Effect of Using 2 Throat Swabs vs 1 Throat Swab on Detection of Group A Streptococcus by a Rapid Antigen Detection Test

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Objective: To assess the effect of using 2 throat swabs vs 1 on rapid detection of group A streptococcus by the STREP A OIA MAX (hereafter, OIA MAX) test.

Methods: Children aged 5 to 18 years with acute pharyngitis were randomized to 1 of 2 study groups. In group 1, one throat swab was obtained, streaked first on sheep blood agar, and then used for OIA MAX testing. In group 2, two throat swabs were obtained simultaneously. One swab was streaked first on sheep blood agar and then joined with the other swab for OIA MAX testing. In both groups, the pledgets in the collection-transport tube were incubated in Todd-Hewitt broth. A positive group A streptococcus culture either by sheep blood agar or Todd-Hewitt broth was confirmed by a latex agglutination test.

Results: Three hundred sixty-three patients were enrolled, 177 in group 1 and 186 in group 2. Cultures were positive for group A streptococcus in 154 (42.4%) of 363 patients. The sensitivity and specificity of OIA MAX testing were 94.7% and 100.0%, respectively, in group 1, and 92.4% and 96.3%, respectively, in group 2. There was no statistical difference between the sensitivity, the specificity, and the predictive values of the OIA MAX test performed with 1 swab compared with those performed with 2 swabs (P> .10). There was no association between OIA MAX test sensitivity and the severity of pharyngitis as measured by the modified Centor criterion (history of fever, absence of cough, presence of pharyngeal or tonsillar exudates, and presence of cervical lymphadenopathy) scores.

Conclusions: The OIA MAX test yielded comparable sensitivity and specificity in both study groups. The use of 2 throat swabs instead of 1 swab did not increase the sensitivity of the OIA MAX test. The performance of the OIA MAX test did not depend on the severity of pharyngitis.

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A CUTE PHARYNGITIS IS ONE of the most common illnesses in children. Group A streptococcus (GAS) is isolated from the throat of approximately 30% of children with acute pharyngitis.1-4 Laboratory confirmation of GAS is recommended in patients with acute pharyngitis because it is difficult to clinically differentiate between viral and GAS pharyngitis.5-7 The 2 methods for diagnosing GAS pharyngitis are a throat culture and a rapid antigen detection test. The rapid antigen detection test is simple to perform in both laboratory and office settings, and the results are available at the point of care in less than 15 minutes. While the specificity of the rapid antigen test has generally been regarded as acceptably high (90%-99%),3,8-18 there has been a great deal of controversy regarding its sensitivity with respect to the conventional culture methods. 3,8-21 The sensitivity of the rapid antigen test varies depending on the type of commercial kit used and the study design. For example, the optical immunoassay has a sensitivity level ranging from 75% to 95%,8,16,20,21 whereas the enzyme-linked immunosorbent assay has a sensitivity level of 68% to 91%.3,15-18 Although some studies have shown that the sensitivity of the rapid antigen test is less than that of sheep blood agar (SBA) plate culture,3,9,11,13,14,16-19 others have revealed that the sensitivity of some rapid antigen detection tests is either equivalent or superior to SBA culture.10,12,15,20

Owing to the variation in the reported sensitivity of the rapid antigen test, the American Academy of Pediatrics and the Infectious Diseases Society of America recommend a backup throat culture for all negative rapid antigen detection test results in children.7,22 The recommendation was based on the concern that false-negative rapid antigen detection test results may lead to misdiagnosis of GAS pharyngitis, which could result in supplicative and nonsupplicative complications and in the increased risk of transmitting the or-
ganism to others. However, the conventional culture methods have some drawbacks, which include a longer lag period between specimen collection and final microbiologic diagnosis. This could delay initiation of antibiotic therapy and alleviation of the patient’s symptoms. In addition, some physicians may elect to prescribe antibiotic therapy while they wait for the culture results, and this could lead to an inappropriate use of antibiotics and an increase in health care cost. The alternative option, waiting for a culture result to determine the need for treatment, would not alter the risk for rheumatic fever because treatment is effective when initiated as late as 7 to 10 days after onset. However, some patient populations such as those seen at emergency departments or urgent care centers may not be reachable after they leave the point of care, making it difficult to communicate the culture result to the patients and to treat them appropriately.

