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Comparison of Gen-Probe Group A Streptococcus Direct Test with Culture for Diagnosing Streptococcal Pharyngitis

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The Group A Streptococcus Direct Test (GP-ST test; Gen-Probe, Inc., San Diego, Calif.) was compared with culture for the detection of *Streptococcus pyogenes* from throat swabs of 767 patients with pharyngitis. Swabs were tested by the GP-ST test after inoculating a 5% sheep blood agar (SBA) plate. SBA plates were incubated at 35°C in room air for 72 h. SBA plates with no evidence of beta-hemolytic colonies after 18 to 24 h of incubation were subcultured by taking a swipe across the primary inoculum from the SBA plate to an agar selective for *Streptococcus* spp. In a low-prevalence (11.9%) population and in comparison with the number of positive cultures detected by the 72-h single-culture method (SBA plate method), the GP-ST test had a sensitivity of 88.6%, a specificity of 97.8%, a positive predictive value of 83.9%, and a negative predictive value of 98.5%. In comparison with the growth of any colonies of *S. pyogenes* on the 72-h SBA plates plus a subculture onto selective blood agar, the sensitivities and specificities were as follows: 72-h SBA plate method, 96.7 and 100%, respectively; GP-ST test, 85.7 and 97.8%, respectively. The GP-ST test is an easy-to-perform, reliable test for batch screening of throat swabs for *S. pyogenes*.

The Group A Streptococcus Direct Test (GP-ST test; Gen-Probe, Inc., San Diego, Calif.) is a DNA probe assay which uses nucleic acid hybridization to detect *Streptococcus pyogenes* from throat swabs. Specific rRNA sequences unique to *S. pyogenes* are detected by using a chemiluminescent single-stranded DNA probe. The labeled DNA probe combines with the rRNA present in the sample to form a stable double-stranded hybrid. The labeled DNA-RNA hybrids are measured in a luminometer in relative light units (RLUs), which are directly proportional to the amount of hybridization that has occurred.

An evaluation was conducted at Mayo Medical Center to determine the utility of the GP-ST test compared with that of a 72-h sheep blood agar (SBA) plate culture method and a selective agar subculture method for the detection of *S. pyogenes* from throat swabs.

(This study was presented in part at the 93rd General Meeting of the American Society for Microbiology, Atlanta, Ga., 16 to 20 May 1993.)

MATERIALS AND METHODS

A total of 767 throat swabs were obtained from patients presenting with pharyngitis at the Mayo Medical Center in Rochester, Minn., during a 12-week period between 3 August and 23 October 1992. A Culturette swab (Becton Dickinson Microbiology Systems, Cockeysville, Md.) was used for specimen collection. The throat swabs were transported to the microbiology laboratory at ambient temperature and were cultured upon receipt. The swabs were then frozen at −70°C for later batch testing by the GP-ST test.

The throat swabs were inoculated onto 5% SBA plates (Becton Dickinson Microbiology Systems) and were streaked onto the plates for isolation. Stabs were made into the agar with the inoculating loop to facilitate detection of oxygen-labile β-lysin. These SBA plates were incubated at 35°C in room air for 72 h and were examined daily for the presence of beta-hemolytic *Streptococcus* spp. Beta-hemolytic streptococci were serogrouped by using the Streptex latex agglutination reagent (Murex Diagnostics Limited, Dartford, England).

When the SBA plates showed no growth of beta-hemolytic streptococci after 18 to 24 h of incubation, a swipe of the growth from the first quadrant was subcultured onto a selective streptococcal agar (Becton Dickinson Microbiology Systems) or onto SXT-BA (Remel, Lenexa, Kans.). This plate was incubated at 35°C in room air for 48 h and was examined daily for beta-hemolysis. Any beta streptococci isolated were serogrouped by using the Streptex latex agglutination reagent.

