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Comparison of Several Laboratory Media for Presumptive Identification of Enterococci and Group D Streptococci

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Bile-esculin (Difco), modified bile-esculin (Difco), selective enterococcus (Pfizer Co.), and eosin-methylene blue agar media were evaluated for accuracy in identifying group D streptococci. The regular and modified bile-esculin media performed equally well, but the selective enterococcus and eosin-methylene blue agars did not accurately differentiate the group D from non-group D streptococci. A modified 6.5% NaCl broth was compared with unmodified 6.5% NaCl broth and *Streptococcus faecalis* (SF; Difco) broth for accuracy in differentiating enterococci from non-enterococci. The modified and unmodified broths worked equally well in the salt tolerance test, but the lot-to-lot variability of SF broth made this medium unusable as an indicator for enterococci. With all seven media, the number of strains giving positive tests decreased when the tests were incubated at 45 C as compared with 35 C, and the number of strains giving negative tests increased. Thus, the number of false-positive identifications decreased, but the number of false-negative identifications increased. Variability in the susceptibility of group D non-enterococcal streptococci to oxacillin and methicillin sensitivity disks limited the usefulness of these tests for presumptive identification of either enterococci or group D streptococci.

New tests for differentiating between the group D or enterococci streptococci, or both, and the non-group D streptococci have been advocated in several recent publications. In our laboratory, we evaluated bile-esculin agar (BEM; reference 3) that had been formulated by Swan (11) for its capacity to differentiate between group D and non-group D streptococci. Isenberg et al. (5) advocated using a modified bile-esculin medium, selective enterococcus medium (SEM; Pfizer Co.), for isolation of enterococci from clinical material. Lorian and Zanon (7) described oxacillin in a disk test for differentiating enterococci from staphylococci. Von Graevenitz et al. (13) proposed using susceptibility to oxacillin and methicillin as a differential test for recognizing enterococci. Toala et al. (12) used eosin-methylene blue agar (EMBM) to differentiate between enterococcal and non-enterococcal streptococci. Braunstein et al. (1) used eosin-methylene blue to differentiate group D from non-group D streptococci. We have reported preliminary evaluations of *Streptococcus faecalis* (SF) broth, a modified 6.5% NaCl broth, and an unmodified NaCl broth as indicators of enterococci (Bacteriol. Proc. 5:105, 1971).

Our studies show that non-enterococcal group D streptococci are isolated from a significant number of group D streptococcal infections (2). There is evidence that the antibiotic suceptibility of these non-enterococcal group D streptococci is not like that of enterococci (unpublished data).

These facts indicate that the clinical bacteriologist should differentiate the enterococci from the non-enterococci to help the physician prescribe effective treatment. It is not sufficient to differentiate the group D from the non-group D streptococci. We present data here comparing the efficacy of eight tests used to differentiate (i) the group D from non-group D streptococci and (ii) the enterococci from the non-enterococci. Seven of these eight tests were evaluated at two incubation temperatures (35 and 45 C) for this capacity.

MATERIALS AND METHODS

Cultures. A total of 636 strains of streptococci were tested (Table 1). The diagnostic strains were isolated

TABLE 1. *Streptococcal strains used*

Identification	No. of stock strains	No. of diagnostic strains	Total
Group D streptococci			
Streptococcus faecalis	3	63	66
S. faecalis var. *liquefaciens*	3	19	22
S. faecalis var. *zymogenes*	13	20	33
S. faecium	3	19	22
S. durans	4	10	14
Subtotal (enterococci)	26	131	157
S. faecium var. *casseliflavus*	15	0	15
S. avium (group Q)	6	2	8
Subtotal (enterococcal-like)	21	2	23
S. bovis	3	28	31
S. equinus	4	2	6
Subtotal (group D non enterococci)	7	30	37
Alpha- and non hemolytic, nongroupable streptococci (viridans)			
S. mitis	7	67	74
S. sanguis	3	46	49
S. salivarius	2	17	19
S. MG	2	28	30
S. mutans	7	24	31
S. uberis	7	0	7
Unspeciated	0	30	30
Subtotal (viridans streptococci)	28	212	240
Groupable streptococci			
Group A	3	14	17
Group B	8	17	25
Group C	6	11	17
Group E	6	0	6
Group F	10	4	14
Group G	8	20	28
Group H	6	0	6
Group K	4	3	7
Group L	4	0	4
Group M	7	0	7
Group N	6	0	6
Group O	4	1	5
Group P	7	0	7
Group R	8	0	8
Group S	4	0	4
Group T	2	0	2
Group U	3	0	3
Subtotal (groupable strains)	96	70	166
Total no. of cultures tested	191	445	636

from human sources and were received for identification from private institutions, state health departments, and other federal agencies. Each strain was serologically grouped by the Lancefield method and tested for hemolytic and catalase activity (3). In addition, the group D and viridans streptococci were tested for their physiological characteristics by a battery of tests previously described (2). We speciated 98% of the group D streptococci and 87% of the viridans streptococci with these methods.

