Effect of Medium and Cultivation Conditions on Comparisons between Latex Agglutination and Culture Detection of Group A Streptococci

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In the laboratory diagnosis of pharyngitis, results from latex agglutination tests (LAT) performed directly on throat swabs are often compared with the isolation of group A beta-hemolytic streptococci (GABHS) from simultaneously obtained swabs cultivated on a variety of media under different atmospheric conditions. In this study, results of an LAT, Directigen, were compared with those of two different media: sheep blood agar (SBA) and group A selective strep agar (ssA). Specimens inoculated on SBA were incubated in three different atmospheres: air, 3 to 5% CO₂, and anaerobically. Those inoculated on ssA were incubated in 3 to 5% CO₂ only. Isolation of GABHS was confirmed by coagglutination. The standard for true positivity was the isolation of GABHS from at least one of the simultaneous cultures. Comparisons were made with samples from 693 adult patients. GABHS was isolated on at least one of the three cultures in 143 patients, demonstrating an isolation rate of 20.6%. LAT exhibited a sensitivity of 95.1%. SBA incubated in air, in CO₂, or anaerobically had sensitivities of 86.2, 85.9, and 93.7%, respectively. The ssA detected 99.3% of the positive specimens. Single SBA culture proved to be inferior to LAT and therefore was a poor standard for measuring LAT performance. Single ssA cultures demonstrated the greatest sensitivity in GABHS detection and therefore could serve as a standard for measuring LAT performance.

The advent of latex agglutination tests (LAT) promises to simplify laboratory diagnosis of streptococcal pharyngitis. These simple, rapid methods allow for documentation of a specific etiological agent 1 to 2 days sooner than do standard culture methods. Depending on the LAT, group A beta-hemolytic streptococci (GABHS) can be detected within 7 min to 1 h after receipt of a specimen. This contrasts with 24 to 48 h to detection by standard culture techniques.

Recently, a number of reports have appeared in the literature evaluating LAT (5, 9, 13). In each case, the performance of a particular LAT was compared with results obtained from a single throat culture. However, controversy continues regarding optimal media and incubation conditions for the isolation of GABHS for throat cultures (2, 6, 11).

In this study, we compared an LAT, Directigen (Hynson, Westcott and Dunning, Baltimore, Md.), with sheep blood agar (SBA) prepared by our laboratory incubated in three different atmospheres: air, 3 to 5% CO₂, and anaerobic conditions. We also included in this comparison selective strep agar (ssA; BBL Microbiology Systems, Cockeysville, Md.) for GABHS, reported by some investigators to be superior to SBA (4). The ssA combines SBA with antibacterial agents colistin, crystal violet, and trimethoprim-sulfamethoxazole. The goal of this study was to demonstrate the impact of the reference method on the interpretation of new product evaluation.

Dual pharyngeal specimens were collected on Dacron swabs (Culturette; Marion Scientific Corp., Kansas City, Mo.) from adult patients at the Medical College of Virginia Hospital emergency room and transported to the clinical microbiology laboratory. Specimens were inoculated with both swabs simultaneously on the day of receipt randomly onto two SBA plates (trypctic soy agar [Difco Laboratories, Detroit, Mich.] with 5% sheep blood) and ssA in that order. Throat swabs were inoculated on the first quadrant of each plate. This inoculum was streaked for isolation of colonies with a wire loop so that all four quadrants of the plate were covered. A total of 253 duplicate SBA culture were incubated in air and anaerobically in an anaerobe jar (Scott Laboratories, Inc., Richmond, Calif.) and 440 cultures were incubated in approximately 5% CO₂ (candle jar) and under anaerobic conditions. All 693 specimens were inoculated onto ssA plates in the same manner and incubated in approximately 5% CO₂. The temperature of incubation for all plates was 35°C. Following an 18- to 24-h incubation period, each plate was examined for the presence of beta-hemolytic colonies. Such colonies were subcultured to fresh SBA plates. Because of its selective nature, beta-hemolytic colonies on ssA were identified from the primary plate without subculturing. Identification was confirmed in all cases by Phadebact (Pharmacia Diagnostics, Piscataway, N.J.) coagglutination testing.

The Directigen protocol, which followed manufacturer instructions, was as follows. A swab from each patient was placed in 0.5 ml of Directigen enzymatic extraction reagent, agitated for approximately 5 s, and incubated for 60 min at 35°C. Each swab was pressed and rolled against the side of its test tube for maximum recovery of liquid and discarded. A 50-μl sample of each extracted specimen was placed on a glass slide from the Directigen kit in the circles designated anti-group A latex and control latex for each sample. One free-falling drop (approximately 15 μl) of anti-group A streptococcus latex was dispensed on each circle containing extracted specimen and designated anti-group A latex. Positive and negative controls provided by the LAT kit were also tested. After mixing each extract and latex suspension,
TABLE 1. Comparison of the sensitivities of LAT and single-culture techniques in the detection of GABHS with a three-culture standard

