Rapid Deoxyribonuclease Test with Methyl Green

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A 4-h deoxyribonuclease test using methyl green to differentiate *Serratia* from other *Enterobacteriaceae* was developed. The tests agreed 100% with an overnight plate test for 100 *Serratia*, 83 *Enterobacter*, and 6 *Klebsiella* species.

Serratia species have become an important group of microorganisms clinically and are reported from cases of pneumonia, meningitis, abscesses, septicemia, and wounds (2, 5, 7). Current kit methods of identification in use in many laboratories sometimes require the deoxyribonuclease (DNase) test to differentiate *Serratia* (usually DNase positive) from *Enterobacter*, *Klebsiella*, and *Citrobacter* species (DNase negative). Methods currently in use frequently take 18 to 24 h to perform, delaying results a full day (4, 9, 10). The rapid DNase method described in this report was designed to be completed within 4 h.

The test depends on the fact that most *Serratia* species produce DNase (8). This enzyme will act upon deoxyribonucleic acid (DNA), causing it to depolymerize. If methyl green is added to a medium containing DNA, it will form a stable blue-green complex with the DNA. As the DNA is depolymerized, it releases the methyl green and, at the pH of the medium, free methyl green fades and becomes colorless.

Serratia isolates and some of the *Enterobacter* species used in this study were stock cultures obtained from the Diagnostic Microbiology Laboratory of the University of Minnesota. The cultures had been stored on paraffin-sealed Trypticase soy agar (BBL) slants in the dark at room temperature. Stock *Serratia* (100) and *Enterobacter* (52) isolates were identified by the method of Durand and Blazevic (1). An additional 31 isolates of *Enterobacter* and all other organisms tested were recent laboratory isolates, identified by use of API 20E strips (Analytab), and stored on triple sugar iron slants (Grand Island Biological Co. [GIBCO]) at 4°C or on tryptic soy agar slants (GIBCO) at room temperature. All organisms were subcultured to MacConkey agar plates (GIBCO) prior to testing.

All strains were checked for DNase activity by growing the organisms on DNase agar with toluidine blue (GIBCO) for 18 to 24 h at 35°C.

These plates were then checked for a pink area surrounding the organism, indicating the production of DNase.

The DNase base medium for the rapid test was prepared by combining 31.5 mg of DNA (Difco), 5.0 ml of 0.1 M CaCl₂, and 5.0 ml of 0.1 M MgCl₂ in 100 ml of 0.05 M Tris buffer, pH 7.4 (Sigma Chemical Co.). Methyl green indicator was prepared by the method of Kurnick (6) by dissolving 0.05 g of methyl green (Chroma Gesellschaft, Schmid and Co., Stuttgart-Unterturkheim) in 10 ml of distilled water. The solution was extracted eight times with equal volumes of chloroform or until the chloroform layer became colorless. The chloroform layer was discarded each time. The final working concentration of methyl green was prepared by adding 1.6 ml of the extracted solution to 100 ml of distilled water.

The rapid test was performed by making a heavy (milky) suspension of the organism to be tested in 0.5 ml of DNase base medium in a test tube (13 by 100 mm). The suspension was incubated for 2 h in a 37°C heating block. After incubation, 2 drops of methyl green were added with a Pasteur pipette, and the tubes were lightly shaken to mix. The tubes with added methyl green were incubated an additional 2 h at 37°C. A positive reaction was indicated by a colorless tube, whereas a negative reaction remained blue-green. Positive (*Serratia*) and negative (*Enterobacter*) controls were run with each test.

Results obtained with the rapid test and the plate method are shown in Table 1. Excellent agreement was achieved with all *Enterobacteriaceae* isolates tested. Thirty of the *Serratia* isolates were retested after incubation at room temperature for 3 days on a MacConkey plate, and identical results were obtained, indicating that a fresh isolate is not necessary. The test does not appear to be satisfactory with a weak DNase producer such as *Pseudomonas maltophilia*. Of the four strains tested, one was

TABLE 1. Comparison of rapid and conventional DNase tests

Species	No. tested	No. positive on DNA plate ^a	No. positive with rapid test
<i>Serratia</i>	100	100	100
<i>Enterobacter</i>	83	0	0
<i>Klebsiella</i>	6	0	0
<i>P. maltophilia</i>	4	1	0

^a Incubated 18 to 24 h at 35°C.

weakly DNase positive on the plate after 1 day of incubation, two strains required 2 days of incubation, and one strain showed no DNase production at 2 days. All four strains were negative when the rapid test was used.

For differentiating *Serratia* from various other *Enterobacteriaceae* such as *Klebsiella* or *Enterobacter*, the rapid DNase test described here is both quick and accurate. The reagents are stable for at least 3 months at 4 to 10°C. It could prove useful as an addition to the growing battery of rapid tests for the identification of *Enterobacteriaceae*.

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