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Clinical Investigations

COMPARISON OF DIRECTIGEN FLU A+B WITH REAL TIME PCR IN THE DIAGNOSIS OF INFLUENZA

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СРАВНЕНИЕ ИММУНОХРОМАТОГРАФИЧЕСКОГО МЕТОДА (DIRECTIGEN FLU A+B) И ТЕСТА RT-PCR ПРИ ИНФЕКЦИЯХ ГРИППА

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ABSTRACT

Early diagnosis and treatment of patients with influenza is the reason why physicians need rapid high-sensitivity influenza diagnostic tests that require no complex lab equipment and can be performed and interpreted within 15 min. The **Aim** of this study was to compare the rapid Directigen Flu A+B test with real time PCR for detection of influenza viruses in the Republic of Macedonia. **Materials and methods:** One-hundred-eight respiratory samples (combined nose and throat swabs) were routinely collected for detection of influenza virus during influenza seasons. Forty-one patients were pediatric cases and 59 were adult. Their mean age was 23 years. The patients were allocated into 6 age groups: 0 - 4 yrs, 5 - 9 yrs, 10 – 14 yrs, 15 – 19 yrs, 20-64 yrs and > 65 yrs. Each sample was tested with Directigen Flu A+B and CDC real time PCR kit for detection and typisation/subtypisation of influenza according to the lab diagnostic protocol. **Results**: Directigen Flu A+B identified influenza A virus in 20 (18.5%) samples and influenza B virus in two 2 (1.9%) samples. The high specificity (100%) and PPV of Directigen Flu A+B we found in our study shows that the positive results do not need to be confirmed. The overall sensitivity of Directigen Flu A+B is 35.1% for influenza A virus and 33.0% for influenza B virus. The sensitivity for influenza A is higher among children hospitalized (45.0%) and outpatients (40.0%) versus adults. **Conclusion**: Directigen Flu A+B has relatively low sensitivity for detection of influenza viruses in combined nose and throat swabs. Negative results must be confirmed.

Key words: *influenza, rapid test, real time RT-PCR*

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РЕЗЮМЕ

Для ранней диагностики и лечения пациентов с гриппом нужны высокочувствительные диагностические тесты, применение которых не требует сложного лабораторного оборудования, а продолжительность проведения и читки реакции не превышает 15 минут. **Целью** данного исследования является сопоставительное сравнение быстрого теста Directigen Flu A+B с тестом PCR в режиме реального времени при выявлении вирусов гриппа в Республике Македонии. **Материалы и методы:** В течение нескольких месяцев распространения сезонного гриппа было проведено 108 дыхательных проб (комбинированные мазки из носа и горла). Среди пациентов был 41 ребёнок и 59 взрослых, средний возраст которых составлял 23 года. Пациенты были разделены на следующие возрастные группы: 0 - 4, 5 – 9, 10 – 14, 15 – 19, 20 – 64 лет и старше 65 лет. Каждая проба была протестирована с использованием Directigen Flu A+B и тестового набора в режиме реального времени PCR CDC на выявление и определение типа и подтипа вируса гриппа в соответствии с лабораторным диагностическим протоколом. **Результаты:** Тест Directi-

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gen Flu A+B выявил 20 (18.5%) проб с положительным результатом на вирус гриппа А и 2 пробы (1.9%) с положительным результатом на вирус гриппа В. Высокая степень специфичности (100 %) и высокая степень положительного прогнозируемого значения, которые продемонстрировал тест Directigen Flu A+B во время нашего исследования являются показателем того, что положительные результаты не нуждаются в подтверждении. Общая чувствительность теста Directigen Flu A+B составляет 35.1 % на вирус гриппа типа А и 33.0 % на вирус гриппа типа В. Тест на вирус гриппа типа А проявил более высокую чувствительность при детях (госпитализировано 45.0 %, амбулаторных – 40.0 %) по сравнению с аналогичной при взрослых. **Заключение:** Тест Directigen Flu A+B обладает относительно низкой чувствительностью при выявлении вирусной инфекции в мазках из горла и носа. Отрицательные результаты подлежат верификации.

