Suitability of a Throat Culture Method for Evaluation of Group A Streptococcal Antigen Detection Kits

JAMES A. KELLOGG, PH.D., DAVID A. BANKERT, B.S., AND JOHN S. LEVISKY, M.A., M.S.

Previous reports have indicated a wide variation in observed sensitivity of antigen-detection kits for group A streptococci. Before undertaking an evaluation of these new kits, the sensitivity of the throat culture technic routinely used by this laboratory was reexamin ed. Each throat swab was directly inoculated to sheep blood agar containing trimethoprim–sulfamethoxazole (SXT·BA) and drug-free sheep blood agar (SBA) plates. Swabs were then washed in saline and the saline used to inoculate one more of each type of medium. SXT· BA cultures were incubated aerobically (5 to 10% CO₂), and SBA cultures were incubated anaerobically, both for two days at 35 °C. From 726 patients, 164 (22.6%) of the specimens contained group A streptococci, 99% detected on directly inoculated cultures and 100% on cultures inoculated with the saline wash. Either an aerobically (CO₂) incubated SXT·BA or an anaerobically incubated SBA, directly inoculated and held for two days, appears to offer a satisfactory reference culture method for the recovery of group A streptococci.

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IN PREVIOUSLY PUBLISHED reports, the sensitivity of commercially available kits for detection of group A streptococcal antigen has ranged widely from 62 to 95%. One possible explanation for the variation in these results is the variation in culture technology employed in the studies.

Radetsky and Todd37 have indicated that the meaningful evaluation of any new diagnostic test depends on the investigator’s ability to confidently determine the presence or absence of disease by the best reference test possible, or the “gold standard.” Although the agar culture method has been the most practical procedure for determining the presence of group A streptococci in the throat, it is probably one of the least standardized technics in the field of clinical microbiology. The routine inoculation of each throat swab to two or more media has been found to significantly improve the recovery of Streptococcus pyogenes, and yet only one of the published studies31 on the antigen-detection kits used more than one plate per swab. Incubation of the cultures for a second day has frequently been reported to substantially increase the yield of group A streptococci, but only five of the antigen studies incubated their cultures for more than one day. One report has indicated that only about 3–5% of the bacteria on seeded swabs were recovered from each swab-inoculated agar plate.10 In that study, inoculating the broth with which the swabs were moistened after direct plate inoculation resulted in up to a tenfold increase in number of colony-forming units (CFU) from each swab. Sautter and colleagues found a 24% increase in recovery of group A streptococci by placing throat swabs in sterile saline, mixing thoroughly, and inoculating the saline onto blood agar plates.42

Before undertaking an evaluation of the antigen-detection kits, the throat culture technic routinely used by this laboratory was reevaluated. The results of direct inoculation versus a saline wash of the swab, as well as the effect on recovery of medium type, atmosphere, and duration of incubation, were analyzed so that a suitable reference culture method could be documented for subsequent evaluation of the rapid serologic tests.

Materials and Methods

Oropharyngeal specimens from patients (90% of them children) with pharyngitis were collected on rayon-tipped Culturette® swabs (Marion Scientific, Kansas City, MO). The specimen swabs were processed by the laboratory within four hours of receipt. Each swab was first inoculated in a random fashion to two culture plates: a 5% sheep blood agar plate containing trimethoprim and sulfamethoxazole (SXT·BA) and a 5% sheep blood agar plate (SBA) plate without anti-microbial agents (Scott Laboratories, Fiskeville, RI), using a 360 degree roll of the swab over about 20% of the agar surface. The swabs were then immersed in tubes containing 0.3 mL sterile, 0.9% saline, vortexed for 5 seconds and removed from the saline, expressing as much fluid as possible back into each tube. All of the fluid remaining in each tube was equally divided between a second set of randomly inoculated SXT·BA and SBA plates. All four plates from each swab (two directly inoculated; two inoculated with the saline wash) were streaked for isolation. Each SXT·BA culture was

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incubated aerobically (5–10% CO₂) and each SBA culture was incubated anaerobically at 35 °C.

