Evaluation of the Bacti-Lab Streptococci Culture Systems for Selective Recovery and Identification of Group A Streptococci

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Twenty-nine lots of Bacti-Lab streptococci culture plates containing nalidixic acid, neomycin, and amphotericin B were inoculated with 25 strains of group A, B, C, F, and G streptococci, 13 strains of nonstreptococcal organisms representative of throat flora, and 198 fresh clinical throat specimens to determine the accuracy of the plates in identifying group A streptococci. With the Bacti-Lab test plates 98.87% of the beta-hemolytic streptococcal strains tested produced growth, with 95.51% yielding more than 50 colonies. Of the nonstreptococcal beta-hemolytic and non-beta-hemolytic organisms tested, 79.54% were inhibited by the selective media. When bacitracin disks were used for presumptive identification of group A streptococci from clinical throat specimens, 12.00% false positive and 0.00% false negative results were obtained as measured by the direct fluorescent-antibody and Lancefield grouping methods.

It has been reported by Vincent et al. (13) that beta-hemolytic streptococci cultured from throat specimens on conventional sheep blood agar are sometimes not recovered or identified because of colonial overgrowth by normal throat flora. They report that the prime problem of overgrowth and obscuration of beta-hemolytic streptococcal colonies on plated media can be averted through the use of antibiotic-impregnated blood agar. When they incorporated neomycin and nalidixic acid into conventional blood agar they found better yields of beta-hemolytic streptococci. *Staphylococcus*, gram-negative rods, and *Neisseria* sp. were completely inhibited. This combination of neomycin and nalidixic acid did not appear to affect the growth of beta-hemolytic streptococci or their hemolytic activity. Even small numbers of beta-hemolytic streptococcal colonies were detectable on the selective media due to markedly improved visualization of colonies. Blanchette and Laurence (1) also reported the increased probability of detecting beta-hemolytic streptococci through the use of neomycin blood agar.

Bacti-Lab (Mountain View, Calif.) has introduced a commercially prepared plated blood agar containing antibiotics for selective isolation of beta-hemolytic streptococci under the name Bacti-Lab streptococcal culture systems. The systems use 5% sheep blood agar containing neomycin, nalidixic acid, and amphotericin B.

The ability of the systems to support growth of laboratory stock strains of beta-hemolytic

streptococci and to isolate and identify beta-hemolytic streptococci from fresh clinical pharyngeal specimens is evaluated in this report.

MATERIALS AND METHODS

Description of Bacti-Lab systems. The Bacti-Lab streptococci culture systems consist of sectioned plastic blood agar plates with accessories for collecting specimens (cotton swabs and ANAERO-DISCS [Bacti-Lab, Mountain View, Calif., patent no. 3,632,478]).

The four types of plates evaluated were the "Strep-plate", which contains one 54 mm by 45 mm section; the "Strep Duet" plate, which is divided into two 54 mm by 45 mm sections; and the "Strep-5" and "Strep-10" plates containing, respectively, 5 and 10 sections measuring 38 mm by 32 mm. Each section filled to a depth of 3 to 4 mm contains 5% sheep blood agar treated with neomycin, nalidixic acid, and amphotericin B (Fig. 1).

The blood agar base contains 2% soy and milk protein digest (Bacti-Lab), 0.5% NaCl, and 1.5% agar. A 2-ml amount of a solution containing 3 mg of neomycin sulfate (Sigma Chemical Co.) and 1.5 mg of nalidixic acid (Sterling-Winthrop) is added to 100 ml of tempered blood agar base for the antibiotic medium. The final concentration is 15 μ g of nalidixic acid and 30 μ g of neomycin per ml. To inhibit fungal growth amphotericin B (Squibb) is also added in a final concentration of 3 μ g/ml.

Stock cultures. Axenic stock cultures used in the evaluation were 24-h broth cultures containing approximately 1.5×10^8 organisms per ml as determined on a Coleman spectrophotometer. The stock cultures of group A, B, C, F, and G streptococci were isolated and identified in our laboratory, as were group A streptococcal T types 1, 3, 4, 5, 6, 9, 11, 12, and 28. The acid-extraction capillary precipitin

FIG. 1. Two types of Bacti-Lab streptococci culture plates showing growth on individual sections inoculated with clinical pharyngeal specimens. Beta-hemolysis resulting from group A streptococcal growth may be discerned in sections A and B in the top plate and sections C, D, E and F on the bottom plate.

method of Lancefield (7, 8, 9) was used to group streptococcal isolates and the T typing was carried out according to Moody et al. (11). Antistreptococcal grouping sera were obtained from Bioquest and T-typing sera from the Communicable Disease Center, Atlanta, Ga.