One of the reasons for variation in the sensitivity of the rapid antigen test for GAS is the number of bacteria or the inoculum size present in a throat swab. Using 2 swabs instead of 1 could theoretically increase the inoculum size and improve the performance of the rapid antigen test. This may help clinical practitioners reach a prompt and accurate diagnosis and initiate an appropriate therapy in a larger number of patients at the point of care. We therefore performed the study to assess whether using 2 throat swabs instead of 1 would improve the sensitivity of the STREP A OIA MAX (hereafter, OIA MAX) rapid antigen test (Thermo Electron Corp, Louisville, Colo).

METHODS

PATIENT POPULATION

The study was conducted at Children’s Hospital of Michigan, Wayne State University School of Medicine, Detroit, from December 2001 to January 2003. Children aged 5 to 18 years who were seen in the emergency department with the diagnosis of acute pharyngitis, either with or without pharyngeal exudates, fever, or cervical node enlargement, were included in the study. Fever was defined as the presence of an axillary temperature greater than or equal to 100.4°F (38°C), or subjective fever reported by a caregiver. Children with rhinorrhea or conjunctivitis (suggestive of a viral infection), and those with rales, wheezing, or breathing difficulty (suggestive of a lower respiratory tract disease) were excluded from the study. Children who had received systemic antibiotics within the previous 10 days were also excluded. The study was approved by the Human Investigation Committee of Wayne State University School of Medicine. Consent from parents or legal guardians and verbal assent from children were obtained prior to their participation in the study.

STUDY DESIGN

All subjects were evaluated and screened for study eligibility by the first author (E.N.E.) prior to study entry. This was a convenience sample of children with pharyngitis; the subjects were enrolled when the first author was present in the emergency department. Demographics and clinical information associated with pharyngitis of the eligible subjects were collected using a data collection form. The subjects were randomized in a 1:1 ratio according to a computer-generated randomization list to 1 of 2 study groups. In group 1, a single throat swab was obtained by rubbing it on the posterior pharynx and both tonsils. In group 2, two throat swabs were obtained by rubbing them simultaneously on the posterior pharynx and both tonsils. All throat swab samples were collected by the first author using 1 or 2 sterile rayontipped applicators. They were transported in the S/P Culturette System (Baxter Diagnostics, Deerfield, Ill) to the clinical microbiology laboratory within 1 hour after sampling for culture and the OIA MAX test. In group 1, the swab was first streaked on TSA II 5% SBA plate (Becton Dickinson, Cockeysville, Md). The same swab was then used to perform the OIA MAX test. In group 2, one swab was streaked on SBA for culture and then joined with the other swab for the OIA MAX test. The OIA MAX test was performed by 1 of 2 trained technicians in both study groups. The test instruction according to the OIA MAX test package insert was followed, except for the use of 2 swabs in group 2.

To optimize the detection of GAS, the pledget contained in the collection-transport tube was removed aseptically in all cases and was incubated for 18 hours at 35°C in the Todd-Hewitt W/CNA Lim Broth (THB) (Remel Inc, Lenexa, Kan), which is an enrichment broth for bacterial growth. After incubation, part of the broth was streaked on SBA for further culture. All SBA plates were incubated at 35°C in 6% to 8% carbon dioxide for 48 hours. The culture, grown in either SBA or THB/SBA, was considered the gold standard test to evaluate the performance of OIA MAX test in the 2 study groups. All positive SBA or THB/SBA cultures for GAS were confirmed using the PathoDx Strep growing latex agglutination test (Diagnostic Products Corp, Los Angeles, Calif). The test results were communicated to patients and treating physicians. The subject’s treatment and clinical outcomes were not evaluated in the study.