Cultures that did not contain group A streptococci after overnight incubation were reincubated in their original atmospheres for another 24 hours. Group A streptococci were identified either with a fluorescent antibody procedure (Clinical Sciences, Inc., Whippany, NJ) or with the Streptex® latex-bound antibody test (Wellcome Reagents, Ltd., Beckenham, England). The quantitative recovery of the organism was categorized as either 1–9, 10–99, or greater than or equal to 100 colonies per plate. The Z-test for differences between proportions was used for statistical analysis of the results.8

**Results**

Between May 7 and July 30, 1985, 726 specimens from patients with pharyngitis were processed according to the study protocol and 164 (22.6%) were found to contain group A streptococci. There was no significant difference between the 163 isolates recovered from those plates directly inoculated with the swabs and the 164 recovered from a saline wash of the same swabs after two days of incubation (Table 1).

At least one of the two plates had positive results within 24 hours from 155 (95%) of the swabs found to contain group A streptococci after direct swab inoculation and from 153 (93%) of the same swabs after inoculation of the saline wash of the same swabs after two days of incubation (Table 1).

<table>
<thead>
<tr>
<th>Culture Result*</th>
<th>No. (%) of Positive Cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct Swab Inoculation</td>
<td>Saline Wash of Swab</td>
</tr>
<tr>
<td>Both SXT-BA and SBA positive in 24 hours</td>
<td>133 (82)</td>
</tr>
<tr>
<td>SXT-BA positive in 24 hours; SBA positive in 48 hours</td>
<td>0</td>
</tr>
<tr>
<td>SXT-BA negative in 48 hours; SBA positive in 24 hours</td>
<td>0</td>
</tr>
</tbody>
</table>

The quantitative recovery of *S. pyogenes* did not vary significantly between the two methods of inoculation (direct or saline wash) or the two media-atmosphere combinations (Table 2). More than 80% of the cultures with group A streptococci contained 100 or more colonies of the organism per plate. High colony counts were also frequently encountered when recovery of the organism on either type of medium directly inoculated with a swab

<table>
<thead>
<tr>
<th>Type of Culture*</th>
<th>No. (%) of Positive Cultures with Following Number of Colonies per Plate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct inoculation</td>
<td></td>
</tr>
<tr>
<td>SXT-BA (158)</td>
<td>3 (2)</td>
</tr>
<tr>
<td>SBA (161)</td>
<td>8 (5)</td>
</tr>
</tbody>
</table>

Saline wash

<table>
<thead>
<tr>
<th>Type of Culture*</th>
<th>No. (%) of Positive Cultures with Following Number of Colonies per Plate</th>
</tr>
</thead>
<tbody>
<tr>
<td>SXT-BA (158)</td>
<td>6 (4)</td>
</tr>
<tr>
<td>SBA (160)</td>
<td>9 (6)</td>
</tr>
</tbody>
</table>

SXT-BA = 5% sheep blood agar cultures with sulfamethoxazole and trimethoprim incubated aerobically (5 to 10% CO₂); SBA = 5% sheep blood agar cultures (drug-free) incubated anaerobically.

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**Table 1. Recovery of Group A Streptococci from Swabs Directly Inoculated and Washed in Saline**

<table>
<thead>
<tr>
<th>Culture Result*</th>
<th>Direct Swab Inoculation</th>
<th>Saline Wash of Swab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Both SXT-BA and SBA positive in 24 hours</td>
<td>133 (82)</td>
<td>133 (82)</td>
</tr>
<tr>
<td>SXT-BA positive in 24 hours; SBA positive in 48 hours</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SXT-BA negative in 48 hours; SBA positive in 24 hours</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Table 2. Quantitative Recovery of Group A Streptococci**

<table>
<thead>
<tr>
<th>Type of Culture*</th>
<th>1–9</th>
<th>10–99</th>
<th>≥100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct inoculation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SXT-BA (158)</td>
<td>3 (2)</td>
<td>24 (15)</td>
<td>131 (83)</td>
</tr>
<tr>
<td>SBA (161)</td>
<td>8 (5)</td>
<td>21 (13)</td>
<td>132 (82)</td>
</tr>
</tbody>
</table>

