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Rapid Sodium Chloride Tolerance Test for Presumptive Identification of Enterococci

S. M. HUSSAIN QADRI,[1]* CHARLES W. NICHOLS,[1] AND S. G. M. QADRI[2]

Department of Pathology and Laboratory Medicine, The University of Texas Medical School, Houston, Texas 77030,[1] and St. Michael's Hospital, Texarkana, Arkansas 75501[2]

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A rapid test is described which distinguishes enterococci from other group D streptococci in 8 to 24 h by use of a medium containing brain heart infusion agar, NaCl, dextrose, and bromocresol purple.

Enterococci are found as part of the normal flora of human intestine. Their ability to cause severe disease in humans and their relative resistance to antimicrobial agents necessitate their differentiation from other group D streptococci that are not enterococci. Since all group D streptococci are bile-esculin positive, enterococci are differentiated by their ability to grow in the presence of 6.5% NaCl (1, 2). However, growth on conventional NaCl broth is difficult to interpret and requires 24 to 48 h to complete. In this paper, we describe a modified NaCl medium which yields rapid and easy-to-read results.

The organisms used in this study consisted of 211 strains of enterococci and 68 strains of non-enterococcus group D streptococci which were isolated from the clinical laboratories of Hermann Hospital and Ben Taub General Hospital in Houston, Tex., and St. Michael's Hospital, Texarkana, Ark. These isolates were presumptively identified by conventional bile-esculin and 6.5% NaCl broth tolerance tests (1, 2). The test medium contained per liter: 52 g of brain heart infusion agar (Difco, Detroit, Mich.), 10 g of dextrose, 60 g of NaCl, and 20 mg of bromocresol purple. The ingredients were dissolved by heating and adjusted to pH 7.0. The medium was dispensed into screw-cap tubes, autoclaved at 121°C for 15 min, and allowed to cool in a slanted position. Tubes containing the test medium were inoculated by streaking the surface of the slant with an inoculating needle containing a small amount of the 24-h culture. The tubes were incubated at 35°C and observed at 6, 8, and 24 h. Ability to tolerate 6.5% NaCl was indicated by growth accompanied with a color change of purple to yellow.

Of the 211 enterococcus isolates, 84 (39.8%) produced a color change in the medium within 6 h and 194 (92%) within 8 h. The remaining 17 strains were negative at 8 h but became positive within 24 h. The 68 isolates of group D streptococci other than enterococci remained negative after 48 h.

With the growing workload at most clinical microbiology laboratories, it is desirable to use methods that hasten the identification of significant organisms and that are easy to interpret. A number of different methods have been described in literature for the isolation and identification of enterococci (1-3). Facklam (1) reported that a previously described azide medium (3) was not sensitive or selective enough to be useful in the differentiation of enterococci. Facklam and Moody (2) tested a 6.5% NaCl broth and found that it was slow and difficult to interpret. A modified broth containing 0.1% dextrose and bromocresol purple as a pH indicator was found to be easier to interpret (2). The method described herein is a modification of Facklam's medium (2) in which addition of 1.0% dextrose and agar makes the test rapid and easier to read. In this study, addition of 0.1% dextrose did not produce reliable and reproducible results. Increasing the concentration to 1.0% gave good results. Agar was added to prevent convection currents, thus allowing the acid produced to remain concentrated in the medium and not be diluted by mixing throughout the medium. This resulted in hastening the color change due to growth and acid production by enterococci to within 8 h in 92% cases. Often the results can be obtained the same day if the culture is inoculated at the beginning of the day. The method described in this paper showed 100% correlation with the conventional medium of Facklam and Moody (2).

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