

NOTES

Butyrate Esterase (Tributyryl) Spot Test, a Simple Method for Immediate Identification of *Moraxella (Branhamella) catarrhalis*

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A total of 47 *Moraxella (Branhamella) catarrhalis* strains, 89 strains of *Neisseria* spp., and 82 strains of miscellaneous bacteria and yeasts were studied by using a fluorogenic spot method which detects butyrate esterase. A positive butyrate esterase spot test correctly differentiated *M. catarrhalis* from *Neisseria* spp., which had a negative butyrate esterase spot test reaction. The test is rapid, simple, and easy to perform. The butyrate esterase spot test was useful for direct identification of *M. catarrhalis* from primary cultures. However, false-positive reactions may occur with mixed cultures.

Moraxella (Branhamella) catarrhalis is now recognized as a major pathogen that causes otitis media, sinusitis, conjunctivitis, bronchitis, and pneumonia. In addition, a significant proportion of *M. catarrhalis* strains produce the enzyme β -lactamase. This organism shares several characteristics with the saprophytic strains of *Neisseria* spp. Both are found in the same habitat, and both are asaccharolytic. Conventional tests may require 24 to 48 h for completion. Alternative rapid, easy-to-perform identification methods are desirable.

The differences in the esterase activities of members of the family *Neisseriaceae* have been known for several years. From the early studies of Berger (1), tributyrin (glycerol tributyrate) hydrolysis has been used as a key test for differentiation and identification of *M. catarrhalis* (4, 8). Several substrates and techniques have been proposed for detecting butyrate esterase (2, 3, 6). Vanechoutte et al. (7) recently described a fluorogenic method that allowed accurate results after 5 min of incubation. The aim of this study was to evaluate the usefulness of a spot fluorescence technique which simplifies and shortens this method.

A total of 47 *M. catarrhalis* strains, 89 strains of *Neisseria* spp., and 82 strains of other bacteria and yeasts were studied (Table 1). All of the strains were isolated from human sources at different hospitals, except for 26 strains that were kindly supplied by the reference laboratory Centro Nacional de Microbiología, Majadahonda, Madrid, Spain. Most of the strains were maintained frozen at -70°C or lyophilized. Stock strains were subcultured twice to appropriate media before testing.

Neisseria spp. and *M. catarrhalis* strains were identified by using standard methods (5, 8). The tests which we used included Gram staining and tests for oxidase, catalase, superoxol (4), DNase, nitrate reduction, susceptibility to a 10- μg colistin disk, and production of acid from glucose, maltose, lactose, sucrose, and fructose. γ -Glutamyl aminopeptidase and *o*-nitrophenyl- β -D-galactopyranoside detection tests were performed when they were needed. The

remaining bacterial strains and yeasts were identified by using conventional techniques. Butyrate esterase activity was detected by using the following methods: the acidimetric method, the tube fluorogenic method, and the butyrate esterase spot test (BST).

In the acidimetric method (substrate, tributyrin), tests were performed by using the technique of Riou et al. (6) and commercially available tributyrin tablets (Rosco, Taastrup, Denmark). A heavy suspension of an organism that was equivalent to a no. 2 McFarland standard was made in a screw-cap tube containing 0.5 ml of saline. One tributyrin tablet was added to the suspension, and the preparation was mixed and incubated at 35°C . Tests were read after 2, 3, and 4 h of incubation (Table 1). The development of bright yellow was considered to be a positive result. Each batch of tablets was quality controlled with the following reference strains: *M. catarrhalis* ATCC 25238 (positive) and *Neisseria meningitidis* ATCC 13090 (negative).

In the tube fluorogenic method (substrate, methylumbelliferyl butyrate), tests were performed as described by Vanechoutte et al. (7). Briefly, the reagent was prepared as follows: a stock solution was made by dissolving 100 mg of 4-methylumbelliferyl butyrate (Sigma Chemical Co., St. Louis, Mo.) in 10 ml of dimethyl sulfoxide (E. Merck AG, Federal Republic of Germany) and 100 μl of Triton X-100 (Sigma). The stock solution was diluted 1:10 in 0.1 M McIlvaine citrate buffer (pH 5.0); this preparation could be stored in 500- μl portions in glass tubes at -70°C for at least 1 month. Two to three colonies from a recent culture plate (not older than 24 h) were suspended in 0.25 ml of fluorogenic reagent and incubated at room temperature. A positive test was indicated by the development of blue fluorescence after exposure to a 366-nm UV source. Tests were read after 5 min, 15 min, and 18 h of incubation. The reference strains mentioned above and a reagent blank were used as controls in each test.