For the GP-ST procedure, throat swabs were removed from the freezer (−70°C) and were allowed to thaw at room temperature. The GP-ST kits were removed from the refrigerator and were allowed to warm to room temperature. Bacterial cell controls were prepared by pipetting 5 ml of sterile saline into two clean culture tubes. The respective tubes were inoculated with a 1-μl loopful of *S. pyogenes* ATCC 12344 (positive cell control) and *Streptococcus agalactiae* ATCC 13873 (negative cell control) from fresh SBA cultures (18 to 24 h) and were vortexed thoroughly. A total of 10 μl of the respective cell suspensions was pipeted into two labeled tubes containing 300 μl of lysis reagent, and the tubes were vortexed thoroughly. A total of 50 μl of the positive and negative controls provided in the kit was pipeted into two labeled tubes, and the tubes were set aside for the hybridization step.

The throat swabs were placed into 300 μl of lysis reagent in labeled tubes. All tubes containing swabs and the cell controls were incubated at 95°C for 10 min in a heating block; they were then removed and allowed to cool for 5 min. While the tubes cooled, the swabs were thoroughly expressed against the side of the tube and discarded. Within 1 h, 50 μl of the lysates was pipeted into labeled tubes. (The remainder of the lysate was frozen at −70°C for possible repeat testing to resolve discrep-

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TABLE 1. Comparison of the GP-ST test and 72-h SBA plate culture with any positive culture[a]

Test	Percent (95% confidence interval)			
	Sensitivity	Specificity	Positive predictive value	Negative predictive value
GP-ST	85.7 (78.0, 92.3)[b]	97.8 (96.7, 100)	83.9 (76.3, 91.4)	98.1 (97.0, 99.0)
72-h SBA plate culture	96.7 (92.3, 100)[b]	100	100	99.6 (99.0, 100)

[a] Prevalence rate of positive cultures was 11.9%. Both the GP-ST test and the 72-h SBA plate culture were compared with any positive culture detected by the 72-h SBA plate culture method or on subcultures by using selective streptococcal agar or SXT-BA (see text).
[b] Significantly different by McNemer's test ($P = 0.002$).

ant results.) A total of 50 μl of probe reagent was pipeted into each tube, including the positive and negative control tubes provided with the kit. The tubes were covered, the contents were mixed by shaking gently, and the tubes were incubated at 60°C for 30 min to allow hybridization to occur. After the tubes were removed from the heating block, 300 μl of selection reagent was added and the tubes were vortexed thoroughly. The tubes were incubated at 60°C in a heating block for 7 min, removed, and allowed to cool at room temperature for at least 5 min. The tubes were read within 1 h in a Leader luminometer by using a 2-s read time. The labeled DNA-RNA hybrids were measured in the luminometer, and the results are reported in RLUs.

The test results of the GP-ST test were based on the following cutoff values recommended by the manufacturer: positive, ≥4,500 RLUs; negative, <4,500 RLUs.

When the results of the GP-ST test were positive and the results of the 72-h SBA plate cultures and the selective streptococcal subculture were negative, the GP-ST assay was repeated by using the remaining stored lysate. If the discrepancy still existed, the culture plates were examined for possible nonhemolytic strains of *S. pyogenes*. In addition, the rayon pledget from the bottom of the Culturette sheath was incubated in a tube of Todd-Hewitt broth with SXT-BA (Remel) for 18 to 24 h at 35°C. The broth was subcultured onto SBA plates and a selective streptococcal agar plate or SXT-BA. These plates were incubated at 35°C for 72 h and were checked daily for beta-hemolysis.

When the GP-ST test result was negative and the 72-h SBA plate culture or selective streptococcal subculture result was positive for *S. pyogenes*, the probe assay was repeated by using the remaining stored lysate. *S. pyogenes* was tested by the probe assay to verify its reactivity by placing a 1-μl loopful of the cells into 300 μl of lysis reagent in a test tube and proceeding with the assay as described above for throat swabs. The serogrouping of the organism was repeated, and a Gram stain was performed.

