

Comparison of the Gen-Probe Group A Streptococcus Direct Test with Culture and a Rapid Streptococcal Antigen Detection Assay for Diagnosis of Streptococcal Pharyngitis

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The Gen-Probe Group A Streptococcus Direct Test (GP-ST) is a new assay which utilizes a nucleic acid probe to detect group A streptococci directly from pharyngeal swabs. In this study, 1,103 specimens were cultured and tested by GP-ST. The sensitivities and specificities were as follows: culture, 98.8 and 100%; GP-ST, 92.4 and 99.6%. Of the 1,103 specimens, 808 were also tested with the TestPack Strep A assay. For the specimens tested by all three methods, the sensitivities and specificities were as follows: culture, 99.5 and 100%; TestPack Strep A assay, 76.3 and 99.7%; GP-ST, 93.5 and 99.7%. The GP-ST is a very user-friendly assay which has the potential to replace culture for the diagnosis of streptococcal pharyngitis.

Laboratory diagnosis of group A streptococcal pharyngitis in the United States is made primarily by culture or rapid streptococcal antigen detection methods. Recently, a commercially manufactured kit was developed which permits detection of group A streptococci (GAS) directly in pharyngeal specimens with a nucleic acid probe. The kit is manufactured by Gen-Probe Inc. (San Diego, Calif.) and is named the Group A Streptococcus Direct Test (GP-ST).

The purpose of this study was to examine the performance characteristics of the GP-ST in comparison with those of culture and a rapid streptococcal antigen test for the diagnosis of GAS.

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MATERIALS AND METHODS

In our institution, Culturette brand swabs (Becton Dickinson Microbiology Systems, Cockeysville, Md.) are utilized for collection of specimens for culture of GAS. If a direct streptococcal antigen test is also ordered, a Culturette II is used to collect the specimen.

All specimens were collected from emergency room or pediatric or adult clinic patients at the Geisinger Medical Center. When two swabs were collected, one was used for culture and the GP-ST. The second swab was used only for the direct streptococcal antigen assay. When only a single swab was collected, no direct streptococcal antigen assay was performed.

Culture was performed on Group A Selective Strep Agar (SSA) (Becton Dickinson Microbiology Systems) with incubation at 35°C in 5 to 10% CO₂. Cultures which were negative for GAS after overnight incubation were reincubated for a second day and re-examined. GAS were identified either by serotyping with Streptex Latex A reagent (Wellcome Diagnostics, Dartford, England) or biochemical confirmation with PYR reagent (Remel, Lenexa, Kans.).

The direct streptococcal antigen assay used in the study was the Abbott TestPack Strep A assay (TP-ST; Abbott Laboratories, Abbott Park, Ill.). This test was performed by

following manufacturer's instructions. During this study, all specimens on which a TP-ST was performed were also cultured, regardless of the TP-ST result.

Nucleic acid hybridization assays are based on the ability of complementary strands of nucleic acid to anneal, forming stable double-stranded complexes. The GP-ST uses a chemiluminescent, single-stranded DNA probe that is complementary to the rRNA of the target GAS. The selection reagent used in the assay differentiates the nonhybridized probe from the hybridized probe in a liquid-phase reaction.

The GP-ST was performed by following the manufacturer's instructions. Briefly, Culturette swabs were stored at 4°C for up to 72 h before testing. Each swab was placed into a polypropylene tube containing 300 µl of lysis buffer. (The Culturette holders were then returned to a 4°C refrigerator for possible use to resolve discrepancies.) Tubes were heated for 10 min at 95 ± 3°C in a dry bath heating block. Following a 5-min cooling period, swabs were thoroughly rolled on the inside of the tubes to express as much of the liquid as possible. A 50-µl volume of the lysate was combined with 50 µl of the labeled probe in a clean polypropylene tube. The remaining lysate was retained at 4°C for any required retesting. Tubes were incubated for 30 min at 60 ± 1°C. After the tubes were removed from the water bath, 300 µl of the selection reagent was added to each tube. Following vigorous vortexing, the tubes were incubated for 7 min at 60 ± 1°C. Tubes were allowed to cool for 5 min and read in a Leader 50 luminometer. This instrument, which can be programmed for this assay, dispensed detection reagents into each tube and measured chemiluminescent output in relative light units (RLU). A firm cutoff of 4,500 RLU for Culturette swabs has been established by the manufacturer to differentiate between negative and positive results.

Some of the pledgets (rayon plugs) in the Culturette holders were cultured in broth to resolve discrepant results. This was done by carefully cutting the Culturette holder with a pair of scissors just above the pledget. The pledget was removed with sterile forceps and placed in a 5-ml tube of Todd-Hewitt broth. Following overnight incubation at 35°C, the Todd-Hewitt broth was subcultured to SSA and tryptic soy agar with 5% sheep blood and examined in the same manner as routine cultures.

