JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 1979, p. 236-238
0095-1137/79/02-0236/03$02.00/0

Vol. 9, No. 2

Comparison of Media and Techniques for Detection of Group A Streptococci in Throat Swab Specimens

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Received for publication 11 December 1978

Detection of group A streptococci in primary throat cultures was compared by using aerobic and anaerobic incubation with selective and nonselective media. Sheep blood agar plates incubated anaerobically detected 98% of the group A streptococci, whereas aerobically incubated blood agar plates which had been stabbed at the time of inoculation detected only 63%. Blood agar plates containing sulfamethoxazole and trimethoprim (23.75 and 1.25 μg per ml, respectively) detected only 70% of group A streptococci when incubated aerobically and 84% when incubated anaerobically.

The serious sequelae of group A streptococcal pharyngitis require that culture methods for detection of these organisms in throat specimens be as sensitive as possible. The Center for Disease Control recommends that throat swab specimens be inoculated into molten sheep blood agar from which pour plates are prepared (3). Anaerobiosis below the surface of the agar permits maximum expression of beta-hemolysis by subsurface colonies. McGonagle (7) found that routinely streaked blood agar plates (BAP) which were anaerobically incubated were as sensitive as agar overlay plates in detecting group A streptococci in throat specimens. A less laborious method in which BAP are swabbed with throat swab specimens and then streaked with inoculating loop and incubated aerobically was recommended by Wannamaker (13). The stabbing of the medium places some of the inoculum below the surface of the agar where more anaerobic conditions exist, providing some of the attributes of the pour plate method. Murray et al. (9) felt that stabbed BAP incubated aerobically were as sensitive as anaerobically incubated BAP for detecting group A streptococci. Furthermore, the stabbed aerobic plates were considered more specific for group A streptococci because fewer non-group A streptococci were isolated. Increased specificity of the primary plating medium might reduce cost because fewer cultures would demonstrate beta-hemolysis and require further identification.

Increasing the specificity of primary plating medium for throat cultures while maintaining, or even increasing, sensitivity has been attempted by adding antibiotics to BAP, thus making primary plating medium somewhat selective for group A streptococci (1, 2, 12). Gunn et al. (6) recently reported that incorporation of trimethoprim and sulfamethoxazole into BAP increased both sensitivity and specificity for isolation of group A streptococci.

The purpose of this study was to evaluate the importance of incubation atmosphere and to compare regular BAP with BAP containing sulfamethoxazole and trimethoprim (BAPSXT) for isolation of group A streptococci from throat swab specimens.

MATERIALS AND METHODS

BAP were prepared by adding defibrinated sheep blood (5% vol/vol) to Trypticase soy agar (Baltimore Biological Laboratory [BBL]). BAPSXT contained 23.75 μg of sulfamethoxazole and 1.25 μg of trimethoprim per ml and were prepared by the method of Gunn et al. (6). BAP and BAPSXT were 4-mm thick and were stored at 4°C in plastic bags until use.

All of the specimens examined in this study were routine collections submitted to our laboratory. Throat specimens were collected on 90% cotton-10% rayon swabs (Cheeseborough Ponds, Inc.), transported in Amies transport medium (Difco), and processed within 10 h of collection. Because two plates were inoculated from a single throat specimen, at weekly intervals the order of inoculation was reversed, thus assuring that one type of plate would not benefit from consistently being the first to be inoculated. After plates were inoculated, the swabs were put into tubes containing 2 ml of Streptosel broth (BBL). All plates and tubes were incubated at 35°C, and when anaerobiosis was required, plates were incubated in a GasPak jar (BBL). Hemolysis on each type of plate was determined daily by various technologists without the assistance of microscopy. About half of the plates (including those demonstrating beta-hemolysis) were rechecked at 40× with a stereomicroscope by one of the authors; in no instance was there a discrepancy be-

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tween the macroscopic and microscopic observation. After overnight incubation, Streptosel broths corresponding to specimens showing beta-hemolytic colonies resembling streptococci on one or both plates were examined by fluorescence microscopy for presence of group A streptococci (8) by using conjugate supplied by the Connecticut State Department of Health.

Sensitivity and specificity for each procedure were calculated by the method of Galen and Gambino (5). Sensitivity, defined as the frequency with which group A streptococci demonstrated beta-hemolysis when the particular procedure was used, was calculated by dividing the number of specimens exhibiting beta-hemolysis due to group A streptococci (as determined by FA) by the total number of specimens in which group A streptococci were detected.

Specificity, defined as the frequency that a procedure was negative (demonstrated no beta-hemolysis) in the absence of group A streptococci, was calculated by dividing the number of specimens not demonstrating beta-hemolysis and not containing group A streptococci by the number of specimens in which group A streptococci were not detected.

RESULTS

A total of 1,263 throat swab specimens were inoculated onto two BAP. One BAP was routinely streaked, stabbed a few times in all quadrants (without flaming the inoculating loop), and incubated aerobically. The other BAP was routinely streaked and incubated anaerobically. Of the 1,263 throat cultures, 500 demonstrated beta-hemolysis on one or both of the plates. By using fluorescence microscopy it was apparent that 149 of the throat cultures demonstrating beta-hemolysis contained group A streptococci, whereas the remaining 351 did not. Stabbed BAP which had been incubated aerobically detected only 63.1% (94/149) of the group A streptococci, whereas anaerobically incubated BAP detected 98.0% (146/149) (Table 1). Aerobic stabbed BAP detected far fewer beta-hemolytic non-group A streptococci than did anaerobic BAP. The anaerobic BAP demonstrated 69.2% (771/1,114) specificity, whereas the aerobically incubated BAP with stabs were 92.3% (1,028/1,114) specific.