STATISTICAL ANALYSIS

We hypothesized that a 15% difference in sensitivity of the OIA MAX test would occur between groups 1 and 2 in identifying GAS. Based on a hypothesized 80% sensitivity of the OIA MAX test for group 1 compared with 95% sensitivity in group 2, and β of .20, an estimated sample size of 76 patients with culture-proven GAS pharyngitis in each study group was required. The criterion for α was set at .05, 2-tailed. Demographic and clinical characteristics were compared using t test for continuous variables and χ² analysis for categorical variables. The sensitivity, specificity, and positive and negative predictive values of the OIA MAX test were calculated on the basis of Centor scores in each study group. To compare the sensitivity of the OIA MAX test with respect to the culture in group 1 was compared with its sensitivity in group 2 and the difference between the 2 groups was interpreted using the χ² analysis.

We examined the performance of the OIA MAX test and whether it varied in relationship to disease severity. The modified Centor criteria (history of fever, absence of cough, presence of pharyngeal or tonsillar exudates, and absence of cervical lymphadenopathy) were used to define the clinical spectrum of acute pharyngitis. The score was thus defined as the number of modified Centor criteria present. The sensitivity estimates and the negative predictive values of the OIA MAX test were calculated on the basis of Centor scores in each study group. To compare the sensitivity of the OIA MAX test across groups of patients with an increasing number of Centor scores, the Cochran-Armitage test for trend was performed. Statistical significance was accepted at α ≤ .05, 2-tailed. Statistical analyses were performed using SPSS 11.0 software (SPSS Inc, Chicago, Ill).

RESULTS

There were 373 eligible patients with pharyngitis who were approached for participation in the study, 5 of whom declined to take part. The remaining 368 patients (212 fe-
Group A streptococcus was identified more frequently in the younger age group than in the older age group, with the mean of 8.8 years in patients with GAS vs 10.3 years in those without GAS (P < .01). There were no statistical differences in clinical presentations between patients with GAS and those without GAS either by OIA MAX testing or culture. However, scarlatiniform rash, defined by generalized sandpaper-like red papular eruption, was observed more often in patients with GAS than in those without GAS (25.3% vs 0.9%, P < .01).

Table 2 shows the microbiologic findings and the performance of the OIA MAX test in the 2 study groups. There were no statistical differences in the sensitivity, specificity, positive predictive value, and negative predictive value of the OIA MAX test done with 2 throat swabs vs those performed with a single swab (all P > .10). When we excluded the patients whose OIA MAX test was performed at more than 24 hours after sample collection, the sensitivity and the specificity of the OIA MAX test remained unchanged (93.8% and 100.0% for group 1, and 92.1% and 93.5% for group 2, respectively).

We found no association between the modified Centor scores and the OIA MAX test sensitivity in either group 1, group 2, or both groups combined (Table 3). The trend of OIA MAX test sensitivity in relation to the modified Centor scores was not statistically significant (P = .76). Additional analysis was also performed by stratifying the modified Centor scores into those with scores of less than or equal to 2 vs those with scores of greater than or equal to 3. The OIA MAX test sensitivity of those with the modified Centor scores of less than or equal to 2 vs those with scores of greater than or equal to 3 were 95.9% (95% confidence interval [CI], 91.1%-95.9%) vs 92.3% (95% CI, 83.6%-92.3%) in group 1, 92.7% (95% CI, 85.7%-94.7%) vs 92.1% (95% CI, 83.3%-96.3%) in group 2, and 94.4% (95% CI, 90.9%-95.4%) vs 92.2% (95% CI, 86.6%-95.0%) in combined groups, respectively. The negative predictive value of the OIA MAX test was not related to the modified Centor scores in this study.

