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Rapid Identification of Enterococci by Reduction of Litmus Milk

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Of 100 strains of enterococci, 83% reduced litmus milk within 4 h. None of the 100 non-enterococcal streptococci tested were positive.

The most widely used tests for identification of enterococci in clinical laboratories are hydrolysis of esculin in the presence of 40% bile and growth in 6.5% NaCl (1). Because these tests depend upon growth, they take from 1 to 2 days to perform. In addition to the delay, problems are frequently encountered with demonstration of growth on 6.5% NaCl media (4). This study was undertaken to develop a rapid test for the identification of enterococci that would not depend on growth of the organism.

Most enterococci reduce litmus milk (5) by utilizing the litmus as an electron acceptor (3), thus producing a white color. The reaction is enzyme mediated and so may occur in the presence of preformed enzyme. Previously described tests for litmus milk reactions have been incubated for 24 h or more. We developed a 4-h test by decreasing the volume of the medium and using a large inoculum of organisms.

Several lots of litmus milk were used (Difco Laboratories and BBL Microbiology Systems) and were prepared in accordance with the manufacturers' directions. The medium was refrigerated until used. Amounts (0.5 ml each) were dispensed into sterile tubes (13 by 100 mm) with caps. A series of smaller tubes (6 by 50 mm) filled to a level of 1/4 in. (ca. 0.6 cm) were also used.

One hundred fresh clinical isolates of enterococci were tested. All of these strains grew on 6.5% NaCl medium and blackened bile esculin medium. One hundred clinical isolates of non-enterococcal streptococci were also tested; these included three strains of *Streptococcus anginosus-constellatus*, six of *S. sanguis*, six of *S. bovis*, six of *S. mitis*, five of *S. pneumoniae*, one of *S. salivarius*, one of *S. milleri*, one of *S. equinus*, and three of group D streptococci, not enterococci. The speciated strains were identified by using biochemical reactions described by Facklam (2). The remaining isolates in this group produced alpha-hemolysis on sheep blood agar, did not grow on 6.5% NaCl agar or blacken bile esculin medium, and were considered to be viridans streptococci.

All organisms tested were scraped from overnight cultures on sheep blood agar plates by using a loop. The large tubes (13 by 100 mm) were inoculated with a large loop that was scraped three times across a heavy area of growth. The 6-by-50-mm tubes were inoculated with a 0.001-ml loop that was half full. An uninoculated tube of litmus milk, a known enterococcus strain, and a viridans streptococcus were used as controls for color comparison with each lot tested. Controls and tests were incubated in a 37°C heating block and read at 0.5 h and at hourly intervals for 4 h. Reactions were recorded as positive if a definite white to cream color was observed. A pink color change (acid reaction) was recorded as negative.

Of the enterococci, 83% reduced the litmus milk within 4 h in both the 13-by-100- and the 6-by-50-mm tubes. None of the non-enterococci reduced the litmus milk. The smaller tubes required a smaller inoculum, and reactions occurred slightly faster than in the larger tubes. All 83% of the enterococci gave a clear-cut positive test for reduction in 0.5 h in the small tubes, compared to 47% in the 13-by-100-mm tubes. Attempts to reduce the inoculum to five or six colonies resulted in a low percentage of positive reactions in the 4 h period, and the reactions were not clear cut. Incubation overnight did not increase the number of positive reactions when a large inoculum was used. However, because viridans streptococci may reduce litmus milk during prolonged incubation (2), tests should not be read after 4 h.

Although only 83% of the enterococci reduced litmus milk by this rapid method, there were no false positives by non-enterococci. The specificity and rapidity of the test would thus enable the identification of most enterococci on the same day they are isolated. Only those isolates

which were negative for reduction would need to have further work done. This would result in a significant savings in both technologist time and media as well as yield a more clinically relevant identification.

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