To remove any unintentional bias in the study, the GP-ST

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TABLE 1. Comparison of culture with GP-ST for detection of GAS

| Test | No. of results ^a | | | | Sensitivity (%) | Specificity (%) | Predictive value of: | |
|---------|-----------------------------|----------------|---------------|----------------|-----------------|-----------------|----------------------|-----------------|
| | True positive | False negative | True negative | False positive | | | Positive result | Negative result |
| Culture | 261 | 3 | 839 | 0 | 98.8 | 100 | 100 | 99.6 |
| GP-ST | 244 | 20 | 836 | 3 | 92.4 | 99.6 | 98.8 | 97.7 |

^a There were 264 true-positive results. The prevalence of positive test results was 23.9%.

was always performed by one of us (B.J.H.) while the TP-ST and cultures were performed by personnel in the microbiology laboratory. Results were not reviewed until all tests on a specimen were completed.

RESULTS

Specimens from 1,103 patients were cultured and tested with the GP-ST. Of these specimens, 808 were also tested with the TP-ST.

When each day of testing was completed, the results were compared. If the culture and GP-ST results did not agree, two steps were taken. First, the SSA plate was carefully reexamined by a senior technologist. Second, the GP-ST was repeated with the reserved lysate. For specimens which remained disparate, the pledgets from the Culturette holders were cultured in Todd-Hewitt broth and worked up as previously described.

Of the 1,103 specimens tested, 836 were negative and 241 were positive by both culture and the GP-ST. Twenty were positive by culture only and were classified as falsely negative by GP-ST. Three isolates were GP-ST positive, positive for GAS from pledget culture, and SSA culture negative; these three were classified as false-negative culture results. Table 1 summarizes the performance characteristics of culture and the GP-ST.

Table 2 summarizes the results obtained with the 808 specimens tested by GP-ST, TP-ST, and culture. There were 215 true-positive and 593 true-negative test results among the 808 specimens.

DISCUSSION

Laboratory diagnosis of streptococcal pharyngitis is made primarily by culture or some variation of a streptococcal antigen test. Culture is generally accepted as the definitive test but requires up to 2 days for completion. Streptococcal antigen detection methods have the potential to provide rapid results when performed as a point-of-care test, yet some investigators have found the demands of performing these discrete tests burdensome and have chosen to batch the tests instead (1, 7). This negates, to various degrees, the potential rapidity of the test. In addition, the low sensitivity of the streptococcal antigen tests, in comparison with cul-

ture, has led to recommendations that all negative streptococcal antigen test results be confirmed by culture (2, 5, 9).

Commercially available nucleic acid probe assays for GAS are a recent development. Limited investigations have been performed with nucleic acid probes for detection of GAS from specimens enriched for 4 to 12 h in broth cultures (4, 8). An even more recent development is a commercial kit for performance of tests for GAS directly with pharyngeal swabs. The purpose of this study was to evaluate this kit, the GP-ST, in comparison with culture and a streptococcal antigen test.

The performance characteristics of the GP-ST in the study were excellent, particularly in comparison with those of the TP-ST. For specimens in the study that were tested by all three methods, the sensitivities for GP-ST, TP-ST, and culture were 93.5, 76.3, and 99.5%, respectively. The specificities of all methods were >99%.

Although the performance characteristics of the GP-ST are excellent, it is not clear what role this test should have or will have in the marketplace. The GP-ST is very easy to perform and is especially well adapted to batching. Despite significantly greater sensitivity than the TP-ST, the GP-ST assay cannot replace streptococcal antigen tests for point-of-care testing. The GP-ST assay requires water baths or heating blocks at two different temperatures and a luminometer for interpretation. These requirements, as well as the 2-h total performance time, make the assay unacceptable for most physician office laboratories or emergency room-clinic settings.

For larger laboratories, in particular very large-volume commercial laboratories, the more rapid turnaround time compared with culture coupled with the potential labor savings of batching may elicit interest in the GP-ST. This interest will obviously be strongly influenced by the cost of this assay, which has not been established by Gen-Probe.

If the GP-ST assay is priced comparably to direct streptococcal antigen assays, it may replace direct streptococcal antigen assays in situations in which direct streptococcal antigen assays are currently batched for same-day reporting. One advantage of the GP-ST is a numerical result, eliminating the need for any subjective interpretation of visual end points. In our laboratory, we batch the TP-ST several times each day. Our physicians have expressed a strong interest in

TABLE 2. Comparison of culture with GP-ST and TP-ST for detection of GAS

| Test | No. of results ^a | | | | Sensitivity (%) | Specificity (%) | Predictive value of: | |
|---------|-----------------------------|----------------|---------------|----------------|-----------------|-----------------|----------------------|-----------------|
| | True positive | False negative | True negative | False positive | | | Positive result | Negative result |
| Culture | 214 | 1 | 593 | 0 | 99.5 | 100 | 100 | 99.8 |
| GP-ST | 201 | 14 | 591 | 2 | 93.5 | 99.7 | 99.0 | 97.8 |
| TP-ST | 164 | 51 | 591 | 2 | 76.3 | 99.7 | 98.8 | 92.1 |

^a There were 215 true-positive results. The prevalence of positive test results was 26.6%.

