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Evaluation of Anaerobic Incubation for Recovery of Group A Streptococci from Throat Cultures

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No statistical differences were found in the recovery of group A streptococci from throat culture specimens after overnight incubation of blood agar plates in 5% CO_2 compared with anaerobiosis. Anaerobic incubation required many more subcultures and resulted in considerably greater technical time and expense.

Although throat cultures are considered essential for the diagnosis of group A streptococcal pharyngitis, reports have disagreed on the optimal culture medium to be utilized and the necessity for anaerobic incubation. Murray et al. found that sheep blood agar containing gentamicin was inferior to plain blood agar and that group A streptococcal recovery was equivalent in three atmospheres (air, 3 to 5% CO_2, and anaerobiosis) after both 24 and 48 h of incubation (8). Dykstra et al. (2) reported that blood agar containing trimethoprim and sulfamethoxazole did not produce the higher yield of group A streptococci from throat specimens reported by other investigators (4, 6). Dykstra et al. also found that blood agar plates produced a significantly higher number of group A streptococci after overnight anaerobic incubation than when they were incubated aerobically (2).

While performing an evaluation of primary plating media for throat cultures (9), our laboratory staff noted that anaerobic incubation produced statistically higher yields of both group A and non-group A beta-hemolytic streptococci than did incubation in 95% air-5% CO_2 (unpublished data). However, there were certain methodological problems with our initial evaluation, including: (i) our specimens were received by mail with a 2- to 4-day transportation delay from several interisland public schools; (ii) because of the large number of cultures processed (4,000), half plates were inoculated directly with Dacron throat swabs; and (iii) only 70% of beta-hemolytic, bacitracin-susceptible isolates were serologically grouped. To eliminate these problems, we repeated an atmospheric incubation study by using 725 outpatient and hospital patients (usual time between collection and plating, 2 to 4 h). Dacron swabs (Culturette; Marian Health and Safety) were blended in a Vortex mixer in 0.5 ml of Todd-Hewitt broth, and 0.1 ml of this broth was used to inoculate each of two plates of 5% sheep blood agar (Bennett-Bakte). These plates were streaked with a wire loop in four directions, and stabs were made in the initial streak area of the plate to be incubated in 5% CO_2. Since bacitracin sensitivity is the commonly employed presumptive test for group A streptococci and these bacteria are rarely completely resistant to 0.04 U of bacitracin (10), a Taxo A disk (BBL Microbiology Systems) was placed in the area of initial streaking. The blood agar plates were then incubated at 35°C in either an anaerobic jar (GasPak; BBL) or a 5% CO_2 incubator (Labline). After overnight incubation, the plates were inspected for the presence of beta-hemolytic streptococci and for a zone of inhibition around the bacitracin disk. Beta-hemolytic streptococci were quantitated in a manner modified from Breese et al. (1) and Kaplan et al. (5), as follows: rare, 1 to 10 colonies of beta-hemolytic streptococci; 1+, 11 to 50 colonies of beta-hemolytic streptococci, comprising less than 25% of the total colonies on an agar plate; 2+, 11 to 50 colonies of beta-hemolytic streptococci, comprising 25 to 50% of the total colonies; 3+, >50 colonies, comprising 50 to 75% of the total colonies; 4+, >50 colonies, comprising more than 75% of the total colonies.

Inhibition of throat flora in anaerobic incubation was compared with growth in 5% CO_2 and graded roughly as 25, 50, 75, or 100% reduction. Suspicious streptococcal colonies were subcultured to sheep blood agar for hemolytic reaction and purified plate bacitracin testing. All beta-hemolytic, bacitracin-susceptible streptococci were grouped by the Rantz-Randall modification of the Lancefield precipitin procedure (3). Statistical analysis was performed by using the McNemar test for comparison of paired samples (7).

Table 1 compares isolation rates of beta-he-

TABLE 1. *Recovery of beta-hemolytic and group A streptococci from 725 throat culture specimens*

Incubation atmosphere	No. of isolates	
	Beta-hemolytic	Group A
5% CO_2–95% air	125	64
Anaerobic	206	72

TABLE 2. *Effect of incubation atmosphere on estimated recovery of group A streptococci*

Grade of recovery	No. of positive cultures	
	In CO_2	Anaerobically
Rare	8	6
1+	12	16
2+	9	9
3+	12	12
4+	23	29

molytic streptococci and group A streptococci grown in 5% CO_2 and under anaerobic conditions. A higher number of beta-hemolytic streptococci were isolated anaerobically than under 5% CO_2 ($P < 0.001$). Although there was a higher number of group A streptococci isolated anaerobically, this was not statistically significant at the 5% level and was much less than the 55% higher recovery noted by Dykstra et al. (2) and the 50% higher rate obtained in our unpublished initial study. Blood agar plates incubated anaerobically required considerably more subcultures for suspicious colonies than did cultures incubated in 5% CO_2 (311 versus 134 subcultures; $P < 0.001$). Table 2 shows the results of quantitating group A streptococci. Although more colonies were recovered under anaerobic conditions, there was no statistically significant difference in any quantitation category. Normal flora was grossly similar in amount when the two incubation atmospheres were compared.

Several factors seem to have contributed to the conflicting results obtained not only in our throat culture studies, but also in those by other investigators. These include differences in patient population, time between collection and processing of specimens, degree of bacterial overgrowth, method of streaking plates, means of identification of group A streptococci, and technologist skills. There is probably no single optimal throat culture method, and each laboratory should determine its own results with incubation atmospheres and culture media.

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