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

PETTERI CARLSON* AND SIRKKA KONTIAINEN1,2
Department of Medical Microbiology, Aurora Hospital,1 and Department of Bacteriology and Immunology,
University of Helsinki,2 Helsinki, Finland

Received 6 August 1993/Returned for modification 13 October 1993/Accepted 14 December 1993

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,
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, septicaemia, 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-mannopy-
ranoside. All five A. haemolyticum strains tested were alpha-
mannosidase positive, while practically all Corynebacterium 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. pyo-
genese.

One hundred thirty-eight clinical A. haemolyticum isolates,
including 36 from pharyngeal exudate, 92 from wound infec-
tions, 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 identi-
fied 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% H2O2),
nitrate reduction (heart infusion broth [Difco, Detroit, Mich.] with
0.2% KNO3 [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), re-
verse CAMP (with a sheep blood agar plate [2]), and fer-
mation of carbohydrates. For fermentation tests, 1.0% final
concentrations of the following carbohydrates in a fermenta-
tion 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 domes-
tic 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 Coryne-
bacterium pseudodiphtheriticum from pharyngeal exudate.
In addition, the type strains of Erysipelothrix rhusiopathiae, Liste-
ria 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% CO2 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.
turbidity. The alpha-mannosidase diagnostic tablet (p-ni-
trophenyl-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 McFar-
land 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.

* Corresponding author. Mailing address: Department of Medical
Microbiology, Aurora Hospital, Nordskjöldinkatu 20, SF-00250
Helsinki, Finland. Phone: 358-0-4702548. Fax: 358-0-4702932.
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*.

## REFERENCES


