

# Rapid Ornithine Decarboxylase Test for the Identification of *Enterobacteriaceae*

GUNNAR D. FAY AND ARTHUR L. BARRY

*Section of Infectious and Immunologic Diseases, Department of Internal Medicine, School of Medicine, University of California, Davis, California 95616, and Clinical Microbiology Laboratory, Sacramento Medical Center, Sacramento, California 95817*

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Conventional methods for detecting ornithine decarboxylase activity require an extended period of incubation. However, with a few simple modifications, accurate results were obtained within a few hours rather than several days. The broth medium was modified, primarily by omitting glucose and by decreasing the pH to 5.5. A 1-ml amount of this broth was inoculated with one colony and then overlaid with sterile mineral oil. Within 2 to 4 hr, the pH increased if ornithine was decarboxylated, thus changing the color of the internal pH indicator to a dark purple. If the amino acid was not decarboxylated, the pH decreased to pH 5.0 to 5.2, enough to give a definite yellow color. With 347 selected clinical isolates, the rapid test gave results identical to those obtained in 1 to 4 days with Moeller's decarboxylase medium. Less reliable results were obtained with Difco's decarboxylase medium with 0.3% agar which was stab-inoculated and read after 18 to 24 hr without a mineral oil seal. The rapid ornithine decarboxylase test represents a simple, accurate technique which is well suited for the clinical microbiology laboratory.

In the identification of *Enterobacteriaceae*, the ornithine decarboxylase test is of paramount importance, especially for separating members of the *Klebsiella-Enterobacter-Serratia* group and for identifying species of *Proteus*. Details of decarboxylase tests are somewhat varied (1, 3-5, 7). In general, the microorganisms first ferment glucose to lower the pH so that the optimal hydrogen ion concentration for decarboxylase activity is reached. Decarboxylation results in the formation of amines and a consequent increase in pH. Positive results are usually obtained after 18 to 24 hr of incubation, but with some strains the tests must be held for as long as 4 days. Oxygen is generally excluded by overlaying the broth with paraffin or mineral oil or by the addition of 0.3% agar (12). A medium with agar cannot be held longer than 24 hr, and thus a few false-negative results should be expected.

To obtain more rapid results, the conventional decarboxylase medium was modified (no added glucose; pH 5.5), and a small volume of broth with a mineral oil seal was inoculated heavily (one colony in 1 ml of broth). With these slight modifications, highly reliable results could be obtained after only 2 to 4 hr at 35 to 37 C.

## MATERIALS AND METHODS

All cultures used in this study were recent clinical isolates and were identified with the rapid screening tests described elsewhere (2) and with the more conventional methods described by Edwards and Ewing (7) and by Martin (3). Motility was detected after 18 to 24 hr at 37 C in G-I motility medium (Difco); nonmotile cultures remained negative after an additional 5 days at room temperature.

Three methods for detecting ornithine decarboxylase activity were compared by testing 347 isolates (107 *Klebsiella*, 62 *Enterobacter*, 24 *Serratia*, 73 *Escherichia*, and 81 *Proteus* strains). The following methods were compared.

(i) Moeller's decarboxylase broth with 1.0% ornithine (Difco) was prepared in 5-ml volumes (13 by 100 mm tubes), inoculated, overlaid with sterile mineral oil, and incubated at 37 C, along with an inoculated control base medium without amino acid. The tests were examined daily for as long as 4 days.

(ii) Difco's decarboxylase base with 0.5% ornithine and 0.3% agar in 5-ml volumes (13 by 100 mm screw-cap tubes) was stab-inoculated and read after 18 to 24 hr at 37 C, with the caps screwed tight.