Saline wash

<table>
<thead>
<tr>
<th>Type of Culture*</th>
<th>1–9</th>
<th>10–99</th>
<th>≥100</th>
</tr>
</thead>
<tbody>
<tr>
<td>SXT-BA (158)</td>
<td>6 (4)</td>
<td>22 (14)</td>
<td>130 (82)</td>
</tr>
<tr>
<td>SBA (160)</td>
<td>9 (6)</td>
<td>20 (13)</td>
<td>131 (82)</td>
</tr>
</tbody>
</table>

SXT-BA = 5% sheep blood agar cultures with sulfamethoxazole and trimethoprim incubated aerobically (5–10% CO₂); SBA = 5% sheep blood agar cultures (drug-free) incubated anaerobically.

* Numbers within parentheses indicate numbers of positive cultures.
that 8-50% of the inoculum could be recovered on each
after two days of incubation but positive results from the
Escherichia coli) Collee and associates.10 Those authors reported that only
the quantitative results of the direct inoculation and those
saline wash of the swab yielded one colony of 5.
A streptococci, using an incubation time of 48 hours and
3-5% of the 1,000 or more organisms (anaerobes and
ism, 92 (53%) and 146 (83%) were detected on the
SXT • BA and SBA cultures, respectively, after direct swab
inoculation with the saline wash.

Discussion

Direct swab inoculation of either medium (SXT • BA
or SBA) appeared to be effective for the recovery of group
A streptococci, using an incubation time of 48 hours and
the atmospheres employed in this study. The only spec-
imen that had negative results by direct swab inoculation
after two days of incubation but positive results from the
saline wash of the swab yielded one colony of S. pyogenes
on one plate (SXT • BA) after 48 hours.

These data showing a very high correlation between
the quantitative results of the direct inoculation and those
of a wash of the swab could be expected to miss as much as 16% of the group
A streptococci was reported from saline used to
wash throat swabs after direct plate inoculation. The swabs
and saline were inoculated to single SBA plates, incubated aerobically (5% CO₂), and examined at 24 and 48 hours.
In the present study, only 1% increase (one additional positive culture) in recovery of group A streptococci re-
sulted from the use of the saline wash. These differing
results may be due to our use of an inhibitory medium
(SXT • BA) in the aerobic (5-10% CO₂) environment and
our incubation of SBA cultures anaerobically.

Several reports have indicated a significantly improved
detection of group A streptococci on SXT • BA plates in-
cubated in air, air supplemented with 5-10% CO₂, or ox-
gen-free conditions15,21,22 This improved recovery ap-
parently resulted from suppression of other aerobic or
facultatively anaerobic bacteria. A study by Dykstra and
colleagues,11 however, concluded that SXT • BA plates
could be expected to miss as much as 16% of the group
A streptococci in throat specimens, especially if cultures
were incubated anaerobically.

Similarly conflicting reports have appeared concerning
the relative merits of the atmosphere of incubation. Mur-
ray and associates35 reported that the isolation of S. py-
genese was equivalent in aerobic, aerobic with 3-5% of
CO₂, and anaerobic atmospheres using drug-free SBA.
More recently, however, Libertin and colleagues25 found
that a higher percentage of group A streptococci were re-
covered on aerobically incubated SBA than on the same
medium incubated in either of the other two atmospheres.
On the other hand, an anaerobic atmosphere used for one
or two days of incubation of either SXT • BA or SBA cul-
tures has been found to provide significantly greater re-
cover of S. pyogenes in numerous reports.1,2,11,16,22,24,38

In the present study, no attempt was made to compare
the efficiency of either SXT • BA or SBA incubated in each
possible atmosphere because of the volume of such data
already available. Selection of SXT • BA incubated aer-
obically (5-10% CO₂) and SBA incubated anaerobically
was made because, from a review of the literature, these
conditions appeared to offer an optimal potential for re-
cover of group A streptococci. The findings of this study,
indicating an equivalent detection of the organism with

