

Presumptive Identification of Group A, B, and D Streptococci

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A battery of five tests was used for presumptive identification of the pathogenic streptococci. The non-serological methods included determination of hemolysis for all strains, bacitracin susceptibility for group A streptococci, hippurate hydrolysis by group B streptococci, and bile-esculin reaction for group D streptococci. Enterococcal group D streptococci were differentiated from non-enterococcal group D streptococci by 6.5% NaCl tolerance. Two other categories of streptococci resulted: beta-hemolytic streptococci non-groups A, B, or D; and alpha- or nonhemolytic streptococci, not enterococci, not further identified (viridans streptococci). The tests were used as a battery and not as single entities. In this manner more than 99% of the group A, 99% of the group B, 81% of the beta-hemolytic streptococci non-group A, B, or D, 99% of the group D enterococci, 97% of the group D non-enterococci, and 94% of the viridans streptococci were correctly identified.

Several recent studies have pointed out the importance of group B streptococci as human pathogens (2, 3, 12, 16, 23). Because streptococci of groups A, B, and D differ in their susceptibility to antibiotics, including penicillin, reliable methods are needed for differentiating these streptococci from one another and from other streptococci (14, 18, 21). Many laboratories that do not perform sero-grouping of streptococci rely on presumptive tests, such as bacitracin susceptibility for group A streptococci, to identify the pathogenic streptococci. Two presumptive tests for group B streptococci have been proposed. The CAMP reaction is based on an enhancement of the hemolytic action of group B streptococci in the presence of beta-hemolytic staphylococci (7). Hydrolysis of sodium hippurate by group B streptococci was described by Ayers and Rupp (1). Braunstein et al. (4) made use of hippurate hydrolysis to identify group B streptococci, but they did not mention the fact that some group D streptococci can hydrolyze sodium hippurate (5, 19). This report describes how sodium hippurate hydrolysis can be used to presumptively identify beta-hemolytic group B streptococci. It also contains information on non-serological identification of group A (bacitracin susceptibility), group D (bile-esculin reaction), and enterococcal streptococci (bile-esculin and salt tolerance).

MATERIALS AND METHODS

Cultures. Cultures received by the Streptococcus Laboratory, Center for Disease Control (CDC) from

federal, state and city public health departments, various private investigators, and health officials of several foreign countries during the period July 1967 to May 1973 (4,560 cultures) were used in this study. In addition, 358 strains of streptococci and related bacteria were obtained from the CDC stock culture collection of type strains representing all known species of streptococci.

Methods for determining hemolytic and catalase activity, serological grouping, and Gram staining have been previously described (11). Serological grouping and determination of hemolytic activity were performed on all streptococci used in this study. Determination of catalase activity and Gram stains were performed on all group D, alpha-hemolytic non-group D (viridans), and beta-hemolytic non-groupable streptococci.

Commercial bacitracin disks were purchased from Bioquest (Taxo A disks). Neopeptone infusion agar (Difco) blood plates (4% rabbit blood) were prepared. The blood agar plates were marked into quarters, and each quarter was streaked with a loop (3 mm) of an overnight broth culture. A bacitracin disk was placed in the upper third (heaviest part) of the inoculum. The plates were inverted and incubated aerobically at 35 C for 24 h. Plates were considered positive when there was a zone of inhibition and negative where there was growth up to the disk, or no inhibition.

Broth for determining hippurate hydrolysis was prepared by adding 10 g of sodium hippurate to 1 liter of heart infusion broth (Difco). The broth was dispensed in 5-ml amounts into screw-cap tubes (15 by 125 mm) and sterilized by autoclaving (15 lbs, 15 min). The caps were tightened to prevent evaporation. The medium was inoculated with 1 to 2 drops of an overnight broth culture and incubated 42 to 66 h at 35 C. Cultures were centrifuged to sediment the growth, and 0.8 ml of the clear supernatant was pipetted into a Kahn tube. Ferric chloride reagent was

prepared by adding 12 g of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ to 100 ml of aqueous HCl (5.4 ml of concentrated HCl [37%] to 94.6 ml of distilled water. A 0.2-ml portion of the ferric chloride reagent was added to the 0.8 ml of culture supernatant. A heavy cloudy precipitate that persisted after 10 min (with occasional shaking) indicated that the sodium hippurate had been hydrolyzed. A clearing of the initial precipitate indicated that sodium hippurate had not been hydrolyzed.

