

## NOTES

### Rapid Oxidase Method for Testing Oxidase-Variable *Aeromonas hydrophila* Strains

LARRY K. HUNT,<sup>1</sup>† TIMOTHY L. OVERMAN,<sup>2,3\*</sup> AND RAYMOND B. OTERO<sup>1</sup>

Department of Biological Sciences, Eastern Kentucky University, Richmond, Kentucky 40475<sup>1</sup>; Department of Pathology, College of Medicine, University of Kentucky, Lexington, Kentucky 40536<sup>2</sup>; and Pathology Service, Veterans Administration Medical Center, Lexington, Kentucky 40511<sup>3\*</sup>

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A rapid, same-day oxidase test procedure which obviates the problem of false-negative oxidase reactions of *Aeromonas hydrophila* removed from the surface of differential media such as MacConkey agar is described. This method allows oxidase testing to be performed within 3 h, rather than delaying the oxidase test for an additional 18 to 24 h. This procedure is applicable to any rapidly growing gram-negative rod.

Some strains of *Aeromonas hydrophila* are oxidase negative by the Kovacs method (3) when colonies are removed from the surface of a differential medium such as MacConkey agar (4, 5). This same phenomenon has been reported for strains of *A. hydrophila* and *Aeromonas punctata* isolated from environmental sources (1). These strains ferment the lactose in the medium and produce acidic metabolic end products which lower the pH (1, 2). When the pH falls below 5.2 the oxidase reaction is inhibited (2). It is possible to overcome the false-negative reaction by buffering the oxidase reagent at pH 5.0 to 7.0. However, this results in rapid auto-oxidation of the reagent, and new reagent must be prepared daily (1). To properly identify *A. hydrophila* the oxidase test must be performed on colonies growing on a general-purpose medium such as blood agar (1, 4, 5).

It is not always possible to find colonies of the same morphological type on both the general-purpose and differential media used for primary isolation. When a suspect *A. hydrophila* colony is present only on the differential medium, it may be subcultured to a general-purpose medium for overnight incubation before determining the oxidase activity. This may result in a delay in the identification process, especially if rapid identification systems such as the 5-h API 20E (Analytab Products, Plainview, N.Y.) or Micro-ID (General Diagnostics, Morris Plains, N.J.) are used. This report describes a rapid,

same-day oxidase test which detects the positive oxidase reaction of *A. hydrophila* colonies from differential media. This procedure can be used to check the oxidase reaction of any rapidly growing gram-negative organism which has been isolated only on a differential medium.

A suspect colony from the surface of a differential medium is inoculated into a test tube (16 by 125 mm) containing 5 ml of Mueller-Hinton broth (Difco Laboratories, Inc., Detroit, Mich.) and incubated at 35°C for 3 h. The broth culture is then centrifuged for 15 min at 3,200 × *g* to sediment the bacteria. The supernatant fluid is carefully removed so as not to disturb the sedimented bacterial pellet. A portion of the pellet is removed with a platinum loop (6) and tested for oxidase activity with the Kovacs filter paper method (3).

Six previously described strains of *A. hydrophila*, one oxidase constant, two oxidase variable, and three weakly oxidase positive (2), were grown overnight on MacConkey agar and tested by this method. All six strains were strongly oxidase positive. *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 served as oxidase-positive and oxidase-negative controls, respectively, and gave the proper reactions.

#### LITERATURE CITED

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† Present address: School of Dentistry, University of Louisville, Louisville, KY 40232.

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