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Performance of rapid influenza testing in hospitalized children

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Title: PERFORMANCE OF RAPID INFLUENZA TESTING IN HOSPITALIZED CHILDREN

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P. Gratsia; I. Christodoulou; M. Tsolia, Ph.D, M.D.

Abstract: PURPOSE:Influenza infection is associated with high hospitalization rates among young children.Rapid diagnosis of influenza infection is particularly useful in order to prevent nosocomial infection and allows for timely initiation of antiviral treatment.We evaluated the performance of a rapid influenza test in hospitalized children during the influenza season.

METHODS:All children(6mos-14yrs)hospitalized with fever and/or respiratory symptoms,admitted during the 2005 influenza season,participated in the study.A multiplex RT-PCR,able to identify IFV-A H1N1,H3N2 and IFV-Bsubtypes was performed on nasopharyngeal aspirates.The nasal swab was tested with a lateral-flow immunoassay(QuickVue Influenza Test).The performance of the rapid test was compared with the results of PCR.

RESULTS:Influenza infection was diagnosed by PCR in41/217(19%)patients.Infection with influenza A virus(H3N2) was diagnosed in all cases.The performance of the QuickVue Influenza Test was estimated as follows:sensitivity67.5%, specificity96%, positive predictive value79% and negative predictive value93%.The sensitivity of the test was higher in infants 6-12months,in those with short duration of symptoms and in the peak phase of the epidemic.

CONCLUSION:The QuickVue Influenza Test is useful and reasonably accurate to detect influenza infection in hospitalized children during the influenza season. Infection with influenza virus is unlikely if the test is negative. A positive result suggests that infection is probable if influenza virus circulates in the community.

Response to Reviewers: Alex van Belkum
Editor-in-Chief
European Journal of Clinical Microbiology & Infectious Diseases

Ref.: Ms. No. EJCMIID-D-09-00502
"PERFORMANCE OF RAPID INFLUENZA TESTING IN HOSPITALIZED CHILDREN"

Dear Editor,

Please find enclosed for consideration the revised manuscript entitled "Performance of rapid influenza testing in hospitalized children" (Ref.: Ms. No. EJCMIID-D-09-00502)

The manuscript has been modified along the lines suggested in your letter and taking into consideration all the suggestions raised by the reviewer. We appreciate the constructive comments by the reviewer that have allowed us to improve the presentation of this study. The changes introduced are specified in the enclosed response to the comments of the reviewer. In addition, all changes have been highlighted in the text for ease of re-review.

We believe that we have satisfactorily answered all your queries as well as those of your reviewer. We would be pleased if you find that this revised manuscript is now suitable for publication in European Journal of Clinical Microbiology & Infectious Diseases

Sincerely yours,

STRIPELI FOTINI MD, PhD
Pediatrician

Point by point response to the reviewer

We thank the reviewer for his/her most kind comments and for the attention paid to our manuscript. His/her constructive comments have allowed us to improve the presentation of this study. Answers to his/her specific comments and suggestions follow:

1. Abstract1: We acknowledge the reviewer for this specific comment. We added the study period to the methods section (page 2, line 3).
2. Abstract2: As suggested, we added in the results that all cases had influenza A (page 2, line 9).
3. Introduction: As suggested, we have deleted the last sentence of the introduction
4. Methods1: We thank the reviewer for pointing out this point; we have made the appropriate correction (page 3, line 18).
5. Methods2: As suggested, we added in the methods section how the vaccination status of each patient was determined (page 3, line 3).
6. Results 1: We thank the reviewer for pointing out this point; by mistake the mean temperature was written to be 37.9 ± 0.9 instead of 38.9 ± 0.9 . The appropriate correction has been made (page 6, line 4).
7. Results 2: As suggested we reversed the order of 5th and 6th paragraph and also the tables 2 and 3.
8. Results 3: We thank the reviewer for pointing out this point; we have made the appropriate correction to the tables.
9. Discussion 1: We acknowledge the reviewer for this specific comment; we have made the appropriate correction (page 9, line 13).