Ключевые слова: *грипп, быстрый тест, RT-PCR*

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INTRODUCTION

Influenza infections caused by the influenza A and B viruses are the most common respiratory infections. They can be self-limiting in healthy individuals. In very young (< 2 yrs), and elderly (> 65 yrs) individuals, people with co-morbidities such as chronic pulmonary, cardiovascular and metabolic disorders and immunocompromised individuals, influenza can be the cause for influenza-associated complications such as sinusitis, pharyngitis, bronchitis, pneumonia, croup and otitis media; prolonged hospitalization and mortality.[1,2]

Antiviral drugs are effective and well tolerated in a variety of low- and high-risk patients when used within the first 48 h from onset of the diseases and prophylaxis of influenza. Many studies have demonstrated that early initiation of oral oseltamivir therapy increased its therapeutic effects reducing the duration of the symptoms, severity of the illness, incidence of secondary complications, and duration of the hospitalization.[3-5]

Influenza can be difficult to diagnose based on clinical signs and symptoms alone because influenza illness can be similar to illness caused by other infectious agents including parainfluenza viruses, adenovirus, respiratory syncytial virus and *Legionella spp*.[1,2,6] Timely diagnosis and treatment of patients with influenza is the reason why physicians need rapid influenza diagnostic tests with high sensitivity requiring no complex lab equipment and being able to be performed and interpreted easily within 15 minutes.[6-9]

AIM

The aim of this study was to compare the rapid Directigen Flu A+B test with real time PCR for detection of influenza viruses in the Republic of Macedonia.

MATERIALS AND METHODS

The samples included in this study were tested as part of a public health surveillance. The patients were informed about the need for taking samples and testing for influenza. The sampling procedure was performed after obtaining informed consent from the patients. The study was conducted in full conformity with the Helsinki declaration. The 108 respiratory samples that were routinely collected to detect influenza virus were nose and throat swabs taken during influenza seasons. They were placed immediately in a transport medium, kept at 2-8°C and transported to the laboratory as soon as possible. The nose and throat samples from each patient were combined in one single sample. Forty-one (37.9%) patients were pediatric cases (0-18 years old) and 59 (54.6%) were adult. Their mean age was 23 years (range 0-87 years). The patients were allocated into several age groups as follows: 0 – 4 yrs (n = 16), 5 – 9 yrs (n = 8), 10 – 14 yrs (n = 8), 15 – 19 yrs (n = 11), 20 – 64 yrs (n = 54) and > 65 yrs (n = 3). The samples were taken from 87 (80.5%) hospitalized patients and from 21 (19.4%) outpatients.

RNA extraction was performed with QIAamp viral RNA kit (Qiagen, Germany) according to the manufacturer's instructions and used for real time PCR reaction for detection and typisation/subtypisation of influenza virus. CDC real time RT-PCR was used with specific matrix and HA gene primers and probes for influenza virus typing and subtyping. The reaction was performed according to published laboratory diagnostic protocol (World Health Organization). CDC Protocol of rtRTPCR for swine influenza A (H1N1), 28 April 2009. The WHO Collaborating Centre for influenza at CDC Atlanta, United States of America).[10] Diagnostic kits were globally available via WHO Global In-

fluenza Surveillance Network (GISN).[11] The assay was performed on a IQ5 (Biorad). The turnaround time in real time machine is 2 hours.

The specimens were aliquot and stored at -70°C. They were tested with Directigen Flu A+B (Becton, Dickinson and Company, Maryland, USA).

Directigen Flu A+B is a membrane-based enzyme immunoassay for direct, simultaneous and qualitative detection of influenza A and B viral antigens. We performed it according to the instructions of the manufacturer. The sample was mixed with detergent and mucolytic agent and applied to the test membrane. After washing, the enzyme conjugated monoclonal antibody was added and the reactivity was determined by the appearance of a purple triangle on the membrane. A purple control dot only with no visible triangle indicates a negative test. The test took 15 minutes to perform.

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the Directigen flu A+B test results were calculated using results of the rRT-PCR assay as reference.