Other isolates used in the evaluation were *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Klebsiella pneumoniae* ATCC 13883, *Streptococcus pneumoniae* ATCC 6303, *Neisseria meningitidis* ATCC 13077, and *Candida albicans* ATCC 10231 obtained from the American Type Culture Collection, Rockville, Md. One isolate each of *Corynebacterium renale*, *Bacillus subtilis*, and *Pseudomonas aeruginosa* was supplied by Colorado State University, Fort Collins. Non-beta-hemolytic organisms were isolated from clinical cultures.

Stock cultures of beta-hemolytic streptococci were maintained at 4 C in Todd-Hewitt broth (Difco). Other stock cultures except *E. coli* and *N. meningitidis* were maintained at 4 C in Trypticase soy broth (BBL). *E. coli* was maintained in lauryl sulfate broth (Difco). *N. meningitidis* was held on Martin Lester agar and transferred to Trypticase soy broth before testing.

Inoculation and processing of test plates. Test plates supplied by the manufacturer were first evaluated for their ability to inhibit growth of stock cultures representative of normal throat flora and to produce growth with beta-hemolytic streptococci. The manufacturer's instructions for inoculating plates were observed with one exception. A 3-mm loop was substituted for the normally used cotton swab to provide a more consistent inoculum. The

inoculum was spread across the length of the entire section starting at the outside edge and streaked downward covering the section. This was made possible through the use of a specially fashioned loop. A 90° bend was made about 10 mm from the loop end followed by another 90° bend in the opposite direction close to the loop end. This formed an inverted capital L which allowed the loop to fit over the plastic rib barrier, insuring even spreading of the inoculum. After inoculation a 29-mm-square plastic disk (ANAERO-DISC) molded with a handle on top and a 2-mm prong on the underside was stabbed into the heaviest inoculated area and then placed securely on the moist agar surface to form an air-tight seal. In effect this simple device provided a convenient means of reducing oxygen tension. After air drying, a 6-mm bacitracin disk (Bioquest) containing 0.02 mg was placed below the ANAERO-DISC to aid in differentiating groups of beta-hemolytic streptococci (10). Concomitantly, as a purity and viability control, all stock cultures were inoculated with a 3-mm loop on quarter sections of 5% sheep blood agar without antibiotics (tryptic soy agar, Difco). Bacitracin disks were similarly placed on each inoculated quadrant.

A second series of tests was conducted with fresh clinical throat swabbings which had been inoculated onto test plates with cotton swabs. The placement of the ANAERO-DISC and bacitracin disk was carried out as described above. Both test and control plates were incubated aerobically for 24 h at 35°C and read visually without magnification using a 100-W microscope lamp. Beta-hemolytic streptococcal colonies on test plates were counted and resultant growth was recorded as "confluent" when more than 50 colonies grew out, "isolated" when 1 to 49 colonies appeared, and "none" when no colonies were evident. Because bacitracin disks on test plates were placed below rather than on the inoculated area, inhibition zones were measured on both test and control plates from the outer zone perimeter to the disk center, hereafter referred to as "half-zones." However sections were considered positive for group A streptococci when any zone of inhibition appeared around the bacitracin disk and negative when there was growth up to the disk or noninhibition. This method of interpretation, which has been used by others, considerably simplified the reading of results on test and control plates. The frequency of false positive results due to other groups of beta-hemolytic streptococci is discussed later (4).

Beta-hemolytic streptococci isolated from clinical specimens on test plates were subcultured on 5% sheep blood agar without antibiotics. Isolates were subcultured in Todd-Hewitt broth and grouped with the capillary tube extraction method of Lancefield (7, 8, 9) and the direct fluorescent-antibody method (5).

RESULTS

A total of 1,022 inoculations with 42 stock cultures were applied to 29 lots of test media. Seven-hundred and fourteen tests were made

with 25 strains of beta-hemolytic streptococci (Table 1). Confluent growth was obtained on 682 tests or 95.51%. Isolated growth was found on 24 tests or 3.36%. No growth was experienced in eight tests or 1.12%.

