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Evaluation of Sulfamethoxazole-Trimethoprim Blood Agar Plates for Recovery of Group A Streptococci from Throat Cultures

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We compared the selective blood agar medium of Gunn et al. (J. Clin. Microbiol. **5**:650-655, 1977) which contains sulfamethoxazole plus trimethoprim (SXT-BA) to the conventional blood agar surface plate (SBA) and a modified blood agar pour plate plus broth method for the recovery of group A streptococci from throat swabs. The influence of CO_2 and ambient air incubation of the SXT-BA and SBA plates was also evaluated. A total of 696 throat swabs from symptomatic children were cultured simultaneously by the five methods and observed after overnight incubation; 204 positive cultures were detected overall. Recovery rates of each individual method were: SXT-BA (CO_2), 90.7%; SXT-BA (air), 87.7%; pour plate plus broth, 83.3%; SBA (CO_2), 79.4%; and SBA (air) 77%. Approximately one-half of the false-negative cultures in the SXT-BA (CO_2) and SXT-BA (air) methods had colony counts of $\geq$10 to 100 colonies per plate. In contrast, for the SBA (CO_2), SBA (air), and pour plate plus broth methods, approximately 70% of the false-negative cultures had colony counts of $\geq$10 to 100/plate. False-positive cultures obtained by the SXT-BA (CO_2) and SXT-BA (air) methods were 11 and 12.7%, respectively—one-half as high as the rates obtained by the remaining methods. Beta-hemolytic streptococci, groups C, F, and G, are inhibited on the SXT-BA plates and were the primary cause of the higher false-positive rates on SBA and pour plate plus broth methods. An additional 3% positive cultures were obtained by incubating SXT-BA (CO_2) plates up to 48 h before discarding as negative. We recommend either the SXT-BA (CO_2) or the SXT-BA (air) method with up to 48 h of incubation for routine use in throat cultures.

Recently, Gunn et al. (6) introduced a new selective surface plating medium, which contains sulfamethoxazole plus trimethoprim (SXT-BA), for the enhanced recovery of group A streptococci from throat cultures. We have compared the SXT-BA plate to the conventional sheep blood agar (SBA) method and to a modification of a pour plate plus broth (PP+B) method. We also found the SXT-BA plate to be superior to the SBA plate in this and in a previous study (8a). The SXT-BA was also more sensitive than the modified PP+B method for the isolation of group A streptococci.

MATERIALS AND METHODS

SXT-BA and SBA plates were made in our laboratory by using Trypticase soy agar (Baltimore Biological Laboratories [BBL]). The stock solution of sulfamethoxazole plus trimethoprim (Burroughs Wellcome Co.) was made as originally described (6), except that the final volume was made up to 1 liter instead of the recommended 100 ml. This greater dilution was used to avoid working with the suspension of the two drugs that occurs with lesser dilution. Quantities of sulfamethoxazole-plus-trimethoprim stock solution were stored at −20°C until the day of use. For the SXT-BA plates, 10 ml of stock solution was added per liter of agar after melting but before autoclaving. After autoclaving and cooling to 55°C, 7% sheep blood was added, and 15 ml of SXT-BA was dispensed into plastic petri plates (100 by 15 mm). SBA plates were prepared in the same manner, but without the sulfamethoxazole plus trimethoprim. Plates were stored under refrigeration for up to 1 week, but we have found them to be stable for at least 2 weeks if kept in a plastic bag.

A total of 696 throat swabs (cotton tipped) from symptomatic children between the ages of 2 and 15 years old were cultured. Specimens were submitted in a modified Stuarts transport medium by physicians located throughout Wisconsin. Most specimens were in transit only 1 day, but some were 2 days old upon culture.

Each swab was first inoculated upon two SBA and then upon two SXT-BA plates which were then streaked for isolation. Several stabs were made in both the primary and secondary areas of streaking to promote beta hemolysis. One set of plates consisting of an SBA and an SXT-BA plate was incubated at 37°C under 5 to 10% CO_2 [SBA (CO_2) and SXT-BA

(CO_2)]; the second set was incubated at 37°C under ambient atmosphere [SBA (air) and SXT-BA (air)]. This was done to determine whether the CO_2 incubation originally used (6) was necessary. Swabs were then used for the PP+B culture method.

It has been determined that by swabbing a single plate directly with the swab, approximately 3 to 5% of the organisms present are removed from the swab (2). Since four plates were swabbed, approximately 12 to 20% of the organisms had been removed before the swab was used in the PP+B method. Practically speaking, performing the PP+B method last should have had a negligible effect on the detection and quantitation of group A streptococci by the PP+B method.