For statistical analysis of the data, confidence intervals for sensitivity, specificity, and positive and negative predictive values were based on exact binomial probabilities. The difference between sensitivities was evaluated by McNemer's test.

RESULTS

The results of the study are summarized in Table 1. Calculations are based on the results of the initial GP-ST test and do not reflect the results of subsequent tests performed to resolve discrepant results.

The sensitivity and specificity of the GP-ST test compared with that of the 72-h single SBA culture method for throat swabs were 88.6 and 97.8%, respectively. The predictive value for a positive result in our patient population was 83.9%, and the predictive value for a negative result was 98.5%. All beta-streptococci detected by the culture method were recovered within 48 h. For the purposes of the present study, SBA plates were incubated for 72 h. Our routine method for *S. pyogenes* detection uses the same plate incubated for 48 h only. Table 1 shows the comparison of GP-ST with culture by using the 72-h SBA plate method and a subculture on selective agar. The sensitivity and specificity of GP-ST were 85.7 and 97.8%, respectively. The 72-h SBA culture compared with any positive culture gave a sensitivity of 96.7% and a specificity of 100%. The difference in the sensitivity of the GP-ST test compared with that of the SBA culture method was highly significant ($P = 0.002$).

The 72-h SBA culture and subcultures also grew beta streptococci other than group A streptococci, including 20 group B streptococci, 7 group C streptococci, 12 group G streptococci, and 1 group D streptococcus. All beta streptococci detected by the culture method were recovered within 48 h on either SBA plates or a subculture. The GP-ST test was negative for all specimens containing these streptococci except one group B streptococcus, which, on repeat Streptex testing, was confirmed to be of the group B serogroup. The GP-ST assay for this specimen gave readings of 4,758 RLUs and gave readings of 4,208 and 4,435 RLUs on repeat testing.

Table 2 shows the results of a comparison of GP-ST readings with culture results. The quantity of *S. pyogenes* organisms isolated on culture when the GP-ST test result was negative was as follows: for growth on subculture only and quadrant growth of 1+, 2+, 3+, and 4+, there were 3, 3, 7, 0, and 0 cultures, respectively (see footnote *b* of Table 2 for definitions of quadrant growth). Of 13 GP-ST-negative, culture-positive throat swabs, 12 were retested, and the results of all repeat tests were also negative. One specimen had insufficient lysate for repeat testing. When testing was performed on these culture isolates of *S. pyogenes*, the GP-ST test result was positive. Culturette pledgets were available for further testing for 8 of 15 GP-ST test-positive, culture-negative specimens. Subculture of the pledgets in Todd-Hewitt broth yielded only one beta-hemolytic *Streptococcus* sp. which was subsequently identified as a group B streptococcus. Of the 78 GP-ST test-positive, culture-positive throat swabs, 8 throat swabs grew two to eight colonies of *S. pyogenes* on blood agar culture.

DISCUSSION

The rapid direct detection of group A streptococcal pharyngitis is preferred by physicians and patients. If the diagnosis of group A streptococcal pharyngitis can be accurately provided during an office visit, therapy can be administered on the same day. Proper therapy for group A streptococcal pharyngitis is desired so that sequelae, including rheumatic fever, are prevented (2). The traditional approach for diagnosing group A streptococcal pharyngitis involves the isolation of *S. pyogenes*

TABLE 2. Comparison of GP-ST test readings with culture results

RLUs (10^3)	No. of GAS[a]	No. of cultures with result of[b]:				
		Subculture only	1+	2+	3+	4+
<1.6	42					
1.6–2.0	214			1		
2.1–2.5	236		1			
2.6–3.0	103	1	1	2		
3.1–3.5	33	1	1	4		
3.6–4.0	27	1				
4.1–4.4	6					
4.5–5.5	10		1			
5.6–10	5		5	6	1	
11–20			2	4		
21–50				4	3	1
51–100					1	1
110–200				3	6	
210–300				3		
310–400						2
410–500					1	1
510–600					1	1
610–700						
710–800						
810–900						1
910–1,000						1
1,100–1,500				1	2	9
1,600–2,000					1	8
>2,100					2	6

[a] GAS, *S. pyogenes*.