RESULTS

Out of the 108 samples tested with rRT-PCR, 72 (66.7%) were positive. Of these 57 (79.2%) were positive for influenza A virus and 15 (20.8%) for influenza B virus. From the influenza A positive samples 33 (57.9) were flu A/H1pdm09, 8(14.0%) flu A/H3 and 16 (28.1%) were not subtyped.

Out of 108 samples tested with Directigen Flu A+B, 20 (18.5%) samples were positive for influenza A and 2 (1.9) were positive for influenza B .

Test parameters of the Directigen Flu A+B test as compared with the rtRT-PCR (CDC) assay are shown in Tables 1, 2 and 3 by age groups.

DISCUSSION

Clinicians need fast, accurate and sensitive tests for detection of influenza virus in their daily routine work. Rapid tests are important for optimal timing of antiviral use, they eliminate the unnecessary prescription of antibiotics, and appropriate management of patients with influenza.

Real time RT-PCR is a method with high sensitivity (99.4%) and specificity of diagnosing influenza virus infections, but it is too laborious and time consuming, requiring special complex laboratory infrastructure and equipment, and trained, highly skilled staff. It is not rapid and far from easy to perform if one has a low level of expertise.[12]

Directigen Flu A+B is an easy rapid test taking only 15 minutes to perform. It does not require laboratory expertise and is able to be performed in the physician's office.

When evaluating the performance of rapid tests it is important to consider the following factors related to specimen (type, quality and transport), patient (age and immune status) and influenza type.[6]

According to the manufacturer specimens acceptable for influenza testing with Directigen Flu A+B are nasopharyngeal washes, nasopharyngeal aspirates, nasopharyngeal swabs, throat swabs, and bronchoalveolar lavages. We used nose and throat swabs as these are quite simple to take and can practically be collected in a physician's office.[6] However, swab specimens are the least sensitive and the least specific in comparison with nasopharyngeal washes and aspirates due to higher viral loads in nasopharyngeal washes.[13]

Manufacturer reports give sensitivity of 76.7% for influenza A and 0.00% for influenza B virus from combined nose/throat swabs compared

Table 1. Test parameters of the Directigen Flu A+B test as compared with the rtRT-PCR (CDC) assay

	Sensitivity in % (95% CI)	Specificity in % (95% CI)	PPV in % (95% CI)	NPV in % (95% CI)
Total for Influenza A	35.1 (24.0 - 48.1)	100 (93.0 - 100)	100 (83.9 - 100)	58.0 (56.4 - 74.0)
for Influenza A/H1pdm09	48.5 (32.5 - 64.8)	100 (94.9 - 100)	100 (80.6 - 100)	80.7 (71.2 - 87.6)
for Influenza A/H3	37.5 (13.7 - 69.4)	100 (95.6 - 100)	100 (43.85 - 100)	94.3 (87.4 - 97.5)
for Influenza B	13.3 (3.7 - 37.9)	100 (96.0 - 100)	100 (34.2 - 100)	87.7 (80.1 - 92.7)

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Table 2. Test parameters of the Directigen Flu A+B test as compared with the RT-PCR (CDC) assay according age groups