The PP+B method used was a modification recommended to our laboratory by Elaine Updyke (then at the Center for Disease Control, Atlanta, Ga.). We did not use either preincubation of the swabs in broth as is now recommended for greater sensitivity (4), or surface streaking of plates. Swabs were placed in 3 ml of Todd Hewitt broth and mixed vigorously on a Vortex mixer for 5 s. Next, a sterile 6-inch (ca. 15.2-cm) applicator stick was inserted into the broth, removed, and reinserted into a tube of melted veal infusion agar (15 ml/tube; BBL). The agar had been cooled to 55°C and contained 7% defibrinated sheep blood. Approximately 0.001 ml of inoculum was transferred from the broth to the melted agar. The agar tube was then mixed by inversion, poured into plastic petri plates, and allowed to solidify. Pour plates and corresponding broth cultures were incubated at 37°C overnight in room air.

All throat swabs were cultured before noon each day and observed for beta-hemolytic streptococci-like colonies by 8:30 a.m. the following day. Plates were observed by using a fluorescent light source located behind the upheld plate.

All cultures positive for beta-hemolytic streptococci-like colonies were screened for group A streptococci by using a standard fluorescent-antibody (FA) technique (9). Smears were made from the broth culture used in the PP+B method, as is usual for that method, or by making a direct smear from isolated colonies on one of the surface streak plates (3). The Lancefield precipitin test was used to confirm our FA results whenever differences were noted in the ability of the various methods to recover group A streptococci.

In general, for all methods group A streptococci colonies were quantitated on the basis of the following categories: negative; <10; 10 to 100; and >100 colonies per plate. However, if <10 colonies per plate were only visible on a surface streaked plate but they occurred in the area of the secondary streaking, the colony count was recorded as being 10 to 100 colonies per plate. Additionally, if 1 to 100 colonies were visible on the streak plate but they occurred in the area of the third or fourth cross streak, the colony count was recorded as being >100 per plate. This policy was felt to be the most consistent and representative but it did result in reporting higher colony counts than were actually observed with the SBA (CO_2) and SBA (air) methods.

The quantitative recovery rates are presented as a rough comparison by using a nonstandardized inoculum for each of the five media. However, preliminary studies in our laboratory have shown that when a throat swab is plated to the surface of six plates, there was no detectable difference in either the types of bacteria isolated or in their relative numbers.

RESULTS

Of the 696 throat swabs cultured, 204 (29.3%) were positive for group A streptococci by at least one of the five methods used for each specimen. No single method recovered all of the 204 group A streptococci recovered by all five methods as a whole (Table 1). The SXT-BA (CO_2) method yielded the highest recovery rate (90.7%) followed by the SXT-BA (air) (87.7%), the PP+B (83.3%), the SBA (CO_2) (79.4%), and the SBA (air) (77%) methods.

Colony counts obtained by surface streak plate methods were generally higher than those obtained by the PP+B method (Table 1). This was to be expected, however, from the considerably larger inoculum size used in the former methods and is an advantage of the surface streak plate methods over the PP+B method.

It is important to note that the colony counting procedure described in Materials and Methods resulted in our reporting higher colony counts by the SBA methods than were actually visible. If, however, the actual number of colonies present are tabulated, the enhanced sensitivity of the SXT-BA method is even more

TABLE 1. *Recovery rates of group A streptococci from 696 throat cultures by five culture methods*

Colony count of group A streptococci	No. of positive throat cultures for group A streptococci as detected by each culture method[a]				
	SXT-BA (CO_2)	SBA (CO_2)	SXT-BA (air)	SBA (air)	PP+B
<10	12	27	10	11	42
10 to 100	28	12	30	14	27
>100	145	123	139	132	101
Total positive cultures	185 (90.7)	162 (79.4)	179 (87.7)	157 (77)	170 (83.3)

[a] Values in parentheses indicate percent based on a total of 204 cultures positive for group A streptococci as detected by all five methods as a whole.

apparent: there were 77 instances noted in which the SXT-BA (CO_2) method raised the colony count by one or more quantitation categories over that obtained by the SBA (CO_2) method. Similarly, there were 52 such instances noted in which the SXT-BA (air) method raised the colony count by one or more quantitation categories over that obtained by the SBA (air) method.

Table 2 shows the level of colony counts which went undetected by each method (false-negative cultures). Significantly, only about one-half of the false-negative cultures which occurred for the SXT-BA (CO_2) and SXT-BA (air) methods showed high colony counts (i.e., 10 to 100 or >100 colonies per plate). In contrast, approximately 70% of the false-negative cultures which occurred for the SBA (CO_2), SBA (air), and PP+B methods were in the high range. That is, the SXT-BA (CO_2) and SXT-BA (air) methods were the least likely methods to yield false-negative results when the colony counts were high. False-negative cultures where the colony count was <10 colonies per plate probably represent chance variation expected when the swab contains low numbers of group A streptococci; we have, therefore, excluded them from consideration.

