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NOTES

Improved Reliability of the Primary Plate Bacitracin Test on Throat Cultures with Sulfamethoxazole-Trimethoprim Blood Agar Plates

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The primary plate bacitracin differentiation disk susceptibility test identified 85% of group A streptococci from throat cultures on SXT-BA(CO_2) plates within 24 h, as compared to only 26% on a conventional sheep blood agar plate.

In a study soon to be published, our laboratory will report on the improved isolation rate of group A streptococci from throat cultures using the selective sulfamethoxazole-trimethoprim blood agar SXT-BA(CO_2) plate of Gunn et al. (5). We now report on the improved reliability of the primary plate bacitracin differentiation disk susceptibility test for rapid identification of group A streptococci from throat cultures using the SXT-BA(CO_2) method.

SXT-BA and conventional sheep blood agar (SBA) plates were prepared by our method (manuscript in preparation), using Trypticase soy agar (Baltimore Biological Laboratory). A total of 804 throat swabs from symptomatic children between the ages of 2 and 15 years old were cultured. Specimens were mailed in and had been in transit for up to 2 days in modified Stuart's transport medium before being cultured.

Swabs were first inoculated upon two SBA and then upon two SXT-BA plates, all of which were then streaked for isolation and stabbed several times. A bacitracin differentiation disk (Difco Laboratories) was applied to the center of the primary inoculation area on one of both types of plates. Incubation was at 37°C under 5 to 10% CO_2. All plates were incubated up to 2 days before being discarded as negative.

After overnight incubation, all four plates were observed for beta-hemolytic streptococci, and bacitracin susceptibility results were read. Bacitracin test results were interpreted as follows: any zone of inhibition of beta-hemolytic streptococci indicated that the isolate was a group A streptococcus; no zone of inhibition of beta-hemolytic streptococci indicated a non-group A streptococcus. Occasionally yeast and nonhemolytic or weakly beta-hemolytic gram-negative rod colonies occurred within the zone of inhibition of beta-hemolytic streptococci. These colonies were easily identified macroscopically or with the use of a Gram-stained smear and were disregarded in the interpretation of the primary plate bacitracin test results. If growth of beta-hemolytic streptococci was too sparse to accurately detect presence of a zone of inhibition, or if overgrowth by normal flora bacteria prevented reading of a zone, the bacitracin test was repeated by using the conventional pure subculture technique.

Plates showing beta-hemolytic streptococci-like colonies were screened for group A streptococci by using a standard fluorescent-antibody (FA) technique (3). Smears for FA staining were made from a lightly turbid suspension of several colonies. The Lancefield precipitin test was only used to group isolates for which the bacitracin and FA staining results disagreed with one another.

Group A streptococcus colonies were quantitated by our method (manuscript in preparation), using only the colony counts actually observed for comparisons.

In total, both culture methods detected 201 cultures positive for group A streptococci, a positivity rate of 25.1%. Of the beta-hemolytic streptococci isolated on the SXT-BA(CO_2) plate, 82.2% were group A, as compared to 66.8% on the SBA plate (Table 1). The SXT-BA(CO_2) plate detected 199 of a possible 201 cultures positive for group A streptococci, a false-negative rate of 1%. One of these false negatives had a colony count of >100 per plate, and the second had a colony count of <10 per plate. The SBA plate, in comparison, only detected 149 of a

TABLE 1. *Relative effectiveness of the SXT-BA(CO_2) and SBA methods in detecting group A streptococci in throat cultures*

Method	Total no. of cultures positive for beta-hemolytic streptococci	Group A streptococcus cultures			
		Total no.[a]	% Detected[b]	False negative	
				Total no.	Rate (%)[b]
SXT-BA(CO_2)	242	199 (82.2)	99	2	1
SBA	223	149 (66.8)	74	52	26

[a] Each value in parentheses indicates percentage based on total number of cultures positive for beta-hemolytic streptococci.

[b] Each value indicates percentage based on total of 201 cultures positive for group A streptococci as determined by both methods.

possible 201 cultures positive for group A streptococci, yielding a false-negative rate of 26%. Of the SBA false-negative cultures, 86% had colony counts of 10 to 100 colonies per plate by the SXT-BA(CO_2) method. The 25% increased isolation rate observed for the SXT-BA(CO_2) method as compared to the SBA method is in closer agreement with the 28 to 42% range reported by Gunn et al. (5) than we found in another study (manuscript in preparation).