The procedures for preparing and reading the following tests have been previously described (10, 11): bile-esculin and modified bile-esculin, 6.5% NaCl (salt broth) and modified 6.5% NaCl (modified salt broth), and *Streptococcus faecalis* (SF) broth. Unmodified bile-esculin medium (BEM) and salt broth were used from July 1967 to July 1971, and modified bile-esculin and modified salt broth were used during the latter part of this study.

A battery of physiological tests (9) was used to identify the group D and other alpha-hemolytic streptococci. In a separate study in which silica gel-transported swabs were used, the presumptive test reactions of beta-hemolytic streptococci were determined before the sero-grouping results were known. Nose, throat, vaginal, and umbilical swabs were placed in 5 ml of Todd-Hewitt broth (THB, Difco) and incubated for 24 h at 35 C. Pour plates were made by transferring one loop of the overnight THB culture to 15 ml of sterile physiological saline in a 40-ml centrifuge tube. The loop was sterilized, and one loop of the saline-diluted organisms was placed in 15 ml of melted neoptone infusion agar in a 40-ml centrifuge tube. A 0.6-ml portion of defibrinated rabbit blood was then added to the melted agar. The inoculum, blood, and melted agar were mixed thoroughly and poured into a plastic petri dish (15 by 100 mm). Plates were incubated overnight at 35 C. Hemolysis was read with a broad-field microscope according to the method of Brown (6). Beta-hemolytic colonies were "fished" from the pour plate into 30 ml of THB in 40-ml centrifuge tubes. The 30 ml of THB was incubated overnight at 35 C. Inocula for bacitracin susceptibility, sodium hippurate hydrolysis, bile-esculin reaction, and 6.5% NaCl broth tests were taken from the 30-ml THB before extraction. Bacitracin susceptibility, bile-esculin reaction, and salt tolerance tests were read after 24 to 48 h. Hippurate hydrolysis was determined after 2 days of incubation at 35 C. When there were discrepancies between results of serological reactions and results of presumptive tests, the original plates were reexamined and several isolations were made. In several instances, we were able to isolate beta-hemolytic group B and D streptococci from the same plate.

RESULTS

As shown in Table 1, the streptococci were placed into one of six categories according to their serological reactions with group-specific antisera. Group A and group B streptococci each comprise a specific classification, but the category of "beta-strep, non-A, B, or D," contains beta-hemolytic streptococci belonging to

TABLE 1. *Bacitracin susceptibility of 1132 streptococcal strains when tested with commercial bacitracin disks*^a

Streptococci	No. tested	Positive (%)
Group A	571	99.5
Group B	184	6.0
Beta strep non-A, -B, or -D	134	7.5
Group D enterococci	65	0
Group D non-enterococci	25	4.0
Viridans	153	7.8

^a The specimens used in this study were all the streptococci received for identification between July 1972 and May 1973.

serogroups C, F, G, L, M, and non-groupable. The "group D enterococci" category consists of *S. faecalis* and its varieties *zymogenes* and *liquefaciens*, *S. faecium*, and *S. durans* (9). The "group D non-enterococci" category is made up of *S. bovis* and *S. equinus*. The "viridans" streptococci are made up of alpha- and non-hemolytic streptococci whose extracts failed to react with CDC group A, B, C, D, and G antisera, or reacted with one of the CDC group F, H, K, or O antisera. No beta-hemolytic group H, K, or O streptococci were identified during the period of this study. Several alpha-hemolytic streptococcal species are known to have antigens that react with the group F antisera.

Table 1 shows results of testing the susceptibility of 1,132 streptococci to commercial bacitracin disks. Results show that 99.5% of the group A streptococci were susceptible to this bacitracin. Among the group B streptococci, 6.0% were susceptible, and only 7.5% of the beta-hemolytic streptococci non-group A, B, or D were susceptible to bacitracin. None of the enterococcal and only 4% of the non-enterococcal group D streptococci were susceptible to commercial bacitracin. Only about 8% of the viridans streptococci were susceptible to commercial bacitracin.

Table 2 shows the results of the hippurate hydrolysis tests with 3,030 strains of streptococci. None of the group A strains, but 99.6% of the group B strains, hydrolyzed hippurate. Only one group C strain hydrolyzed hippurate among the beta-hemolytic streptococci not in A, B, or D groups. However, 6.9% of the enterococci hydrolyzed hippurate. No one species of enterococci hydrolyzed hippurate more often than another, and there was no correlation of hemolysis to hippurate hydrolysis among these strains. None of the group D non-enterococcal

strains hydrolyzed hippurate. *S. uberis* and *S. acidominimus* were the only strains of the viridans streptococci that hydrolyzed hippurate. Only one strain each was identified from human sources. Not included in the table are 54 stock strains of streptococci representing group E, N, P, R, S, T, and U streptococci. These streptococci are found rarely, if at all, in humans; all failed to hydrolyze hippurate.