(iii) The rapid ornithine broth medium was prepared as follows: 5.0 g of peptone (Difco), 3.0 g of yeast extract (Difco), and 5 ml of 0.2% (w/v) bromocresol purple in 50% (v/v) ethanol were added to sufficient deionized water to make 1,000 ml. The solution was heated until completely dissolved, and

then 10 g of L-ornithine hydrochloride (Difco) was added. The broth was adjusted to pH 5.5 with HCl or NaOH and sterilized at 121 C, 15 psi, for 15 min. The pH was checked and readjusted to 5.5 if necessary.

The day the tests were performed, 1-ml portions of ornithine broth were transferred aseptically to chemically inert, sterile plastic test tubes (12 by 75 mm). To perform the test, colonies were selected from 18- to 24-hr blood-agar plates (Trypticase Soy Agar, Bioquest, with 5% defibrinated sheep blood) or MacConkey (Bioquest) agar plates. The broth was inoculated with one colony, overlaid with at least 0.5 ml of sterile mineral oil, and incubated in a heating block at 37 C. A dark-purple color indicated a positive reaction, whereas negative tests were yellow.

For the initial portion of these studies, the changes in the pH of the test media were monitored continually by use of a model 10 pH meter (Corning Glass Works, Corning, N.Y.) attached to a continuous recording apparatus (Varian Aerograph, Walnut Creek, Calif.) with a semimicro combination electrode (Corning Glass Works) which was left immersed in the inoculated broth medium during incubation.

## RESULTS

To investigate the effects of varying the initial pH and the volume and type of broth media, the pH changes were monitored during incubation, with the use of an ornithine-positive *Enterobacter cloacae* strain and an ornithine-negative *Klebsiella pneumoniae* strain. These studies demonstrated that the most rapid increase in pH with the *Enterobacter* strain could be obtained by adjusting the new ornithine broth to pH 5.5. The *Klebsiella* strain does not decarboxylate ornithine and, once a pH of approximately 5.0 was reached, no further change in pH was discernible.

The strain of *Enterobacter* showed a faster increase in pH in the new, rapid ornithine decarboxylase broth than in either Moeller's or Difco's decarboxylase medium. The results obtained in 5-ml volumes of Moeller's medium (pH 6.0), Difco's medium (pH 6.5), and the rapid test medium (pH 5.5) are shown in Fig. 1. After 3 hr in both Moeller's and Difco's media, the pH was decreasing, but, in the rapid ornithine broth, alkaline products were already accumulating and the culture had reached a pH of 6.2. The sensitive pH range of bromocresol purple ranges from pH 5.2 to 6.8 (yellow to purple). A purple color is just discernible at pH 6.0 and becomes more intense with further increase in pH. A pH of 6.0 was reached in the rapid medium with 2.5 hr, but it took 4.5 hr in Moeller's ornithine medium. In Difco's medium after 5 hr of incubation, the microorganism was just beginning to decarboxylate, and it took 7 hr before pH 6.0 was

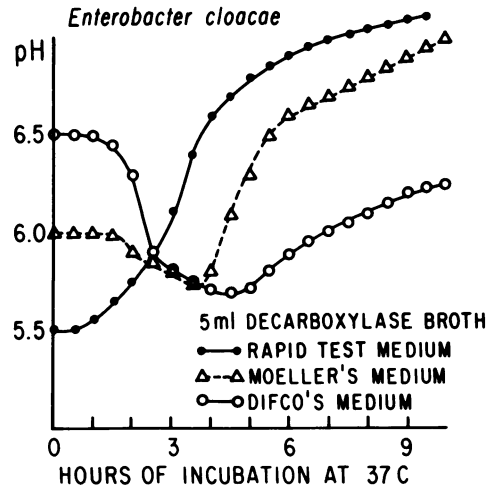


FIG. 1. Changes in pH of *Enterobacter cloacae* broth cultures during incubation at 37 C in 5-ml volumes of three different ornithine decarboxylase broths adjusted to their recommended initial pH.

reached. By reducing the volumes of broth from 5 to 1 ml, the same three media showed somewhat more rapid increases in pH with the *Enterobacter* strain.