Throat cultures which yielded beta-hemolytic streptococci-like colonies that proved not to be group A streptococci were designated false-positive cultures. The SXT-BA (CO_2) and SXT-BA (air) methods yielded the lowest rates of false-positive cultures, 11% and 12.7% respectively (Table 3). In contrast, for the SBA (CO_2), SBA (air), and PP+B methods, the rates of false-positive cultures were 27%, 23.8%, and 22.7%, respectively. Both the SXT-BA (CO_2) and the SXT-BA (air) methods reduced by one-half the number of unnecessary FA staining tests performed on non-group A beta-hemolytic streptococci-like colonies.

Beta-hemolytic streptococci, groups C, F, and G, are inhibited on the SXT-BA plates and were the primary cause of the higher false-positive rates on SBA and PP+B plates. In addition, beta-hemolytic gram-negative rods and beta-hemolytic staphylococci also were responsible for significant numbers of false-positive cultures by the PP+B method.

In a separate study using the SXT-BA (CO_2) method alone, we found that the isolation rate of group A streptococci was significantly increased by incubating plates up to 48 h before discarding as negative. Of 2,536 SXT-BA (CO_2) plates which were negative after overnight incubation, an additional 77 isolates (3%) were positive after an additional day of incubation. In three-fourths of the cases, the colony counts were >10 per plate.

DISCUSSION

It is clear that a positive throat culture for group A streptococci is not a definite indication of streptococcal pharyngitis even in symptomatic patients (12). In most cases of streptococcal pharyngitis, a positive throat culture will show colony counts of >100 colonies per plate by the direct surface streak plate technique.

TABLE 3. *False-positive cultures obtained by each culture method*

Method	Total no. of false-positive cultures[a]
SXT-BA (CO_2)	23 (11)
SBA (CO_2)	60 (27)
SXT-BA (air)	26 (12.7)
SBA (air)	49 (23.8)
PP+B	50 (22.7)

[a] A false-positive culture is a culture which yielded beta-hemolytic streptococci-like colonies that proved not to be group A streptococci. Values in parentheses indicate percent, determined by dividing the number of instances in which a given method detected beta-hemolytic streptococci-like colonies into the number of instances in which the isolates were found to be other than group A streptococci, and then multiplying by 100.

TABLE 2. *Magnitude of colony count of group A streptococci which were undetected by each culture method*

Corresponding highest colony count of group A streptococci observed by a positive method	No. of false-negative cultures[a]				
	SXT-BA (CO_2)	SBA (CO_2)	SXT-BA (air)	SBA (air)	PP+B
<10	10	12	11	12	10
10 to 100	5	17	8	21	20
>100	4	13	6	14	4
Total no. of false-negative cultures	19 (9.3)	42 (20.6)	25 (12.3)	47 (23)	34 (16.7)

[a] A false-negative culture is one which is negative for group A streptococci by the method indicated but which was positive by at least one of the remaining 4 methods. Values in parentheses indicate percent.

However, occasionally the same colony counts may be found in cultures from carriers of group A streptococci (12). Practically speaking, however, if a positive culture is from a symptomatic patient, most physicians will treat, regardless of the amount of group A streptococci found.

The use of sulfamethoxazole plus trimethoprim in throat culture media to reduce the inhibitory effect of normal throat flora bacteria on the growth and/or beta hemolysis of group A streptococci was excellently discussed by Gunn et al. (6). This selectivity of SXT-BA plates is the reason for their superiority over the SBA plate. The superiority of the SXT-BA method over the PP+B method that we found in this study is due both to the selectivity of the SXT-BA method and to the larger inoculum size it employs. The minimal inoculum size recommended for the PP+B method is based upon two assumptions. First, that use of a small inoculum size will prevent normal flora bacteria from overgrowing and, thereby, from preventing detection of group A streptococci which can occur by using a heavier inoculum size. Second, that colony counts will always be high in cases of streptococcal pharyngitis. From our own experience with the PP+B method, the first assumption appears to be correct. In many throat cultures there is a very heavy growth of alpha-hemolytic streptococci, facultative gram-negative rods, and *Staphylococcus aureus*. These organisms often overgrow and prevent detection of the group A streptococci unless a smaller inoculum size is used, as in the PP+B method (10). The second assumption, however, contradicts the previous report of Kaplan et al. (7) who found that one-third of the patients with pharyngitis had less than 10 colonies per plate, but had a serological response indicative of infection. Our data indicate that the PP+B method with its low inoculum size is obviously too insensitive to detect these cases. In fact, Pike (10) has calculated that there would have to be 4,000 colony-forming units of a pure culture of streptococci per ml of broth before there was a 98% probability that a 0.001-ml portion contained streptococci. Preincubation of swabs for 2 h in broth followed by plating has been shown to yield up to 17% more positive cultures than can be obtained by immediate plating (9). We did not use this enrichment technique, since we feel that it is too expensive and impractical for routine use and that it may yield misleading information by failing to differentiate between the carrier and the infected state (11). This differentiation might ultimately be made by direct, selective quantitation of group A streptococci in throat cultures, such as by using the SXT-BA (CO_2) method. In this manner, it may be possible to show a closer correlation between high colony counts and a positive serological response than observed by Kaplan et al., who used a nonselective plating method.

