Comparison of Three Methods for Detection of Group A Streptococci in Throat Swabs

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Group A streptococci are generally detected in throat swabs by (i) rapid antigen tests, (ii) conventional culture, or (iii) combinations of both. Direct fluorescent-antibody testing of a 2-h enrichment broth (FA/EN) was an accepted method for same-day results before the advent of rapid antigen tests. We compared FA/EN in Todd-Hewitt Broth (THB) with conventional culture and a rapid antigen test, TestPack Strep A (TPS). Nine hundred seventy specimens were evaluated in this study. Cultures were performed for 48 h on sheep blood agar (SBA) incubated aerobically and on a selective agar for group A streptococci (SSA) incubated in 5 to 10% CO₂. Following a 2-h incubation, the fluorescent-antibody test was performed. A subculture of the centrifuged sediment from the THB enrichment was also done. In comparison with a positive culture on SBA or SSA or subculture of the THB pellet, the sensitivities and specificities of the different methods were as follows: SBA, 92 and 100%; SSA, 92 and 100%; TPS, 68 and 99%; FA/EN, 88 and 98%. The FA/EN method offers the potential for definitive finalized reports on the same day as specimen collection with greater sensitivity than TPS. This study included sequential plating and rapid antigen testing of a single swab. In a separate set of experiments to validate this study design, it was shown that recovery of streptococci from swabs plated sequentially on five plates did not vary with the order of plating and the actual proportion of organisms recovered from a swab on a single plate was only 1%.

Rapid tests for detection of group A streptococci (GAS) have gained widespread acceptance during the past decade. They are generally simple to perform, and results theoretically can be available within 10 min after a throat swab is obtained. These characteristics make the tests attractive candidates for use during clinic visits, with results available and definitive therapy begun before a patient leaves. Nevertheless, this is not a universal practice. In many laboratories, including our own, testing is performed several times a day in batches of various sizes. Most often, the patient has left the office or clinic and is simply informed of the results by telephone. In fact, some practices in which the rapid tests were once performed while the patient waited reverted to batch testing because of disruptions to work flow (11). Batch testing is probably even more common at larger medical centers with centralized testing facilities because of improved work flow, lower costs, and the inherent delays in specimen transportation and result reporting that would make patient waiting times excessive.

Another consideration in the use of rapid tests for GAS is the lack of adequate sensitivity for reliance on the tests alone. This factor has led some authorities to recommend that negative tests be confirmed with culture (1, 5). This necessity adds expense, complexity, and delay. We wondered whether same-day definitive test results might be as useful as the combination of a direct antigen test and a follow-up culture in the management of patients in a large medical practice. To begin to answer this question, we needed first to determine the adequacy of available methods for same-day results.

Before the advent of direct antigen tests, Cherry and Moody (2) showed that a direct fluorescent-antibody test of a centrifuged pellet from a 2- to 4-h blood broth culture gave sensitivity equivalent or even superior to that of culture on plated media available at that time. Waters and Makens (13) confirmed the results of Cherry and Moody and reported a sensitivity of 95% for a modified fluorescent-antibody test of a broth enrichment culture (FA/EN method). Our study was designed to re-evaluate the fluorescent-antibody test in comparison with a rapid antigen test and two common methods of culture. To provide same-day results, we set the enrichment time at 2 h rather than the range of 2 to 4 h used in the earlier studies.

MATERIALS AND METHODS

Study design. From 13 March 1991 to 13 September 1991, all throat cultures from the emergency room, pediatric clinics, and family practice clinic of the Geisinger Medical Center were collected on Culturette II swabs (Becton Dickinson Microbiology Systems, Cockeysville, Md.). Upon receipt in the laboratory, one swab was used first to inoculate each of two primary plated media and then used for a rapid streptococcal antigen test. The second swab was utilized for the FA/EN method.

Staffing limitations dictated that the test protocol be followed from Monday to Thursday of each week during the study. The only exceptions to this were for certain holiday and vacation periods.

