Performance of Rapid Streptococcal Antigen Testing

Varies by Personnel

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Abstract

**Background:** Rapid carbohydrate antigen tests are frequently used to diagnose group A streptococcal (GAS) pharyngitis. Despite evidence of modest sensitivity in medical settings, rapid antigen tests are available to the public for self-testing. We sought to determine if the personnel performing a rapid streptococcal antigen test influences the test’s performance characteristics.

**Methods:** Throat swabs of pediatric patients performed in a tertiary-care children’s hospital network for GAS pharyngitis were included during two study periods in 2004 and 2005. Performance characteristics of a rapid carbohydrate antigen test were evaluated in three clinical settings against a nucleic acid probe test method according to the personnel performing the test (laboratory technologist versus non-laboratory personnel). Between the study periods, non-laboratory personnel from one site underwent re-training. Subsequently, performance characteristics of the rapid antigen test were reassessed.

**Results:** The sensitivity of the rapid antigen test varied widely among the different testing sites (56-90%). Notably, test sensitivity was consistently greater when performed by laboratory technologists when compared to non-laboratory personnel (p<0.0001). Although rapid antigen test sensitivity significantly improved after re-training non-laboratory personnel at one testing site (sensitivity before versus after re-training, p<0.0001), sensitivity remained greater in the laboratory technologist cohort (p<0.0001).

**Conclusions:** These data confirm the important relationship of the operator performing a rapid streptococcal antigen test with the test’s accuracy, even in a clinical setting where operator training is mandated. Therefore, its use cannot be recommended outside the medical setting by lay persons without culture back-up.
Introduction

Group A streptococcus (GAS) is a common cause of pharyngitis, resulting in more than 10 million physician visits each year (6) and accounting for 15-30% of sore throats in children (3,13,21,33). Accurate diagnosis is necessary to permit targeted administration of antimicrobial therapy to prevent suppurative (peritonsillar and retropharyngeal abscesses) and non-suppurative (rheumatic heart disease and acute glomerulonephritis) complications, to hasten symptom resolution, and to reduce the transmission of GAS in the community, while limiting the use of antibiotics in viral-mediated infections (12,25,30,36,41).

Unfortunately, clinical findings and simple scoring systems based on these findings are unreliable for identifying patients with GAS pharyngitis (2,15,20,27,35). For this reason, laboratory tests are commonly used to confirm the diagnosis in children that present with throat pain. Rapid streptococcal carbohydrate antigen tests are widely used in the clinical setting because results are available during a patient’s visit. The ability to provide treatment at the point of care is a distinct advantage of this test when compared to standard culture. Importantly, a primary drawback of carbohydrate detection systems is modest sensitivity (55-90% in post-marketing trials) (1,7,17,19,22,26,34,39). As such, the American Academy of Pediatrics (10), the American Heart Association (11), and the Infectious Disease Society of America (4) all recommend backup throat cultures for negative rapid antigen detection tests in pediatric patients to achieve greater overall diagnostic sensitivity.

Although the sale of rapid antigen test kits directly to individuals for use in the home setting is not approved by the United States Food and Drug Administration (FDA), select kits may be purchased in a pharmacy or over the internet (8,9,14,29,31,32,38). Thus these testing devices are being utilized without physician supervision and without the safeguard of a follow-up
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culture. Furthermore, while a positive antigen test result for GAS is likely to prompt
consultation with a physician, a negative test result may falsely imply illness due to another
etioologic agent, resulting in a delayed diagnosis or no diagnosis at all. Regrettably, as self-
testing kits become increasingly available to the public, undiagnosed and untreated GAS
infections are likely to increase, serving as a reservoir for the spread of this infection to the
community and putting patients with these infections at risk for complications of streptococcal
tonsillopharyngitis.

