

## Alpha-Mannosidase: a Rapid Test for Identification of *Arcanobacterium haemolyticum*

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**A 4-h alpha-mannosidase test for identification of *Arcanobacterium haemolyticum* strains ( $n = 139$ ) and differentiation of *A. haemolyticum* from *Actinomyces pyogenes* strains ( $n = 30$ ) and other gram-positive rods was evaluated. Practically all *A. haemolyticum* strains (138 of 139) and the *Listeria monocytogenes* type strain were alpha-mannosidase positive, while all *A. pyogenes* strains and *Corynebacterium* ( $n = 25$ ) strains as well as the *Rhodococcus equi* and *Erysipelothrix rhusiopathiae* type strains were negative. The rapid alpha-mannosidase test, in conjunction with a Gram stain and catalase and reverse CAMP tests, was useful in identification of *A. haemolyticum* and in differentiation of *A. haemolyticum* from *A. pyogenes* and *Corynebacterium* spp.**

*Arcanobacterium haemolyticum* (formerly *Corynebacterium haemolyticum* [3]) is an aerobic, catalase-negative, gram-positive rod which has been isolated from patients with pharyngitis, wound infections, septicemia, endocarditis, and osteomyelitis (9). Biochemically and by Gram stain, it is not difficult to differentiate *A. haemolyticum* from *Corynebacterium* spp., but it is more difficult to differentiate it from *Actinomyces pyogenes*, another catalase-negative, gram-positive rod (8). Recently, Kämpfer (6) used fluorogenic substrates in the identification of corynebacteria and related organisms. One of the substrates used by Kämpfer was 4-methylumbelliferyl-alpha-D-mannopyranoside. All five *A. haemolyticum* strains tested were alpha-mannosidase positive, while practically all corynebacteria tested were negative. *A. pyogenes* was not studied. A rapid test for alpha-mannosidase activity is commercially available. We wanted to find out whether it would be useful in identification of *A. haemolyticum* and in differentiation of it from *A. pyogenes*.

One hundred thirty-eight clinical *A. haemolyticum* isolates, including 36 from pharyngeal exudate, 92 from wound infections, 6 from peritonsillar abscesses, 3 from maxillary sinuses, and 1 from blood, were tested. *A. haemolyticum* ATCC 9345 was used as a control. All strains were biochemically identified as *A. haemolyticum* as described by Krech and Hollis (8), i.e., by the following tests: beta-hemolysis on horse blood agar (4% Trypticase soy agar II [BBL, Cockeysville, Md.] with 6% defibrinated horse blood), Gram stain, catalase (3% H<sub>2</sub>O<sub>2</sub>), nitrate reduction (heart infusion broth [Difco, Detroit, Mich.] with 0.2% KNO<sub>3</sub> [Merck, Darmstadt, Germany]), urease (3% urea agar base [BBL] with 1.5% Bacto Agar [Difco]), gelatin hydrolysis (11% gelatin [Merck] in peptone broth [Orion Diagnostica, Espoo, Finland]), motility (microscopically), reverse CAMP (with a sheep blood agar plate [2]), and fermentation of carbohydrates. For fermentation tests, 1.0% final concentrations of the following carbohydrates in a fermentation base (1% Bacto Tryptone [Difco] and 0.5% NaCl [Baker Chemicals, Deventer, Holland] with 0.0018% phenol red [Merck]) were used: glucose (Difco), maltose (Merck), sucrose (Difco), mannitol (Difco), and xylose (Difco). API Coryne

(Biomerieux, Marcy l'Etoile, France) biochemical profiles were also obtained for 50 strains (26 from pharyngeal exudate, 20 from wound infections, 3 from peritonsillar abscesses, and 1 from blood) and the type strain. The API Coryne identification strips were read after overnight incubation at 35°C. API Coryne profiles (excellent identification of *A. haemolyticum*) confirmed the identification given by the tests described by Krech and Hollis (8).

Twenty-seven *A. pyogenes* strains (from infections in domestic animals, kindly given to us by the National Veterinary and Food Research Institute, Helsinki, Finland) were also tested for alpha-mannosidase activity. *A. pyogenes* ATCC 19411, ATCC 8104, and ATCC 8108 were used as controls.

Twenty-five *Corynebacterium* strains were also tested for alpha-mannosidase activity. They were either type strains (Table 1) or clinical isolates identified by API Coryne profiles. Seven of the clinical isolates were *Corynebacterium jeikeium* (six from blood and one from a wound) and one was *Corynebacterium pseudodiphtheriticum* from pharyngeal exudate. In addition, the type strains of *Erysipelothrix rhusiopathiae*, *Listeria monocytogenes*, and *Rhodococcus equi* were tested.