T type 1 group A streptococci failed to grow on two lots. One other lot was found toxic for group A T type 6 and 28 and one group G streptococcal strain. All 5% sheep blood agar control plates without antibiotics yielded at least 50 colonies per plate. Some growth of beta-hemolytic streptococci was therefore affected in 706 of 714 tests or 98.87%.

In addition 308 tests were conducted on test media with stock cultures of nonstreptococcal organisms and simulated clinical cultures (Table 2). In 40 tests or 12.98% confluent growth was obtained. Twenty-three tests or 7.46% produced isolated growth. In 245 tests or 79.54% growth was completely inhibited.

Test organisms most often producing confluent or isolated growth on test media were *Streptococcus pneumoniae* and an unknown non-beta-hemolytic clinical isolate. However, when they were mixed with stock beta-hemolytic streptococcal strains no difficulty was encountered in visually identifying beta-hemolytic streptococcal colonies in 15 of 15 trial tests. When *P. aeruginosa* was mixed with group A streptococci complete inhibition of *P. aeruginosa* and confluent growth of group A streptococci occurred in 14 of 15 tests. Control tests with identical cultures on conventional 5% sheep blood agar without antibiotics resulted in overgrowth by *P. aeruginosa* in 12 of 12 tests. Visualization of the group A streptococci was completely obscured. The *S. aureus* and *B. subtilis* strains produced isolated growth on two and four lots, respectively.

In the second study 198 clinical cultures were tested with Bacti-Lab plates (Table 3). Of the 198 clinical cultures, 192 were also tested with the rapid fluorescent-antibody method and 196 with the Lancefield grouping technique. Bacti-Lab test plates as well as control plates without antibiotics supported confluent growth with all clinical specimens tested.

Group A streptococci were isolated from 146 (73.73%), 121 (63.02%), and 121 (61.73%) specimens and grouped with the Bacti-Lab (using bacitracin disks), the fluorescent-antibody, and the Lancefield methods, respectively. Also grouped with the Lancefield method were 12 group B, 21 group C, 1 group F, 12 group G, and 29 nongroupable strains of beta-hemolytic streptococci (38.26%). Bacti-Lab plates with bacitracin disks identified as non-group A streptococci 52 strains (26.26%); 71 strains

TABLE 1. Growth patterns on 29 lots of Bacti-Lab streptococci culture plates containing neomycin, nalidixic acid, and amphotericin B challenged with 25 stock strains of group A, B, C, G, and F streptococci

Challenge streptococcal group	No. of lots tested	No. of tests	No. of tests yielding:		
			>50 colonies	1-50 colonies	0 colonies
Group A streptococci T type					
1	2	3	1 (33.33) ^a	0 (0.00)	2 (66.66)
3	29	42	42 (100.00)	0 (0.00)	0 (0.00)
4	29	42	40 (95.23)	2 (4.76)	0 (0.00)
5	29	42	42 (100.00)	0 (0.00)	0 (0.00)
6	29	42	37 (88.09)	4 (9.52)	1 (2.38)
9	28	39	37 (94.87)	2 (5.12)	0 (0.00)
11	5	5	5 (100.00)	0 (0.00)	0 (0.00)
12	29	42	41 (97.61)	1 (2.38)	0 (0.00)
28	29	42	40 (95.23)	1 (2.38)	1 (2.38)
	29				
Total	29	299 (100.00)	285 (95.31)	10 (3.34)	4 (1.33)
Group B streptococcal strain					
1	17	24	23 (95.83)	1 (4.16)	0 (0.00)
2	9	9	9 (100.00)	0 (0.00)	0 (0.00)
3	9	9	9 (100.00)	0 (0.00)	0 (0.00)
4	9	9	9 (100.00)	0 (0.00)	0 (0.00)
5	9	9	9 (100.00)	0 (0.00)	0 (0.00)
Group C streptococcal strain					
1	29	42	42 (100.00)	0 (0.00)	0 (0.00)
2	29	42	41 (97.61)	1 (2.38)	0 (0.00)
3	28	41	41 (100.00)	0 (0.00)	0 (0.00)
4	17	24	24 (100.00)	0 (0.00)	0 (0.00)
5	17	24	24 (100.00)	0 (0.00)	0 (0.00)
Group G streptococcal strain					
1	29	42	32 (76.19)	7 (16.66)	3 (7.14)
2	29	42	42 (100.00)	0 (0.00)	0 (0.00)
3	28	41	39 (95.12)	2 (4.87)	0 (0.00)
4	17	24	22 (91.66)	2 (8.33)	0 (0.00)
5	17	24	24 (100.00)	0 (0.00)	0 (0.00)
Group F streptococci					
	3	9	7 (77.77)	1 (11.11)	1 (11.10)
Total		415 (100.00)	397 (95.66)	14 (3.52)	4 (1.00)
Grand total		714	682 (95.51)	24 (3.36)	8 (1.12)