Culture. Two primary plated media were utilized, tryptic soy agar with 5% sheep blood (SBA) and Group A Selective Strep Agar, a proprietary medium containing crystal violet, colistin, and trimethoprim-sulfamethoxazole (SSA) (4). All of the media used in the study were obtained from Becton Dickinson Microbiology Systems. The nonselective SBA plate was always inoculated first, followed by the SSA plate. Incubation was at 35°C, in room air for SBA and in 5 to 10% CO₂ for SSA.

 Cultures were examined on the day following inoculation, and those that were negative on day 1 were reincubated for another 24 h and reexamined. To remove any unintentional

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bias, the SBA plate on each culture was examined by one technologist while the SSA plate was examined by a different technologist.

Serotyping was performed on beta-hemolytic streptococci utilizing the Streptex Latex A reagent (Wellcome Reagents Division, Burroughs Wellcome Company, Research Triangle Park, N.C.). Group A streptococci on SBA plates were semiquantitatively measured by growth in quadrants as follows: one or two quadrants, few organisms; three quadrants, moderate number of organisms; four quadrants, many organisms.

**Rapid streptococcal antigen test.** After plating of the SBA and SSA, the swab handle was marked and placed back into the Culturette II holder. Three or four times daily, rapid streptococcal antigen tests were performed by Abbott Test Pack Strept A (TPS) (Abbott Laboratories, Abbott Park, Ill.). The previously marked swab was removed from the Culturette II holder, and the test was performed by following the manufacturer’s instructions. Following completion of the TPS, the Culturette II holder with the second swab was stored at 4°C for later testing.

**FA/EN method.** The FA/EN method was adapted from the method of Cherry and Moody (2). On the morning following receipt of a specimen in the laboratory, the swab was removed from the refrigerator and placed in a sterile polypropylene tube (12 by 75 mm; Fisher Scientific) containing 1 ml of Todd-Hewitt Broth (THB). The tube was incubated at 35°C for 2 h. The swab was wrung out on the inside of the tube, and the tube was centrifuged at 700 x g for 5 min. The supernatant was decanted and discarded. The pellet was suspended in 1 ml of phosphate-buffered saline (PBS), pH 7.4. The centrifugation and decanting steps were repeated. The tube was then allowed to sit for 3 min, which permitted residual PBS and cells to collect on the bottom of the tube. With a sterile Pasteur pipet, 2 drops of the sediment was transferred to an SBA plate. (The inoculum on the SBA plate was streaked out, plates were incubated, and beta-hemolytic colonies were identified in the same manner as for the primary SBA plate.) A small amount of the sediment was thinly spread over a 5-mm-diameter circle on a 10-well slide (catalog no. 10-125; Cel-Line Associates, Newfield, N.J.). The slide was allowed to air dry, placed in a petri dish, and fixed for 1 min with 95% ethanol. It was removed from the ethanol, and after drying, 20 μl of fluorescein isothiocyanate-labeled, polyclonal antistreptococcal globulin (INCSTAR, Stillwater, Minn.) was added to each well. Slides were incubated in a moist chamber at room temperature for 30 min. The slides were held horizontally and rinsed with PBS. They were placed in a Coplin jar filled with PBS for 5 min. The washing with PBS was repeated, after which the slides were rinsed with distilled water and blotted dry. The slides were mounted with FA fluor mounting medium (Ortho Diagnostic Systems, Raritan, N.J.) and examined with a fluorescence microscope using an oil immersion (×100) objective. Wells with at least one pair or chain of bacteria that morphologically resembled streptococci and showed 3+ to 4+ fluorescence were recorded as positive.

Group A, C, and G beta-hemolytic streptococci and *Staphylococcus aureus* controls were run with each batch of slides. Controls were inoculated into THB and processed the same way as patient specimens.