Presumptive tests. SEM (Pfizer Diagnostics) and BEM (Difco) were prepared according to the manufacturer's instructions. They were prepared as 5-ml agar slants in screw cap tubes (16 by 125 mm). The modified BEM (MBEM) was prepared the same as the BEM except that horse serum was

not added to the sterilized medium. EMBM (Difco) was prepared according to the manufacturer's instructions and poured into plastic petri dishes (15 by 60 mm). Single-strength SF broth (Difco) was prepared according to the manufacturer's instructions and used as 5-ml broth in screw cap tubes (16 by 125 mm). The unmodified 6.5% NaCl broth (salt broth) was prepared by increasing the NaCl concentration of Heart Infusion broth (Difco) to 6.5% and tubing the broth in 5-ml quantities in screw cap tubes 16 by 125 mm. The modified 6.5% NaCl broth (modified salt broth) was prepared in the same manner except that 0.1% glucose (final concentration) and an indicator (bromocresol purple) were added to the broth before tubing. Five percent rabbit blood agar plates (Neopeptone Infusion agar; Difco) were used to determine the susceptibility of the strains to oxacillin and methicillin. This technique was not designed to mimic or replace the standard Kirby-Bauer antibiotic susceptibility testing technique, but was intended only as a rapid presumptive test to identify enterococci. All media were sterilized by autoclaving (15 lb/in^2 for 15 min).

By using a Pasteur pipette, duplicate SEM, BEM, MBEM, EMBM, salt broth, modified salt broth, and SF broth media were inoculated at the same time with two drops of a 24-h Todd-Hewitt broth (THB; Difco) culture of the specimen being tested. One tube of each medium and one EMBM plate were incubated at 35 C; the duplicate set of media was incubated at 45 C. Two drops of the same THB culture were placed on the surface of a blood agar plate and smeared over the entire surface of the plate with a sterile glass spreader. One oxacillin disk and one methicillin disk (1 μg and 5 μg respectively; BioQuest) were placed on each half of the plate and incubated at 30 C. All tests were read at 24, 48, and 72 h.

A positive reaction on SEM, BEM, or MBEM was recorded when half or more of the medium was blackened after any time interval. Negative reactions were recorded when no blackening or blackening of less than half of the tube occurred after 72 h. The EMBM plates were recorded as positive when growth was observed by visual inspection after any time interval; no growth after 72 h was designed a negative reaction. The salt, modified salt, and SF broth tests were read by rotating the tubes in front of an incandescent light and observing the contents for growth and indicator change. Obvious growth in any of the three tests was recorded as a positive reaction. In the modified salt and SF broths, a change in indicator was helpful in judging whether growth had occurred. Streptococci were considered resistant to methicillin and oxacillin if the diameter of the zone of inhibition was less than 10 mm (13).

RESULTS

Table 2 shows the percentage of positive reactions for streptococcal strains in four tests used to identify the group D streptococci when incubated at 35 and 45 C. When the media were incubated at 35 C, the results show that nearly all enterococcal strains gave positive reactions at the various readings, and all enterococcal-like

TABLE 2. *Percentage of positive reactions of streptococcal strains on four tests used to recognize group D streptococci*[a]

No. of streptococcal strains at temperature (C)	Positive reactions (%)											
	SEM			BEM			MBEM			EMBM		
	24	48	72	24	48	72	24	48	72	24	48	72
157 Group D enterococci												
35	98	100	100	96	100	100	97	99	100	98	98	98
45	97	97	97	82	86	86	81	88	88	96	96	96
23 Groups D and Q enterococcal-like												
35	100	100	100	100	100	100	100	100	100	100	100	100
45	95	95	95	70	74	78	4	8	22	91	91	91
37 Group D non-enterococci												
35	96	100	100	94	100	100	52	82	98	42	45	45
45	92	94	94	30	58	64	20	30	40	22	22	24
240 Viridans												
35	9	24	33	1	4	6	1	3	4	10	12	13
45	3	4	4	1	1	2	0	0	0	1	2	2
166 Groupable strains												
35	1	5	7	0	0	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0	0	0	0	0	0