The present study showed that 2 throat swabs obtained simultaneously and tested by OIA MAX test did not improve the sensitivity of the test when compared with 1 swab. In fact, OIA MAX test sensitivity, based on the conventional SBA and THB/SBA culture standard, was comparable between the 2 study groups (94.7% in 1-swab group vs 92.4% in the 2-swab group). Our findings contrast with the results of a previous study in which a 2-swab throat sampling significantly improved the sensitivity of the TestPack Plus (Abbott Diagnostics, Abbott Park, Ill) when compared with a 1-swab sampling (94% vs 80%, respectively).26 In that study, 4 drops of extraction reagents were used to perform the test in double-swab specimen as opposed to 3 drops for the single-swab specimen. In the present study, the same amount of reagents was used to perform the test in both study groups despite 2 throat swabs being used in group 2. It may therefore be interesting to evaluate the performance of the OIA MAX test when compared with 1 swab with a larger number of samples.
The sensitivity of the OIA MAX test in the present study was 92.4% to 94.7% when compared with the conventional SBA and THB/SBA culture standard. Our results differ from those of a recent report where the sensitivity of the OIA MAX test was 75.5% when compared with the multiplate culture method, and 79.3% when compared with the same-swab, single-plate culture method.14 The standard used in that study to compare with the OIA MAX test consisted of 4 rigorously performed culture methods. Although the goal was to identify all patients with GAS pharyngitis, it may be impractical to perform such a number of cultures to diagnose GAS pharyngitis in a single patient in clinical practice, given the low rates of complications of GAS pharyngitis currently seen in the United States.

Retrospective studies in adults and children indicate that the sensitivity of rapid antigen detection tests may vary across the clinical spectrum of pharyngitis, which is a property known as spectrum bias or spectrum effect.4,28,29 Prior reports indicate that the sensitivity of the rapid antigen test is only 47% to 65% when applied to patients with the modified Centor scores of 0 or 1 (low probability of GAS pharyngitis). As the probability of GAS pharyngitis increases, based on the higher modified Centor scores, so does the sensitivity of the rapid antigen test. The sensitivity of the test increases to 75% to 97% when the modified Centor scores are 3 or 4.4,28,29 However, this spectrum effect was not observed in our study. We found no association between OIA MAX test sensitivity and the severity of pharyngitis based on the modified Centor scores. One possible explanation for our finding is that the proportion of subjects with a modified Centor score of 0 in the present study is small compared with other studies; this could possibly reflect the selection bias during the recruitment process when children with mild symptoms were not enrolled. Another possible explanation is that the rapid antigen test used in this study differed from those used in other studies. The sensitivity varies according to the test used, therefore the spectrum effect may be observed in some rapid antigen tests but may not be observed in others. A direct prospective comparison of rapid antigen tests to evaluate the spectrum effect is of interest.

Some limitations exist in our study. First, since only 1 rapid antigen test was evaluated, the results of this study should not be generalized to other commercially available rapid antigen tests. Second, although we attempted to perform OIA MAX testing as soon as possible, the test had to be performed by a certified technician under the Clinical Laboratory Improvement Act guidelines. Because only 2 technicians were involved in this study, 10% of the OIA MAX tests were not performed until 24 to 48 hours after specimen collection. When those tests were excluded from the analysis, the sensitivity and specificity of the OIA MAX test remained unchanged. A delay of 24 to 48 hours in OIA MAX testing in a minority of patients in the study did not affect its performance overall. The OIA MAX test can be performed as late as 72 hours after sampling. However, tests that are not regulated by the Clinical Laboratory Improvement Act can be performed at the point of care and would shorten the turnaround time.