[b] 1+, <10 colonies in first quadrant streak area; 2+, ≥10 colonies in first quadrant streak area and <5 colonies in second quadrant streak area; 3+, ≥10 colonies in first quadrant streak area, ≥5 colonies in the second quadrant streak area, and <5 colonies in the third quadrant streak area; 4+, ≥10 colonies in first quadrant streak area, ≥5 colonies in second quadrant streak area, and ≥5 colonies in third quadrant streak area.

on blood agar. In our hands, this method requires a minimum of 18 to 24 h for identification of the organism.

Rapid, easy-to-perform streptococcal antigen immunoassays have been developed recently. Such tests are easily adapted into the work flow of physician's offices. However, a problem with these assays has been a lack of sensitivity compared with that of culture. Wegner and colleagues (7) compared the sensitivity of five group A streptococcal antigen detection systems and two culture methods, a one-plate method with a single 5% SBA plate containing sulfamethoxazole and a two-plate method with a 5% SBA plate containing sulfamethoxazole and a Trypticase soy agar containing 5% sheep blood. The sulfamethoxazole-blood agar plates were incubated anaerobically; the Trypticase soy blood agar plates were incubated aerobically. When compared with the two-plate culture method (sensitivity, 100%), the sensitivity of the single-plate culture method was 72%, and the sensitive for five group A streptococcal antigen tests ranged from 31 to 50%. Wegner et al. (7) concluded that if antigen tests are used without culture backup, physicians may undertreat patients with streptococcal pharyngitis, risking untoward sequelae (7).

Anhalt and colleagues (1) compared the Abbott Testpack Strep A assay (Abbott Laboratories, North Chicago, Ill.) with three culture methods, including sheep blood agar incubated aerobically, selective streptococcal agar incubated in 5 to 10% CO_2, and a subculture in Todd-Hewitt broth. When the Abbott TestPack Strep A assay was compared with any positive culture obtained by these methods, a sensitivity and a specificity of 68 and 99%, respectively, were determined (1).

A recent report by Harbeck and colleagues (3) described a novel rapid optical immunoassay for detecting *S. pyogenes* from pharyngeal specimens. Compared with an enriched broth culture, this new method of antigen detection showed ranges for sensitivity and specificity in two separate studies of 97.4 to 98.9% and 95.6 to 98.6%, respectively (3). Additional studies that include comparison of this rapid antigen method with other rapid detection methods are necessary to confirm these findings.

Heiter and Bourbeau (4) recently reported results of a comparison of the GP-ST test with conventional culture and the Abbott TestPack Strep A rapid antigen assay with respective sensitivities and specificities as follows: culture, 99.5 and 100%; Abbott TestPack Strep A assay, 76.3 and 99.7%; GP-ST test, 93.5 and 99.7%. They concluded that the GP-ST test is easy to use and has the potential to replace culture for the diagnosis of streptococcal pharyngitis; they suggested that evaluations of different patient populations, especially those with lower prevalences of *S. pyogenes*, were needed to corroborate their results. They noted a prevalence of 26.6% positive test results (culture-positive results) at their institution (4). More recently, Steed and colleagues (6) compared the GP-ST test with their conventional culture in specimens from pediatric patients having a 21% prevalence rate of positive cultures. In that study, the sensitivity and specificity of the GP-ST test compared with those of culture were 86 and 95%, respectively (6).