For the laboratory that requires rapid results, the hippurate broth can be inoculated from the original isolation plate. One or two isolated colonies can be used to inoculate the broth, and hippurate hydrolysis can be tested for the following day. We tested 114 group B and 20 non-group B cultures in this manner. Ninety-four percent of the group B streptococci hydrolyzed hippurate after 20 h of incubation. The negative broths were remixed and incubated an additional day. All but one of the group B streptococci were positive after 44 h of incubation. None of the non-group B streptococci hydrolyzed hippurate after 20 or 44 h of incubation.

Table 3 shows the results of the bile-esculin test with 3,143 strains of streptococci. The results show that none of the group A or B

TABLE 2. Hippurate hydrolysis results with 3,030 selected streptococci^a

Streptococci	No. tested	No. positive	Positive (%)
Group A	108	0	0
Group B	973	969	99.6
Beta strep non-A, B, or D	345	1	0.3
Group D enterococci	463	32	6.9
Group D non-enterococci	134	0	0
Viridans	1,007	21 ^b	2.1

^a In addition to several stock strains of each species, the strains used in this study were clinical isolates received for identification. The group B streptococci were the majority of group B streptococci received between July 1967 and June 1972. Among the beta streptococci non-A, B, or D are 98 group C, 103 group G, 79 group F, 6 group L, 6 group M, and 53 beta-hemolytic non-groupable streptococci. The group D and viridans strains tested include the majority of these strains received for identification from July 1967 through May 1973.

^b All strains of *S. uberis* (16) and *S. acidominimus* (5) hydrolyzed hippurate. No other viridans streptococcal species hydrolyze hippurate. Also tested, but not included in this table, were 54 stock strains of streptococci representing groups E, N, P, R, S, T, and U. These strains are found rarely, if at all, in humans. All failed to hydrolyze hippurate.

TABLE 3. Percentage of positive reactions in bile-esculin medium, 6.5% NaCl broth, and SF broth^a

Streptococci	No. tested	Bile-esculin positive (%)	6.5% NaCl tolerant (%)	SF broth tolerant (%)
Group A	108	0	1.9	3.7
Group B	973	0	79.2	>0.6
Beta strep non-A, B, or D	345	>0.3	15.4	9.3
Group D enterococci	515	99.6	99.6	98.1
Group D non-enterococci	134	99.3	2.2	40.3
Viridans	1,068	5.3	4.8	13.1

^a Group A, B and the beta-hemolytic streptococci non-A, B or D were the same strains used in the hippurate hydrolysis experiments. All strains of group D and viridans streptococci received for identification between July 1967 and May 1973 are included in this study.

streptococci were able to blacken BEM. One strain in the beta-hemolytic streptococci non-A, B, or D (a group G) was able to blacken BEM. The results also show that both the enterococcal (99.6%) and non-enterococcal (99.3%) group D streptococci blacken BEM. Among the viridans strains, 5.3% gave positive BEM reactions. Among the 57 positive BEM strains were 24 *S. mutans*. Of 144 *S. mutans* strains tested, 17% gave positive BEM reactions. An additional 35 *S. mutans* strains gave trace or weak reactions on BEM that were read as negative.

Table 3 also shows the tolerance to 6.5% NaCl broth by 3,143 streptococci. Results show that 1.9% of the group A streptococci and nearly 80% of group B streptococci grew in 6.5% NaCl broth; 15.4% of the beta-hemolytic streptococci non-groups A, B, or D grew in 6.5% NaCl broth. Among these streptococci, 25% of the group C's and 17% of the group G's tolerated 6.5% NaCl broth. Nearly all enterococcal group D strains (99.6%) tolerated 6.5% NaCl broth, whereas 2.2% of the non-enterococcal group D streptococci tolerated the salt broth. Of the 4.7% of the viridans streptococci that were tolerant to 6.5% NaCl broth, none gave positive BEM reactions. The largest percentage of strains among the viridans group that were salt tolerant were unidentified. Fifteen of 68 strains (22%) were salt tolerant. None of these strains could be speciated by physiological tests, and only 30 of 68 strains were from human sources.

