# Multi-Biochemical Test System for Distinguishing Enteric and Other Gram-Negative Bacilli

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A multi-biochemical test system consisting of nine tests, entitled Enterotube, was evaluated in parallel with conventional tests to determine its value in the identification of enteric and certain other gram-negative bacilli. The 242 bacterial strains studied were from a variety of pathological specimens and from our culture collection. When the results with individual tests represented in both test systems were compared, no discrepancies were noted in the indole test, and one discrepancy was recorded for dextrose. In 7 of 242 hydrogen sulfide tests, 3 of 242 phenylalanine tests, 22 of 242 urease tests, 15 of 242 dulcitol tests, 12 of 242 lactose tests, 27 of 217 lysine decarboxylase tests, and 5 of 242 citrate tests, the Enterotube results were contrary to those obtained with conventional methods. The lysine decarboxylase test in the Enterotube posed a problem of interpretation and readability and is not an acceptable alternative to the conventional methods. Fifteen of the strains studied were incorrectly identified by the Enterotube system and four could not be differentiated from other closely related strains. *Salmonella* could be identified as to group, whereas *Shigella* strains were frequently misidentified as *Escherichia*. The Enterotube method is simple and convenient, and all media are inoculated at once from a single colony.

Methods used for differential identification of enteric and related gram-negative bacilli have been dependent mostly on biochemical tests. Many of these tests are time-consuming, and 72 hr may elapse before the organism can be properly identified. Investigators have constantly sought simpler and more rapid procedures to differentiate gram-negative bacilli. Until recently, the vast majority of biochemical tests have been diverse and separate in media and methods. Today a number of rapid biochemical test systems are available to the microbiologist. One such test system is the reagent-impregnated paper strip. Several workers (1, 5, 9) have reported on the use of the paper-impregnated strip as a more rapid substitute for conventional methods. Paper strips impregnated with reagents to test for indole, acetylmethylcarbinol production, urease, cytochrome oxidase, phenylalanine deaminase, lysine decarboxylase activity, niacin, and nitrate reduction are available (1, 5, 7, 9, 11). An R/B enteric differential system (Diagnostic Research Inc., Roslyn, N. Y.), which uses two tubes of culture media and various reagents, has been developed for determination of certain biochemical tests. Tube 1 is used to test for phenylalanine deaminase, lactose, glucose, hydrogen sulfide, and lysine

decarboxylase activity. Tube 2 is used to test for indole, ornithine activity, and motility. Another newly formulated test system, Enterotube (Roche Diagnostic Div., Hoffman-LaRoche, Inc., Nutley, N.J.), is a compartmental tube containing a number of different types of biochemical test media used for the identification of *Enterobacteriaceae* (8). This test system is based on the findings of Edwards and Ewing (4) that eight biochemical reactions can serve to separate members of the enteric family into *Shigella-Escherichia*, *Salmonella-Arizona-Citrobacter*, *Klebsiella-Aerobacter-Serratia*, and *Proteus*-Providence divisions. This paper concerns a study which tests the use of a multi-biochemical test system (Enterotube) for distinguishing enteric and certain other gram-negative bacilli and compares the results with conventional tests.

## MATERIALS AND METHODS

**Cultures tested.** Gram-negative bacilli obtained from various kinds of pathological specimens received in the clinical laboratory formed the material for this study. The strains comprised primary isolates from urine, sputa, wounds, body fluids, stool specimens, and isolates from our culture collection. Initially, each clinical specimen was Gram-stained, transferred to

routine culture media consisting of Thioglycollate medium (BBL), blood (human), and MacConkey (Difco) agar except for stool cultures which were planted on MacConkey, S-S (Difco), and Brilliant Green (Difco) agars. Individual colonies with identical colonial morphology were used as inoculum for the Enterotube system and Kligler iron agar (KIA). Conventional biochemical media were inoculated from KIA.