A number of factors have the potential to further lower the sensitivity of antigen tests
when performed by patients in the home setting. These factors include improper collection of
samples from sites other than the pharynx or tonsils, insufficient quantity of sample, and inability
to perform and interpret the test correctly. All but the last of these factors are likely to be
overcome in a cooperative child by adequately instructing parents in proper techniques of
specimen collection. Interpretation of the test, however, is likely to reflect the operators’
experience performing and reading the test. If so, the accuracy of GAS antigen tests may be
relatively low when performed by non-laboratory personnel compared to laboratory personnel.

In this study, we evaluate the impact of education, training and experience on the
performance of a rapid streptococcal antigen detection test. Specifically, we gauge the utility of
testing in the home setting by comparing the accuracy of a simple antigen test when performed
by laboratory versus non-laboratory personnel at a tertiary-care children’s hospital and
associated satellite urgent care facilities.
Materials and Methods

Study Population: Pediatric patients being evaluated for streptococcal pharyngitis in Columbus Children’s Hospital medical system were included in this study. This medical care system consists of a tertiary-care Emergency Department, four Urgent Care Clinics, and ten Primary Care Centers. Annually, nearly 500,000 patient visits are recorded from these sites.

Patients were included if a throat swab for the detection of group A streptococcus was performed. The study was divided into 2 periods. Period 1 included patients evaluated at any of the sites during October and November of 2004, while period 2 included patients evaluated during January 2005.

Sample Collection and testing: A Dacron double-swab collection-transport system (COPAN Venturi Transystem, COPAN Diagnostics Inc., Corona, CA) was used for all sampling. Samples were collected from the patient’s posterior pharynx and tonsillar surfaces by physicians and nurses as recommended by the Infectious Disease Society of America (4).

Immediately after sample collection, one swab from each dual swab collection-transport device was used for rapid antigen detection testing with the Abbott Signify Rapid Strep A test (Abbott Laboratories, Abbott Park, IL) at all locations. The remaining swab was stored in a secured refrigerator and transported to the Columbus Children’s Clinical Microbiology Laboratory within 24 hours. The Gen-Probe group A streptococcus direct test (Gen-probe, Inc., San Diego, CA) was performed on all swab collections by laboratory technologists according to the manufacturer’s instructions on the remaining swab. In the event of a negative DNA probe on a patient with a positive rapid test, a broth-enhanced culture for GAS was performed on the pledget from the collection-transport tube system after the swab was removed for the DNA probe test. Briefly, the plastic transport tube was cut with scissors and the pledget at the base of the

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tube was removed with sterile forceps. The pledget was then placed into LIM broth (Becton-Dickinson, Sparks MD) and incubated overnight at 35°C. A subculture of the broth was performed onto SXT Blood Agar (Becton-Dickinson) and the plate incubated in 5% CO2 at 35°C for 48 hours. Culturing the pledget of a transport system for detection of group A streptococcus has been described previously (7,23). β-hemolytic colonies were identified as GAS by a latex agglutination test (Streptex GAS test; Remel Inc, Lexena, KS) for GAS carbohydrate antigen.

*Disease Status:* A patient with a positive DNA probe or enhanced broth culture was considered to have GAS pharyngitis. A patient with a positive rapid antigen test and a positive DNA probe or culture was considered to have a true-positive antigen test. Conversely, a patient with a positive rapid antigen test and a negative DNA probe and culture was considered to have a false-positive antigen test. Negative rapid strep test results were treated similarly when compared to DNA probe and culture (true and false negative results).

*Intervention:* Between Study Period 1 (10/1/04-10/7/04 and 10/21/04-11/6/04, 22 days) and Study Period 2 (1/3/05-1/29/05, 26 days) non-laboratory personnel performing rapid antigen testing in the Primary Care Centers underwent competency assessment and re-training by Microbiology laboratory personnel.