	Sensitivity in % (95% CI)	Specificity in % (95% CI)	PPV in % (95% CI)	NPV in % (95% CI)
for Influenza A - age 0 - 4 (n = 16)	57.1 (25.1 - 84.2)	100 (70.1 - 100)	100 (51.0 - 100)	75.0 (46.8 - 91.1)
for Influenza A - age 5 - 9 (n = 8)	57.1 (25.1 - 84.2)	100 (20.65 - 100)	100 (51.0 - 100)	25.0 (4.6 - 69.9)
for Influenza A - age 10 - 14 (n = 8)	25.0 (4.6 - 69.9)	100 (51.0 - 100)	100 (20.6 - 100)	57.1 (25.1 - 84.2)
for Influenza A - age 15 - 19 (n = 11)	28.6 (8.2 - 64.1)	100 (51.0 - 100)	100 (34.2 - 100)	44.4 (18.9 - 73.3)
for Influenza A overall age 0 - 19 (n = 43)	44.0 (26.7 - 62.9)	100 (82.4 - 100)	100 (74.1 - 100)	56.3 (39.3 - 71.8)
for Influenza A - age 20 - 64 (n = 54)	28.0 (14.3 - 47.6)	100 (88.3 - 100)	100 (64.6 - 100)	61.7 (47.4 - 74.2)
for Influenza A - age > 65 (n = 3)	50.0 (9.4 - 90.5)	100 (20.6 - 100)	100 (20.6 - 100)	50.0 (9.4 - 90.5)
for Influenza B - age 0 - 4 (n = 16)	25.0 (4.6 - 69.9)	100 (75.7 - 100)	100 (20.6 - 100)	80.0 (54.8 - 92.9)
for Influenza B - age 5 - 9 (n = 8)	NA	100 (67.6 - 100)	NA	100 (67.6 - 100)
for Influenza B - age 10 - 14 (n = 8)	0 (0.0 - 79.3)	100 (64.6 - 100)	NA	87.5 (52.9 - 97.8)
for Influenza B - age 15 - 19 (n = 11)	NA	100 (74.1 - 100)	NA	100 (74.1 - 100)
for Influenza B overall age 0 - 19 (n = 43)	20 (3.6 - 62.4)	100 (90.8 - 100)	100 (20.6 - 100)	95.0 (77.9 - 96.2)
for Influenza B - age 20 - 64 (n = 54)	0 (0.0 - 32.4)	100 (92.3 - 100)	NA	85.2 (73.4 - 92.3)
for Influenza B - age > 65 (n = 3)	NA	100 (43.8 - 100)	NA	100 (43.8 - 100)
for Influenza B - age missing (n = 8)	50.0 (9.4 - 90.5)	100 (61.0 - 100)	100 (20.65 - 100)	85.7 (48.7 - 97.4)

with culture versus sensitivity of 95.7%/87.5% for influenza A and B, respectively when using nasopharyngeal aspirates. The sensitivity of the test when using throat swabs in pediatric cases is 79%, and in adult cases is 63%. The specificity reported by the manufacturer is 90.8% for influenza A and 100% for influenza B from combined nose/throat swabs, and 91.4% - 98.1% specificity for influenza A - influenza B, respectively when using nasopharyngeal aspirates. (Leaflet provided by the manufacturer in the kit).

The high specificity (100%) and PPV of the Directigen Flu A+B test observed in our study indicates that positive results can be the final results allowing the physician to make a decisions for the treatment. That is comparable to previously published studies for rapid tests, including the Directigen Flu A+B.[5,14,15]

Table 3. Test parameters of the Directigen Flu A+B test as compared with the rtRT-PCR (CDC) assay according hospitalization of different groups

	Sensitivity in % (95% CI)	Specificity in % (95% CI)	PPV in % (95% CI)	NPV in % (95% CI)
for Influenza A – children (0 - 18 years) hospitalized (n = 33)	45.0 (25.8 - 67.8)	100 (77.2 - 100)	100 (77.2 - 100)	54.2 (35.1 - 72.1)
for Influenza A - children (0 - 18 years) outpatient (n = 8)	40.0 (11.8 - 76.9)	100 (43.8 - 100)	100 (34.2 - 100)	50.0 (18.8 - 81.2)
for Influenza A – adults hospitalized (n = 49)	29.2 (14.9 - 49.2)	100 (86.7 - 100)	100 (64.6 - 100)	59.5 (44.5 - 72.3)
for Influenza A - adults outpatient (n = 10)	33.3 (6.1 - 79.2)	100 (64.6 - 100)	100 (20.6 - 100)	77.8 (42.3 - 94.3)
for Influenza A total hospitalized (n = 87)	34.7 (22.9 - 48.7)	100 (90.8 - 100)	100 (81.6 - 100	54.3 (42.7 - 65.4)
Total Influenza A outpatient (n = 21)	37.5 (13.7 - 69.4)	100 (77.2 - 100)	100 (43.8 - 100)	72.2 (49.1 - 87.5)
for Influenza B - children (0 - 18 years) hospitalized (n = 33)	25.0 (4.6 - 69.4)	100 (88.3 - 100)	100 (20.6 - 100)	90.6 (75.8 - 96.8)
for Influenza B - children (0 - 18 years) outpatients (n = 8)	0 (0.0 - 79.3)	100 (64.6 - 100)	NA	87.5 (52.9 - 97.8)
for Influenza B – adults hospitalized (n = 49)	0 (0.0 - 39.0)	100 (91.8 - 100)	NA	87.8 (75.8 - 94.3)
for Influenza B - adults outpatient (n = 10)	0 (0.0 - 65.8)	100 (67.6 - 100)	NA	80.0 (49.0 - 94.3)
for Influenza B - total hospitalized (n = 87)	10 (1.8 - 40.4)	100 (95.2 - 100)	100 (20.6 - 100)	89.5 (81.3 - 100)
for Influenza B - total outpatient (n = 21)	20 (3.6 - 62.4)	100 (80.6 - 100)	100 (20.6 - 100)	80.0 (58.4 - 91.9)