When the primary plate bacitracin test was performed on SXT-BA(CO_2) plates, 85% of the group A streptococci were correctly identified after overnight incubation, and 14% had to be tested by replating (Table 2). In all but two instances, replating had to be performed because the number of colonies of group A streptococci were too few to accurately detect the presence of a zone of inhibition by the bacitracin disk. In the two instances in which colony counts were adequate, overgrowth of normal flora prevented reading of the zone. In contrast, when the primary plate bacitracin test was performed on SBA plates, only 24% of the group A streptococci could be correctly identified after overnight incubation, and 50% had to be replated. In this case, replating was always due to overgrowth by normal flora bacteria.

Previously, it has been shown that the primary plate bacitracin test on SBA plates can accurately identify 56 to 63% of group A streptococci from throat cultures (6, 8). Our data for the primary plate bacitracin test on SXT-BA(CO_2) plates significantly exceeds these figures. Inhibition of overgrowth by normal flora bacteria is the obvious reason for the superiority of the primary plate bacitracin test results on SXT-BA plates versus those on SBA plates. The 24% reliability of the primary plate bacitracin test on SBA plates we observed is considerably less than the 56 to 63% that others have reported (6, 8). This difference is probably primarily due to our use of mailed-in specimens in which group A streptococci may have been significantly

TABLE 2. *Identification of group A streptococci by the bacitracin disk susceptibility test*

Method	No. of streptococci		
	Group A, correctly identified[a]		Non-group A, bacitracin positive[b]
	Primary plate	Secondary plate	
SXT-BA(CO_2)	171 (85)	28 (14)	5 (11.6)
SBA	48 (24)	101 (50)	5 (6.8)

[a] Values in parentheses indicate percentages based on 201 cultures positive for group A streptococci as determined by both methods.

[b] Values in parentheses indicate percentages based on total number of non-group A beta-hemolytic streptococci isolated by each method.

overgrown by normal flora bacteria. Although overgrowth by normal flora does frequently occur even when throat swabs are plated after only a few hours of being taken, overgrowth could reasonably be greater for mailed-in specimens that were in transit for 1 to 2 days. Additionally in our study, the isolation rate of group A streptococci on SBA plates was compared to that found by the selective SXT-BA(CO_2) method. In other studies, this was not the case (6, 8). When based only upon the number of group A streptococci isolated by the SBA method alone, the primary plate bacitracin susceptibility test correctly identified 32.1% after overnight incubation.

The rates of bacitracin-positive test results for non-group A beta-hemolytic streptococci were 11.6 and 6.8% for the SXT-BA(CO_2) and SBA methods, respectively. These figures are in general agreement with those of Facklam et al. (4), who reported that 6 to 7.5% of the non-group A beta-hemolytic streptococci will be bacitracin positive. Since the SXT-BA(CO_2) method isolated 43 non-group A beta-hemolytic streptococci, we could expect up to three of them to be bacitracin positive (we observed five). Since the

SBA method isolated 74 non-group A beta-hemolytic streptococci, we could expect up to six of them to be bacitracin positive (we observed five).

We did not detect any group A streptococci which were not susceptible to bacitracin, which is to be expected from the 0.5% incidence previously reported (4, 7). Additionally, as judged by the duplicate plates free of bacitracin disks, in no case did the bacitracin disk on the primary plate completely inhibit minimal growth of group A streptococci. This observation contradicts one objection raised by Murray et al. (6) on performing the primary plate bacitracin susceptibility test.

Finally, it should be noted that we found two isolates of beta-hemolytic streptococci which were interpreted as FA negative because they fluoresced weakly but which were group A streptococci by the Lancefield precipitin and bacitracin tests. We also found two strains of beta-hemolytic streptococci which were interpreted as FA positive but which were bacitracin negative and group G by the Lancefield precipitin test.

In conclusion, we believe that the primary plate bacitracin differentiation disk susceptibility test performed on SXT-BA(CO_2) plates is very reliable for the detection and identification of group A streptococci from throat cultures. Primary plate bacitracin susceptibility testing on SXT-BA(CO_2) plates offers a 24-h advantage for early diagnosis and treatment of streptococcal pharyngitis. This is unlikely to affect the duration or symptoms of pharyngitis (3) or even the incidence of rheumatic fever (1). However, it is a convenience most physicians will appreciate, since it will increase the likelihood of parents returning their children for proper therapy and does save most of the time and materials required for the conventional bacitracin test. The major disadvantage of using SXT-BA(CO_2) plates is that, at present, there is no commercial source of prepoured plates.

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