Alex van Belkum
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INTRODUCTION

1
2 Seasonal influenza infection serves as a major cause of morbidity and mortality,
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4 particularly among high risk population groups. It has been associated with marked
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6 social and economic consequences, including high rates of academic and occupational
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8 absenteeism, as well as significant treatment and hospitalization costs. Influenza
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10 infection is associated with elevated hospitalization rates among young children,
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12 comparable to those observed among older adults or other high risk groups [1, 2, 3].
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14 Influenza is also associated with an enhanced morbidity burden within both the
15
16 outpatient and community settings [4].
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20 The diagnosis of influenza is usually based on patient's history, clinical signs and
21
22 symptoms and on the knowledge of local epidemiological status. The combination of
23
24 fever and cough was reported to increase the likelihood that respiratory disease was
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26 influenza, especially in adults [5, 6] However, the clinical signs and symptoms of
27
28 influenza infection among children frequently overlap with those caused by other
29
30 viral infections present among the community [7]. Rapid diagnosis for influenza is
31
32 useful in order to minimize nosocomial infections, thus allowing the timely institution
33
34 of infection control measures [8]. It also allows for the timely administration of
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36 antiviral treatment which is most effective if initiated within 48 hours since the onset
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38 of symptoms [9-11]. Finally, tests that yield results rapidly can influence clinical
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40 management regarding antibiotic treatment decisions. As a result, the availability and
41
42 utilization of influenza rapid diagnostic tests within both the laboratory and clinical
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44 settings have substantially increased during recent years. The influenza rapid
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46 diagnostic tests, providing results within 15 minutes, have also been applied as
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48 screening tests for influenza infection. More than ten types of rapid influenza
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1 diagnostic tests have been approved by the U.S. Food and Drug Administration
2 (FDA).
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5 The purpose of this study is to evaluate the performance of a rapid influenza test
6 performed at the bedside of hospitalized children during one year's influenza season
7 with the use of reverse transcription polymerase chain reaction (RT_PCR) as the
8 standard diagnostic method. The performance of the rapid test was also compared to
9 the clinical diagnosis of influenza in hospitalized children.
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20 **MATERIALS AND METHODS**

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22 A cross-sectional study design was conducted during the 2005 respiratory-
23 infection season in Athens, Greece. The study period was initiated on Jan. 15, 2005,
24 following the detection of two consecutive pediatric patients with confirmed influenza
25 virus infection, and was concluded on May 15, 2005, following the conclusion of the
26 influenza season within the region. The study was approved by the Ethical Committee
27 of the "P. & A. Kyriakou" Children's Hospital in Athens, Greece. Informed consent
28 for study participation was required from the legal guardians of all eligible
29 participants prior to study participation.
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42 All children, aged 6 months to 14 years, who presented with fever ($\geq 37.8^{\circ}\text{C}$,
43 axillary) and/or respiratory symptoms and were consequently hospitalized at the "P. &
44 A. Kyriakou" Children's Hospital during the period Jan. 15, 2005 through May 15,
45 2005, were eligible (N=311). No exclusion criteria, regarding demographic and/or
46 socio-economic characteristics, for study participation were applied.
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54 All study participants were assessed within 48 hours following hospital
55 admission. A standardized questionnaire was completed by a study physician for all
56 study participants. The questionnaire was applied in order to obtain detailed
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1 information about patients' demographics, previous medical history, the presence and
2 duration of clinical symptoms and signs, physical findings, laboratory and
3 radiological results. Information about the vaccination status against influenza was
4 also obtained by vaccine records of the participants.
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10 A nasopharyngeal aspirate (NPA) of 2 ml was obtained from each participant
11 by a pediatrician through the application of a small flexible tube attached to a sterile
12 mucus trap. If such secretions could not be obtained, 2 ml of sterile saline was
13 introduced and re-aspirated. All collected specimens were stored at -70°C until
14 multiple xRT – PCR test was performed, within one month. A nasal swab of the
15 turbinates was also obtained and tested right after collection for the presence of the
16 influenza antigen, by the same researcher, through the application of the QuickVue
17 Influenza Test.
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29 **Influenza virological diagnosis**