Table 3 shows the tolerance of 3,143 streptococci to SF broth. Only 3.7% of the group A strains tested and less than 0.1% of the group B

strains tolerated SF broth. The 9.3% of the beta hemolytic streptococci non-group A, B, or D strains that tolerated SF broth were equally divided into groups C, G, and F streptococci. Nearly all enterococcal group D streptococci (98%) tolerated SF broth. Forty percent of the non-enterococcal group D streptococci and 13.1% of the viridans tolerated SF broth. These latter two figures are much too high to make SF broth a useful indicator of enterococci. No one species of viridans streptococci was particularly more tolerant than any other to SF broth.

Table 4 shows the presumptive test results of 770 streptococci isolated from a series of specimens from mothers and babies during a study to determine the colonization rates of group B streptococci. The results show that all the group A streptococci were correctly identified. All but one of the group B streptococci were correctly identified. Five group G streptococci did not hydrolyze hippurate or blacken BEM, but all of them were susceptible to bacitracin and were, therefore, erroneously identified as presumptive group A streptococci. All five group G strains were isolates from one mother and her baby, so it is likely that all of the isolates were of the same strain. One group C strain was correctly identified as a beta-hemolytic streptococci, non-group A, B, or D. All group D streptococci were correctly identified on the basis of positive BEM reactions. All group D strains in this study were identified as enterococci on the basis of tolerance to salt.

Table 5 shows the presumptive test reactions of some bacteria that can be commonly confused with some species of streptococci. *S. (diplococcus) pneumoniae* were often (48%) susceptible to bacitracin. None of the 17 strains hydrolyzed hippurate, blackened BEM, grew in 6.5% NaCl broth, or tolerated SF broth.

All six of the *Aerococcus viridans* strains were sensitive to bacitracin, hydrolyzed hippurate, and tolerated 6.5% NaCl and SF broths. Half of the strains blackened BEM. All 27 of the *S. salivarius* (13) strains were susceptible to bacitracin, but failed to hydrolyze hippurate,

blacken BEM, grow in 6.5% NaCl broth, or tolerate SF broth. The majority (24) of these strains were catalase negative and may be readily misidentified. All six *Listeria* strains gave positive BEM reactions, grew in 6.5% NaCl broth, were resistant to bacitracin, and failed to tolerate SF broth. Four of the six strains hydrolyzed hippurate.

DISCUSSION

The best method of identifying the streptococci is to grow isolated pure colonies of the infecting organism, extract the group carbohydrate, and demonstrate a serological reaction between the extracted antigen and specific grouping antiserum. The CDC recommends the serological grouping procedure as the method of choice for identifying the streptococci. However, we realize that this method of growing and extracting the organism is time consuming and that the cost of obtaining specific potent antisera makes the method unacceptable for some laboratories. For these laboratories we recommend an alternative method of identifying the pathogenic streptococci which is based on determination of (i) hemolytic activity, (ii) bacitracin susceptibility, (iii) hydrolysis of sodium hippurate, (iv) hydrolysis of esculin in the presence of 40% bile, and (v) tolerance to 6.5% NaCl broth.

The clinical diagnosis of the patient's illness as well as the source of the culture are helpful information in identifying the streptococci. For example, if the specimen is from the cerebrospinal fluid or blood of a neonate (neonatal sepsis or meningitis), the bacteriologist should suspect group B streptococci (as well as *Escherichia coli* and *Listeria monocytogenes*). If the specimen is a throat or skin culture (pharyngitis or impetigo), the bacteriologist might well expect to find group A streptococci. Urine specimens may contain group D streptococci. The bacteriologist may find the determination of hemolysis and bacitracin susceptibility are sufficient procedures for investigating throat and skin specimens. The complete procedures listed in Table 6 are not necessary if the physician is interested in only group A or non-group A streptococci from the throat and skin.