**Effect of plating sequence on organism recovery.** To evaluate the recovery of streptococci when multiple tests or platings are all done from a single swab, as performed in this study, the following experiment was conducted. Ten recent clinical isolates of beta-hemolytic GAS were each subcultured to a blood agar plate and incubated aerobically overnight at 35°C. A suspension of each strain was prepared in THB at a density of 0.5 McFarland and serially diluted in 10-fold steps to 10⁻⁵.

The approximate lower one-third of a Culturette tip was dipped into selected THB dilutions for each isolate. The swabs were rinsed on the inside of the tubes to remove any large droplets of broth, replaced into the Culturette holders, and allowed to stand at room temperature for 1 h. An additional five swabs were individually weighed, dipped similarly into sterile THB, and weighed again. The weight difference (99 ± 5 [standard deviation] mg) was used to estimate the volume of broth inoculum adsorbed onto each swab.

The 10⁻³, 10⁻⁴, and 10⁻⁵ dilutions for each isolate were subcultured (preliminary experiments had shown that this range of dilutions yielded numbers of bacterial colonies that could be readily counted). Each swab was sequentially rolled in a straight line down the center of five blood agar plates. The inoculum was then spread over the plate perpendicularly to the line of application with a sterile loop in a manner analogous to that used for urine cultures.

Each isolate was tested in duplicate, including preparation of two sets of serial dilutions of the broth inoculum, for a total of 20 separate tests. Lastly, the undiluted cell suspension was quantitatively subcultured to determine initial inoculum concentrations. Inoculated plates were incubated for 24 h at 35°C in air, and plate counts were recorded.

**Statistical analysis.** Sensitivity, specificity, and confidence intervals (C.I.) were calculated on the basis of the definition and formulas presented by Llstrup (6). Comparison of method sensitivities was done by using the chi-square test with two-tailed limits for probability.

For the sequential plating experiment, an analysis of variance was done by using the SAS/STAT statistical program, release 6.03 (SAS Institute, Cary, N.C.).

### RESULTS

**Sequential plating experiment.** Ten strains were tested in duplicate for a total of 20 tests. One test could not be evaluated because of insufficient colonies at the plated dilutions; therefore, results from 19 tests were evaluated. Because of variation in the initial inoculum (range of 8 x 10⁻⁷ to 2 x 10⁻⁹ CFU/ml), the colony count on each plate in a sequential plating series was expressed as a proportion of the highest colony count in the series.

The numerical proportions for all of the no. 1 plates were totaled and divided by 19, yielding the average comparative proportion for plate 1. The same calculation was done for plates 2 through 5. The results are presented in Table 1. The

<table>
<thead>
<tr>
<th>Plate no.</th>
<th>Ave relative recovery</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>73</td>
<td>23</td>
<td>32-100</td>
</tr>
<tr>
<td>2</td>
<td>69</td>
<td>20</td>
<td>28-96</td>
</tr>
<tr>
<td>3</td>
<td>73</td>
<td>22</td>
<td>31-100</td>
</tr>
<tr>
<td>4</td>
<td>77</td>
<td>24</td>
<td>23-100</td>
</tr>
<tr>
<td>5</td>
<td>69</td>
<td>22</td>
<td>37-100</td>
</tr>
</tbody>
</table>

* The plate with the highest colony count in the series was assigned a value of 100; *F* = 0.048, not significant.
averages ranged from 69 to 73%, and the differences between sequential plates were not statistically significant.

**Study.** During the 6 months in which this study was conducted, 970 specimens were tested. Because each specimen in this study was tested by five methods, culture on SBA and on SSA, subculture of the THB pellet, FA/EN, and TPS, the data could be presented and analyzed in several ways.

A true-positive result was defined by isolation of GAS on SBA or SSA or from the THB pellet. The results are shown in Table 2. The number of true-positive results was 213, with sensitivities for FA/EN and TPS of 88 and 68%. There were 16 false-positive results with FA/EN and 6 with TPS, with specificities of 98 and 99%, respectively.