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31 The QuickVue Influenza Test (Quidel, San Diego, USA) was used for the
32 rapid diagnosis of influenza infection. This test is based on a lateral-flow
33 immunoassay and detects the presence of both influenza virus A and influenza virus B
34 but does not differentiate between the two. The test was performed according to the
35 manufacturer's instructions. The researcher was trained in how to perform the test and
36 read the result. Specifically, the patient's specimen was placed in an extraction tube
37 where the virus particles in the specimen were disrupted, thus exposing internal virus
38 nucleoprotein. Following extraction, a test strip was placed within the tube, where
39 nucleoproteins in the specimen reacted with both the lyophilized buffer and mouse
40 monoclonal anti-influenza virus A and anti-influenza virus B antibodies contained in
41 the test strip. A positive test result was obtained if a pink or red test line along with
42 the blue control line appeared on the test strip, indicating that the extracted specimen
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1 contained influenza viral antigens. A negative test result was obtained if only a blue
2 control line appeared, showing that no influenza viruses A or B antigens were present.
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4 Reverse transcription of RNA material isolated from nasopharyngeal samples
5 was performed as described previously [12]. RNA was extracted from 50µl of nasal
6 aspirates using Trizol reagent (Invitrogen, CA, USA). Reverse transcription (cDNA
7 synthesis) was performed in 20µl reactions using 8µl RNA, Superscript III reverse
8 transcriptase (Invitrogen) and random hexamers.
9

10 Multiplex, nested (two rounds) PCRs for influenza virus (serotypes A/H1N1,
11 A/H3N2, B) were conducted in 50-µl reactions consisting of 1x Buffer, 3mM Mg²⁺,
12 0.2mM dNTPs, 2U of Platinum Taq DNA polymerase (Invitrogen), and 0.2µM of
13 each of previously published primers [13]. Four microliters of cDNA were used in
14 the first round PCR reaction mix, while 2µl of the PCR product of the first round were
15 used in the amplification mixture of the second round [14]. Samples were amplified in
16 a PTC-200 DNA Engine thermocycler (MJ Research, MA, USA) with previously
17 described thermocycling conditions [12].
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36 **Statistical analyses**

37 The primary outcome of the study was assessed by comparing the
38 performance of the QuickVue influenza test with that of the multiple xRT – PCR, the
39 currently established criterion standard. The sensitivity, specificity, positive predictive
40 value (PPV), and negative predictive value (NPV) of the QuickVue influenza test
41 were evaluated both among the overall study population and according to the clinical
42 symptoms of influenza. The sensitivity measures the proportion of actual positives
43 which are correctly identified as such and the specificity measures the proportion of
44 negatives which are correctly identified. However in practice, the PPV (the proportion
45 of patients with confirmed influenza among patients with a positive rapid test) and
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1 NPV (the proportion of patients without influenza among patients who had negative
2 rapid test) provide the most useful clinical information. Two other tools for the
3 clinician which can help him to translate the test characteristics into clinically relevant
4 terms are the positive and negative likelihood ratios (LRP and LRN respectively). The
5 LRP (=sensitivity/(1-specificity)) and the LRN ((1-sensitivity)/specificity) are
6 measures of the discriminating power of a test. LRPs of >10 and LRNs of <0.1
7 generate large changes from pretest probability to posttest probability. The translation
8 of pre-test probabilities to post-test probabilities given the likelihood ratio is done
9 with the use of Fagan TJ normograph (15). The chi-square analysis was applied in
10 order to compare patients' characteristics. A p-value (p) of ≤ 0.05 was considered as
11 the criterion for statistical significance for all analyses. Stata (Stata Corporation,
12 Texas, USA) version 8 was used for all analyses.

31 RESULTS

32 Among the eligible study population (N=311), 217 (69.8%) were tested by
33 both PCR and QuickVue Influenza Test for the presence of influenza infection. The
34 ninety-four (30.2%) eligible study participants were excluded from the study due to
35 either denied informed consent for study participation or limited hospital stay duration
36 (less than 48 hours).