*Study groups:* Specimens tested by laboratory-employed personnel were labeled “Lab.” No laboratory-employed technologist was retrained during the study. The remaining specimens were tested by persons not employed by the laboratory. Specimens tested by non-laboratory personnel who underwent retraining between the two study periods were labeled “Non-lab, retrained.” The remaining specimens were tested by non-laboratory employees who did not undergo retraining between the two study periods. These swabs were labeled “Non-lab, control.”
**Outcomes:** Sensitivity and specificity of the rapid antigen test when performed by laboratory and non-laboratory personnel were the primary outcomes. The secondary outcome was the impact of competency training versus testing experience (number of tests run) on the test characteristics of the rapid antigen test performed by the Primary Care network. The number of tests performed during the study periods was tabulated and considered a proxy (surrogate measure) for operator experience during the study.

**Statistical Analysis:** Data was analyzed with the Stata statistical program, version 8 (Stata Inc, College Station, TX). Proportions were compared with the Binomial test for proportions. A nominal p value <0.05 was considered statistically significant.

**Approval:** This study was approved by the Institutional Review Board of the Research Institute of Columbus Children’s Hospital. Because routine patient care was not altered and patient data was de-identified from test results, informed consent was waived.
Results

Tables 1 and 2 show the test characteristics of the rapid antigen test for Study Period 1 (Table 1) and Study Period 2 (Table 2). While test specificity was excellent ($\geq 97\%$) at all sites for both time periods, sensitivity varied from 56-90\%. Sensitivity of the rapid antigen test prior to re-training (Study Period 1) did not differ between the non-laboratory personnel groups ($p = 0.29$). However, sensitivity of the test when performed by laboratory personnel was significantly greater than the sensitivity observed for non-laboratory personnel testing ($p < 0.0001$ for pairwise comparisons, Table 1).

In the second period, sensitivity of the rapid antigen test improved significantly after competency retraining in the Primary Care Centers (“Non-lab, retrained,” $p < 0.0001$). However, a significantly larger improvement was observed in non-laboratory personnel who did not undergo re-training (Absolute increase in sensitivity of 26\% vs. 15\% for “Non-lab, control” and “Non-lab, retrained,” respectively, $p = 0.016$). Despite the improvement in the sensitivity of the rapid antigen test by non-laboratory personnel in the second study period, the sensitivity of the GAS tests remained superior when performed by laboratory personnel ($p < 0.0001$ for pairwise comparisons, Table 2).

The number of tests run in the second period by non-laboratory personnel who did not undergo re-training (843) was over 2 times greater than the number run in the same period by non-laboratory personnel undergoing re-training (377). Additionally, the prevalence was significantly higher in Study Period 2 compared to Study Period 1 ($p < 0.001$).
Discussion

Many variables affect the performance of laboratory tests. For a rapid streptococcal carbohydrate antigen detection system, in particular, predictors include the quality of the specimen obtained from the posterior pharynx and tonsillar tissue—as this relates to inoculum size—and the operators’ ability to appropriately test the throat sample and interpret the result of the test (15,16,18,24,26). Data from our study confirm that the operator performing and interpreting this test is an important determinant of the test’s accuracy even in a clinical setting where training and quality assurance are mandated. Specifically, our data show that even when dealing with a relatively simple test, in a hospital system that cares for nearly 500,000 pediatric patients annually, performance varies widely by the experience of the operator. Additionally, our test operators were medical personnel who are likely to be more cognizant of proper kit storage, expiration dates, and use of controls compared to the lay public. In extrapolating these results to the performance of the test when used for self-diagnosis in the home setting, our data indicate that the accuracy of this test is likely to be lower.