In comparing the sensitivities of culture on SSA or SBA and FA/EN, we found no statistically significant differences when comparing one method with either of the other two. For example, the sensitivity of FA/EN compared with that of SBA culture was 94% (C.I., 91 to 97%) and the sensitivity of SSA culture compared with that of SBA culture was 95% (C.I., 92 to 98%). While the sensitivity of FA/EN declined to 88% (C.I., 84 to 92%) compared with that of culture on SBA or SSA or the THB pellet subculture, the sensitivity of either SSA or SBA alone declined to 92% (C.I., 88 to 96%) when it was compared against this same three-method standard.

Overall, there were more false-negative results with FA/EN than with TPS. However, the false-positive results were not evenly distributed throughout the study. Of the first 138 specimens tested, there were seven FA/EN false-positive results. For these first 138 specimens, 10 (including appropriate controls) were examined per slide, 5 in each of two rows. After the method was changed to use of a single row of five wells per slide, there were only nine false-positive results from the remaining 832 specimens. By comparison, there were no false-positive TPS results from the first 138 specimens and six false-positive results from the remaining 832 specimens.

Table 3 presents a comparison of the sensitivities of FA/EN and TPS as a function of the amount of growth on SBA. When many colonies were present on SBA, the sensitivities of the two methods were comparable; however, with lesser numbers of colonies, the differences became very evident. FA/EN had sensitivities of 97 and 74% for moderate and few colonies, while TPS had sensitivities of 75 and 23% for moderate and few colonies, respectively ($P < 0.05$).

When tested with four controls, batches of greater than 10 clinical specimens required about 10 min per clinical speci-

men for the FA/EN method. At a labor cost of $2.50 (10 min at $15/h) plus an estimated cost of $1.25 for supplies, the FA/EN method cost is about $3.75 per patient specimen tested.

**DISCUSSION**

This study was designed to evaluate an FA/EN method for detection of GAS from throat swabs in comparison with the TPS rapid antigen test and traditional culture on two different media.

The principal reason for the rapid acceptance of commercial antigen tests, such as the TPS, seems to be convenience and the desirability of a laboratory test result which is generated more rapidly than that of a 24- to 48-h culture. However, the rapid antigen tests have not demonstrated adequate sensitivity such that they can be utilized alone as definitive tests to rule out streptococcal pharyngitis (1, 5). Other published studies of TPS have reported sensitivities of 73 to 96% (3, 9, 10, 12). In this study, the sensitivity of the TPS in comparison with that of culture on either SSA or SBA was 73 to 74%.

For our study, we decided to consider definitive any test with at least 90% sensitivity compared with culture. Of course, no firm rules exist to justify this definition, but experiences with single cultures would justify it on the basis of the observed discrepancies between duplicate cultures, either one of which would be considered definitive in usual practice. We have demonstrated that an earlier technique, FA/EN, offers sensitivity comparable to that of either of the primary culture media utilized in this study and superior to that of a rapid direct antigen test. Each of the three methods, FA/EN, SBA culture, and SSA culture, has a sensitivity of 93 to 95% compared with each of the other two methods. This result is consistent with a widely held presumption that the discordance between simultaneously collected throat swabs is approximately 10% (7).

Performance of the FA/EN, more specifically, reading of the fluorescent-antibody slides, is technically more demanding than performance of rapid antigen tests, such as TPS. All of the fluorescent-antibody slides in this study were evaluated by a single technologist; however, this individual had no prior experience in reading fluorescent-antibody slides. The unexpected and relatively high rate of false-positive results early in the study (7 of 138) led us to suspect that there was cross-contamination from adjacent rows on the slide. Without attempting to prove this assumption, we changed our method to use of only a single row of wells per slide and observed a lower false-positivity rate for the remainder of the study. The specificity may also have improved as the