[a] Strains were incubated at 35 and 45 C for 24, 48, and 72 h. SEM, selective enterococcus agar (Pfizer Co.); BEM, bile-esculin agar; MBEM, modified bile-esculin agar; EMBM, eosin-methylene blue agar.

strains were positive after 24 h on all four media. SEM and BEM were very efficient in the identification of non-enterococcal group D strains when incubated at 35 C; 100% of the strains were positive on both media after 48 h. MBEM was less efficient than SEM or BEM in the identification of non-enterococcal group D strains because the positive reaction developed slowly on this medium at 35 C; the non-enterococcal group D strains were 52, 82, and 98% positive after 24, 48, and 72 h, respectively. Only 45% of the non-enterococcal group D strains showed growth on EMBM incubated at 35 C after 72 h. Significant numbers of viridans streptococci gave positive SEM reactions when the medium was incubated at 35 C. Viridans streptococci gave 9, 24, and 33% positive reactions after 24, 48, and 72 h, respectively. The BEM and MBEM were more effective than the other media differentiating group D streptococci from the non-group D streptococci. Only 6 and 4% of the viridans strains were positive after 72 h at 35 C on BEM and MBEM, respectively. Of the viridans streptococci, 10, 12, and 13% grew on EMBM after 24, 48, and 72 h, respectively, when the medium was incubated at 35 C. No groupable strains gave positive reactions on BEM, MBEM, or EMBM at either 35 or 45 C, but 1, 5, and 7% of these strains gave positive reactions on SEM after 24, 48, and 72 h at 35 C.

Fewer strains of all categories of streptococci gave positive reactions on the media when incubated at 45 C than at 35 C (Table 2). The percentage of all group D strains giving positive SEM reactions dropped only 3 to 6% when the medium was incubated at 45 C. More significant decreases were observed with these strains in BEM, MBEM, and EMBM; decreases of 12 to 58% were observed. Fewer viridans streptococci gave positive reactions on all four media incubated at 45 C than at 35 C. Only 4% of the viridans streptococci gave positive reactions on SEM after 72 h at 45 C compared with 33% when the SEM was incubated at 35 C for 72 h. The percentage of viridans streptococci giving positive reactions on BEM and MBEM decreased from 6 to 4%, respectively, at 35 C and to 2% and none at 45 C after 72 h.

Table 3 shows the percentage of positive reactions of streptococcal strains in three media used to differentiate enterococcal from non-enterococcal streptococci when incubated at 35 and 45 C. All the enterococcal strains grew well in the three media; 98, 99, and 100% of the strains grew in salt, modified salt, and SF broths, respectively, when incubated at 35 C for 72 h. Fewer enterococcal-like strains grew in the three broths; 91, 87, and 78% of these strains grew in salt, modified salt, and SF broths, respectively, when incubated at 35 C for 72 h. None of the non-enterococcal group D strepto-

TABLE 3. *Percentage of positive reactions of streptococcal strains on three tests used to recognize enterococci*[a]

No. of streptococcal strains at temperature (C)	Positive reactions (%)								
	Salt broth			Modified salt broth			SF broth		
	24	48	72	24	48	72	24	48	72
157 Group D enterococci									
35	97	97	98	96	99	99	91	99	100
45	85	91	93	93	97	97	90	94	95
23 Groups D and Q enterococcal-like									
35	91	91	91	56	78	87	78	78	78
45	61	65	65	43	43	43	0	0	0
37 Group D non-enterococci									
35	0	0	0	0	0	0	4	4	4
45	0	0	0	0	0	0	0	0	0
240 Viridans									
35	0	2	2	0	0	0	1	1	1
45	0	1	1	0	1	1	0	1	1
166 Groupable strains									
35	11	15	18	17	19	21	0	0	0
45	0	1	1	0	0	0	0	0	0

[a] Strains were incubated at 35 and 45 C for 24, 48, and 72 h. Salt broth, 6.5% NaCl broth; modified salt broth, 6.5% NaCl plus glucose and indicator; SF, *Streptococcus faecalis*.

cocci grew in salt or modified salt broths, and 4% grew in SF broth when the media were incubated at 35 C. Only 2% of the viridans streptococci could grow in salt broth, none could grow in modified salt, and 1% could grow in SF broth when the media were incubated at 35 C for 72 h. Of the groupable streptococci, 18 and 21% grew in salt and modified salt broths, respectively, when the media were incubated at 35 C for 72 h. None of the groupable streptococci grew in SF broth when incubated at 35 C for 72 h.

