

A clinical utility of a strip test for influenza A/B and comparison with detection by RT PCR

Maciej Miarka¹✉, Andrzej Horban¹, Henryka Maliszewska¹, Przemysław Biliński² and Wanda Prus-Kowalczyk¹

¹Regional Infectious Diseases Hospital, Warsaw, Poland; ²Chief Sanitary Inspectorate, Warsaw, Poland

Introduction and objective: In June 2009 the World Health Organization announced influenza pandemic caused by A/H1N1/v virus. It became crucial to recognize new cases of A/H1N1/v infection. An effective screening diagnostic procedure was needed for patients suffering from influenza-like symptoms for making an initial diagnosis and analyzing epidemiological pattern of infection. We used a strip test for influenza A/B as a screening diagnostic procedure for patients suffering from influenza-like symptoms for making an initial diagnosis. For comparison, RT PCR for detecting A/H1N1/v was performed. The aim of this study was to assess the efficacy and sensitivity of the strip test and its value for making initial diagnosis of influenza A/H1N1/v. **Material and methods:** Strip testing for the influenza A/B infection was performed on 1123 patients with influenza-like symptoms in the Admission Unit of the Regional Infectious Diseases Hospital in Warsaw. Strip test results were analyzed according to the age of patients and season of the year. For 97 patients strip test results for detecting A/H1N1 infection were compared with those obtained by RT PCR. **Results:** There were no statistically significant differences found between the methods and strip testing demonstrated sensitivity of 61% and specificity of 71%. **Conclusions:** No statistically significant differences were found between the two methods, however, strip test had low sensitivity and specificity.

Key words: A/H1N1/v influenza virus, strip test, influenza RT PCR

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INTRODUCTION

In June 2009 the World Health Organization announced an influenza pandemic caused by the A/H1N1/v virus. Analysis of A/H1N1/v genome indicated mixed animal and human origins that seemed to be unconnected with the occurrence of the seasonal human H1N1 influenza virus present in the population since 1977. A complete susceptibility of the population to this new strain was due to A/H1N1/v novel immune properties; an exception were those aged over 65, who had acquired cross-immunity from previous times. The A/H1N1 pandemic virus was found to display a different epidemiology and clinical time course compared

to the seasonal influenza strain. Out of the 568967 reported cases of illness or suspected illness in Poland, 2072 samples were taken for analysis (Polish National Institute of Hygiene report between 31.08.2009 and 31.12.2009). Positive results of infection to virus A, B or RSV, para-influenza type 1, 2 or 3 virus and adenovirus were observed in 539 cases (26%). Until 31.12.2009, 304 cases of infection with pandemic A/H1N1/v virus strain had been confirmed in Poland. In the Infectious Diseases Hospital in Warsaw we have observed A/H1N1/v infection in 109 patients (Female — 64, Male — 45, aged 17–71 years), hospitalized between August and December 2009. The influenza specific PCR test (TaqMan A/H1N1) was used for pandemic flu confirmation (Cholewińska *et al.*, 2010a; Cholewińska *et al.*, 2010b). In parallel, a screening strip test, several folds cheaper, was used in the Admission Unit (AU) of the Infectious Diseases Hospital. This paper shows the evaluation of sensitivity and efficacy of a strip test for making an initial diagnosis of A/H1N1/v.

Subject group consisted of 1123 patients aged 8–83 years (average 34.1), admitted to the AU between 14.07.2009 and 31.12.2009, and suffering from influenza-like symptoms (Fig. 1).

METHODS

The test method used was the Influenza A/B 2 Panel Test # 4A470 from GECKO Pharma Vertrieb GmbH. This is a qualitative one-step procedure for detecting group A & B nucleoprotein antigens. In parallel, two molecular biology tests were used to confirm the presence of A/H1N1/v infection: the New Influenza A Virus (H1N1) Real Time RT-PCR (Shanghai ZJ Bio-Tech Co. Ltd) and the TaqMan Influenza A (H1N1) Assay Sets (Applied Biosystems). Both tests are based on fluorescent RT-PCR, where samples containing genetic material from A/H1N1/v result in the RNA strand being subjected to reverse transcription into the complementary DNA strand where it is then amplified by the polymerase chain reaction. Samples of nose swab extracts obtained from the subjects' nasal secretions were subjected to analysis within one hour of being taken; this being recommended by the manufacturers' as having the best diagnostic value. The differences between tests were analyzed by the McNemara test at

✉ e-mail: emem2002@gmail.com

Abbreviations: RT PCR, reverse transcriptase polymerase chain reaction; A/H1N1/v, influenza A virus subtype H1N1 variant, RSV, respiratory syncytial virus, CDC, Centres for Disease Control and Prevention.