<table>
<thead>
<tr>
<th>Method of detection</th>
<th>Total no. of specimens</th>
<th>Ratio of positive results (\times 100)</th>
<th>Sensitivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Directigen</td>
<td>693</td>
<td>136/143</td>
<td>95.1</td>
</tr>
<tr>
<td>SBA-air</td>
<td>253</td>
<td>50/58</td>
<td>86.2</td>
</tr>
<tr>
<td>SBA-CO₂</td>
<td>440</td>
<td>73/85</td>
<td>85.9</td>
</tr>
<tr>
<td>SBA-anaerobiosis</td>
<td>693</td>
<td>134/143</td>
<td>93.7</td>
</tr>
<tr>
<td>ssA</td>
<td>693</td>
<td>142/143</td>
<td>99.3</td>
</tr>
</tbody>
</table>

\* Number positive/total number positive by any of the three culture methods applied to each specimen.

we placed the slide on a mechanical rotator under a moistened humidifying cover and rotated it for 4 min. Aglutination reactions were read immediately thereafter. Positive and negative controls were conducted with each batch of specimens.

GABHS were detected and isolated from 20.6% (143/693) of the specimens submitted. Fifty-eight positive cultures occurred among the 253 specimens cultured aerobically, anaerobically, and on ssA, whereas 85 positive cultures were detected among the 440 specimen cultured in CO₂, under anaerobic conditions, and on ssA. There was no statistical difference in the positive rates between these two subpopulations (chi-square and Fisher exact tests) or between them and the total population of patients. The sensitivities of GABHS detection obtained by each culture method (Table 1) were as follows: air, 86.2% (50/58), CO₂, 85.9% (73/85), anaerobiosis, 93.7% (134/143) and 99.3% (142/143) with ssA. There was one specimen in which GABHS were detected on only the anaerobe plate, and four occurred in which ssA was the only successful means of detection by culture. There were no positive air or CO₂ cultures which were not positive by at least one of the other methods. Of 143 Directigen-positive specimens, 136 (95.1%) were confirmed by at least one of the three culture techniques applied to each specimen. This true-positive rate of LAT was significantly higher than the true-positive rate of SBA in air \((P = 0.03\) by chi-square test) and that of SBA in 3 to 5% CO₂ \((P = 0.015\) by chi-square test). The Directigen LAT also detected two true-positive cultures missed by SBA incubated anaerobically. Only ssA detected more true-positive cultures than did the LAT. The sensitivities of anaerobic SBA, the LAT, and ssA were not statistically significantly different. There were three instances each in which only ssA and the LAT were positive and only SBA in anaerobic conditions and ssA were positive.

Years of research have resulted in a lack of a consensus regarding a standard method of culturing for the recovery of GABHS from throat specimens. This becomes a problem when comparing a new technique, i.e., LAT, with culturing in the laboratory diagnosis of GABHS. Murray et al. (10) recommended aerobic incubation. Their study revealed no significant difference in the isolation rates of GABHS between the three atmospheres. A continuation of this study demonstrated higher isolation rates by aerobic incubation (7). In a study by Pien et al. (11), comparing SBA incubation in CO₂ and anaerobically yielded no statistical difference in the recovery rates of GABHS, but CO₂ was preferred because less technical time and expense were required. Conversely, other authors have recommended anaerobic incubation to enhance the expression of the oxygen-labile beta-hemolysin streptolysin 0 (1, 8) and to suppress the growth of inhibitory viridans group streptococci (12). A recent study by Carlson et al. (4) described ssA as being a superior tool for GABHS isolation. Overnight anaerobic incubation followed by a second overnight incubation period in an enriched CO₂ atmosphere was also described (3).

Commercially available LAT kits reported in recent literature have been evaluated as to their feasibility for the rapid detection of GABHS. In each case, evaluations were based on results obtained from a single throat culture. With some authors, culture occurred in air (5), with others it occurred in CO₂ (9), and yet others favor anaerobic conditions (13). The possibly differing sensitivities of the various culture methods in different studies introduce uncertainty in interpretation of LAT results. This study addresses that uncertainty. The three commonly used atmospheres for incubation of SBA were studied in addition to ssA, the selective medium. The sensitivities of a single culture in air, CO₂, and anaerobically were 86.2, 85.9, and 93.7%, respectively. Directigen was more sensitive (95.1%) than any of these three approaches to culture on SBA. Thus, if a single SBA culture in any of these three atmospheres is used as a standard with which Directigen is compared, true-positive LAT results would be improperly defined as false-positive results. The LAT was, indeed, statistically significantly more sensitive than single culture in air or CO₂. This difference may be attributed in part to our failure to stab SBA plates after streaking. In this study, only the ssA medium was more sensitive than the LAT, thus qualifying as a more suitable standard for comparison.

In summary, quantitation of the sensitivity of the Directigen LAT is determined by the culture method with which it is compared. Of the four culture techniques examined, only the ssA medium was a single-culture standard more sensitive than the antigen test under evaluation. This finding is of interest because several widely quoted evaluations of Directigen and other antigen detection tests for GABHS used single SBA culture standards.

LITERATURE CITED