When the media were incubated at 45 C, reduced numbers of positive cultures were obtained in almost all instances. The percentage of enterococcal strains that grew decreased 5% in salt and SF broths and 3% in modified salt broth when incubated at 45 C. The percentage of enterococcal-like strains shows decreases of 12 to 78% after 72 h when the media were incubated at 45 C. No appreciable changes in growth percentages were noted among the nonenterococcal group D or viridans streptococci when the three media were incubated at 45 C. Incubation at the higher temperature dropped the percentage of groupable strains growing in salt broth from 18% at 35 C to 1% at 45 C. The percentage of groupable strains growing in modified salt broth dropped from 21% at 35 C to none at 45 C. None of the groupable strains grew in SF broth at either incubation temperature.

Table 4 shows the susceptibility of the streptococcal strains to oxacillin and methicillin sensitivity disks. Only two enterococcal strains (1%) were susceptible to either oxacillin or methicillin in our nonstandardized system. These two strains gave small zones of inhibition (12–15 mm). All of the enterococcal-like strains were resistant to both antibiotic disks. Of the non-enterococcal group D strains, 16% were resistant to both oxacillin and methicillin. The diameters of the zones were fairly uniform among the susceptible strains with both disks: 10 to 25 mm with oxacillin and 10 to 28 mm with methicillin. Of the viridans strains, 8% were resistant to oxacillin and 5% were resistant to methicillin. The range of the diameters of inhibition was very large with both disks (10–50 mm). Only 1% of the groupable (two group B streptococci) strains were resistant to both oxacillin and methicillin. The range of diameters of inhibition was 10 to 40 mm among the groupable strains.

DISCUSSION

BEM. Results with BEM show that group D and non-group D streptococci can be differentiated when grown on slants of modified BEM incubated at 35 C. The omission of horse serum from the medium decreased the cost and simplified the preparation of the agar slants. Although the group D streptococci as a whole grew more slowly on modified BEM, most enterococci were positive after 24 h at 35 C.

The results with SEM show that this medium does not inhibit the growth of all the viridans streptococci when incubated at 35 C. Other investigators have reported that significant numbers of non-group D streptococci isolated from food, sewage, and feces grew in and blackened SEM (8). We have also tested BioQuest's bile-esculin agar (enterococcus selective agar) with 100 streptococcal strains and have obtained similar results. Examinations of the technical bulletins distributed by the three companies show two major differences in the media. Pfizer and BioQuest media are similar and contain sodium azide (0.25 g/liter) and 10 g of oxgall (bile salts) per liter. The Difco BEM contains no sodium azide but does contain 40 g of oxgall per liter. These differences cause the microorganisms to react differently in the media. The azide in the agar will inhibit gram-negative organisms and make the medium usable as primary isolation medium for streptococci (5, 6, 8, 10). Difco BEM does not inhibit the growth of organisms by azide inhibition; the Difco product contains four times as much oxgall as do the Pfizer or BioQuest products. A 10% solution of bile salts is equivalent to a fresh

TABLE 4. *Recognition of enterococci by resistance to oxacillin and methicillin sensitivity disks*

No. of streptococcal strains	Oxacillin			Methicillin		
	Resistant strains	Zone sizes (mm) of sensitive strains		Resistant strains	Zone sizes (mm) of sensitive strains	
		Range	Avg		Range	Avg
157 Group D enterococci	99	12–15	13	99	10–12	11
23 Groups D and Q enterococcal-like	100	0	0	100	0	0
37 Group D nonenterococci	16	10–25	17	16	10–28	19
240 Viridans	8	10–50	22	5	10–50	24
166 Groupable strains	1	10–40	20	1	10–40	23

bile solution; thus, the 40 g of bile salts per liter of the Difco product is equivalent to a 40% fresh bile concentration, and the 10 g of bile salts per liter of SEM and BioQuest BEM is equivalent to only a 10% fresh bile concentration. The 40% fresh bile concentration is similar to the concentration of bile early investigators used when they employed bile tolerance as an aid in identifying and differentiating streptococci. Evidently, the bile concentration in the Pfizer and BioQuest products does not inhibit the growth of as many streptococci as does the concentration in the Difco product. Strains of *Streptococcus sanguis*, *S. mutans*, and *S.* MG hydrolyze esculin, but very few of these strains can tolerate the 40% fresh bile concentration and hydrolyze esculin in combination. Many of these strains hydrolyzed the esculin in the Pfizer and BioQuest products. Positive reactions of one-third of the viridans streptococci restricted the use of these media with low bile concentrations as differential media for identifying enterococci or group D streptococci.

