

## B.CAT CONFIRM, a Rapid Test for Confirmation of *Branhamella catarrhalis*

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**B.CAT CONFIRM (Scott Laboratories, Inc., Fiskeville, R.I.), a rapid test for detection of tributyrin hydrolysis, was evaluated for its ability to identify strains of *Branhamella catarrhalis* and to differentiate them from *Neisseria* species and related species. On initial testing, B.CAT CONFIRM was positive for 65 (96%) of 68 *B. catarrhalis* strains within 30 min after inoculation. Retesting of the remaining three strains resulted in their correct identification. B.CAT CONFIRM was negative for all *Neisseria* spp. (130 strains) and for *Kingella* spp. (3 strains). Two of the three *Moraxella* spp. were weakly positive in the B.CAT CONFIRM after 60 min, but these reactions were easily distinguishable from the strong reactions of *B. catarrhalis* strains. This test will be helpful in the clinical laboratory for the rapid identification of *B. catarrhalis* in clinical specimens.**

*Branhamella catarrhalis* (2, 3) constitutes part of the human respiratory tract flora and during the last several years has been recognized as a significant human pathogen. *B. catarrhalis* has been most commonly associated with otitis media, sinusitis, bronchitis, pneumonia, and conjunctivitis (4, 9, 10). It has also been reported as an uncommon cause of endocarditis, acute urethritis, meningitis, and sepsis in the immunocompromised host (4). The recognition and rapid identification of this organism in clinical specimens also have therapeutic importance, since most strains recovered from disease processes produce  $\beta$ -lactamase and are resistant to penicillin and ampicillin (4).

*B. catarrhalis* is generally identified by failure of the organism to utilize carbohydrates, reduction of nitrate, production of DNase, and growth on chocolate and nutrient agars at 22°C (4, 6, 9, 10). Some strains will also grow on selective media used for isolation of the pathogenic *Neisseria* spp. Whereas some of these tests may take 24 to 48 h to perform, some commercial systems can identify these organisms within 2 to 4 h by using modifications of these conventional tests (4, 8, 9, 10). In chromogenic enzyme substrate tests, these organisms are presumptively identified by negative reactions for *o*-nitrophenyl- $\beta$ -D-galactopyranoside, prolyl aminopeptidase, and  $\gamma$ -glutamyl aminopeptidase (5, 7). Conventional or modified conventional tests are then generally used for species identification (4, 9, 10).

In 1962, Berger (1) demonstrated that *B. catarrhalis* was able to hydrolyze the organic substrate tributyrin with the release of butyric acid. In *Bergey's Manual of Systematic Bacteriology*, tributyrin hydrolysis is mentioned as a key test for differentiating the human isolate *B. catarrhalis* and the animal species *Branhamella caviae*, *Branhamella ovis*, and *Branhamella cuniculi* from the *Neisseria* spp. (12). The hydrolysis of tributyrin has not been extensively exploited for identification of *B. catarrhalis* in the clinical laboratory. Vaneechoutte and co-workers (11) recently reported a rapid tributyrin hydrolysis test that used 4-methylumbelliferyl butyrate as the substrate. Production of the fluorescent umbelliferyl moiety on hydrolysis of the substrate was used to detect hydrolysis. *B. catarrhalis* strains produced positive

fluorescence within 5 min, while *Neisseria* spp. failed to produce fluorescence.

Recently, a commercial test for the rapid detection of tributyrin hydrolysis by *B. catarrhalis* has become available. B.CAT CONFIRM (Scott Laboratories, Inc., Fiskeville, R.I.) consists of a small plastic cuvette containing a disk that is saturated with tributyrin. The disk is hydrated by adding 8 drops of a balanced salt-phenol red (pH 7.2 to 7.4) solution to the cuvette. Several isolated, morphologically similar colonies of oxidase-positive, gram-negative diplococci are emulsified in the balanced salt solution, and the cuvette is sealed and incubated for up to 60 min at 35°C in a non-CO<sub>2</sub> incubator. A change in the color of the solution from red to yellow indicates hydrolysis of tributyrin. No color change (i.e., red or red-orange) is interpreted as a negative test.