**Enterotube biochemical test system.** Enterotubes (Hoffman-LaRoche, Inc.) are plastic tubes with one side round and the adjacent side flat. The flat side is covered with a thin plastic seal. Each tube has eight compartments containing a given biochemical test medium. A single inoculating needle extends lengthwise through the center of the media in each compartment and protrudes at each end of the tube. One end of the wire and tube is covered by a blue screw cap, and the other is covered by a white screw cap. The inoculating end of the wire is covered by the white cap. Aerobic conditions during incubation are insured by three small air holes which appear on the side of the tube, and these are covered by a blue strip which is removed at the time of inoculation. Each compartment is labeled with the appropriate name of the media it contains. The Enterotube is inoculated by removing the white plastic cap, touching the center of an isolated colony with the straight wire, and then withdrawing the wire from the tube. The wire inoculates each of the test media in the compartments. The question of sufficient inoculum arises when inoculating (8) test media simultaneously with a single needle from a bacterial colony. With rare exceptions, each of the eight compartments seemed to be inoculated satisfactorily after withdrawing the inoculating needle from the Enterotube. The wire may now be used to inoculate other test media such as gelatin and motility test media which are used routinely in our identification schema. We have inoculated as many as four substrates with success.

The type of media in the compartments and the reactions were as follows.

**Dextrose-agar.** The fermentation of dextrose changed the indicator, phenol red, from pink to yellow.

**Hydrogen sulfide-indole-agar.** Hydrogen sulfide was demonstrated by the formation of a black precipitate along the inoculation line, whereas indole production was tested by injecting 0.3 ml of Kovac's reagent through the plastic film with a needle and syringe onto the culture growth in the compartment. A red color indicated the presence of indole.

**Phenylalanine deaminase-agar.** Deamination was tested by injecting 0.3 ml of a 10% ferric chloride solution through the plastic film with a needle and syringe onto the culture growth in the compartment. The formation of a dark green color on the surface of the culture slant indicated a positive test. One should inject the Kovac's and ferric chloride solutions used in the indole and phenylalanine deaminase tests with the Enterotubes in an upright position to prevent the flow of these reagents into other compartments via the inoculating canal.

**Urea-agar.** The production of ammonia from urea was shown by a change in the phenol red indicator from yellow to pink.

**Dulcitol- and lactose-agar.** The fermentation of these carbohydrates was indicated by a change in the phenol red indicator from pink to yellow in their respective compartments.

**Lysine-lactose-agar.** A positive lysine decarboxylase test was noted by a change in the phenol red indicator from yellow to pink or orange.

**Simmons citrate agar.** A positive test was shown by a change in the bromothymol blue indicator from green to blue. All reactions in the compartments were read after incubation at 37 C for 18 to 24 hr and again at 48 hr.

Conventional biochemical tests were run in conjunction with the Enterotubes. The types of media or methods used were as follows.

**Carbohydrate test media.** Seitz-filtered 10% carbohydrate solutions of dextrose, lactose, and dulcitol were added aseptically to cool autoclaved bromocresol purple broth basal medium (Difco). The media were then dispensed in sterile tubes (125 by 16 mm).

**Hydrogen sulfide.** Formation of hydrogen sulfide was determined in Kligler iron agar.

**Tryptone broth.** Production of indole was tested with Kovac's reagent after 18 to 24 hr of growth in tryptone broth (Difco).

**Phenylalanine deaminase agar.** Production of phenylpyruvic acid from phenylalanine was tested by the method of Ewing, Davis, and Reavis (6) after 24 hr of incubation on phenylalanine agar slants (Difco).

**Urea-agar.** Hydrolysis of urea was tested in Christensen (2) nutrient urea agar.

**Lysine media.** The two lysine decarboxylase methods used were a modified lysine-lactose-agar technique and the lysine broth method of Moeller (10). The Moeller broth was read daily for 4 days.

**Lysine-lactose-agar.** The lysine-lactose decarboxylase test medium slants consisted of lysine decarboxylase basal medium (Difco) with 0.5% L-lysine, 1.0% lactose, 1.5% agar, and bromocresol purple indicator. A control slant without the amino acid was also used.

**Lysine broth.** The lysine decarboxylase test broth consisted of decarboxylase basal medium (Difco) with 0.5% L-lysine and bromocresol purple indicator. A control broth without the amino acid was also used.