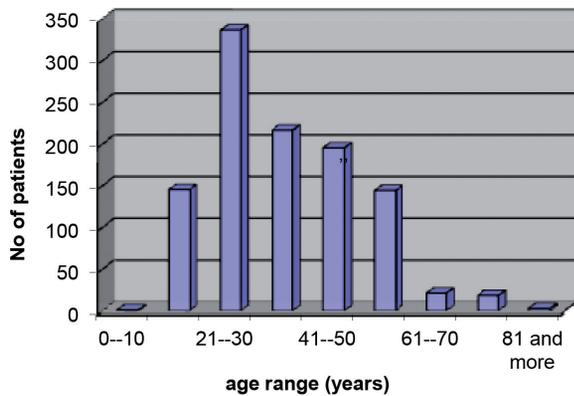


Figure 1. Number of patients with influenza-related symptoms, who had the test for A/B influenza done in the Admission Unit of Regional Hospital for Infectious Diseases in Warsaw in the period between 14.07 and 31.12.2009, in age groups.

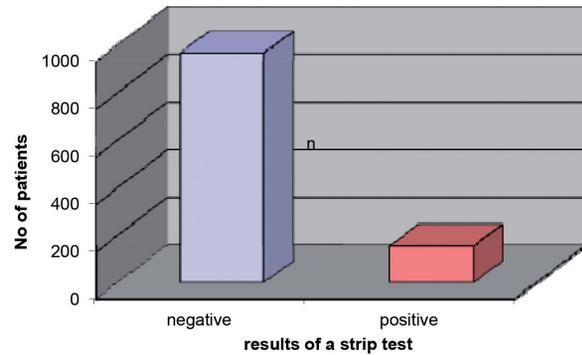


Figure 2. Results of strip test for A/B influenza performed for patients with influenza related symptoms in the Admission Unit of the Regional Hospital for Infectious Diseases in the period between 14.07. and 31.12.2009.

the $P < 0.05$ level using the Stat v.9 program; Stata Statistical Software (College Station, TX, Stata Corporation).

RESULTS

The Influenza A/B 2 Panel Test #4A470 method classifies results as being positive or negative. The manufacturer instructions state that special attention should be given to the variable intensity of the test line colour developed, which is related to the sample concentration of antigen. Therefore, the results showing small intensities ie. low concentrations, should be treated as being positive, as the test is qualitative. Out of the 1123 samples analyzed, 157 (14%) gave positive results (Fig. 2).

The majority of patients were admitted in July, August, November and December of 2009 with the highest amount of positive test results recorded during August, September and November. The lowest number was recorded in October when there were no positives detected (Fig. 3).

An age-related dependency was observed, with the highest number of positives recorded in July and August for those aged 11-20 and 21-31 years and in November and December for those aged 21-30 and 31-40 years. (Fig. 4).

Amongst the 1123 patient group, 97 patients were tested using the RT PCR techniques. Interestingly, only

46 of these patients were tested positive by the strip test, the remaining 51 patients were tested negative by the strip test. Those with a positive strip result were confirmed in 34 (74%) cases using the RT PCR method but were unconfirmed in 12 (26%) samples. Of the 51 patients demonstrating the aforementioned negative results, the A/H1N1/v was detected using the PCR method in 22 patients (43%) whilst 29 (57%) were found to be free of the infection.

The breakdown of the results obtained using the strip test and PCR methods is presented in Table 1.

The amount of patients with a positive result of strip test (47%) was not significantly different from those who obtained a positive result using the PCR test (58%), $P = 0.086$.

The sensitivity of the strip test to detect persons actually sick (a true positive according to the PCR test) amounted to 61% with specificity of 71%.