### Table 3. Sensitivity and Negative Predictive Values of the STREPA OIA MAX Test as Stratified by the Modified Centor Criterion* Scores

<table>
<thead>
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<th>Modified Centor Score</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<td>Group 1</td>
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<td></td>
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<td></td>
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<tr>
<td>No. of patients, %</td>
<td>6</td>
<td>26</td>
<td>74</td>
<td>55</td>
<td>16</td>
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<tr>
<td>No. of positive OIA MAX</td>
<td>1</td>
<td>13</td>
<td>33</td>
<td>19</td>
<td>5</td>
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<tr>
<td>No. of positive cultures</td>
<td>1</td>
<td>13</td>
<td>35</td>
<td>21</td>
<td>5</td>
</tr>
<tr>
<td>Sensitivity, % (95% CI)</td>
<td>100 (25.2-100)</td>
<td>100 (87.1-100)</td>
<td>94.3 (87.7-94.3)</td>
<td>90.5 (79.9-90.5)</td>
<td>100 (70.2-100)</td>
</tr>
<tr>
<td>NPV, % (95% CI)</td>
<td>100 (85.0-100)</td>
<td>100 (87.1-100)</td>
<td>95.1 (89.5-95.1)</td>
<td>94.4 (88.3-94.4)</td>
<td>100 (86.5-100)</td>
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<td>Group 2</td>
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<td>No. of patients</td>
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<td>21</td>
<td>82</td>
<td>51</td>
<td>25</td>
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<td>No. of positive OIA MAX</td>
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<td>27</td>
<td>27</td>
<td>11</td>
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<tr>
<td>No. of positive cultures</td>
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<td>10</td>
<td>28</td>
<td>28</td>
<td>10</td>
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<tr>
<td>Sensitivity, % (95% CI)</td>
<td>100 (59.1-100)</td>
<td>90 (71.7-90.0)</td>
<td>92.9 (83.4-95.7)</td>
<td>89.3 (78.2-94.0)</td>
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<tr>
<td>NPV, % (95% CI)</td>
<td>100 (69.3-100)</td>
<td>91.7 (76.4-91.7)</td>
<td>96.4 (91.6-97.8)</td>
<td>87.5 (75.7-93.0)</td>
<td>100 (86.6-100)</td>
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<td>Both groups</td>
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<td>No. of patients</td>
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<td>47</td>
<td>156</td>
<td>106</td>
<td>41</td>
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<tr>
<td>No. of positive OIA MAX</td>
<td>4</td>
<td>22</td>
<td>60</td>
<td>46</td>
<td>16</td>
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<tr>
<td>No. of positive culture</td>
<td>4</td>
<td>23</td>
<td>63</td>
<td>49</td>
<td>15</td>
</tr>
<tr>
<td>Sensitivity, % (95% CI)</td>
<td>100 (64.8-100)</td>
<td>95.7 (86.7-95.7)</td>
<td>93.7 (88.8-94.9)</td>
<td>89.8 (83.1-92.6)</td>
<td>100 (86.6-100)</td>
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<tr>
<td>NPV, % (95% CI)</td>
<td>100 (84.4-100)</td>
<td>96.0 (87.8-96.0)</td>
<td>95.8 (92.7-96.7)</td>
<td>91.7 (86.2-94.0)</td>
<td>100 (92.0-100)</td>
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</table>

Abbreviations: CI, confidence interval; NPV, negative predictive value.
*Centor criteria are history of fever, absence of cough, presence of pharyngeal or tonsillar exudates, and presence of cervical lymphadenopathy.

MAX test when it is performed with 2 throat swabs using an increased amount of extraction reagents.

The performance of the OIA MAX test using 2 throat swabs was comparable to that using 1 throat swab in rapid detection of GAS in children with acute pharyngitis. Using 2 throat swabs instead of 1 swab did not increase the

### CONCLUSIONS

The performance of the OIA MAX test using 2 throat swabs was comparable to that using 1 throat swab in rapid detection of GAS in children with acute pharyngitis. Using 2 throat swabs instead of 1 swab did not increase the
sensitivity of the OIA MAX test in this study. We found no association between OIA MAX test sensitivity and the severity of pharyngitis as measured by the modified Centor scores in this population. A prospective, comparative study to assess the performance of each rapid antigen test in regard to the spectrum effect in children will be useful.

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REFERENCES