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Rapid Identification of Enterococci

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Enterococci were identified in 4 h with bile esculin agar and the Autobac system (General Diagnostics, Warner-Lambert Co., Morris Plains, N.J.), which was used to incubate and monitor salt broth for growth. Of 86 group D streptococci tested, 41 enterococci grew in salt broth and were bile esculin positive within 4 h; none of 45 group D nonenterococcal streptococci exhibited growth in the salt broth, and only 38 of them were bile esculin positive within 4 h.

Group D streptococci are generally identified by their ability to hydrolyze esculin in the presence of 40% bile (1). Enterococci can be further differentiated by their ability to grow in 6.5% salt (1). In general, these tests require 24 to 72 h of incubation before they are interpreted. Several workers have attempted to shorten this incubation time or develop alternative rapid tests. Qadri et al. (3) modified the salt broth of Facklam (1) by adding agar and increasing the dextrose concentration from 0.1% to 1.0%. This enabled the detection of acid production by enterococci within 6 to 8 h for the majority of strains tested. Facklam (1) used bile esculin agar slants to determine esculin hydrolysis after incubation for 24 h or more. Schierl and Blazevic (4) used detection of litmus milk reduction within 4 h as a criterion for identification of enterococci. However, only 83% of enterococci yielded positive results, and negative results required further testing. In this study we describe a rapid, 4-h technique for identification of enterococci based upon a modification of the standard esculin hydrolysis and salt tolerance biochemicals tests.

A total of 86 group D streptococci were tested. These strains were from a variety of clinical specimens submitted to the medical microbiology laboratory at the University of California Irvine Medical Center, Orange. Isolates were taken from 18- to 24-h cultures; if an isolate had been frozen, it was subcultured twice to 5% sheep blood agar before being tested. Of the 86 isolates tested, there were 41 enterococci and 45 group D nonenterococcal streptococci, as identified by the standard esculin hydrolysis and salt tolerance physiological tests (2).

For determination of salt tolerance, brain heart infusion broth (Scott Laboratories, Inc., Fiskeville, R.I.) with 0.3% glucose, 6.5% sodium chloride, and 0.002% bromcresol purple was used. Three to four colonies from an 18- to 24-h culture on 5% sheep blood agar were inoculated into the salt broth. The contents of the tube were mixed well, and the inoculum was standardized to 1.5×10^7 CFU/ml with the Autobac system (General Diagnostics, Warner-Lambert Co., Morris Plains, N.J.). A portion of this standardized broth was then transferred to a single chamber of a 12-chamber cuvette, and the remaining inoculated salt broth was incubated (35°C, in air) and checked for color change and turbidity at 4, 24, and 48 h. After an initial photometric reading was taken with the Autobac system, the cuvette was placed in the incubator-shaker (35°C, 220 rpm), and another reading was taken at 4 h. With the Autobac system there is an inverse relationship between bacterial growth and voltage change; in this study, a drop of 0.2 V or greater was the parameter used to indicate growth.

For determination of esculin hydrolysis, bile esculin agar (Scott Laboratories, Inc.) was used. Up to 12 isolates per plate were tested by inoculation of the plate with one colony in a single streak. The presence or absence of blackening of the agar surrounding the inoculum was recorded at 4 and 24 h.

Within 4 h, all 41 enterococci grew in the salt broth, as detected with the Autobac system (Table 1). However, turbidity or sugar fermentation could not be reliably interpreted macroscopically after 4 h of incubation of the salt broth. Of the 41 enterococcal isolates, 39 (95.1%) demonstrated turbidity and a pH change in the salt broth after overnight incubation. One isolate became turbid in the salt broth after 24 h, with no change in pH. Another isolate required 48 h of incubation before turbidity and color change became apparent. However, both isolates caused a decrease of more than 0.2 V within 4 h. In contrast, none of the nonenterococcal isolates grew in the salt broth, as detected with either the Autobac system or conventional tests.

TABLE 1. Differentiation of enterococci from non-enterococci by a rapid procedure

Test	No. positive	
	Enterococci	Non-enterococci
Esculin hydrolysis		
4 h	41	38
24 h	41	45
Growth in 6.5% salt		
4 h	41	0
48 h	41	0

Hydrolysis of esculin, as evidenced by blackening of the agar within 4 h, occurred with 100% of the enterococcal isolates (Table 1). Of the non-enterococcal isolates, 84.4% (38 of 45) blackened the bile esculin agar within 4 h, whereas 15.6% (7 of 45) required overnight incubation of the plate before there was evidence of esculin hydrolysis. Therefore, the determination of esculin hydrolysis within 4 h appears to be a reliable test for identification of enterococci but not necessarily for identification of other group D streptococci. Unless both esculin hydrolysis and salt tolerance are positive, we recommend standard testing with extended incubation of the bile esculin plate for identification of group D non-enterococcal streptococci.

Differentiation of enterococci from other group D streptococci is clinically important since enterococci are, in general, more resistant to penicillin (2). Therefore, a rapid method to detect enterococci would be a valuable diagnostic and therapeutic aid for laboratories. With the two tests described in this report, enterococci could be reliably identified within 4 h. Other group D streptococci still required incubation for 24 h mainly because of their inability to hydrolyze esculin within 4 h. In summary, we recommend this inexpensive, rapid procedure for identification of enterococci.

LITERATURE CITED

1. **Facklam, R. R.** 1973. Comparison of several laboratory media for presumptive identification of enterococci and group D streptococci. Appl. Microbiol. **26**:138–145.
2. **Facklam, R. R.** 1980. Streptococci and aerococci, p. 88–110. *In* E. H. Lennette, A. Balows, W. J. Hausler, and J. P. Truant (ed.), Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.
3. **Qadri, S. M., M. J. deSilva, S. G. M Qadri, and A. Villarreal.** 1979. Presumptive identification of enterococci from other D streptococci by a rapid sodium chloride tolerance test. Med. Microbiol. Immunol. **167**:197–203.
4. **Schierl, E. A., and D. J. Blazevic.** 1981. Rapid identification of enterococci by reduction of litmus milk. J. Clin. Microbiol. **14**:227–228.