A number of factors may play a role in explaining differences observed in test accuracy between laboratory and non-laboratory personnel. We believe, however, that the most important determinant of accuracy over time is accumulated operator experience. This may merely reflect the number of tests run by an operator (as suggested by our data for non-laboratory personnel), or echo qualitative factors such as whether or not feedback is provided routinely concerning the accuracy of putative diagnoses when performing this test. Specifically, if there are regular opportunities (particularly in borderline cases) for test operators to observe features of screening tests that reflect a higher likelihood of a positive bacterial culture result, these observations are likely to enhance learning. Such ideal conditions for learning are likely to be present in settings
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with full laboratory support where both antigen testing and culture are often performed by the same operator(s), but less probable in settings where only the initial antigen test is performed without regular culture feedback as is likely to occur in settings that lack full on-site laboratory support. Furthermore, conditions such as these, high frequency of testing and performance enhancement from subsequent culture results, would not be present in the home setting. Therefore, significant improvement in testing performance would not be expected for the lay user.

Another factor that may exert a role in differences in test performance is the severity of streptococcal pharyngitis. Indeed, studies have found that the sensitivity of rapid streptococcal antigen tests improves as the clinical likelihood of streptococcal pharyngitis increases (spectrum bias) (13, 15, 21). In our study, to the extent that the severity of illness was related to the site of the clinical encounter and confounded by the type/experience of personnel running tests at these sites, the difference in the accuracy of tests may have been influenced by spectrum bias. In fact, the sensitivity improved for each testing site during the second study period which enjoyed a higher prevalence of disease (GAS pharyngitis). Nonetheless, in extrapolating this factor to self-testing outside of the medical setting, it is reasonable to expect that rapid antigen tests, if performed more often in children with less severe symptoms—a likely scenario—will perform as poorly due to the same sort of bias.

Interestingly, we observed that rapid antigen test sensitivity improved significantly for both non-lab personnel groups (Table 2) even though only one non-lab group underwent retraining between study periods 1 and 2. This finding may be related to the increased rate of GAS testing during the second study period which provided increased experience from performing the antigen test more frequently. Tests were conducted more frequently among non-laboratory
operators not undergoing re-training and appeared to have a greater impact on test accuracy than re-training. Thus, it is likely that re-training did play a role in the improvement in sensitivity seen in the experimental group but that the effects were masked by the improvement seen in all groups. However, without quality improvement measures, it is highly improbable that the sensitivity of this test for self-diagnosis by patients would approach even the low rates found in our study (sensitivity as low as 56%).

In addition, the improved sensitivity for the non-lab groups may be explained by the “Hawthorne Effect” (28). This theory suggests performance may improve, often transiently, simply because the participant knows s/he is being observed. For our study, in between the study periods, operators may have become aware that the performance of the rapid strep test was under evaluation. As such, the operators may have used more care in performing the tests during Study Period 2, thus affecting the results of this study.

Until the major determinants of test performance accuracy are elucidated and controlled, it appears imprudent to promote home GAS antigen kits for self-diagnosis. We concur with recommendations by the American Academy of Pediatrics, the American Heart Association, and the Infectious Disease Society of America that recommend back-up testing for negative rapid streptococcal antigen tests in children—testing that is presently only available in the clinical setting. The FDA has enforced these recommendations in that “no rapid test has been cleared, approved, or waived through the regulatory process as a stand alone test in the face of locally suppurative disease, lack of a backup method for a negative rapid GAS test result constitutes off label use” (40).

Notably, even with the best sensitivity of 90% measured in this study, one in ten children with a negative rapid antigen result would be at risk for suppurative and non-suppurative
complications of streptococcal pharyngitis without culture confirmation and subsequent
treatment. It is relevant to note that when performing the rapid streptococcal antigen test, 76% of
parents state that they would not seek medical evaluation if the test was negative (5). With the
potential for widening use of the home kits in the absence of back-up cultures, a large number of
such patients are unlikely to be recognized to have GAS pharyngitis and could pose a public
health risk.