37 Children who participated to the study were almost equally males (108/217)
38 and females (109/217). Fifty two per cent of the participants were between the ages of
39 12 months and 5 years (Table 1). One hundred forty five children out of 217 (67%)
40 were Greeks while 47 (22%) were Albanian, and 25 (11%) were of other ethnicities.
41 Fourteen study children (7%) were born at gestational age less than 37 weeks. Of the
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1 study population, 54 (26%) had predisposing conditions for influenza infection,
2 including asthma (20%), seizures (4%) and congenital heart disease (2%).
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5 Among the study population, 176 (86%) presented with fever (mean
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7 temperature: 38.9 ± 0.9 °C) and 29 children (14%) with respiratory symptoms only.
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9 The subgroup of febrile children consisted of 149 patients (85%) with respiratory
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11 symptoms; 9 (5%) had vomiting and / or diarrhea, 2 (1%) had abdominal pain, 1 had
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13 myalgias (0.6%) and 6 (3.5%) seizures. The remaining 9 febrile children (5%) were
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15 admitted with the diagnosis of a febrile illness without any localizing signs. The main
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17 respiratory symptoms were rhinitis (96%) and cough (89%).
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22 According to the PCR conducted, 18% of the study population had influenza
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24 infection, all of which was influenza A (H3N2). The characteristics of patients with
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26 and without influenza infection are shown in (Table 1).
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30 QuickVue Influenza Test detected influenza virus in 27 samples (68%) found
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32 to be positive by PCR but did not detect virus in 13 samples positive by PCR. Seven
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34 samples (4%) were positive by QuickVue Influenza Test but negative by PCR. Based
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36 on these, the performance of the test was estimated as follows: sensitivity 67.5%,
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38 specificity 96%, positive predictive value 79% and negative predictive value 93%
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40 (Table 2). As it shown in the table the LRPs were high, 6.3 to 23, with most between
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42 12 and 18 and the LRNs varied between 0.08 and 0.5 implying conclusive changes
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44 from pretest probability to posttest probability.
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49 The overall sensitivity of the clinical symptoms of influenza (fever and cough)
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51 was 76%, with a positive predictive value of 18%; specificity and negative predictive
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53 value were 20% and 79%, respectively. With regard to the age of participants, the
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55 sensitivity and positive predictive value of clinical symptoms of influenza were higher
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57 among children aged between 6 and 12 months as compared with those children aged
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1 greater than 12 months (80% and 13% to 76% and 19% respectively). Similar results
2 were found for specificity and negative predictive value (30% and 92% to 18% and
3 74% respectively). The positive likelihood ratios and the negative likelihood ratios
4 varied between 0.9 and 1.1 and between 0.7 to 1.4, respectively (Table 3)
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10 During the 4-week peak period of influenza activity which accounted for 70%
11 of all instances of influenza virus identification, the sensitivity increased to 76% and
12 the positive predictive value to 86%. Specificity and negative predictive values
13 remained almost the same, 95% and 91% respectively. Sensitivity and positive
14 predictive values were lower during the first and third phase of the epidemic
15 (sensitivity 57% and 50% and positive predictive value 67% and 66% respectively).
16 Specificity changed to 91% and 98% while negative predictive values changed to
17 87% and 96% respectively (Table 3).
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29 The mean duration of symptoms at the time of admission was 4.57 ± 2.43 days.
30 Stratification by reported disease duration, showed that sensitivity and specificity of
31 the QuickVue Influenza Test among hospitalized children with disease duration <2
32 days were 76% and 100%, respectively. When QuickVue Influenza Test was
33 performed in children with disease duration longer than 2 days an estimated
34 sensitivity and specificity of 65% and 94%, respectively, was calculated. Rapid test
35 had higher PPVs with samples from children with shorter disease duration (100 to
36 71%) but the NPV was almost the same for both groups (94 to 93%) (Table 3).
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48 The sensitivities of QuickVue Influenza Test were high in samples collected
49 from children aged ≤ 12 months (80%), but moderate with samples collected from
50 children aged > 12 months (66%). The test was highly specific with samples from
51 both age categories (95 to 96%). PPV was higher with samples from children >12
52 months (82 vs 67%) while NPV was approximately identical (92 to 97%) (Table 3).
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DISCUSSION

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Rapid identification of influenza cases is essential, particularly among hospitalized patients, for the initiation of specific antiviral treatment administration and consequent diminishment of the length of hospitalization. Moreover, rapid identification of influenza cases may contribute to the limitation of unnecessary antibiotic use and prevention of nosocomial transmission of the virus to high risk hospitalized population groups [16-18].