### Table 2. Comparison of culture on SBA or SSA with FA/EN and TPS for detection of GAS

<table>
<thead>
<tr>
<th>Test</th>
<th>True positive</th>
<th>False negative</th>
<th>True positive</th>
<th>False positive</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBA</td>
<td>195</td>
<td>18</td>
<td>757</td>
<td>92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SSA</td>
<td>197</td>
<td>16</td>
<td>757</td>
<td>92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>THB pellet</td>
<td>194</td>
<td>19</td>
<td>757</td>
<td>93</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TPS</td>
<td>145</td>
<td>68</td>
<td>751</td>
<td>6</td>
<td>68</td>
<td>99</td>
</tr>
<tr>
<td>FA/EN</td>
<td>188</td>
<td>25</td>
<td>741</td>
<td>16</td>
<td>88</td>
<td>98</td>
</tr>
</tbody>
</table>

* The number of true-positive results was 213. The number of true-negative results was 757. The prevalence of positive cultures was 22%.

### Table 3. Comparative sensitivities of FA/EN and TPS as a function of the amount of growth on SBA

<table>
<thead>
<tr>
<th>Test</th>
<th>No. of positive results (% sensitivity) at indicated growth level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Many organisms</td>
</tr>
<tr>
<td>SBA</td>
<td>72</td>
</tr>
<tr>
<td>FA/EN</td>
<td>71 (99)</td>
</tr>
<tr>
<td>TPS</td>
<td>69 (96)</td>
</tr>
</tbody>
</table>

* For definitions of growth levels, see Materials and Methods.
  * Accounted for 37% of the total.
  * Accounted for 47% of the total.
  * Accounted for 16% of the total.
experience of the technologist performing the test increased; however, it was not salient to the purpose of this study to investigate the basis for the improvement further.

One might argue that the order of inoculation of media and use of the swabs in the rapid tests should have been randomized. This process would have added complexity to a study that was already relatively complex and was intended to be done with minimal disruption of routine laboratory activity.

To demonstrate the validity of our nonrandomized inoculation protocol, we performed a sequential plating experiment. By using 10 strains of beta-hemolytic GAS tested in duplicate, we found no statistically significant difference in the recovery of streptococci from swabs on a series of five plates. The actual amount of broth adsorbed onto each swab was approximately 0.1 ml. A comparison of the initial inoculum with the plate counts showed that only about 1% of the inoculum on each swab was transferred to a plate. The fact that such a small percentage of the streptococci present on the swab was transferred to solid media during plating is consistent with our finding that the order of plating used in this study did not affect organism recovery.

For diagnosis of streptococcal pharyngitis, the FA/EN method offers the potential for definitive final reporting on the same day as collection; however, its complexity favors batching of specimens. While some laboratories may not have either the equipment or the personnel necessary to support this methodology, it may be possible to adapt the principle of pretest enrichment to other, nonmicroscopic methods. For example, if a rapid antigen test were performed following a 2-h broth enrichment, would the sensitivity of the test be comparable to what we have demonstrated in this study for the FA/EN method? This concept merits further study.

There is no consensus regarding either the optimal culture medium or the optimum incubation atmosphere for cultivation of GAS. As part of an extensive review of GAS culture, Kellogg (8) summarized studies which compared the sensitivities of SSA and SBA media in different incubation atmospheres. Those studies that specifically compared incubation for 48 h on SSA in 5 to 10% CO2 and SBA incubated aerobically for 48 h either suggested no difference in sensitivity between the two methods or slightly favored SSA. In our study, there was no statistically significant difference in the recovery of GAS from cultures incubated for 48 h on SBA aerobically or on SSA in 5 to 10% CO2. Further studies utilizing SSA are needed for better assessment of performance in different laboratories.

In our laboratory, we use SSA incubated in 5 to 10% CO2 for up to 48 h for culture of GAS. We prefer SSA because of suppression of much of the normal pharyngeal flora, including non-group A beta-hemolytic streptococci. The higher cost of an SSA plate ($0.08 more than an SBA plate) is offset by the ease of use and elimination of workup of most non-group A beta-hemolytic streptococci. We do not use the FA/EN method in our laboratory but are investigating less labor-intensive methods to accomplish the same end.

REFERENCES