B.CAT CONFIRM was evaluated for its ability to identify *B. catarrhalis* strains and to differentiate them from *Neisseria* spp. and similar organisms. Isolates (204) included 68 *B. catarrhalis* (respiratory tract, 41; conjunctiva, 12; stock strains, 8; wounds, 4; blood, 2; peritoneal fluid, 1), 36 *Neisseria gonorrhoeae* (male urethra, 18; endocervix, 16; rectum, 2), 36 *Neisseria meningitidis* (respiratory tract, 18; rectum, 9; cerebrospinal fluid, 4; male urethra, 2; blood, 2; endocervix, 1), 26 *Neisseria lactamica* (respiratory tract, 23; male urethra, 1; rectum, 1; stock strain, 1), 10 *Neisseria cinerea* (respiratory tract, 6; stock strains, 2; male urethra, 1; endocervix, 1), 22 *Neisseria* spp. (respiratory tract, 16; blood, 4; wound, 1; stock strain, 1), 3 *Kingella* spp. (stock strains, 2; blood, 1), and 3 *Moraxella* spp. (conjunctiva, 2; stock strain, 1). Organisms were identified by conventional procedures, including the following: Gram stain morphology; oxidase and catalase reaction; growth on modified Thayer-Martin medium; acid production from glucose, maltose, fructose, sucrose, and lactose by the rapid carbohydrate fermentation technique; reduction of nitrate and nitrite; production of DNase on DNase test agar with toluidine blue O; and susceptibility to colistin (6, 9, 10). Blood or chocolate agar subcultures that had been incubated at 35°C for 18 to 24 h were used to provide inoculum for conventional tests and for the B.CAT CONFIRM as described above. B.CAT CONFIRM results were recorded after 30 and 60 min of incubation at 35°C. All testing was performed in a blinded fashion.

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Of the 68 *B. catarrhalis* strains tested, 65 (96%) were positive in B.CAT CONFIRM after 30 min, producing a bright yellow reaction in the cupule. The other three isolates were yellow-orange after 30 min but were clearly positive after 60 min of incubation. On repeat testing, all three produced clearly positive reactions within 30 min. None of the *N. gonorrhoeae*, *N. meningitidis*, *N. lactamica*, *N. cinerea*, *Neisseria*, or *Kingella* isolates were positive after either 30 or 60 min. Although some of the *N. meningitidis* strains and two of the three *Kingella* spp. turned the salt solution in the cupule slightly red-orange after 60 min, these reactions were still easily distinguishable from positive reactions. Among the *Moraxella* spp., two produced red-orange reactions after 30 min and yellow-orange reactions after 60 min. The third strain was negative at both reading times.

In this study, B.CAT CONFIRM was useful for the rapid identification of *B. catarrhalis*. Inoculation of five to eight morphologically similar colonies of gram-negative, oxidase-positive diplococci into the cupule resulted in clearly positive reactions within 30 min for most *B. catarrhalis* strains. Weak-positive reactions were noted with two of three *Moraxella* spp. In their study with the 4-methylumbelliferyl butyrate substrate, Vaneechoutte et al. (11) also reported that all three *Moraxella* isolates they tested were tributyrin hydrolysis positive after 15 min, while all *B. catarrhalis* strains were positive after 5 min. The weak reactions in B.CAT CONFIRM were easily differentiated from strongly positive reactions. Additional *Moraxella* strains, however, will need to be examined in this regard. Vaneechoutte et al. also reported that tributyrin was hydrolyzed by a few strains of *Acinetobacter calcoaceticus*, nonhuman *Branhamella* spp., *Pseudomonas aeruginosa*, *Staphylococcus* spp., and *Candida albicans*. While organisms representative of these species were not evaluated by B.CAT CONFIRM, the examination of characteristic colony morphology, the careful performance of Gram-stained smears, and spot tests such as for oxidase and catalase reactivity should prevent misidentification of these isolates. In addition, the testing format of B.CAT CONFIRM requires that only oxidase-positive, gram-negative diplococci be tested. While B.CAT CONFIRM is designed to confirm presumptive results obtained with carbohydrate or enzyme substrate tests, use of this test along with Gram-stained smear morphology, oxidase reactivity, and the chromogenic cephalosporin test for  $\beta$ -lacta-

mase may allow direct identification of *B. catarrhalis* directly from isolated colonies on primary cultures.

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