Hemolysis. Correct determination of hemolysis is important for proper identification of pathogenic streptococci regardless of the method used to identify the species. Streptococcal hemolysis was first defined (this definition has not changed) by microscope examination of subsurface growth (6). Taranta and Moody (20) present excellent descriptions of Brown's definitions. Laboratories that do not prepare pour

TABLE 4. Presumptive identification of 770 fresh clinical beta-hemolytic streptococci

Serological group	No. tested	Bacitracin positive (%)	Hydrolyzed hippurate (%)	Bile-esculin positive (%)	6.5% NaCl broth tolerant (%)
A	6	100	0	0	0
B	487	>1	99.9	0	66.5
C/G	6	83	0	0	0
D	271	0	19.1	100	100

TABLE 5. Percentage of positive reactions of streptococcal-related bacteria in five presumptive tests

Bacteria	No. tested	Positive reactions to (%)				
		Bacitracin susceptibility	Hippurate hydrolysis	Bile-esculin reaction	Tolerance to 6.5% NaCl broth	SF broth
<i>Streptococcus (diplococcus) pneumoniae</i>	17	48	0	0	0	0
<i>Aerococcus viridans</i>	6	100	100	50	100	100
<i>Staphylococcus salivarius</i> ..	27	100	0	0	0	0
<i>Listeria</i> (four species)	6	0	67	100	100	0

TABLE 6. Presumptive identification of streptococci

Procedure	Group identification					
	Group A	Group B	Non-group A, B or D	Group D enterococcus	Group D not an enterococcus	Viridans non-group D
Hemolysis	Beta	Beta	Beta	Beta, alpha or none	Alpha or none	Alpha or none
Bacitracin sensitivity	+	- ^a	- ^a	-	-	V
Hippurate hydrolysis	-	+	-	V ^b	-	- ^a
Bile-esculin hydrolysis	-	-	-	+	+	-
Tolerance to 6.5% NaCl	-	V	- ^a	+	-	-

^a An occasional exception occurs.

^b V, variable.

plates should incubate streaked plates under reduced oxygen tension to detect oxygen-labile streptolysin O as well as the oxygen-insensitive streptolysin S. Streptococci that produce very little or no streptolysin S appear as "poor surface hemolyzers" or nonhemolytic when incubated under aerobic conditions. The method of stabbing the blood agar plate described by the American Heart Association (22) is not as acceptable as the pour-plate method of determining hemolysis, but it can be used when it is impossible to make pour plates or to incubate anaerobically.

Bacitracin. Maxted (15) noted that some alpha-hemolytic streptococci were susceptible to bacitracin. We found about 8% of the viridans strains susceptible. This finding indicates that correct determination of hemolysis is necessary when bacitracin is being used to presumptively identify group A streptococci. It is especially important for throat swabs where alpha- and nonhemolytic streptococci abound.

Our data indicate that more than 99% of the group A streptococci are susceptible to bacitracin and are correctly identified if the test is performed properly. A heavy inoculum of a pure isolate, as suggested by Maxted, is advisable. Minimum zones of inhibition are not required

for presumptive group A identification. Any zone of inhibition is considered positive, and no zone is considered negative. Reports of zone size requirements are in the literature (8, 17), and the technical bulletin of one manufacturer implies that a zone of a certain size is necessary for presumptive identification of group A streptococci. The originator of the test (15) did not specify that zones should be a certain size. No experimental data are available to show that measurement of zones of inhibition is necessary for the differentiation of group A from non-group A streptococci. By requiring zone sizes of 10 mm or more for presumptive identification of group A streptococci, at least one group of investigators increased the false negative error of the tests by 10% (8). It is preferable to have more false positive results (an over-diagnosis) rather than false negative results. The users of bacitracin should realize that bacitracin inhibits growth of some strains of beta-hemolytic streptococci other than group A and should report their findings as follows: (i) "presumptive beta-hemolytic group A by bacitracin," or (ii) "beta-hemolytic streptococci non-group A by bacitracin."

Hippurate hydrolysis. The data from this study show that more than 99% of the group B

streptococci can be correctly identified by sodium hippurate hydrolysis. The beta-hemolytic streptococci that hydrolyze hippurate are either group B or group D streptococci. Group B streptococci can be readily differentiated from those of group D by the bile-esculin test; group B streptococci do not blacken BEM, but 99% of group D streptococci do. Beta-hemolytic, BEM-negative, hippurate-hydrolyzing streptococci can be reported as presumptive group B streptococci. *S. acidominimus* and *S. uberis* are members of the viridans streptococci that hydrolyze hippurate, but are found rarely, if at all, in specimens from human infections. Non-hemolytic group B streptococci have recently been isolated from humans (24). These strains hydrolyzed hippurate and could be identified as presumptive group B streptococci on the basis of hippurate hydrolysis and the fact that *S. acidominimus* and *S. uberis* are rarely found in humans. Unusual strains such as alpha- or nonhemolytic streptococci that hydrolyze hippurate should be sent to state health departments for confirmation and definitive identification.