The time lag before decarboxylation becomes visible was reduced for each of the three media when the pH was adjusted to 5.5 and when the volume of broth was reduced to 1 ml (Fig. 2). Again, the increase in pH was most rapid for the new ornithine broth. To reach pH 6.0, the *Enterobacter* required 9 hr in Difco's medium, 4.5 hr in Moeller's medium, and only 2.5 hr in the rapid ornithine broth.

The new, rapid ornithine broth was compared with Moeller's ornithine decarboxylase broth and with Difco's ornithine decarboxylase medium with 0.3% agar. At the same time, motility tests were performed with the *Klebsiella-Enterobacter-Serratia* group (Table 1). With the first 191 isolates, two rapid tests were inoculated: one with colonies selected from blood-agar plates and the other with colonies selected from MacConkey agar plates. No noticeable advantage or disadvantage was noted regarding the source of inoculum. Thus, the remaining isolates were picked from MacConkey agar plates. Of the 347 isolates that were tested, 213 were found to be ornithine decarboxylase-positive in Moeller's medium. All but two *Escherichia coli* strains were positive after 1 day of incubation; the two exceptions were positive after 2 days. Of the 213 organisms positive in Moeller's medium, 210 were also positive in Difco's medium with agar. The three negative strains were all *En-*

*terobacter* and were retested in Moeller's and Difco's media with and without 0.3% agar. The three *Enterobacter* strains were positive in Moeller's ornithine decarboxylase medium with and without agar after only 1 day of incubation. In Difco's medium without agar, two of the three *Enterobacter* strains were positive only after 2 days; the other was negative after 4 days.

The rapid ornithine decarboxylase test agreed completely with the results obtained after 1 to 2 days in Moeller's medium. The 213 ornithine-positive organisms were all positive in the rapid test medium, and the 134 negative organisms were all negative in the rapid test medium. Occasionally, an ornithine-positive

strain of *E. coli* or *Proteus* sp. gave a faint purple color less intense than that usually seen after 4 hr in the rapid broth, but such equivocal results were eliminated by reincubation for an additional 30 to 60 min. Occasionally *E. coli* may reduce the indicator, producing a light-gray color which is difficult to interpret. A drop of bromocresol purple will alleviate this problem. Equivocal results or indicator reduction was not very common and did not occur at all with the *Klebsiella*, *Enterobacter*, or *Serratia* strains.

Of the 62 *Enterobacter* strains used in this study, 2 motile strains were ornithine-negative by all three methods and 1 was negative in Difco's medium but positive in the other two media. Four nonmotile *Enterobacter* strains were encountered. These were ornithine decarboxylase-positive in Moeller's medium and in the rapid test medium, but two of the four nonmotile strains were negative in Difco's medium with agar.

## DISCUSSION

In clinical microbiology, the identification of microorganisms must be made as rapidly and efficiently as possible. Several test schemes have been advocated to make the identification of *Enterobacteriaceae* expeditious (2, 15-18). Most of the simplified schemes that have been suggested, as well as the standard conventional methods, depend heavily upon the ornithine decarboxylase test. A simple, accurate method for performing this test would be highly desirable, especially if the results could be obtained rapidly. Goldschmidt et al. (11) recently described a rapid test for decarboxylase activity which involves the detection of amines by extraction with chloroform followed

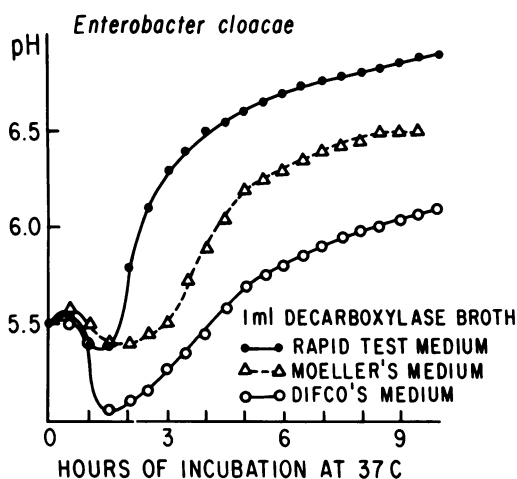


FIG. 2. Changes in pH of *Enterobacter cloacae* broth cultures during incubation at 37 C in 1-ml volumes of three different ornithine decarboxylase broths adjusted to an initial pH of 5.5.