However, Gordis et al. (5) found in their study that one-third of the patients who develop rheumatic fever had been recently cultured and had negative results. The negative cultures were probably due to variables such as poor collection technique, inadequate survival and/or overgrowth in transport media, and poor culture and isolation techniques. This indicates that microbiologists should do all they can to recover as many group A streptococci as possible.

Although the 11.4% greater recovery rate of group A streptococci obtained with the SXT-BA (CO_2) method as compared to the SBA (CO_2) was significant, it was not as great as the 28% to 42% values previously reported (6). The reason for these differences may be related to the fact that our throat cultures were all from children, whereas 60% of the patients studied by Gunn et al. were adults. It is possible there may be differences between the concentrations of normal flora and group A streptococci colony-forming units found in infected children and infected adults. More recently, Gunn has found that the differences in his isolation rates, using SXT-BA (CO_2) and SBA (CO_2) plates, are now running between 11 to 20%—much closer to what we encountered in this study (personal communication).

An apparent paradox of the SXT-BA method is that 92% of group A streptococci, when tested in thymidine-free media, cannot grow in the presence of the 25 μg of sulfamethoxazole-trimethoprim per ml used in the SXT-BA medium (1). However, the Trypticase soy agar used for the SXT-BA plates contains thymidine, which group A streptococci are able to utilize, circumventing the metabolic inhibitory effect of 25 μg of sulfamethoxazole-trimethoprim per ml. Viridans streptococci and other normal throat flora bacteria are less able to use the thymidine and are thus generally inhibited (S. R. M. Bushby, personal communication).

Unfortunately, the precise amount of thymidine required to achieve the optimal selective effect is unknown. Various commercial media have been shown to vary markedly in thymidine content (8), and only Trypticase soy agar has been proven to be reliable for the SXT-BA (CO_2) method (6). Of some concern is the finding that various lots of Trypticase soy broth also vary considerably in thymidine content (S. R. M. Bushby, personal communication). Our data showed that when incubation was limited to 24

h, in 9 instances the SXT-BA (CO_2) method failed to detect group A streptococci in quantities of ≥10 to 100 colonies per plate, a false-negative rate of 4.4%. Gunn et al. (6) reported a false-negative rate of 1.4% (7/508) for the SXT-BA (CO_2) method. His value is considerably less than what we observed and may indeed be due to differences in thymidine content among different lots of commercial Trypticase soy agar used.

We found, however, that incubating our SXT-BA (CO_2) plates for up to 48 h resulted in an additional 3% recovery of group A streptococci. The 1.4% false-negative rate we could expect, then, after 2 days of incubation is identical to that reported by Gunn et al. using 24 h of incubation of SXT-BA (CO_2) plates. Thus, prolonging the incubation of SXT-BA (CO_2) plates to 48 h may reduce the effects of variable thymidine content in lots of Trypticase soy agar. In view of the already discussed importance of using as sensitive a culture method as possible, microbiologists may wish to include an SBA (CO_2) plate to detect an additional 1.4% positive cultures.

In our experience, use of *Streptococcus pyogenes* ATCC 19615 (Bactrol, Difco Laboratories) to serve as a positive control for growth and for beta hemolysis, as has been suggested (6), is satisfactory. It may be possible and desirable, however, to accurately establish the amount of thymidine necessary to add to a thymidine-free basal medium to maximize inhibition of normal throat flora by sulfamethoxazole-trimethoprim, without inhibiting growth of any group A streptococci.

Our laboratory processes an average of 37,000 throat cultures per year. By using the SXT-BA (CO_2) method instead of the PP+B method in our laboratory, we have calculated a minimum savings of $5,000 per year. This savings is based upon elimination of the accompanying broth culture and extra materials needed in the PP+B method and upon saving an average of at least 8 s of work time per culture. The SXT-BA (CO_2) method is also considerably less tedious and requires less hand manipulations than the PP+B method. Additionally, we find that smears made directly from colonies on SXT-BA (CO_2) plates for FA staining generally show greater numbers of fluorescing cells, and individual cell walls stain more uniformly positive than with the PP+B method (3). Based upon the substantial economic and logistic advantages and upon the superior sensitivity for detection of group A streptococci, we recommend the SXT-BA (CO_2) throat culture method of Gunn et al. (6). Hopefully, commercial media producers will offer SXT-BA plates for laboratories unable to prepare their own.

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