EMBM. The results indicate that EMBM could not be used as a differential medium for either enterococci or group D streptococci because growth of the non-enterococcal group D strains varied. These results are similar to the results we obtained with methylene-blue milk (2, 3). Since about half of the *S. bovis* strains grow on EMBM or in methylene-blue milk, neither medium could be used as a differential test for identifying enterococci or group D streptococci.

Salt tolerance. We have presented preliminary data on the standardization of salt tolerance test (Bacteriol. Proc. 5:105, 1971). Our previous results and these results show that the modified salt tolerance test can be used to accurately differentiate between enterococcal and non-enterococcal streptococci when incubated at 35 C. The 6.5% NaCl broth without indicator was also an excellent medium for differentiating bile-esculin-positive streptococoi into enterococci and non-enterococci. The fact that some strains of groupable streptococci can grow in broth containing 6.5% NaCl (group B streptococci) necessitates using an additional test to identify the enterococci. Other workers (1) have noted that beta-hemolytic group B streptococci will grow in broth containing 6.5% NaCl. None of the groupable streptococci that grew in salt broth gave positive SEM, BEM, and MBEM, or EMBM reactions. We prefer to use the modified salt broth instead of the salt broth without indicator because it is easier to read.

SF broth. Our results with SF broth were disappointing. Not all enterococcal strains grew in this broth, and a few non-enterococcal group D strains did grow in it. Furthermore, when this study was completed we noted that several diagnostic strains of viridans streptococci could grow in the SF broth. A check of the supply of dehydrated media revealed that the study was completed with a single lot of SF medium (control 551201), and we had just begun using a second lot of medium (control 565144). We tested the new lot of SF broth for use as an indicator of enterococci with 200 strains of streptococci received in the diagnostic laboratory over a 6-month period (Table 5). This second lot of SF broth did not perform in the same manner as the previous lot. Of the non-enterococcal group D strains and the viridans streptococci, 84 and 31%, respectively, grew in the broth. Braunstein et al. (1) reported that their beta-hemolytic group B streptococci grew in SF broth. None of our 25 group B strains grew in lot 551201 SF broth. These discrepancies indicate differences in the media used in the laboratories. This variability between lots of the broth made the product completely unreliable as a differential medium for enterococci or group D streptococci.

Effect of incubation at 45 C. When Hajna and Perry (4) described SF broth, they indicated that incubation of the broth at 45 C would yield a highly specific reaction, that is, positive reactions only with strains of *S. faecalis*. To improve the selectivity of the tests, we decided to incubate all of our tests at 45 C.

Incubation of SEM at 45 C improved the performance of the medium. The percentage of enterococci and non-enterococcal group D streptococci giving positive reactions decreased somewhat (3% for enterococci and 6% for non-enterococcal group D streptococci), but the percentage of viridans streptococci also decreased (from 33% at 35 C to 4% at 45 C). The percentage of strains of enterococci and group D non-enterococci giving positive reactions on BEM and MBEM at 45 C decreased so much that neither medium could be used as an indicator of enterococci or group D streptococci when incubated at 45 C.

TABLE 5. *Number and percent-positive reactions of streptococcal strains in Streptococcal faecalis broth, lot 565144*

Streptococcal strains	No. tested	Positive reactions (%)
Group D (enterococci)	35	100
Group D (non-enterococci)	26	84
Viridans	139	31

The percentage of all streptococcal strains growing on EMBM when incubated at 45 C decreased, but the capacity the different categories of streptococci to grow remained variable, rendering the medium unreliable as an indicator of enterococci or group D streptococci.

The results with the salt tolerance broths and SF broth were similar. The percentages of enterococci growing in the three broths decreased somewhat (2-5%) when tests were incubated at 45 C. The enterococci-like strains growing in salt and modified salt broths decreased significantly (28% in salt broth and 54% in modified salt broth) when incubated at 45 C. Although these strains are probably not found in human infections (2), the accuracy of identification of all enterococci was decreased when these tests were incubated at 45 C.

