Rapid Test for Lysine Decarboxylase Activity in Enterobacteriaceae

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A 4-h lysine decarboxylase test was performed on 241 clinical isolates of Enterobacteriaceae. There was 100% agreement between the rapid-test and reference methods.

In 1972 Fay and Barry (2) published a simple, rapid ornithine decarboxylase broth test for the identification of Enterobacteriaceae, which yielded accurate results when compared with the standard Möeller decarboxylase test. Results from the rapid test were available within 4 h after inoculation from primary media as compared with the 4-day required incubation time of Möeller (4).

By using a modification of the Fay-Barry medium we evaluated a rapid test for lysine decarboxylase. The medium was prepared by adding 0.5 g of peptone (Difco) and 0.3 g of yeast extract (Difco) to 100 ml of deionized water. After gentle warming to dissolve the ingredients, 1 g of 1-lysine monohydrochloride and 0.5 ml of 0.5% (vol/vol) alcoholic bromothymol blue were added to the cooled medium. The pH was adjusted to 5.0 to 5.2. After 1 ml was dispensed into disposable borosilicate tubes, the tubes were capped and autoclaved. Membrane filter (Millipore Corp.) sterilization was equally effective. The pH after autoclaving was 5.2 to 5.4. Organisms tested were clinical isolates identified by the standard methods of Edwards and Ewing (1). Rapid-test assays were performed from three primary media incubated for 24 and 48 h. One heavy loopful of growth was used from triple sugar-iron (TSI) slants. Single colonies were selected from MacConkey and sheep blood-agar plates. A Salmonella and Citrobacter strain were included as positive and negative controls with each run, as well as an uninoculated tube of medium. A 1-ml amount of sterile mineral oil or melted paraffin was added to overlay each inoculated tube. The tests were incubated at 37°C in a dry block, dry-air incubator, or a water bath. Tests were read at 1, 2, 3, and 4 h. A yellow color was recorded as negative; green or blue was considered positive.

Colony counts were determined on freshly inoculated tests to determine inoculum effect on test results. Measurements of pH were also carried out to determine the pH necessary for a positive test. Organisms were also inoculated from TSI slants into Möeller lysine decarboxylase medium (Difco) with a control and lysine-iron-agar (LIA) slants. The Möeller test medium was incubated for 4 days, and the LIA slants were incubated for 18 to 24 h.

Table 1 shows the results obtained after 4 h of incubation from the various primary media. Within 1 to 2 h of incubation, 97% of Salmonella, Edwardsiella, Arizona, and Enterobacter were positive. False-negative results occurred at 4 h only if colonies from sheep blood or MacConkey plates were too small to achieve an inoculum of 10^6 or greater in the rapid-test medium. This occurred with two strains of Salmonella from MacConkey agar and with six strains of Arizona from MacConkey and one strain of Arizona from sheep blood-agar.

The 139 Citrobacter were uniformly negative at 4 h of incubation. However, 95% of these organisms were positive when the rapid test was read after 24 h. Because of this, the rapid test should be read within the 4-h limit. Tubes can be read for up to 8 h without the possibility of false-positive results.

An inoculum of 10^6 colony-forming units (CFU)/ml or greater in organisms with lysine decarboxylase activity was necessary to give a definite positive green or blue result in the rapid-test 4-h limit. If the original inoculum of a lysine decarboxylase positive organism is <10^6 CFU/ml, as might occur with a small Arizona colony from a 24-h MacConkey plate, incubation time and temperature are critical. A definite green color may not be achieved in 4 h with an inoculum of <10^6.

Results with LIA and Möeller decarboxylase medium were in 100% agreement with the rapid test if the inoculum produced greater than 10^6.
The medium to yellow violet, presumably because of metabolism of substrates in yeast extract and peptone.

The pK of bromothymol blue is 7.0. The rapid-test 6.1 end point occurs just as the yellow-to-green breakpoint appears. This color change remains in the sensitive range of bromothymol blue, and false positives due to nonspecific alkalization are less likely to occur.

In summary, this test, with proper inoculum size, incubation time, and pH indicator, can be used for detecting lysine decarboxylase activity within the family Enterobacteriaceae and can be substituted for the longer methods in any identification scheme for these organisms.

**LITERATURE CITED**

1. Edwards, P. R., and W. H. Ewing. 1972. Identification of Enterobacteriaceae. Burgess Publishing Co., Minneapolis.

2. Fay, G. D., and A. L. Barry. 1972. Rapid ornithine decarboxylase test for the identification of Enterobacteriaceae. Appl. Microbiol. 23:710–713.

3. Gale, E. F. 1946. The bacterial amino acid decarboxylases, p. 1–32. In F. F. Nord (ed.). Advances in enzymology and related subjects of biochemistry, vol. 6. Interscience Publishers, Inc., New York.

4. Møller, V. 1955. Simplified tests for some amino acid decarboxylases and the arginine dihydrolase system. Acta Pathol. Microbiol. Scand. 36:158–172.