Interpretation

No statistically significant differences were observed between the values obtained with the strip test in comparison with the PCR tests. This does not necessarily rule out that the same applies to the general population, as the numbers sampled were relatively small. But the INSIGHT group created two big studies, FLU 002 and FLU 003, in which there were involved 62 sites

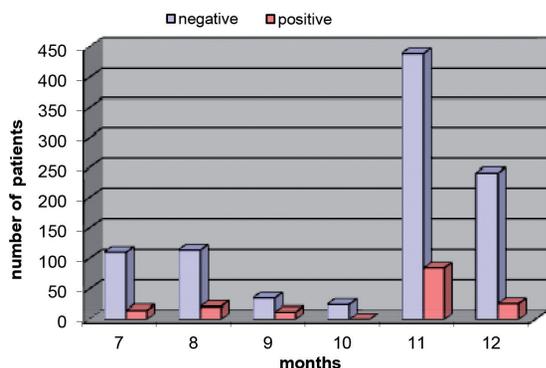


Figure 3. Strip test results for A/B influenza performed for patients with influenza-related symptoms in the Admission Unit of the Regional Infectious Diseases Hospital in the period between 14.07. and 31.12.2009

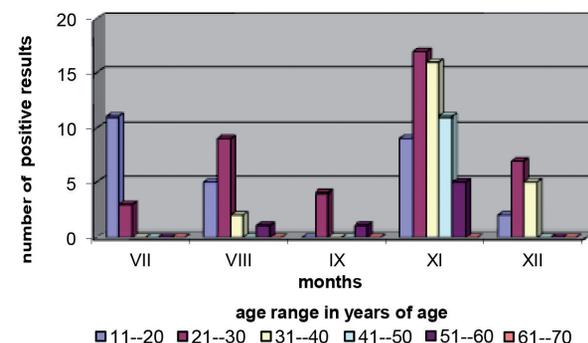


Figure 4. Age-related dependency of influenza illness, revealed with strip tests for A/B influenza performed for patients with influenza-related symptoms in the Admission Unit of the Regional Hospital for Infectious Diseases, in the period between 14.07. and 31.12.2009.

Table 1. Comparison of the results obtained by strip tests and PCR test in patients with influenza symptoms, admitted to wards of the Regional Infectious Diseases Hospital in the period between 14.07.2009 and 31.12.2009.

	PCR (-)	PCR (+)	Total
Strip test (-)	29 (30%)	22 (23%)	51 (53%)
Strip test (+)	12 (12%)	34 (35%)	46 (47%)
Total	41 (42%)	56 (58%)	97 (100%)

in 14 countries on several continents. The purpose of these studies was to estimate the percent of adult patients with illness due to laboratory-confirmed pandemic (H1N1) 2009 infection that experienced clinically significant outcomes, and to study factors related to these outcomes. In FLU 002 for outpatient cases, positive AH1N1/v results according to age distribution and seasonal incidence were similar to ours. In FLU 003 for hospitalized cases with suspected or confirmed influenza positive for AH1N1/v in PCR method there were 46.7% (total) and 50% (from Poland only).

Our group consisting of 97 pairs of results ensures a power of only 38% to recognize that the 11% difference may be statistically significant where the proportions of results disagreeing are 23% and 12%. The results therefore cannot be taken as confirming agreement between the strip and PCR tests. As the strip test used by us was designed to detect only the influenza A/B virus whereas the PCR test detected A/H1N1/v, it is crucial to emphasize that the sensitivity and efficacy of a strip test estimated here refers only to the detection of A/H1N1/v.

CONCLUSIONS

The study results show that the Influenza A/B 2 Panel test #A470 strip test demonstrates low sensitivity and efficacy for A/H1N1/v detection and therefore should not be recommended for excluding A/H1N1/v group virus infection for persons with influenza or influenza-like symptoms, nor for making therapeutic decisions showing a positive test result.

Sensitivity and efficacy of the strip test for detection of the A/H1N1/v influenza virus infection in other studies amounted to respectively: 47%, 86% (CDC, 2009a), 27%, 97% (Uyeki *et al.*, 2009), 63%, 99% (Faix *et al.*, 2009) or was highly differentiated (Ginocchio 2009). Interpretation based on such data was that the negative result of a strip test cannot exclude A/H1N1/v influenza infection in persons with influenza or influenza-related symptoms (CDC, 2009a; CDC, 2009b). The positive result of the strip test cannot be used to undertake therapeutic decisions, contrary to results obtained in another evaluation of a rapid test (CDC, 2009c). Pursuant to CDC recommendations, the strip test should not be used in proceeding algorithms with persons suspected of A/H1N1/v infection (CDC, 2009a).

Among the patients tested by us for A/H1N1/v with the strip test only 14% were positive. Based on the sensitivity of the strip test for A/H1N1/v detection, a larger proportion of positive results in the A/H1N1/v influenza pandemic period should be expected. This may prove an erroneous assumption of an inflated level of A/H1N1/v influenza pandemic intensity in our population in the period subjected to test or on the shift of the epidemic zenith in time. This might justify the introduction of A/H1N1/v influenza virus strain to a seasonal vaccine against influenza in the year 2010/2011.

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