Hajna and Perry (4) suggested that positive reactions in SF broth, when tests were incubated at 45 C, were assurance of the presence of *S. faecalis*. Our results with the one lot of SF broth tested at 45 C support this suggestion. We did not test the second lot of SF broth at 45 C and therefore cannot comment about the reliability of this media at 45 C. If SF broth must be used, each new lot should be tested for differentiating capacity with several known streptococcal strains that will give known positive and negative reactions. Since not all enterococci are *S. faecalis*, SF broth was too restrictive as an indicator of enterococci when incubated at 45 C.

Oxacillin and methicillin as indicators of enterococci. Our results show that the variability of susceptibility of the non-enterococcal group D streptococci (mainly *S. bovis*) to the antibiotic disks made the test an unreliable indicator of enterococci. Even though most of the enterococci (99%), were identified correctly by both disks, several non-enterococcal group D streptococci (16%) and viridans streptococci (8%) were resistant and were presumptively identified as enterococci. Although standardized Kirby-Bauer susceptibility tests were not performed on all of these isolates, some of the *S. bovis* strains gave minimal inhibitory concentrations of 5 μg or greater with methicillin in a standardized tube dilution antibiotic susceptibility test. These values are low enough to indicate that these strains would be resistant to methicillin sensitivity disks. These results are similar to those of other investigators (9, 13). Pleceas (9) indicated that oxacillin could not be used as a single test to identify the enterococci. She also stated that the *S. bovis* strains tested varied (most of them were resistant) in susceptibility to oxacillin in her testing system. Von Graevenitz et al. (13) reported that the susceptibility of *S. bovis* to oxacillin and methicillin varied.

Several important facts should be brought to the attention of the bacteriologist concerned with identifying enterococci and group D streptococci. There are differences in bile-esculin media, and the user must be aware of how the media will perform. For example, SEM and other BEM containing low bile concentrations and azide probably work better than do BEM or MBEM as selective primary media; however, BEM or MBEM will work better than the media containing low bile concentrations for differentiating group D streptococci from non-group D streptococci.

Tests with EMBM will yield a considerable number of false-positive enterococcal identifications. We do not recommend this agar for use in a differential test for enterococci or group D streptococci.

There is no single test that will accurately identify the enterococci. The salt tolerance test can be used very effectively with the BEM test to identify the enterococci. A positive SEM, BEM, or MBEM reaction and growth in 6.5% salt broth means that the strain is an enterococcus. A positive BEM or MBEM and negative salt tolerance reaction means that the streptococcus is a group D streptococcus but not an enterococcus. This identification cannot be made with SEM and other BEM with low concentrations of bile because 25 to 33% of the viridans streptococci would fall in this category. The *S. bovis* strains have a susceptibility to antibiotics not like the enterococci or viridans streptococci (unpublished data), and the laboratory may need the tools to recognize these strains for the best management of patients with *S. bovis* infections. Negative SEM, BEM, or MBEM and negative salt tolerance reactions mean that the streptococcus is not an enterococcus or group D streptococcus; if the culture is alpha- or non-hemolytic, it is a viridans streptococcus; if the strain is beta-hemolytic, then other tests must be performed to identify the Lancefield group of the strain. A positive salt tolerance test will occur occasionally when the tests with SEM, BEM, or MBEM are negative. Beta-hemolytic group B streptococci will often grow in 6.5% salt broth; other beta-hemolytic streptococci generally will not. A positive reaction to salt tolerance and negative reactions in BEM, SEM, or MBEM should occur less than 1% of the time among the alpha- and non-hemolytic strains. When this does occur, the broth from the salt tolerance test should be tested for purity by streaking the growth onto a blood agar plate and comparing the morphology to that of the original strain.

Incubating the tests at 45 C did not improve

the performance to any great extent of any of the media in identifying the enterococci. We do not recommend incubation of these tests at 45 C for differentiating streptococci. Our reason is that the number of false-negative reactions increase with all tests, and we believe it is better to "overidentify" than to "underidentify" the enterococci.

The methicillin and oxacillin susceptibility tests do not satisfactorily differentiate the enterococci or group D streptococci. We recommend modified BEM and modified salt tolerance tests for the identification of these two groups of organisms.

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