TABLE 3. RLU values for all true-negative and true-positive test results

| No. of RLU ^a | No. of true-negative results | No. of true-positive results |
|-------------------------|------------------------------|------------------------------|
| <1,999 | 199 | 1 |
| 2,000-2,499 | 396 | 5 |
| 2,500-2,999 | 169 | 4 |
| 3,000-3,499 | 43 | 5 |
| 3,500-3,999 | 18 | 3 |
| 4,000-4,499 | 11 | 2 |
| 4,500-4,999 | 2 | 4 |
| 5,000-5,499 | 0 | 3 |
| 5,500-5,999 | 0 | 7 |
| 6,000-6,499 | 0 | 2 |
| 6,500-6,999 | 0 | 5 |
| 7,000-7,499 | 0 | 3 |
| >7,500 | 1 | 220 |

^a All values below the gap are interpreted as positive test results.

a same-day definitive test for GAS. Rather than a TP-ST with a sensitivity of 70 to 80% with all negative test results confirmed by culture, the physicians prefer a finalized same-day result. The interest in this approach to patient management certainly reflects the compliance and follow-up issues of the patient population that we serve.

In comparison with our experience with other Gen-Probe assays, we found the GP-ST easier to perform. This is most noticeable when the GP-ST is compared with assays with radioactive labels, such as the *Mycoplasma pneumoniae* assay. The GP-ST is also simpler to perform than the Pace 2 assay for *Chlamydia trachomatis*.

We have previously conducted two studies, evaluating broth enrichment techniques designed to facilitate same-day reporting of specimens collected in the morning or afternoon (1, 3). The sensitivity of the GP-ST in this study is comparable to those of the broth enrichment methods which we have previously evaluated.

The ideal use of the GP-ST may be as a definitive replacement for testing of specimens for which both a direct streptococcal antigen assay and culture are ordered. Speculation on the cost effectiveness of this approach is complicated because the cost of the GP-ST has not been established. If priced comparably to the direct streptococcal antigen assays, a GP-ST would certainly be less expensive to perform than a direct streptococcal antigen assay and a culture of a direct streptococcal antigen assay-negative specimen. This approach obviously is predicated on the acceptance of the GP-ST as a replacement for culture. There is no consensus as to what the sensitivity of a test must be to be an acceptable replacement for culture. We suspect that most experts would consider a sensitivity of 90 to 95% compared with culture acceptable.

An approach to enhancing the sensitivity of the GP-ST would be to alter the cutoff between negative and positive test results. The present cutoff of 4,500 RLU, as shown in Table 3, yielded only 3 false-positive GP-ST results among 836 true-negative test results (specificity, 99.6%). The number of test results falling around the 4,500-RLU value, listed in 500-RLU increments, is given in Table 3. These data demonstrate how well the GP-ST differentiates true-positive from true-negative specimens. Ninety-one percent of all true-negative tests had <3,000 RLU, while 83% of all true-positive tests had >7,500 RLU. The mean for specimens with >7,500 RLU was 817,046 RLU.

On the basis of our data, if the cutoff for a positive test result were lowered from 4,500 to 3,500 RLU, the sensitivity of the GP-ST would increase from 92.4 to 94.3%; however, the specificity would drop from 99.6 to 96.3%. There would also be a decrease in the predictive value of a positive test result from 98.8 to 88.4%, a decrease that we deem unacceptable. The data from this study support the cutoff value of 4,500 RLU.

Another approach to improving sensitivity would be to establish an indeterminate range and culture the pledgets from specimens falling in that range. In this study, there were 35 specimens (3.2% of all specimens) with values between 3,500 and 4,499 RLU. Six of these 35 specimens were culture positive on SSA as well as pledget culture positive. On the basis of the data from this study, culturing of the pledgets from all specimens with values of 3,500 to 4,499 RLU would require 30 to 35 cultures per 1,000 tests. We believe that this selective culture technique is merited if the increased sensitivity demonstrated in this study (92.4% without the indeterminate zone; 94.7% with the indeterminate zone) can be substantiated by further studies. If this protocol were followed, approximately 99% of all test results would remain unchanged by culture of the pledget. In this study, pledgets were cultured in Todd-Hewitt broth overnight before subculture to SSA and tryptic soy agar with 5% sheep blood. However, our experience with enrichment broths for GAS in previous studies leads us to believe that a shorter incubation period may be adequate (1, 3).

The performance characteristics of comparative test methodologies for detection of GAS are influenced by a variety of characteristics, including patient age, population selection, the prevalence of GAS in the population at the time of the study, and perhaps most importantly, the culture technique (6). Therefore, caution should be exercised in assessing the performance characteristics of a new assay, such as the GP-ST, on the basis of a study in one laboratory.

In two previous studies performed in our laboratory, the sensitivities of the TP-ST in comparison with culture on SSA were 72.6 and 70.4%. The prevalences of positive test results for these same two studies were 22 and 23%, respectively. In this latest study, the sensitivity of the TP-ST was 76% in comparison with culture on SSA while the prevalence of GAS was 26.6%. We do not know whether the variables which produced a higher sensitivity than previously observed in our laboratory for the TP-ST also positively influenced the GP-ST.

Additional studies of the GP-ST are needed to evaluate its clinical performance more fully. Evaluations of different patient populations, in particular those with lower prevalences of GAS-positive cultures, are needed.

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