immunofluorescent assay. The sensitivity of DFA for the pandemic virus A (H1N1)pdm09 ranged from 32.35–38.7% to 65% [18,22,23]. The sensitivity of DFA in our study was and our result is consistent with the results of other researchers. Sandora found the sensitivity of DFA in detection of influenza A (H1N1)pdm09 infection as high as 53%, specificity 99% [30]. Bakerman et al. reported the sensitivity of DFA of 65% (specificity 99.6%) [3].

In our study the accuracy of RIDT was comparable with the accuracy of DFA, and we conclude that in young children aged less than 5 years, consulted with symptoms of ILI in primary care settings, the RIDT may be a sound tool in the diagnosis of influenza and the method of choice for screening for influenza infections and there is no need to conduct immunofluorescence assays as they are more time consuming, more expensive, and require access to a well-equipped laboratory and highly trained laboratory personnel. RIDT should be especially used in resource-limited settings where equipment, reagents and laboratory staff are not always available.

In our study both methods “missed” one-third of infected individuals. Clinicians must be aware of the limitations of RIDT and the results should be interpreted cautiously. In an epidemic or pandemic season false negative results occur more often than false positive results. The physician should consider sending respiratory specimens of patients with negative results received in RIDT for influenza testing by RT-PCR or viral culture or DIA, especially when community influenza activity is high, and particularly when the patient is in the risk group of severe course of disease and complications [9,11,12,33]. For children at high risk with influenza-like illness during high-prevalence periods of influenza, empiric initiation of antiviral therapy should be considered for patients with negative RIDT results [33].

Both methods – the rapid influenza detection test and direct immunofluorescent assay – allow one to differentiate

between influenza type A and type B infection, but they do not provide information concerning the subtypes of the virus [2,3]. The gold standard for diagnosis of influenza and for evaluation of diagnostic tests is still viral culture, but molecular biology methods (PCR and RT-PCR) are also considered as gold standard, especially for clinical diagnosis [9,11,33]. 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 real time when the diagnostic, isolation and therapeutic questions need to be addressed and solved, not hours later. Laboratory methods based on molecular biology should be recommended to all patients with a severe course of the disease suspected for influenza, even when RIDT provided a negative result. Laboratory-based surveillance is also needed in order to obtain information on currently circulating influenza viruses, and the emergence and prevalence of antiviral resistant strains [33].

One advantage of our study is the homogeneous group of patients (children younger than 59 months) while most studies dedicated to evaluation of rapid influenza tests have been conducted on adult, mixed adult and pediatric patients or among patients aged 0–18 years [2,7,8,24,31].

Another advantage of our study is that it was conducted in out-patient settings while most other studies were performed in hospital settings or emergency units [24,30,31].

There are several limitations of our study. The sample size for calculation of the accuracy of RIDT and DFA was relatively small, although limited to a very age-related homogeneous group of patients. Another limitation is that DFA was not conducted for each sample (samples were collected in the primary care setting, in the evening hours and at weekends too; laboratory staff and equipment required for DFA testing were not available every time).

CONCLUSIONS

The rapid influenza detection test BD Directigen EZ Flu A+B[®] is characterized by moderate accuracy which is comparable to the direct immunofluorescence assay accuracy. RIDT may be used as a screening test in a differential diagnosis of influenza caused by type A virus in children younger than 59 months consulted in the primary care setting with symptoms of ILI.

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The authors have no potential conflicts of interest to declare.