**Simmons citrate agar.** The use of citrate as a sole carbon source was indicated by the production of ammonia and a change in the color of the medium (Difco) from green to blue. The test reactions were read after incubation for 24 and 48 hr except when otherwise stated.

## RESULTS

In all, 242 cultures of various groups of enteric and other gram-negative bacilli were studied. The organisms were identified by the two test systems and the results were compared as shown in Table 1. Additional biochemical or serological tests were sometimes necessary to confirm the identity of the organisms.

Fermentation reactions by the Enterotube and conventional methods correlated well with dex-

TABLE 1. *Comparison of a number of biochemical tests by conventional tests and the Enterotube system*

| Organism | No. of strains | Dextrose | | H₂S | | Indole | | Phenyl-alanine | | Urease | | Dulcitol | | Lactose | | Lysine | | Citrate | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | C[a] | E | C | E | C | E | C | E | C | E | C | E | C | E | C | E | C | E |
| *Shigella* | | | | | | | | | | | | | | | | | | | |
| *Shigella* group A | 2 | 2 | 2 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| *Shigella* group B | 2 | 2 | 2 | 0 | 0 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Shigella* group D | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Alkalescens-Dispar | 2 | 2 | 2 | 0 | 0 | 2 | 2 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 2 | 0 | 0 |
| *Escherichia*[b] | 60 | 60 | 60 | 0 | 0 | 59 | 59 | 0 | 0 | 0 | 0 | 30 | 26[c] | 59 | 56 | 0 | 3 | 2 | 2 |
| *Klebsiella pneumoniae* | 60 | 60 | 60 | 0 | 0 | 2 | 2 | 0 | 0 | 51 | 46 | 7 | 6 | 60 | 58 | 3 | 2 | 60 | 60 |
| *Enterobacter aerogenes* | 6 | 6 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 3 | 1 | 0 | 6 | 6 | 1 | 0 | 6 | 6 |
| *E. cloacae* | 4 | 4 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 1 | 4 | 4 | 0 | 0 | 4 | 4 |
| *E. hafniae* | 12 | 12 | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 2 | 3 | 6 | 4 | 0 | 5 | 12 | 12 |
| *Serratia marcescens* | 4 | 4 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 4 | 4 | 4 | 4 |
| *Proteus mirabilis* | 27 | 27 | 27 | 23 | 19 | 0 | 0 | 26 | 26 | 27 | 27 | 0 | 0 | 0 | 0 | 0 | 12 | 20 | 19 |
| *P. rettgeri* | 10 | 10 | 10 | 0 | 0 | 10 | 10 | 8 | 9 | 10 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 4 |
| *P. morganii* | 4 | 4 | 4 | 2 | 0 | 4 | 4 | 4 | 3 | 4 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *P. vulgaris* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Providence group | 3 | 3 | 3 | 0 | 0 | 3 | 3 | 3 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 3 | 3 |
| *Salmonella* | | | | | | | | | | | | | | | | | | | |
| *S. paratyphi* B | 2 | 2 | 2 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 0 | 0 | 2 | 2 | 2 | 2 |
| *S. typhi* | 2 | 2 | 2 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 0 | 0 | 2 | 1 | 2 | 2 |
| *S. gallinarum* | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 |
| *Salmonella* group E | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 |
| *Citrobacter* group | 11 | 11 | 11 | 2 | 1 | 2 | 2 | 0 | 0 | 4 | 1 | 9 | 9 | 6 | 3 | 3 | 3 | 11 | 11 |
| *Arizona arizonae* | 2 | 2 | 2 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 2 | 2 | 2 |
| *Pseudomonas aeruginosa* | 20 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 5 | 0 | 0 | 0 | 0 | | | 20 | 20 |
| *Herellea* | 5 | 5 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 0 | 0 | 0 | 0 | | | 5 | 5 |

[a] C, Conventional; E, Enterotube.

[b] Includes *E. coli* and atypical *Escherichia* (*Paracolon coliforme, E. intermedia*).

