Constricted Tube System for Presumptive Identification and Differentiation of Group D Streptococci

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A single, constricted tube containing two differential media to identify and differentiate group D streptococci was developed. Test results with a limited number of group D streptococcal isolates were in complete agreement with results of conventional procedures.

Group D streptococci are comprised of two categories of bacteria, the enterococci and the non-enterococci (5). The differentiation of these two categories is of clinical significance because of their difference in antibiotic susceptibilities (3). Generally, two physiological characteristics determined with two test media in separate receptacles are used to presumptively identify the group D streptococci. This group is identified by the ability to hydrolyze esculin in a bile-esculin medium (5). Within this group, the enterococci are differentiated from the non-enterococci by growth in a high-salt medium (5). This report describes a constricted tube system for the presumptive identification and differentiation of group D streptococci and presents test results with a limited number of streptococcal isolates.

Sixty-five enterococcal strains and 13 non-enterococcal strains (*Streptococcus bovis*) of group D streptococcus, 48 streptococcal strains other than group D (groups A, B, C, E, F, G, H, K, L, M, N, O), and 4 nongroupable streptococcal strains were tested. All strains were previously characterized stock culture strains or recently identified clinical isolates obtained from the following sources: Center for Disease Control (Atlanta, Ga.), American Type Culture Collection (Rockville, Md.), Corning Medical, Microbiology (Roslyn, N.Y.), Massachusetts General Hospital (Boston, Mass.), Ohio State University (Columbus, Ohio), and Montgomery County Public Health Laboratory (Rockville, Md.).

The basal medium used in the constricted tube was PPLO broth without crystal violet (Difco Laboratories, Detroit, Mich.). The esculin medium contained, per liter of water: 21 g of PPLO broth, 15 g of agar, 40 g of oxgall, 1 g of esculin, and 0.5 g of ferric ammonium citrate. The salt medium contained, per liter of water: 21 g of PPLO broth, 15 g of agar, 65 g of NaCl, 10 g of dextrose, and 0.014 g of phenol red. Commercially available media, including bile esculin medium (BEM, Difco), PSE agar (Pfizer

Inc., New York, N.Y.) and SF broth (Baltimore Biological Laboratory, Cockeysville, Md.) were prepared and used according to manufacturers' directions. An unmodified salt medium was also prepared and used as described by Facklam (4). The constricted tube system was comprised of an elongated, substantially cylindrical transparent glass tube (13 by 100 mm, Corning Glass Works, Corning, N.Y.), having upper and lower chambers joined by a conduit having a diameter smaller than either chamber. The lower chamber contained the salt medium, and the upper chamber contained the esculin medium. One half of both chambers (approximately 1.5 ml) were filled with medium. Media in the two chambers were unslanted and were separated by air within the conduit. Inoculation of the media was accomplished by passing a bacteriological needle, containing morphologically identical colonies from a primary isolation medium or from a pure culture, through both media to the bottom of the tube. The inoculated tube was then incubated at 35°C in a non-CO₂ atmosphere for 18 to 24 h. Results obtained with this system were compared to results obtained with the conventional test media.

This constricted tube system and conventional test media were tested with 127 isolates of previously identified streptococci (Table 1). Ninety-nine percent (77/78) of the group D isolates showed positive reactions, indicative of group D streptococcus, in BEM, PSE, and in the constricted tube. One of the group D designates displayed negative reactions in all three media. Two non-group D streptococcal isolates (one group N and one nongroupable) hydrolyzed esculin in the constricted tube and in the BEM; these same strains also grew in PSE. One group E isolate grew in PSE but did not hydrolyze esculin in the constricted tube or in BEM. Of the 78 group D isolates, 65 were identified previously as enterococci. Positive reactions with 95% (62/65) of these enterococcal isolates were

TABLE 1. Reactions of streptococcal strains on three test systems for group D recognition

Streptococcal group	No. of strains	No. of positive reactions ^a		
		Constricted tube	Difco BEM ^b	Pfizer PSEM ^c
D	78	77	77	77
A	14	0	0	0
B	6	0	0	0
C, E, F, G, H, K, L, M, N, O	25	1	1	2
Nongroupable	4	1	1	1

^a Incubation of 18 to 20 h.^b Bile esculin medium.^c PSEM, Pfizer selective enterococcus medium.

observed in the constricted tube, SF broth, and an unmodified salt broth after 18 to 20 h of incubation (Table 2). Positive reactions with group D non-enterococcal isolates or with other streptococcal groups were not observed in the constricted tube or the two conventional media.

Constricted tube systems for bacterial identification are not new. Such systems have been developed for identifying *Enterobacteriaceae* (6-8) and for determining the oxidative or fermentative metabolism of bacterial isolates (2). The present study with a limited number of streptococcal strains indicated that the constricted tube concept can also be used with appropriate media to provide accurate presumptive recognition and differentiation of group D streptococci. The system reported here utilized esculin and salt tolerance media, modified slightly in composition from more conventional media. Incorporation of conventional esculin and solidified salt tolerance media into the respective chambers of the constricted tube would also produce a suitable system (unpublished data). Blackening of the esculin medium indicated that esculin was hydrolyzed in the constricted tube. This reaction, also observed in conventional esculin media, is a result of the formation of an iron-esculetin complex which is not yet fully defined (1). Salt tolerance was detected by a red to yellow color change of the medium due to streptococcal growth and their subsequent fermentation of dextrose.

Excellent agreement was observed between the results of the constricted tube system and the results of conventional test media in recognizing and differentiating the group D streptococcus. Since serological testing of streptococcal isolates was not performed in our laboratory, the identity of the one group D streptococcal isolate which did not hydrolyze esculin could not be confirmed or denied. However, it is speculated that this isolate was a group D streptococcus which failed to hydrolyze esculin in this inves-

TABLE 2. Reactions of streptococcal strains on three test systems to identify enterococci

Streptococcal group	No. of strains	No. of positive reactions ^a		
		Constricted tube	BBL SF broth	NaCl broth ^b
D, enterococci	65	62	62	62
D, non-enterococci	13	0	0	0
A	14	0	0	0
B	6	0	0	0
Miscellaneous (C, E, F, G, H, K, L, M, N, O)	25	0	0	0
Nongroupable	4	0	0	0

^a Incubation of 18 to 20 h.^b See reference 4.

tigation. Failure of enterococcal strains to hydrolyze esculin has been reported previously (3). Hydrolysis of esculin by non-group D streptococci, as observed in this study, has also been reported (4).

Results of this study clearly demonstrated that this constricted tube system was as reliable as the more conventional test media for the presumptive identification and differentiation of group D streptococci. A major advantage of the constricted tube system is convenience; both prepared media located in one tube can be inoculated simultaneously with a bacteriological needle. Considerable saving of laboratory personnel time in media preparation would also be realized if such a system became available commercially.

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