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Previous studies have shown that during a local outbreak, a clinical presentation including both fever and cough was most indicative of influenza infection among the study population. Specifically, the detection of influenza infection presenting with both fever and cough was observed to have sensitivities ranging between 64 and 86%, specificities between 55 and 67%, and PPVs ranging between 48 and 87% [5, 6, 19, 20]. This contrasts other studies which reported that no clinical signs or symptoms are specific for influenza virus infection [21, 22]. The present study indicates that the clinical diagnosis of influenza, based on the combined presence of fever and cough, is difficult among children in everyday clinical practice. The observed limited PPV indicates that only 18% of the true cases of influenza were identified as such based on patients' symptoms, while the specificity of 20% precludes the use of fever and cough for predicting influenza virus infections. The decreased LRPs values and the elevated LRNs values make the influenza infection unlikely with the use of clinical symptoms alone. The symptoms were least accurate among children aged less than one year. This finding is in accordance with the clinical experience of diagnosis of respiratory infections among infants, although the burden of influenza is greater in this age group [23, 24].

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In the present study, influenza infection was identified in 18% of children aged 6 months to 14 years who were hospitalized with fever and/or respiratory symptoms. Influenza infections were detected with positive PCR for influenza A and B. PCR was used as the standard criterion because it detects more influenza cases than viral culture and has a greater accuracy [18, 19, 25, 26]. Similarly, our study showed that the QuickVue Influenza Test has a high diagnostic yield for the detection of influenza infection among hospitalised children during the influenza season. The observed performance of the QuickVue Influenza Test is similar to that from previous studies with reported sensitivities and specificities varying between 74 and 93% and between 76 and 98% respectively [19, 27-29]. The observed PPV (79%) indicates that the majority of patients with positive rapid test truly have influenza infection, while the even greater NPV (93%) indicates that influenza infection is unlikely if the test is negative. Moreover the LRP's were very high indicating that a positive result impacts the probability for influenza more than a negative result. For example if a child has 50% chance of influenza after history and physical, this chance increases to 90-95% (LRP 12-18) with a positive test and decreases to 25-30% (LRN 0.2-0.5) with a negative test. Notice also must be given to the rapid test negative / PCR positive subgroup. It is currently unknown whether these patients are truly able to transmit the infection to others due to low viral load that is detected only by the highly sensitive PCR; consequently it is a matter of further investigation whether these patients should be isolated to prevent transmission to others.

Viral shedding is observed to be elevated and more prolonged in children, especially the younger ones, compared to adults. Moreover, viral shedding is enhanced during the first days of infection [30, 31]. As a result, infected young children having the test performed during the first days of the disease are more likely

1 to have a positive test as compared to older children with extended disease duration.
2 To this effect, the present study findings indicated that the sensitivity of the rapid test
3 increased to 80% among children aged 6 to 12 months and to 75% among children
4 with symptom onset less than 2 days.
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10 With regard to the performance of the QuickVue Influenza Test according to
11 the time phase of an influenza epidemic, the study findings indicated that during the
12 peak of the influenza epidemic, a positive rapid test result is highly indicative (86%)
13 of influenza infection, while a negative test result highly suggests the exclusion of
14 infection (91%). In contrast, during the troughs of the influenza epidemic, the study
15 findings indicated that only half of the positive rapid tests were confirmed influenza
16 cases (PPV 50%). As a result, during such periods a confirmatory test, such as PCR or
17 viral culture, for detecting influenza cases is necessary. Additionally, it should be
18 noted that even during periods of low influenza activity a negative rapid test virtually
19 excludes the diagnosis of influenza (NPV 96%).
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34 Several points however must be interpreted with some caution. First, only
35 influenza A was identified in the study samples, so the rapid test characteristics for
36 influenza B infections could not be assessed. Second, the study was conducted in the
37 inpatient setting and the conclusions from the QuickVue Influenza Test cannot be
38 extrapolated to the ambulatory setting where some differences in disease
39 characteristics may exist, such as viral titers and shorter duration of illness before
40 hospital admission,.
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51 Nowadays, under the threat of the ongoing novel influenza A (H1N1)
52 pandemic it must be added that the use of point-of-care rapid influenza antigen
53 detection tests may also improve the timeliness and appropriateness of decisions
54 regarding the management of hospitalized or ambulatory children. Decisions
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1 regarding initiation of antiviral treatment and application of patient isolation measures
2 may be greatly facilitated if reliable rapid diagnosis is available. Existing rapid
3 antigen detection tests are generally targeted against preserved viral antigens and they
4 have comparable analytical and clinical sensitivity for seasonal influenza A virus and
5 for the new influenza A (H1N1) [32, 33]. Further studies are required to examine the
6 performance characteristics of the antigen detection tests for the diagnosis of
7 influenza caused by this new virus.
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18 In conclusion, our study suggests that the clinical case definition of influenza
19 based on the presence of fever and cough is inaccurate for prediction of influenza
20 virus infection in children. The QuickVue Influenza Test on the other hand is useful
21 and reasonably accurate for the detection of influenza infection in hospitalised
22 children during the influenza season. Infection with influenza virus is less likely if the
23 test is negative. A positive result suggests that infection is probable if influenza virus
24 circulates in the community. On the other hand, in accordance with the WHO
25 statement for the use of rapid testing for influenza diagnosis (July 2005) during
26 periods of low influenza activity, if rapid tests are used, positive results must be
27 interpreted with caution and confirmed by a definitive influenza test. The sensitivity
28 of the test is higher in younger children and in patients with shorter duration of
29 symptoms. However, the results of this study cannot be extrapolated to patients
30 presenting during different seasons.
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57 REFERENCES