TABLE 1. Ornithine and motility results for 347 selected isolates of *Enterobacteriaceae*

Final identification	Total no. of isolates	Motility	Ornithine decarboxylase			No. of isolates giving indicated reaction
			Moller's broth	Difco's with agar	Rapid test	
<i>Klebsiella</i> sp. ....	107	- <sup>a</sup>	-	-	-	107
<i>Enterobacter</i> sp. ....	62	+	+	+	+	55
		-	+	+	+	2
		+	-	-	-	2
		+	+	-	+	1
<i>Serratia</i> sp. ....	24	+	+	+	+	24
<i>Escherichia</i> ....	73	ND	+	+	+	61
		ND	-	-	-	12
<i>Proteus</i> sp. <sup>b</sup> ....	81	ND	+	+	+	68
		ND	-	-	-	13

<sup>a</sup> Symbols: +, positive; -, negative; ND, not done.

<sup>b</sup> Includes 58 *P. mirabilis*, 9 *P. rettgeri*, 8 *P.morganii*, and 6 *P. vulgaris*.

by the addition of a ninhydrin reagent, a series of procedures which are relatively complex.

Gale found that ornithine decarboxylase activity for *Proteus* sp. is optimal at pH 5.5 (10), and for *E. coli* the optimal pH is 5.0 (9). Moeller mentioned that the greatest changes in pH are often observed without glucose and with the initial pH of the medium at 5.5 (14), but noted as well that a small amount of glucose and an initial pH of 6.0 gave better growth. Thus, the optimal pH for demonstrating ornithine decarboxylase activity seems to vary slightly between organisms, and our choice of pH 5.5 was motivated by the initial experiment with an *Enterobacter cloacae* strain.

The new rapid ornithine test is most valuable in two areas. Firstly, the test separates *Klebsiella* from the *Enterobacter-Serratia* group of organisms. *K. pneumoniae* cells are nonmotile (6) and ornithine-negative (8, 15). Strains of *Enterobacter* sp. are usually motile and ornithine-positive. The occasional *Enterobacter* strain that is nonmotile or ornithine-negative, or both, poses a problem in identification and may be easily misidentified as *Klebsiella* (13). Secondly, the rapid ornithine test is helpful in the separation of *Proteus* species. According to Edwards and Ewing (7), 98.3% of *P. mirabilis* and 100% of *P. morganii* strains are ornithine decarboxylase-positive, whereas *P. vulgaris* and *P. rettgeri* are negative. Traub et al. (15) reported that 2.1% of their *P. vulgaris* and none of their *P. rettgeri* strains were ornithine decarboxylase-positive. Tests by Matsen (Bacteriol. Proc., p. 96, 1970) on swarming *Proteus* sp. led to his conclusion that at least the ornithine test and the indole test should be performed to identify swarming *Proteus* sp. By use of a rapid indole test (2) and the new rapid ornithine test, swarming *Proteus* sp. can be identified reliably the same day that they are isolated in the laboratory.

In summary, ornithine decarboxylase activity can be detected after 2 to 4 hr by use of the modified broth described in this report. The end points are clear-cut and easy to read, and the results compare favorably with those obtained with more conventional tests which require from 1 to 4 days of incubation. The test requires a minimal number of manipulations and requires no unusual reagents or equipment. For these reasons, the test is recommended for routine use in the clinical laboratory.

#### ACKNOWLEDGMENT

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