An additional 578 throat swab specimens were inoculated and stabbed onto anaerobically incubated BAP and BAPSXT which were incubated aerobically. Of the 578 throat cultures, 244 were beta-hemolytic on one or both kinds of media and of the 244 beta-hemolytic cultures, 81 were found to contain group A streptococci. Anaerobically incubated BAP detected 98.8% (80/81) of the group A streptococci, whereas the aerobic BAPSXT detected only 70.4% (57/81) (Table 2). The anaerobic BAP were considerably less specific (68.2%, 339/497) than the antibiotic-containing plates (92.1%, 458/497).

A total of 731 additional throat swab specimens were inoculated (and stabbed) onto BAP and BAPSXT; both plates were incubated anaerobically. Although anaerobic incubation improved the performance of BAPSXT, BAP still detected a greater percentage of group A streptococci (97.7%, 130/134) than did BAPSXT (83.6%, 112/134). Nonetheless, the BAPSXT were considerably more specific (80.2%, 479/579) for group A streptococci than were BAP (57.7%, 333/579) (Table 3).

DISCUSSION

The superiority of anaerobic incubation over aerobic incubation for detection of group A streptococci in throat cultures is well-documented (4, 7, 11, 14). The advantages of anaerobiosis are reportedly twofold: expression of hemolysis due to oxygen-labile hemolysins is permitted, and antagonism by other bacterial species is prevented (10) because many interfering

TABLE 1. *Comparison of anaerobically incubated BAP and stabbed BAP (incubated aerobically) for detection of group A streptococci in 1,263 throat swab specimens by beta-hemolysis*

Determination	Anaerobic BAP		Aerobic stabbed BAP	
	Beta-hemolysis	No beta-hemolysis	Beta-hemolysis	No beta-hemolysis
Group A	146[a]	3	94	55
No group A	343	771	86	1028

[a] Number of specimens.

TABLE 2. *Comparison of anaerobic BAP and aerobic BAPSXT for detection of group A streptococci in 578 throat swab specimens by beta-hemolysis*

Determination	Anaerobic BAP		Aerobic BAPSXT	
	Beta-hemolysis	No beta-hemolysis	Beta-hemolysis	No beta-hemolysis
Group A	80	1	57	24
No group A	158	339	39	458

TABLE 3. *Comparison of BAP and BAPSXT (both incubated anaerobically) for detection of group A streptococci in 731 throat swab specimens by beta-hemolysis*

Determination	Anaerobic BAP		Anaerobic BAPSXT	
	Beta-hemolysis	No beta-hemolysis	Beta-hemolysis	No beta-hemolysis
Group A	130	4	112	22
No group A	264	333	118	479

species do not tolerate anaerobic conditions. In the course of other studies in this laboratory (data submitted for publication), it has been noted that all of 500 beta-hemolytic streptococcal isolates in pure culture were capable of producing beta-hemolysis on the surface of aerobically incubated BAP. Thus it appears that bacterial interference is a far more important factor than is the oxygen-labile hemolysin.

This study fails to confirm observations by Murray et al. (9) that the marginal anaerobiosis achieved by stabbing the agar is sufficient for detecting group A streptococci. Our data does support their contention that stabbed aerobic plates are significantly more specific, and this could result in considerable cost savings to the laboratory, but sensitivity is sacrificed to gain this increase in specificity. This is not acceptable in view of the serious sequelae which may follow undiagnosed group A streptococcal pharyngitis.

These data fail to support the observations by Gunn et al. (6) that BAP containing trimethoprim and sulfamethoxazole are useful in detecting group A streptococci. Had Gunn and his associates compared BAPSXT incubated in CO_2 to pour plates or anaerobically incubated plates instead of to stabbed plates incubated in CO_2, their data probably would have been similar to ours. (It is unlikely that incubation of plates in CO_2 provided some advantage over aerobic and anaerobic incubation as performed in our study.)

In the studies of Gunn and his co-workers, and in our study, sulfamethoxazole and trimethoprim were added to blood agar prepared with commercial tryptic soy base which contains an unstandardized amount of thymidine. S. M. R. Bushby of Wellcome Laboratories has noted that group A streptococci will not grow on BAPSXT with a Mueller-Hinton base which contains no thymidine (personal communication). On the other hand, considering the mechanism of action of SXT, an excess of thymidine in the medium would yield no selectivity at all; and all throat flora would be permitted to grow. A problem with Gunn's and our studies is that the thymidine in the medium was not quantitated. Although the practice of using BAPSXT may eventually be sound, the optimum amount of thymidine is yet to be determined. Our data clearly show that BAPSXT prepared from commercially available tryptic soy base, regardless of incubation conditions, might be expected to miss as much as 16% of group A streptococci in throat cultures.

It is apparent from the data presented in our study that in order to achieve maximum sensitivity in isolating group A streptococci in throat cultures, one must employ media and techniques which provide a significant degree of anaerobiosis, such as anaerobically incubated BAP. Furthermore, BAPSXT is not sufficiently sensitive for detection of group A streptococci.

ACKNOWLEDGMENTS

We are indebted to the technologists in the Division of Microbiology at Hartford Hospital and to their section head, Jane Evers, for their assistance during this study. We thank S. R. M. Busby, Wellcome Laboratories, for the sulfamethoxazole and trimethoprim used in this study.

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