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NOTES

Sodium Chloride-Esculin Hydrolysis Test for Rapid Identification of Enterococci

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The ability of enterococci to cause severe disease in humans and their relative resistance to chemotherapeutic agents make it desirable to rapidly differentiate these organisms from other streptococci. We developed and evaluated a test that within 2 h distinguishes enterococci from other alpha-, beta-, or nonhemolytic streptococci in a buffered solution containing 0.2% esculin and 5% sodium chloride. All 239 strains of enterococci tested gave a positive reaction within 2 h, whereas 95 of 96 isolates of other streptococci remained negative at 4 h.

The abilities of Lancefield group D enterococci to hydrolyze esculin to esculetin and to grow in the presence of high salt concentration are used for the presumptive identification of these bacteria (6, 7). Whereas esculin hydrolysis can be determined in growth-supporting media like Vaughn-Levine, bile-esculin, or Pfizer selective enterococcus agar or by non-growth-supporting methods like PathoTec, spot test, etc. (3, 5, 6, 8, 10, 11), sodium chloride tests have always been growth dependent (5, 9). Growth-supporting methods generally require 4 to 48 h for completion of the test. In this note we describe a growth-independent method that utilizes familiar bacteriological methods of inoculation and color change reaction and yields positive results within 1 to 2 h.

The test medium contained 2.0 g of esculin (Sigma Chemical Co., St. Louis, Mo.), 0.5 g of ferric ammonium citrate, 50.0 g of NaCl, 0.4 g of K_2HPO_4, and 0.1 g of KH_2PO_4 in 1,000 ml of distilled water. The pH was adjusted to 5.6 ± 0.2. A pH value of less than 5.4 delayed the reaction. Esculin hydrolysis was completely inhibited at pHs below 4.0, whereas values of more than 5.8 caused yellow discoloration of the medium that interfered with the interpretation of results. The solution was dispensed in 0.5-ml quantities in test tubes (12 by 75 mm) and stored at 4°C. Under these conditions, the stock solution was stable for at least 8 weeks. With refrigeration, a precipitate was formed which went into solution when heated to about 55°C. The heating had no adverse effect on the ability of bacteria to hydrolyze esculin rapidly. All cultures were inoculated to a density of approximately McFarland standard 3 in 0.5 ml of the buffered esculin solution and incubated at 35°C for up to 4.0 h. A positive test was indicated by a color change of the solution to dark brown or black. It is not necessary to use sterile solutions or glassware because the density of inoculum and lack of nutrients in the test solution prevent the growth of any contaminants in the short time required for the completion of the reaction.

All strains of streptococci were isolated from clinical specimens at Oklahoma Memorial Hospital and Veterans Administration Medical Center, Oklahoma City, Okla., and St. Michael's Hospital, Texarkana, Ark. The streptococci were identified by conventional methods (2, 7). All cultures were maintained on 5% (vol/vol) sheep blood agar (tryptic soy agar base). Young cultures (24 to 48 h) were used in this evaluation.

A total of 335 strains of streptococci were tested to determine the ability of the test to distinguish enterococci from other streptococci. The results are shown in Table 1. All 239 isolates of enterococci yielded a 2+ to 4+ reaction within 2 h. A 1+ reaction was observed with more than 95% of the cultures within 1 h. All 96 isolates of other streptococci remained negative at 4 h, with one strain of nonenterococcal group D giving a 1+ reaction in 6 h. Three other isolates of nonenterococcal streptococci yielded 1+ to 2+ reaction in 24 h. Since all the enterococci became positive within 2 h, termination of the test at 4 h would avoid any false-positive reactions by other streptococci.

Batch-to-batch variation of esculin powder was tested by using three lots, with excellent correlation in all cases. Although the evaluation was done with cultures grown on

TABLE 1. Sensitivity and specificity of rapid NaCl-esculin hydrolysis

Organism	No. of isolates	No. (%) positive in:			
		1 h	2 h	6 h	24 h
Lancefield group D Enterococcus	239	126 (53)	239 (100)		
Nonenterococcus	28	0	0	1 (4)	2 (7)
S. pyogenes	13	0	0	0	0
Streptococcus agalactiae	4	0	0	0	0
Streptococcus pneumoniae	15	0	0	0	0
Viridans group streptococci	36	0	0	0	2 (5)

[a] All 28 isolates of nonenterococcal group D streptococci were esculin positive by both the conventional bile-esculin test (4) and the rapid test (7). Seven isolates of *S. pyogenes* and 21 strains of viridans group streptococci yielded positive esculin reactions with the rapid test (7), but all were bile-esculin negative.

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blood agar plates, colonies obtained from Mueller-Hinton plates, where applicable, gave similar results.

In the diagnostic laboratories, enterococci are presumptively identified by determining the salt tolerance of bile-esculin-positive streptococci. Even when serological tests are performed for definitive identification, the sodium chloride tolerance test is needed to differentiate enterococci from nonenterococcal group D streptococci. Conventional growth-dependent tests used for this purpose require 24 to 48 h for completion. Since over 95% of Lancefield group D streptococci isolated from clinical specimens are enterococci, a single tube growth-independent test, as described in this paper, provides a rapid, simple, and easy-to-interpret method for the identification of these bacteria.

Bosley et al. (1) described a 4-h test for the presumptive identification of *Streptococcus pyogenes* and enterococci based on the production of pyroglutamyl aminopeptidase by these organisms. Ellner et al. (4) recently reported that a commercial product (Identicull-AE; Scott Laboratories, Inc., Fiskerville, R.I.) detects the production of this enzyme in 10 min. Both groups of investigators found the test reliable in the presumptive identification of enterococci. Since both *S. pyogenes* and enterococci yield a positive reaction with this test, bile-esculin, latex agglutination, or other tests are still required to differentiate these organisms. A combined sodium chloride-esculin test not only differentiates these bacteria within 2 h, but is simple and costs less than 10 cents per test.

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