Performance Characteristics and Utilization of Rapid Antigen Test, DNA Probe, and Culture for Detection of Group A Streptococci in an Acute Care Clinic

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In 1998, the National Center for Health Statistics reported 12.2 million patient visits for cases of pharyngitis. Of these visits, 2.4 million were due to group A streptococcal (GAS) sore throat, the most common form of acute pharyngitis for which antibiotic therapy is indicated (3, 20). While other bacterial infectious agents such as members of streptococcal groups C and G and Arcanobacterium haemolyticum can occur, they are more typically sought in specific clinical settings (23). As a result, the use of rapid antigen tests (RATs) for the diagnosis of GAS pharyngitis has become common in many office and clinic settings. Given good performance parameters, a point-of-care (POC) test whose results are directed to the physician before the patient leaves the healthcare setting has many benefits. Positive results allow appropriate and directed therapy, are satisfying to the patient, and eliminate phone calls about follow-up results (8, 15). A standard practice for a patient with a negative POC RAT result remains undefined. The lack of a standard is reflected in the conflicting recommendations from the American Academy of Pediatrics and the American Thoracic Society on RAT-negative patients who present with acute pharyngitis (1, 4).

Published articles support many options in the diagnosis of GAS, including the use of the RAT alone, follow-up with culture on all RAT-negative patients, and the use of culture as a single-test option (2, 6, 8, 15, 16, 25). There are advantages, disadvantages, and variable results for both RAT and culture methodologies, depending on the type of test used, the personnel performing the test, and the history of the patient. The DNA probe test adds yet another option to consider in the algorithm for use in GAS diagnosis, because the test offers benefits not available with other technologies.

The present study was conducted to address two issues. The first was to evaluate the GAS Direct probe test (Gen-Probe, Inc.) compared to the optical immunoassay (OIA) RAT (Thermo BioStar) and to culture with a 5% sheep blood agar plate (BAP) incubated anaerobically for 48 hours and subsequently inoculated into Todd-Hewitt enrichment broth (THB). The second was to define the optimal use of the various technologies for GAS diagnosis in a large pediatric acute care clinic.

MATERIALS AND METHODS

Study population. A total of 520 patients were enrolled in the study. Consecutive patients attending the pediatric outpatient clinics at three sites (one on-site...
TABLE 1. Comparison of GAS test methods

<table>
<thead>
<tr>
<th>Parameter</th>
<th>GAS Direct probe test</th>
<th>OIA RAT</th>
<th>Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of specimens</td>
<td>%</td>
<td>No. of specimens</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>164 (173)</td>
<td>94.8</td>
<td>149 (173)</td>
</tr>
<tr>
<td>Specificity</td>
<td>347 (347)</td>
<td>100</td>
<td>337 (347)</td>
</tr>
<tr>
<td>PPV</td>
<td>164 (164)</td>
<td>100</td>
<td>149 (159)</td>
</tr>
<tr>
<td>NPV</td>
<td>347 (358)</td>
<td>96.9</td>
<td>337 (361)</td>
</tr>
</tbody>
</table>

* A total of 520 specimens were evaluated.
* PPV, positive predictive value; NPV, negative predictive value.
* FN probe test specimens had plate colony counts of <10 (3 specimens), 10 to 25 (5), and 25 and 50 (1).
* FN OIA specimens had plate colony counts of <10 (6 specimens), 10 to 25 (8), 25 to 50 (8), and >50 (2).