In the present study, a prevalence of 11.9% (culture positives) was noted, and this prevalence was considerably less than that reported by Heiter and Bourbeau (4) and Steed et al. (6). Specimens were not included from high-incidence areas of our institution, including urgent care, ambulatory pediatrics, and family medicine clinics. Many cultures were obtained from adult patients at a tertiary-care diagnostic clinic, with pharyngitis being an incidental and not a primary complaint. Furthermore, all samples were obtained during a time when the incidence of streptococcal pharyngitis was the lowest at our institution (August through October). Such a low prevalence of streptococcal pharyngitis allowed for a more rigorous evaluation of the GP-ST method.

In the present study, the performance characteristics of the GP-ST test compared favorably with those of our routine culture method. Noteworthy was the fact that the GP-ST test was positive for eight patient specimens which grew eight or fewer (range, two to eight) colonies of *S. pyogenes* on the agar culture. These results were seen, even though fewer *S. pyogenes* organisms may have been available on the swab for the GP-ST test following inoculation onto culture plates. However, this may not be important, as demonstrated by Libertin and colleagues (5). They showed that sequential inoculation of six culture plates with the same swabs showed no discernible difference in the number of *S. pyogenes* organisms isolated between the first and last plates (5). In addition, the freeze-thaw batching procedure used in the current evaluation may have adversely affected the bacterial organisms and the rRNAs present on the throat swab.

Additional comparisons of the GP-ST test with other rapid antigen testing methods are necessary. The lower level of sensitivity of GP-ST for detecting *S. pyogenes* that we observed compared with that observed by Heiter and Bourbeau (4) may have resulted from the lower prevalence of positive cultures and, hence, disease in the population that we surveyed. Indeed, the presence of group A streptococci in some of our patients may have reflected colonization and not active disease. In fact, of the GP-ST test-negative, culture-positive results, 6 of 13 cultures grew fewer than 10 colonies of *S. pyogenes*.

On the basis of our experience, the GP-ST test would be

difficult to adapt to an office practice because of the multiple steps and equipment involved and the time required to perform the test (usually 2 h for a large batch tested with a single-tube model luminometer). However, the GP-ST test may be useful for large-scale batch screening of throat swabs for *S. pyogenes* in a referral laboratory setting and, providing that the GP-ST test is cost-effective, may potentially replace culture-based methods.

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REFERENCES

1. **Anhalt, J. P., B. J. Heiter, D. W. Naumovitz, and P. P. Bourbeau.** 1992. Comparison of three methods for detection of group A streptococci in throat swabs. J. Clin. Microbiol. **30:**2135–2138.
2. **Bisno, A.** 1991. Group A streptococcal infections and acute rheumatic fever. N. Engl. J. Med. **325:**783–793.
3. **Harbeck, R. J., J. Teague, G. R. Crossen, D. M. Maul, and P. L. Childers.** 1993. Novel, rapid optical immunoassay technique for detection of group A streptococci for detection of group A streptococci from pharyngeal specimens: comparison with standard culture methods. J. Clin. Microbiol. **31:**839–844.
4. **Heiter, B. J., and P. P. Bourbeau.** 1992. Comparison of the Gen-Probe Group A Streptococcus Direct Test with culture and a rapid streptococcal antigen assay for diagnosis of streptococcal pharyngitis. J. Clin. Microbiol. **31:**2070–2073.
5. **Libertin, C. R., A. D. Wold, and J. A. Washington II.** 1983. Effects of trimethoprim-sulfamethoxazole and incubation on isolation of group A streptococci. J. Clin. Microbiol. **18:**680–682.
6. **Steed, L. L., E. K. Korgenski, and J. A. Daly.** 1993. Rapid detection of *Streptococcus pyogenes* in pediatric patient specimens by DNA probe. J. Clin. Microbiol. **31:**2996–3000.
7. **Wegner, D. L., D. L. Witte, and R. D. Schrantz.** 1992. Insensitivity of rapid antigen detection methods and single blood agar plate culture for diagnosing streptococcal pharyngitis. JAMA **267:**695–697.