^a Numbers in parentheses indicate percentages.

(36.97%) were identified as non-group A with the fluorescent-antibody method. The false positive and negative rates as measured with the Lancefield method were 12.00% and 0.00% for the Bacti-Lab method and 1.29% and 0.00% with the fluorescent-antibody method.

During the study 13 different lots of bacitracin disks (Bioquest) were evaluated in 555 tests with group A, B, C, and G streptococci on Bacti-Lab plates (Table 4). Four of 53 tests (7.54%) with four lots of disks yielded false positive results with one strain of group B streptococci, producing average half-zones of 5.75 mm. Of 137 tests with three strains of group C streptococci, 34 (24.81%) yielded false positive results with eight lots of disks. Average half-zones were 4.64 mm. Also 27 of 128 tests (21.09%) with four strains of group G streptococci using six

lots of disks yielded false positive results with average half-zones of 5.67 mm. A total of 65 false positive results were found with the 13 lots of bacitracin disks (11.71%). The average half-zones found with group A streptococci on Bacti-Lab plates was 10.69 mm, as compared with a slightly smaller zone of 7.96 mm on control plates.

DISCUSSION

The Bacti-Lab streptococci culture systems were able to support the growth of 95 to 98% of all group A streptococci tested. Only 1% of test plates partly failed to support the growth of the 25 strains of beta-hemolytic streptococci tested. Some strains (T types 1, 6, and 28) seemed to be inhibited by certain lots of media. However, the overall high percentage of recovery (95.51%)

TABLE 2. Growth inhibition patterns on 29 lots of Bacti-Lab streptococci culture plates containing neomycin, nalidixic acid, and amphotericin B when challenged with nine single strains of nonstreptococci and eight mixed strains of streptococci and nonstreptococci

Challenge organism	No. of lots tested	No. of tests	No. of tests yielding:		
			>50 colonies	1-50 colonies	0 colonies
<i>Corynebacterium renale</i>	29	39	0 (0.00) ^a	0 (0.00)	39 (100.00)
<i>Escherichia coli</i> ATCC 25922	29	42	0 (0.00)	0 (0.00)	42 (100.00)
<i>Staphylococcus aureus</i> ATCC 25923	29	42	0 (0.00)	2 (4.76)	40 (95.23)
<i>Bacillus subtilis</i>	29	42	1 (2.38)	3 (7.14)	38 (90.47)
<i>Pseudomonas aeruginosa</i>	29	42	0 (0.00)	0 (0.00)	42 (100.00)
<i>Klebsiella pneumoniae</i> ATCC 13883	15	15	0 (0.00)	1 (6.66)	14 (93.33)
<i>Streptococcus pneumoniae</i> ATCC 6303	5	5	3 (60.00)	1 (20.00)	1 (20.00)
<i>Neisseria meningitidis</i> ATCC 13077	6	6	0 (0.00)	0 (0.00)	6 (100.00)
<i>Candida albicans</i> ATCC 10231	15	15	0 (0.00)	0 (0.00)	15 (100.00)
Mixed non-beta culture	15	16	4 (25.00)	6 (37.50)	6 (37.50)
Mixed non-beta and group A streptococci	14	14	7 (50.00)	6 (42.85)	1 (7.14)
Mixed non-beta and group A T type 12 streptococci and <i>Pseudomonas aeruginosa</i>	15	15	13 (86.00)	1 (6.66)	1 (6.66)
Mixed non-beta and group A T type 12 streptococci	15	15	12 (80.00)	3 (20.00)	0 (0.00)
Total		308	40 (12.98)	23 (7.46)	245 (79.54)

^a Numbers in parentheses indicate percentages.