RESULTS

A total of 520 patient samples were evaluated. Of those patients, 172 were considered infected for GAS, based on positive culture results, and 1 additional patient was considered positive, based on positive rapid antigen GAS and positive probe results. The total of 173 infected GAS patients represented a disease prevalence of 33% during the study period from all clinical sites evaluated. Results for performance of the rapid antigen test and the probe test compared to culture are shown in Table 1. Colony counts for the culture-positive—antigen-negative tests and culture-positive—probe-negative specimens are footnoted at the bottom of Table 1. Based on infected patient status as described above, sensitivity, specificity, and positive and negative predictive value results for the Thermo BioStar RAT were 86.1, 97.1, 93.7, and 93.4%, and 94.8, 100, 100, and 96.9% for the GAS Direct probe test, respectively. Culture was 99.4% sensitive. The THB did not identify additional positive specimens compared to those identified by the primary BAP culture at 48 h. False-positive (FP) results with the RAT were seen in 10 patients. Of those FP results, five were from patients previously treated for GAS and evaluated because of symptoms of pharyngitis. No FP results were noted with the probe test, including those for tests of patients who were previously treated and reviewed. FN results were seen with all methods, 24 with the Thermo BioStar OIA, 9 with the GAS probe test, and 1 with culture. Colony counts for the FN RAT and probe test results were distributed throughout the colony count range evaluated. The FN antigen test specimens exhibited higher colony counts (Table 1) than those of the probe test result. The 9 specimens FN by probe testing were also FN by the RAT. Cross-reactivity was not seen with either the RAT or the probe test in the 2 patients with group C streptococcus.

DISCUSSION

The results seen in this study are similar to those previously published for both the Thermo BioStar RAT and the GAS Direct probe assay (5, 8, 9, 10, 11, 15, 17; Bourbeau, Editorial; W. M. Dunne, Jr., J. C. Mohla, and J. M. Campos, Abstr. 93rd Gen. Meet. Am. Soc. Microbiol. 1993, abstr. C-340, p. 506,
1993). However, no peer-reviewed paper has been published presenting a side-by-side comparison of these two methods from the same patient sample. One abstract describing this type of comparison corroborates the results seen here (S. Wood, H. Takahashi, and J. Fusco, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1567, p. 224, 1999).

Interestingly, the Thermo BioStar RAT performed nearly identically to that used in a study performed 3 years earlier at this institution, when the test was introduced to the market (87.1% sensitive, 97.4% specific) (15). Subsequent review of this institution, when the test was introduced to the market identically to that used in a study performed 3 years earlier at Antimicrob. Agents Chemother., abstr. D-122, p. 134, 2002). Such results and site-dependent variable interpretations (4; K. C. Chapin and M. A. Flintoff, Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. D-122, p. 134, 2002). Such difficulties were not an issue in this study. In addition, it should be noted that RATs have also been shown to correctly identify patients that had unusual GAS isolates not identified by culture (19, 22).

FP results of RATs for patients returning with continued pharyngitis or recently recurrent pharyngitis are unrecognized and underreported. The manufacturer does not recommend the use of the OIA RAT for patients previously treated for GAS or reinfected with GAS or to determine chronic carriers of GAS. This is because the test detects both viable and nonviable antigen, which may result in possible FP results. Typically, a 4-week delay after GAS diagnosis by RAT and/or treatment is recommended as a guideline before reusing a RAT (OIA package insert, Thermo BioStar). As demonstrated in other studies (11, 18), this study showed a number of FP RATs. All of these FP RATs had both negative probe test and culture results, and many were from patients previously diagnosed with GAS and treated with penicillin. The shedding of GAS antigen postdiagnosis and -treatment is well documented, and as such, it should be clear to physicians that all RATs exhibit this trait and would not be appropriate for patients with suspected GAS treatment failure or subsequent reinfection.

Performance of any RAT is most satisfying while the patient is still within the healthcare setting and so can be treated immediately upon a positive result. Critically, relative acceptable sensitivity and specificity with a RAT allows for a great proportion of patients to be treated appropriately (24). However, the time per test, when performed adequately, requires a dedicated technical person in a high-volume setting and one experienced in interpreting the membrane technology. Individual cassettes are costly. These unfavorable features have been noted to be of importance when considering the appropriateness of the test for a given practice setting (8). Most importantly, the prevalence of disease in this study was very high (33%), and the predictive value of the RAT was 93.7%. For a prevalence of 10%, the predictive value of this test would be only about 75%. Physicians using only RATs for diagnosis of GAS should be aware of this critical limitation.