For the alpha-mannosidase test, bacteria from horse blood agar plates incubated for 48 h at 35°C in a humidified atmosphere of 5% CO<sub>2</sub> in air were suspended in 0.25 ml of 0.9% saline. Two different suspensions were prepared from each strain, one with a McFarland no. 2 turbidity and another with a McFarland no. 6 turbidity by visual comparison. The *A. haemolyticum* strains not tested by API Coryne ( $n = 88$ ) were, however, tested only with the suspension with a McFarland no. 2 turbidity. The alpha-mannosidase diagnostic tablet (*p*-nitrophenyl-alpha-D-mannopyranoside; final concentration, 1 mg/ml; Rosco Diagnostica, Taastrup, Denmark) was then added. The tubes were incubated in air at 35°C for 4 h. The results were read independently by each of us.

The results of the alpha-mannosidase tests were clear-cut. When the test was negative the supernatant remained clear, while a distinct yellow color developed when the test was positive. The yellow color was stronger when suspensions with a McFarland no. 6 turbidity rather than those with a McFarland no. 2 turbidity were used. When the test was negative, there was no shade of yellow even when higher-turbidity suspensions were used. There was no discrepancy between our individual interpretations of the test.

The results obtained with the strains tested are in Table 1.

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TABLE 1. Alpha-mannosidase positivity of *A. haemolyticum* and other gram-positive rods

Organism(s)	No. positive/ no. tested
<i>Actinomyces pyogenes</i> .....	0/30
<i>Arcanobacterium haemolyticum</i> .....	138/139
<i>Corynebacterium</i> strains <sup>a</sup> .....	0/25
<i>Erysipelothrix rhusiopathiae</i> ATCC 19414 .....	0/1
<i>Listeria monocytogenes</i> ATCC 19111 .....	1/1
<i>Rhodococcus equi</i> ATCC 6939 .....	0/1

<sup>a</sup> *C. bovis* ATCC 7715; *C. diphtheriae* ATCC 27010, 11049, 11050, and 11051; *C. genitalium* ATCC 33034; *C. jeikeium* ATCC 43734 and CDC F2704 and seven clinical isolates; *C. kutscheri* ATCC 15677; *C. matruchotii* ATCC 14266; *C. minutissimum* ATCC 23348; *C. pseudodiphtheriticum* NCTC 231 and one clinical isolate; *C. pseudotuberculosis* ATCC 19410; *C. renale* ATCC 19412; *C. striatum* ATCC 6940; *C. ulcerans* NCTC 7910; and *C. xerosis* ATCC 373.

One hundred thirty-eight of the 139 *A. haemolyticum* strains (99%) were alpha-mannosidase positive, and all 30 *A. pyogenes* strains and all 25 *Corynebacterium* strains as well as the *R. equi* and *E. rhusiopathiae* type strains were negative. *L. monocytogenes* was alpha-mannosidase positive.

Our study confirmed that *A. haemolyticum* strains are alpha-mannosidase positive, as reported by Kämpfer (6). Likewise, as reported by Kämpfer, all corynebacteria tested were alpha-mannosidase negative. *A. pyogenes* strains were alpha-mannosidase negative, suggesting that the test could be used to differentiate *A. haemolyticum* and *A. pyogenes*. *A. pyogenes* was not studied by Kämpfer. *L. monocytogenes*, which was alpha-mannosidase positive, can be differentiated from *A. hemolyticum* by the catalase test.

The alpha-mannosidase test is not included in the API Coryne or RapID ANA II (Innovative Diagnostic Systems, Norcross, Ga.) identification strips. API Coryne, has, however, been shown to correctly identify *A. haemolyticum* strains (4, 5), while *A. haemolyticum* has been reported to be misidentified as *A. pyogenes* by the RapID ANA II test (1).

The alpha-mannosidase test we used was rapid and easy to perform and interpret. Alpha-mannosidase diagnostic tablets

are stable, inexpensive, ready-to-use reagents with a shelf life of 4 years. This makes the test simpler to perform than the one reported by Kämpfer et al., as no instruments or reagents are required for reading the results (6, 7). The alpha-mannosidase test can be used together with typical colony morphology, beta-hemolysis, Gram stain, catalase, and reverse CAMP tests not only to identify *A. haemolyticum* from clinical samples but also to differentiate *A. haemolyticum* from corynebacteria and *A. pyogenes*.

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