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Characteristics	Influenza (-)	Influenza (+) ³	P
	N=177 ¹	N=40 ¹	
Male gender	88 (50.3) ²	20 (51.3)	0.877
Mean age ±SD, months	49.6±42.5	60±48.4	0.177
Nationality Greek	119 (71)	26 (68)	0.747
Tobacco exposure	106 (63)	15 (60)	0.734
Underlying illness			
Bronchial asthma	31 (19)	10 (26)	0.289
Other	14 (8)	1 (3)	0.256
Duration of symptoms, mean ±SD, days	4.9±7	2.9±2.2	0.065
Vaccinated against influenza	25 (14)	0	0.012

¹Data were available for 205/217 patients

²Numbers in parentheses, percent

³Influenza diagnosed with RT-PCR

Table 1. Patients' characteristics

	N	Influenza ¹	Prevalence of influenza (%)	Sensitivity ² (%)	Specificity ² (%)	PPV ² (%)	NPV ² (%)	LRP (95% CI)	LRN (95% CI)
All study children	217	40	18.5	67.5	96	79	93	17.1(8-36.4)	0.34(0.22-0.53)
Children 6-12 mos	43	5	11.6	80	95	67	97	15.2(3.7-62.8)	0.21(0.04-1.2)
Children > 12 mos	173	35	20.2	66	96	82	92	18.3(7.5-44.6)	0.08(0.04-0.13)
Symptoms dur<2 d	57	12	21.1	76	100	100	94	-	-
Symptoms dur≥2 d	146	26	17.8	65	94	71	93	12(5.6-26)	0.38(0.23-0.62)
Phase of the epidemic									
Early	29	7	24.1	57	91	67	87	6.3(1.45-7.3)	0.47(0.19-1.12)
Peak	88	25	28.4	76	95	86	91	16(5.2-49.2)	0.25(0.13-0.5)
Late	100	8	8	50	98	66	96	23(4.9-10.7)	0.51(0.25-1.02)

¹Diagnosis made by RT-PCR

²Sensitivity, specificity, PPV, NPV, LRP, LRN of the QuickVue Influenza Test

Table 2. Results of patient testing with QuickVue Influenza Test and PCR

	N	Influenza ¹	Prevalence of influenza (%)	Sensitivity ² (%)	Specificity ² (%)	PPV ² (%)	NPV ² (%)	LRP ² (95% CI)	LRN ² (95% CI)
All study children	205	38	18.5	76	20	18	79	0.9(0.8-1.2)	1.2(0.6-2.2)
Children 6-12 mos	43	4	11.6	80	30	13	92	1.1(0.7-1.9)	0.7(0.1-4.2)
Children > 12 mos	162	25	20.2	76	18	19	74	0.9(0.8-1.1)	1.4(0.7-2.8)

¹Diagnosis made by RT-PCR

²Sensitivity, specificity, PPV, NPV, LRP, LRN of the QuickVue Influenza Test

Table 3. Comparison of patients symptoms (fever and cough) with PCR