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 10⁶ 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 10⁶ 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 <10⁴ 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 <10⁴.

Results with LIA and Möeller decarboxylase medium were in 100% agreement with the rapid test if the inoculum produced greater than 10⁶.
CFU/ml in the rapid-test medium. Tubes inoculated with control Salmonella and Citrobacter gave uniformly reproducible results from day to day. Uninoculated control tubes were always negative at 4 and 24 h. Dry-air incubators gave results comparable to the dry block. Water baths and Thrombitron units (Clay-Adams, Parsippany, N.J.) were more effective instruments for heat exchange. By using these as incubators, decarboxylation proceeded at a faster rate (up to a 42 C limit), and positive results were obtained in a shorter period of time.

Organisms differ in their capacity to effect decarboxylation and subsequent alkalization of media (3). In the rapid assay, a rise in pH to 6.1 or higher was usually achieved in 4 h by organisms which were positive in the standard Møller decarboxylase medium. Thus, the pK of the indicator in the rapid-test medium should be such that at pH 6.1 a definitive color change occurs. In preliminary tests, with bromocresol purple as an indicator, the color changes were difficult to read, leading to both false-negative and false-positive results.

The pK of bromocresol purple is 6.3. A yellow-to-purple change does occur between pH 5.8 and 6.1, but in the presence of a heavy inoculum the 6.1 end point is indistinct. Organisms lacking lysine decarboxylase can change 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

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**Table 1. Comparison of 4-h results of the rapid lysine decarboxylase test with reference tests**

| Organism                  | No. tested | Age of inoculum (h) | No. positive | Rapid test* | Reference tests |
|---------------------------|------------|---------------------|--------------|-------------|----------------|
|                           |            |                     | TSI     Sheep blood | Mac- Conkey | LIA | Möller |
| Salmonella                | 49         | 24                  | 49 49 47    | 49 49       | 49 49       |
| Arizona                   | 16         | 24                  | 49 49 49    | 15 15 47    | 16 16       |
| Edwardsiella              | 7          | 24                  | 7 7 7 7     | 7 7 7       | 7 7         |
| Citrobacter diversus     | 51         | 24                  | 0 0 0 0     | 0 0 0       | 0 0         |
| C. freundii-H₂S⁺          | 51         | 24                  | 0 0 0 0     | 0 0 0       | 0 0         |
| C. freundii-H₂S⁻          | 37         | 24                  | 0 0 0 0     | 0 0 0       | 0 0         |
| Enterobacter-lysine⁺      | 15         | 24                  | 15 15 15    | 15 15       | 15 15       |
| Enterobacter-lysine⁻      | 15         | 24                  | 0 0 0 0     | 0 0 0       | 0 0         |

*Tests were performed by using inocula from three different media.
*False negative because of small inoculum (<10⁸ CFU/ml).