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Evaluation of Bacto TB Hydrolysis Reagent (Tween 80) for the Identification of *Branhamella catarrhalis*

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An investigation of the hydrolysis of Tween 80 reagent by *Branhamella catarrhalis* and related organisms (*Neisseria* and *Moraxella* species) revealed that only *B. catarrhalis* gave a positive result. A total of 226 strains, including reference organisms and clinical isolates, were studied. *B. catarrhalis* changed the color of the reagent from amber to pink-red after overnight incubation. We recommend this simple and cost-effective test as an alternative procedure to DNase testing or tributyrin hydrolysis or as a supplemental procedure for the identification of *B. catarrhalis* in clinical specimens.

Branhamella catarrhalis, previously classified as *Neisseria catarrhalis* (6), is now considered a major pathogen. It has been reported as causing respiratory infections (1, 8), otitis media in children (5), and wound infections (3) and as being the etiologic agent in systemic infections (2).

Various tests are used to separate this organism from closely related *Neisseria* species (4, 6) and occasionally from *Moraxella* species (7).

Because of our previous findings that only *Serratia* organisms among various members of the family *Enterobacteriaceae* are able to hydrolyze Bacto TB hydrolysis reagent (9), we investigated the Bacto TB hydrolysis test by using various species of *Neisseria* and *Moraxella* and *B. catarrhalis*. Our findings are presented in this report.

The following organisms were used: *Neisseria gonorrhoeae* ATCC 19424, *N. lactamica* ATCC 23970, *N. meningitidis* group B ATCC 13090, *N. meningitidis* group C ATCC 13102, *N. sicca* ATCC 9913, *N. cinerea* ATCC 14685, *N. flavescens* ATCC 13115, *N. mucosa* ATCC 19696, *N. subflava* ATCC 10555, *N. elongata* ATCC 25295, *B. catarrhalis* ATCC 25238, *Moraxella lacunata* ATCC 11748, *N. osloensis* ATCC 19957, and *M. phenylpyruvica* ATCC 23333. In addition, we tested strains of *N. lactamica* and *N. cinerea* kindly provided by Yvonne C. Faur, General and Special Bacteriology, New York City Department of Health. All other organisms tested were obtained in our laboratory from respiratory and urogenital specimens, blood cultures, cerebrospinal fluids, and eye and ear swabs.

As the organisms were recovered from the specimens, the identifications were made by standard procedures (6, 7), using morphological appearance; Gram stain; oxidase; catalase; growth on Thayer-Martin plates; acid production from glucose, lactose, maltose, and sucrose; nitrate reduction; DNase production on a DNA agar with toluidine blue (BBL Microbiology Systems, Cockeysville, Md.); and the hockey puck test for *B. catarrhalis* (gliding of the colonies on the surface of the agar when pushed with a loop or wooden stick).

The American Type Culture Collection strains were subcultured every week on slants of brain heart infusion agar with 6% sheep cells (Remel, Lenexa, Kans.), incubated overnight in a CO_2 incubator, overlaid with sterile mineral oil, and kept at room temperature. All other organisms were subcultured once like American Type Culture Collection strains, kept 1 week, and then discarded.

A total of 226 strains were investigated, including 36 *N. gonorrhoeae*, 18 *N. meningitidis*, 26 *N. sicca*, 19 *N. flavescens*, 8 *N. mucosa*, 14 *N. lactamica*, 2 *N. elongata*, 10 *N. cinerea*, 19 *N. subflava*, 33 *B. catarrhalis*, 1 *M. lacunata*, 1 *M. osloensis*, 1 *M. phenylpyruvica*, and 38 *Moraxella* species strains.

The reagent used in our investigation was commercially obtained from Difco Laboratories, Detroit, Mich., and was available in 5-ml bottles under the name TB Hydrolysis Reagent (Tween 80). According to Difco technical insert no. 1440, Feb. 1985, it is a phosphate-buffered solution of Tween 80 and neutral red.

The test for Tween 80 hydrolysis was performed as follows. Approximately five colonies were suspended in 1.0 ml of sterile distilled water in small tubes (10.0 by 75.0 mm) placed in racks and homogenized in a vortex for 10 s. Then 0.1 ml of Bacto TB hydrolysis reagent was added to each tube, each tube was covered with tape, and the contents were homogenized again by gently shaking the racks holding the tubes for 5 s.

The tubes were placed in a regular incubator without CO_2 and incubated for 24 h at 35°C. A positive reaction was considered to have occurred when the color of the tube contents changed from the original amber to pink-red. The tubes that showed no change were reincubated for 24 h, checked for change in color, and then discarded.

The results revealed that all 33 isolates of *B. catarrhalis* hydrolyzed Tween 80 after 24 h of incubation, producing a clear-cut change in color from amber to pink-red. All other organisms tested remained negative even after an additional 24 h of incubation.

The testing for Tween 80 hydrolysis was performed at the time of the identification, as the organisms were recovered from the clinical specimens. The test was performed on colonies growing on one of the following media: chocolate agar plates (BBL), tryptic soy agar with 5% sheep cells (BBL), or brain heart infusion agar slants with 6% sheep cells (Remel).

When we employed a very heavy suspension of the organisms, with turbidity at a no. 4 McFarland standard (as we did with some of our subcultures), the test became positive in 3 to 4 h. We did not pursue this aspect, because most of the time only a few colonies were obtained on

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primary plates and would have required another day for reisolation.

Because of the increased role of *B. catarrhalis* in human pathology, newer tests have been developed, such as the rapid DNase RIM Kit produced by Austin Biological Laboratories, Austin, Tex. (3), and tributyrin hydrolysis (4).

Our study revealed that Tween 80 hydrolysis is reliable, simple to perform, and cost-effective (approximately 16¢ per test, including the cost of the tubes). We recommend it as an alternative procedure to DNase testing or tributyrin hydrolysis or as a supplemental procedure for the identification of *B. catarrhalis* in clinical specimens.

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