The overall sensitivity of Directigen Flu A+B in the present study using combined nose and throat swabs is 48.5% for influenza A and 13.3% for influenza B which is lower than what has been found in other studies. In general, rapid tests show lower sensitivity than the sensitivity of PCR.[13,14]

Previous studies described variable low sensitivity ranging from 31.7% to 89% depending on the brand of rapid test.[14-20]

The sensitivity of Directigen Flu A+B is higher in detecting influenza A versus influenza B virus which is a result consistent with the results in other studies.[21] Possible reasons is the higher number of samples positive for influenza A (57 samples) versus 15 influenza B positive samples included in the study.

Because the test is based on an antigen-antibody reaction with monoclonal antibodies specific for nucleoprotein (NP) with high homology in type A and B viruses, the sensitivity of the rapid test depends on the level of cross-reactivity of the NP-specific antibodies.[22]

For influenza pandemics it is important to find an assay able to detect influenza virus type A and a range of virus subtypes, particularly those with pandemic potential.

Among influenza A subtypes in our study better sensitivity was displayed in the detection of

H1pdm09. This can be accounted for by the possible domination of Influenza A/H1pdm09 subtype in the influenza seasons and study group. However, due to a limited number of samples, the difference in sensitivity for H1N1pdm versus H3N2 did not reach statistical significance. The test's sensitivity depends on time of collection during the illness and the type of sample.[23]

The sensitivity for influenza A is higher among children hospitalized (45.0%) and outpatients (40.0%) especially among age groups 0-4 and 5-9 years, and was especially low in hospitalized and outpatient adults. Sensitivity is decreasing with age.[5,20] The difference of the sensitivity between children and adults can be result of the longer virus shedding by the children.[24,25] Also, children with influenza virus type A infections have higher viral loads in the nasopharynx than older patients.[21]

The false negative results are possible at the early stage of infection when the virus load is relatively low and NP is insufficient to be detected with rapid test. Other reasons could be low quality of sample or a small number of studied patients.[22,23]

Negative tests can be the reason for delay in administration of antivirals which can lead to increased severity of influenza and likelihood of mortality. Negative result must be verified.

We didn't confirm the findings in Chan et al. where the sensitivity, specificity, PPV and NPV of the Directigen Flu A+B for influenza A were 96%, 99.6%, 96% and 99.6% and for influenza B these were 87.5%, 96.8%, 80% and 98%, respectively.[21]

LIMITATIONS OF THE STUDY

Specimen type affects rapid test performance, but we were not able to compare different specimens from one and the same patient. Multiple testing was not performed with individual samples. Most of the samples were kept frozen at -70°C for different time until the rapid test became available. In our study the samples were relatively few, as well as samples divided by age groups and type/subtype of the virus which can influence the results. These rises the need for larger sample size in further studies.

CONCLUSIONS

Our findings indicates that the available rapid test in our lab for detection of influenza A and B - Directigen Flu A+B - has a relatively low sensitivity for detection of influenza viruses in combined throat and nose swabs. The low sensitivity indicates that it produces high false negative results. I this respect, negative results obtained with this test should be confirmed with rtRT-PCR and clinicians should not rule out influenza, or change the decision for treatment based on a negative test result. There is a need to use more sensitive rapid test.

On the other hand, high specificity of the Directigen Flu A+B test for detection of influenza A and B implies that physicians can consider the positive results final and use them to plan further treatment.

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