<table>
<thead>
<tr>
<th>Culture Discrepancies*</th>
<th>1–9</th>
<th>10–99</th>
<th>≥100</th>
</tr>
</thead>
<tbody>
<tr>
<td>SXT • BA positive; SBA negative</td>
<td>0</td>
<td>2 (100)</td>
<td>0</td>
</tr>
<tr>
<td>SBA positive; SXT • BA negative</td>
<td>3 (60)</td>
<td>2 (40)</td>
<td>0</td>
</tr>
<tr>
<td>SBA positive in 24 hours; SXT • BA positive in 48 hours</td>
<td>1 (6)</td>
<td>5 (29)</td>
<td>11 (65)</td>
</tr>
<tr>
<td>Both SXT • BA and SBA negative in 48 hours; both positive in 48 hours</td>
<td>0</td>
<td>1 (17)</td>
<td>5 (83)</td>
</tr>
<tr>
<td>Total (30)</td>
<td>4 (13)</td>
<td>10 (33)</td>
<td>16 (53)</td>
</tr>
</tbody>
</table>

*SXT • BA = 5% sheep blood agar cultures with sulfamethoxazole and trimethoprim incubated aerobically (5-10% CO₂); SBA = 5% sheep blood agar cultures (drug-free) incubated anaerobically.
* Numbers within parentheses indicate numbers of positive cultures.
either medium–atmosphere combination, appear to support this selection.

One culture parameter on which most authors (and the current study) agree is the superiority of the recovery of group A streptococci when most media are incubated for a second day. Kurzinski and van Holten, although noting a significant increase in SXT–BA-positive cultures after 48 hours in an aerobic (CO₂) atmosphere, found that all positive cultures were detected within only one day on the same medium in an anaerobic environment. Their results are similar to those in the present study (Table 1), in which 24 of 158 (15%) of the group A streptococci recovered from aerobically (CO₂) incubated SXT–BA, but only 7 of 161 (4%) of those detected on anaerobically incubated SBA were detected only after the second day at 35 °C, after direct-swab inoculation.

It has been reported that there is no single optimal method for recovery of S. pyogenes: any one method by itself is frequently inadequate. Saslaw and Streifeld found that from 20 to 33% of all cultures with S. pyogenes would have been missed if a single culture, not duplicate plates, had been used, regardless of which medium was used alone. In contrast, however, detection sensitivities of 98–100% for single-culture systems have been reported. The results of the present study support those findings, since 97% and 99% of the group A streptococci were recovered from aerobic (CO₂)-SXT–BA and anaerobic-SBA, respectively, after direct-swab inoculation and incubation for 48 hours.

Perhaps the most controversial question related to throat culture and streptococcal antigen detection technology has been the relative clinical significance attached to small numbers of colonies of S. pyogenes recovered in culture. It appears inappropriate to consider the throat culture as a quantitative assay. In the present study, the great majority (87%) of cultures found to yield counts of greater than or equal to 100 colonies per plate. These findings are very similar to those of numerous reports published previously. It is quite apparent from these studies that technic plays a major role in the outcome of a throat culture, in determining whether it will be falsely negative as well as in the relative number of colonies on positive cultures. Factors that have a direct bearing on the quantitative recovery (or false-negative cultures) of the pathogen include the thoroughness of specimen collection, the use of multiple swabs, antibiotic pretreatment and the rapid response of S. pyogenes to antibiotics, poor technic in inoculating or processing the cultures, and the preincubation of specimen swabs in broth, in addition to the previously discussed considerations of selection of media, atmospheres, and duration of incubation. Perhaps because of these variations in technique, it has been observed that differentiation of infection from the group A streptococcal carrier state cannot be made on the degree of culture positivity alone; there is no consistent relationship between the number of colonies on a positive culture and the subsequent antibody response in the patient.

In selecting a culture method for evaluation of the sensitivity of group A streptococcal antigen-detection methods, a prime consideration should be that the reference culture technic has a well-documented sensitivity of at least 95% (acknowledging that many positive cultures come from patients merely colonized) to ensure accurate calculations of antigen test sensitivity, specificity, and predictability of results. From the results of the present study, as well as those of many of the previously mentioned reports, the selection of a single SXT–BA incubated aerobically (5–10% CO₂) or SBA incubated anaerobically appears to provide adequate sensitivity. Neither two plates per specimen nor a saline wash of the swab provided significantly increased recovery of S. pyogenes. Two days of incubation, however, especially in an aerobic atmosphere, appear to be essential.

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References