The probe assay exhibited performance parameters comparable to those of culture and was easy to perform as a batch test in the laboratory during both first and second shifts. Sensitivity reported in this comparison for the GAS Direct probe test is similar to that of other studies, for which reported sensitivities were between 86 and 98% compared to those of culture (11, 17; Dunne et al., Abstr. 93rd Gen. Meet. Am. Soc. Microbiol., 1993; Wood et al., 39th ICAAC). Reported specificity has always been high (98 to 100%), and this level of specificity was seen in this evaluation as well (11, 17; Dunne et al., Abstr. 93rd Gen. Meet. Am. Soc. Microbiol., 1993; Wood et al., 39th ICAAC). Despite the high prevalence of GAS in this pediatric population, other studies with the probe test have shown comparable performance in low-prevalence-of-disease populations and for adults with pharyngitis as an incidental and not a primary complaint (17). There were no FP probe test results, either in those patients returning with repeat symptoms who had previously been treated or in those cross-reacting with other beta-hemolytic streptococci. We found no reported studies of evaluations of the life of the rRNA target for GAS after treatment. However, studies evaluating mycobacteria show that the rRNA target may be an adequate hallmark of acute infection (7, 14). Testing with the probe after treatment failures or reinfection may be an option equal in usefulness to culture for these patients, since the target disappears quickly after treatment (7, 14). Additional studies are necessary to confirm the use of the probe after treatment or GAS and to assess the length of time that the rRNA is present. The probe test did miss some positive cultures over the range of colony counts. The lower colony counts (<10 and 10 to 25) were also missed by the OIA in these patients. Specificity of the probe was higher than that of the OIA RAT in this direct comparison. The 100% specificity level also assures a high positive predictive value, regardless of the prevalence of disease.

Results from the probe test indicate that it can be used as the single primary test or as a backup test for less-sensitive methods and that it offers benefits not provided by other technologies. As a backup test to less-sensitive subjective methods, the probe test offers the ability to report an objective result. In contrast to adequate culture, which takes at least 24 to 48 h from the time of specimen receipt in the laboratory, the probe test can be reported on the same day as specimen collection. The use of culture is warranted to detect group C and G streptococci for patients who persist with pharyngitis after GAS testing results have been found to be negative or in specific epidemiological settings (23). The downside of the probe as a primary test is that it is not a POC test and necessitates follow-up with patients for relaying results.

The DNA Direct probe test as an alternative to culture was chosen for use after discussions between the departments of Pediatrics and Laboratory Medicine. Excellent performance parameters, a <24-h turnaround time to a final result, a greater number of patients receiving directed and appropriate antibiotic therapy, and less cost in labor allowed for an easy consensus for the use of the probe test over culture. Costs for each of the tests in our particular setting were fairly equivalent.
Specific costs related to the institution would need to be evaluated for each practice setting.

The suggestion of using a RAT alone without a backup test method and using culture for all RAT negative test results exhibits the two extreme sides of the diagnosis and testing issue. Clinicians and laboratory administrators should be aware of exuberant arguments for both sides of this issue, since the clinical and diagnostic settings are not always equal when it comes to resources, testing options, clinical judgment, and test performance. In addition, inappropriate antibiotic treatment for FP results and the risk of rheumatic fever or subsequent suppurative and nonsuppurative sequelae (15, 16, 23). In this study, 73% of FNs and the risk of rheumatic fever or subsequent suppurative infection is key when using the testing algorithm for GAS. Physicians should be aware of the POC performance parameters of the particular test used in their setting and other available options. In conjunction with the laboratory, physicians can assess the best plan for the resources available and needs of the clinical setting. In our acute care setting, the current algorithm is the use of a double swab as the standard throat specimen collection. Physicians have a choice for GAS test diagnosis. While the POC RAT remains the primary test, physicians can choose the RAT only, the DNA probe test only, or the RAT with a follow-up DNA probe test for RAT-negative patients. Further evaluation of Direct GAS probe test, RAT, and culture results from patients with presumed treatment failure and/or reinfection is necessary to address RNA persistence.

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REFERENCES