Bile-esculin test. Bile-esculin medium can be used to differentiate between group D streptococci and non-group D streptococci (10, 11). Results with 920 strains of group D streptococci show that 99% of the strains will blacken BEM. Non-enterococcal group D streptococci, mainly *S. bovis*, are found in appreciable numbers of streptococcal infections (9). Thus, BEM cannot be used to differentiate between enterococcal and non-enterococcal streptococci. An additional test, the 6.5% NaCl broth tolerance test, is necessary to complete the identification of enterococci. All of the viridans streptococci that blackened BEM (5.3%) failed to grow in 6.5% NaCl broth. They were erroneously identified as group D streptococci, not enterococci. A large number of viridans streptococci that blackened BEM were *S. mutans*. More than half of the *S. mutans* strains were isolates from dental plaque. The routine clinical laboratory will not receive the high number of *S. mutans* strains used in this study, and therefore fewer false positive BEM reactions should occur. The antibiotic therapy regimens for enterococcal and non-enterococcal infections are generally dissimilar, so some physicians want a definitive answer as to whether or not the infecting agent is an enterococcus. To ascertain this, the laboratorian must determine salt tolerance of BEM-positive strains. BEM-positive streptococci that are salt tolerant are enterococci; BEM-positive streptococci that fail to grow in 6.5% NaCl broth are group D streptococci, but not enterococci.

NaCl tolerance. The alpha-hemolytic streptococci that are BEM negative and fail to grow in 6.5% NaCl broth are members of the viridans streptococci. The species *S. mitis*, *S. MG*, *S. sanguis*, *S. salivarius*, and *S. mutans* can be differentiated by additional physiological reactions; however, because they do not differ in susceptibility to antibiotics, clinical microbiologists do not need to speciate them. Alpha-hemolytic streptococci which are BEM and salt tolerance negative can be reported as viridans streptococci or alpha-hemolytic streptococci, not enterococci, not further identified.

The high percentage of group B streptococci that grow in media containing 6.5% NaCl will not interfere with the presumptive identification procedures. All group B streptococci are bile-esculin negative, and the majority hydrolyze hippurate. This combination of reactions is unique to the beta-hemolytic groups B streptococci.

Salt-tolerant group A streptococci occur occasionally. A strain that is salt tolerance positive and that is not a presumptive group B or presumptive group D streptococcus should be tested for purity by streaking the growth from the salt tolerance test medium onto a blood agar plate and comparing the morphology of this organism with that of the original strain. If the morphology differs, a Gram stain and a catalase test should be performed.

SF broth. We have previously reported (11) that SF broth could be used to differentiate the BEM-positive enterococci from the BEM-positive non-enterococci. The results of our 6-year study show that 40% of the non-enterococcal group D streptococci grew in SF broth, and 13% of the viridans streptococci grew in SF broth, thus making this medium a poor indicator of group D streptococci and an even less successful indicator of enterococci. Braunstein et al. (4) reported some strains of group B streptococci that grew in SF broth, and in a recent study (10), we have found a lot-to-lot variation in SF broth. The variable reactions of the streptococci in a single lot of SF broth and the lot-to-lot variation in the media make the medium useless as a reliable indicator of either enterococci or group D streptococci.

The reactions of bacteria other than streptococci in these presumptive tests point out some of the pitfalls of presumptive test identification. The microbiologist must be certain that he is testing streptococci when he uses the tests listed in Table 6. Determining Gram strain characteristics and catalase activity is important and necessary. *Listeria* and *Aerococcus* may be confused with group D or enterococcal strepto-

cocci in these presumptive tests. Bacitracin susceptibility of both pneumococcal and staphylococcal strains points out the need for the microbiologist to recognize the differences in colonial morphology and hemolytic activity of different kinds of bacteria.

In summary, the streptococci can be presumptively identified with acceptable accuracy by using the tests listed in Table 6. Our results show that: 99.5% of 577 group A, 99.7% of 1,460 group B, 92.6% of 351 beta-hemolytic streptococci non-group A, B, or D, 99.7% of 786 enterococcal group D, 97.8% of 134 non-enterococcal group D, and 94.7% of 1,068 viridans streptococci were correctly identified according to the test results listed in Table 6. The largest error in overall identification was in determining the susceptibility of 140 strains of beta-hemolytic, non-group A, B, or D to bacitracin. Fifteen of these strains were erroneously classified as group A by bacitracin, an overdiagnosis.

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