Our study has a number of limitations. A number of factors that may have affected the
outcomes of this investigation were not studied. These included differences in collection
techniques for obtaining throat swab specimens, differences in the interval to performing the
rapid antigen test, and differences in patient characteristics at the various sites during the study
period. All of these factors may have acted as confounders when comparing test performance at
the difference sites. Future studies should evaluate the variability in test performance between
paired laboratory and non-laboratory personnel using the same specimens and after controlling
for these factors. In addition, our study extrapolates findings for the test from the clinical setting
to the home setting for patients that intend to or already utilize the rapid antigen test for self-
diagnosis. Such guarded extrapolation of study findings is reasonable. However, future studies
are needed to evaluate directly the performance characteristics of these rapid antigen tests against
culture results in this setting. Finally, we investigated the performance of a single rapid antigen
test. There are a number of rapid streptococcal antigen tests on the market, and it is possible
another test may perform differently than reported here. Future studies should be performed to
investigate this potential discrepancy.

In conclusion, our study results suggest that the accuracy of rapid antigen tests for
detecting GAS in throat specimens varies widely by personnel performing the test even in a
clinical setting that mandates training and re-training—ostensibly reflecting the accumulated experience of test operators. Pertinently, the accuracy of antigen tests when performed by laboratory-employed personnel consistently surpasses that by non-laboratory personnel, and among the latter echoes testing frequency over re-training. Because rapid tests fail to exclude GAS pharyngitis in a modest fraction of patients, and because both positive and negative results mandate evaluation by a medical professional, we do not recommend the use of these tests for self-evaluation of throat pain in the home setting. Back-up culture of negative rapid antigen tests should be performed in all Pediatric settings to reliably diagnose GAS pharyngitis.

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Note:
The rapid streptococcal antigen test studied here is no longer available through Abbott Laboratories. The same test is now sold by Genzyme Diagnostics (San Diego, CA) as the Genzyme OSOM Strep A test.
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**Table 1.** Performance characteristics of rapid antigen testing by personnel prior to the re-training of a non-laboratory cohort; Period 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>Tests run (n)</th>
<th>Prevalence</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-lab, retrained&lt;sup&gt;a&lt;/sup&gt;</td>
<td>261</td>
<td>16%</td>
<td>60%&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>99%</td>
</tr>
<tr>
<td>Non-lab, control&lt;sup&gt;d&lt;/sup&gt;</td>
<td>497</td>
<td>16%</td>
<td>56%&lt;sup&gt;b&lt;/sup&gt;</td>
<td>98%</td>
</tr>
<tr>
<td>Lab&lt;sup&gt;d&lt;/sup&gt;</td>
<td>434</td>
<td>22%</td>
<td>88%</td>
<td>99%</td>
</tr>
</tbody>
</table>

<sup>a</sup> re-training before period 2  
<sup>b</sup> p < 0.0001 pairwise comparison to “Lab” group  
<sup>c</sup> p > 0.05 pairwise comparison to “Non-lab, control” group  
<sup>d</sup> no re-training before period 2  
(All p values calculated by Binomial test for proportions)

**Table 2.** Performance characteristics of rapid antigen testing by personnel after the re-training of a non-laboratory cohort; Period 2.

<table>
<thead>
<tr>
<th>Group</th>
<th>Tests run (n)</th>
<th>Prevalence</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-lab, retrained&lt;sup&gt;a&lt;/sup&gt;</td>
<td>377</td>
<td>25%</td>
<td>75%&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>97%</td>
</tr>
<tr>
<td>Non-lab, control&lt;sup&gt;d&lt;/sup&gt;</td>
<td>843</td>
<td>20%</td>
<td>82%&lt;sup&gt;c&lt;/sup&gt;</td>
<td>98%</td>
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<tr>
<td>Lab&lt;sup&gt;d&lt;/sup&gt;</td>
<td>475</td>
<td>31%</td>
<td>90%</td>
<td>97%</td>
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</tbody>
</table>

<sup>a</sup> re-training before period 2  
<sup>b</sup> p < 0.05 pairwise comparison to “Non-lab, control” group  
<sup>c</sup> p < 0.0001 pairwise comparison to “Lab” group  
<sup>d</sup> no re-training before period 2  
(All p values calculated by Binomial test for proportions)