In the BST (substrate, methylumbelliferyl butyrate), the reagent was the same as that used for the tube fluorogenic method. A few drops of the reagent were placed onto a piece of filter paper. Two to three colonies from a recent culture were streaked onto the paper, which was immediately ex-

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TABLE 1. Detection of butyrate esterase by three methods

Taxon (no. of strains tested)	Cumulative no. of positive results ^a								
	Acidimetric			Tube fluorogenic			BST method		
	2 h	3 h	4 h	5 min	15 min	>18 h	30 s	1 min	3 min
<i>Moraxella catarrhalis</i> (47)	46	47		47			47		
<i>Neisseria gonorrhoeae</i> (27)						27			8
<i>Neisseria meningitidis</i> (28)						28			7
<i>Neisseria lactamica</i> (12)						12			3
<i>Neisseria polysaccharea</i> (5)						5			1
<i>Neisseria subflava</i> (7)						7			3
<i>Neisseria sicca</i> (5)						5			
<i>Neisseria denitrificans</i> (1)						1			
<i>Neisseria cinerea</i> (3)						3			1
<i>Neisseria mucosa</i> (1)						1			
<i>Moraxella</i> spp. (7)	2	2	3	3	3	5	3	3	5
<i>Acinetobacter</i> spp. (27)	23	25	25	14	24	27		25	25
<i>Pseudomonas aeruginosa</i> (10)	6	7	9	10				10	
<i>Staphylococcus aureus</i> (10)	8	10		10			10		
Coagulase-negative staphylococci (10)	4	10		10			10		
<i>Candida albicans</i> (10)	9	10		10			10		

^a Positive results within the times indicated. There were no positive results for eight *Eikenella corrodens* strains.

posed to a UV lamp. A blue fluorescent spot was considered positive. The tests were read after 30 s, 1 min, and 3 min. Positive and negative reference strains were used as controls.

Table 1 shows the results obtained with the three methods at the intervals of incubation used. As expected, the acidimetric and tube fluorogenic techniques clearly differentiated between *Neisseria* spp. and *M. catarrhalis* strains when the tests were read after 3 h and 5 min, respectively. All *M. catarrhalis* strains gave positive results with the BST within 30 s. In contrast, enzymatic activity was not detected in *Neisseria* spp. by the BST, except for a few strains that showed weak reactions after 3 min, probably because of nonspecific hydrolysis of the substrate. Three of seven strains of *Moraxella* spp. other than *M. catarrhalis* also gave positive results with each of the three test methods. We found complete agreement between the BST results and the results of the reference techniques.

Among the remaining miscellaneous bacterial and fungal isolates, butyrate esterase activity was observed in *Acinetobacter* spp., *Pseudomonas aeruginosa*, staphylococci, and *Candida albicans*. Both staphylococci and *C. albicans* gave more rapid positive results than gram-negative rod-shaped bacteria.

In order to evaluate the possible interference of other microorganisms when the BST was performed, two to three colonies of six different strains of *Neisseria* spp. (three *N. meningitidis* strains, one *N. lactamica* strain, and two *N. subflava* strains) were mixed with two or three colonies of either *Staphylococcus aureus* or *C. albicans*. The mixed cultures were tested by using the BST. Each combination of microorganisms was tested in triplicate. False-positive results were obtained with all combinations after mean times of ≤ 42 s. In the majority of cases, a fluorescent spot was observed within 30 s.

In this study we demonstrated the utility of the BST. This is a technique that is simple and easy to perform and that gives immediate and accurate results. Moreover, it is also inexpensive in reagents and handling times. At current prices and exchange rates in Spain, each test (including two controls) costs approximately \$0.10. The proposed test may be used with confidence to differentiate *M. catarrhalis* from

Neisseria spp. in pure cultures. The BST is also useful for identifying *M. catarrhalis* from primary isolation plates. In this case, however, caution must be taken with mixed cultures. The tests should be performed only on colonies of oxidase-positive, gram-negative diplococci. The morphology of the colonies and Gram staining results may also be helpful for differentiating *M. catarrhalis* from other *Moraxella* spp. that give positive BST results. The possibility of false-positive results with the BST must be kept in mind when staphylococci and *C. albicans* are present, which may be difficult to detect when the colonies are immature.

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