TABLE 3. Determination of streptococcal group on 198 clinical specimens using three grouping methods

Streptococcal group isolated	Bacti-Lab method	Fluorescent-antibody method	Lancefield precipitin method
A	146 (73.73) ^a	121 (63.02)	121 (61.73)
B	0 (0.00)	0 (0.00)	12 (6.12)
C	0 (0.00)	0 (0.00)	21 (10.71)
F	0 (0.00)	0 (0.00)	1 (0.51)
G	0 (0.00)	0 (0.00)	12 (6.12)
Non-groupable	52 (26.26)	71 (36.97)	29 (14.79)
Total	198 (100.00)	192 (100.00)	196 (100.00)

^a Numbers in parentheses indicate percentages.

indicated the ability of most lots of media to support the growth of group A beta-hemolytic streptococci. The majority (79%) of nonstreptococcal beta-hemolytic and non-beta-hemolytic strains tested were inhibited by the selective media, with the exception of the *S. pneumoniae* strains and several unknown strains of non-beta-hemolytic organisms isolated from clinical specimens. However, when they were inoculated onto test plates together with beta-hemolytic streptococci, the latter produced luxuriant growth with distinct colonies resulting in unobscured visualization of colonies.

Although *P. aeruginosa* overgrew beta-hemolytic streptococcal strains on control media, it was completely inhibited in 86% of tests on Bacti-Lab media. This may indicate that some clinical cultures containing beta-hemolytic streptococci may escape detection on conventional blood agar because of masking by *P. aeruginosa*.

When tested with clinical pharyngeal specimens, Bacti-Lab plates with bacitracin disks yielded 12% false positives. Similar results with the presumptive bacitracin method have been reported by other investigators who have reported from 4 to 13% false positive results using the measured zone method of interpretation (2, 3, 4, 12).

A commonly used alternate method for collecting pharyngeal specimens for examination for group A streptococci is the delayed filter paper recovery method of Hollinger and Lindberg (6). They inoculated sterile filter paper strips with pharyngeal material and planted them on blood agar several days after collection. They reported a 90% recovery rate using this method and when pharyngeal material was immediately plated on blood agar without antibiotics they found only a 74% recovery rate. The 88% recovery rate obtained with the Bacti-Lab antibiotic-impregnated blood agar is at least as good as that obtained with these two methods in common use and has the added advantage of rapid and selective recovery.

Some questions remain unanswered, such as why were a few lots apparently unable to support growth of some strains of group A streptococci and why are inhibition zones on Bacti-Lab plates larger? Blood agar plates from the particular lots in question were the only test plates made from more than one source of sheep blood. This may have had an inhibitory effect. All other lots involved the use of a single source and did not appear to inhibit growth. Bacitracin inhibition half-zones on Bacti-Lab plates

TABLE 4. *Bacitracin inhibition patterns on Bacti-Lab and control plates with 13 lots of bacitracin disks (Bioquest) in 555 tests with four streptococcal groups*

Streptococcal group tested	No. of lots tested	No. of tests run	No. false positive	No. false negative	Avg half-zone of inhibition (mm) ^a	
					Bacti-Lab plate	Control plate
A	13	237		0	10.69	7.96
B ^b	4	53	4 (7.54)		5.75	0.71
C ^c	8	137	34 (24.81)		4.64	1.35
G ^d	6	128	27 (21.09)		5.67	4.70
		555	65	0	6.68	3.68
Total	13	(100.00) ^e	(11.71)	(0.00)	(Avg)	(Avg)

^a Half-zones of inhibition were determined by measuring from outer perimeter of inhibition to center of disk.

^b One strain.

^c Three strains.

^d Four strains.

^e Numbers in parentheses indicate percentages.

may have been larger because of a media composition different from that used in the control plates, resulting in different antibiotic diffusion rates.

Bacti-Lab streptococci culture systems lend themselves well for use in laboratories where elaborate facilities such as fluorescent-antibody microscopes and highly trained personnel are not readily available. The data obtained from this study indicate that the Bacti-Lab method is a valid procedure for presumptive identification of group A streptococci in clinical pharyngeal specimens.

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