[c] With *Escherichia*, 23 strains were positive by both methods.

trose and fairly well with dulcitol and lactose. All of the organisms examined except *Pseudomonas aeruginosa* fermented dextrose both by the conventional method and by the Enterotube. One of 20 *P. aeruginosa* strains produced acid in the conventional dextrose broth but was acid-negative in the Enterotube. When retested this strain gave the same results. There were 15 discrepancies between the two systems among the 242 strains in the fermentation of dulcitol. Three *E. coli*, one *Enterobacter cloacae*, and one *E. hafniae* strain failed to ferment dulcitol by the conventional method but did so in the Enterotubes. One Alkalescens-Dispar strain, seven *Escherichia* including two atypical *Escherichia* (late lactose-fermenters), one *K. pneumoniae*, and one *Enterobacter aerogenes* fermented this alcohol in the conventional tubes but not in the Enterotubes. With lactose, 230 of 242 tests were in agreement by both test systems. Three atypical *Escherichia* (two *Paracolon coliforme*; one *E. intermedia*), two *K. pneumoniae*, two *E. hafniae*, three *Citrobacter*, and two *Arizona* produced acid in the conven-

tional broth but not in the Enterotubes. One atypical *Escherichia* culture failed to utilize lactose by both methods.

**Hydrogen sulfide.** Hydrogen sulfide formation was in accord most of the time on both types of test media. Four *Proteus mirabilis* cultures and two *P. morganii* strains produced a small amount of hydrogen sulfide in KIA but not in the Enterotubes. It appears that small amounts of hydrogen sulfide and late production of the gas cannot be determined in the hydrogen sulfide-indole-agar after the addition of the indole reagent. A number of organisms produced a weak brown color in the Enterotubes which could not be detected after the addition of Kovac's reagent. The nine *Citrobacter* organisms which failed to produce hydrogen sulfide on KIA were *C. freundii* strains.

**Indole.** The Enterotube indole reactions of the strains were identical with those of the conventional tests. One of the *E. coli* strains was repeatedly indole-negative but all other criteria for *Escherichia* were met, including a positive methyl red and a negative Voges-Proskauer reaction.

Two of 60 *K. pneumoniae* strains consistently gave a positive indole test, and all other characteristics were those of *Klebsiella* including a negative motility. One of these strains gave a positive serological reaction in group 2 typing serum.

**Phenylalanine deaminase.** The results of the two methods correlated very well. In only three instances out of 242 total tests was there a discrepancy between the two methods. In one instance, the Enterotube was positive with a strain of *P. rettgeri*, whereas the conventional test was equivocal. With one *P. morganii* and one Providence strain, the conventional test was positive and the Enterotube was negative. One *P. mirabilis* and one *P. rettgeri* strain failed to produce phenylpyruvic acid by both techniques. Repeated testing of these organisms with inocula from the same source gave similar results.

**Urea-agar.** There were 60 *K. pneumoniae* strains tested. Of these strains, 51 gave positive conventional urease tests and 9 were negative, whereas 46 were positive in the Enterotubes. Of the 46 positive strains, 10 were positive only after 48 hr of incubation. Three of six *E. aerogenes* organisms urease-positive by the conventional tests were negative on Enterotubes. All four *E. cloacae* strains showed negative Enterotube tests, but two were positive with the conventional tests. There was one positive *Serratia* strain by both test systems. Of the 12 *E. hafniae* strains tested, 7 produced positive tests only by the conventional method, reactions being confined to the culture slants after overnight incubation. All 42 strains of *Proteus* produced positive urease tests by both of the test methods. Four of 11 *Citrobacter* strains showed positive reactions on Christensen agar slants, whereas none was positive by the Enterotube test after 48 hr of incubation. However, one of these strains was positive after 72 hr of growth. All but two of the *P. aeruginosa* strains gave negative urease tests in 48 hr. The other urease-positive strains, five on conventional slants and three on Enterotubes, showed reactions from 48 hr to 5 days.

**Lysine decarboxylase.** There was some difficulty encountered in the interpretation of the decarboxylase reaction in the Enterotubes. In a number of tests, the color of the medium changed to purple, the intensity of which deepened after 48 hr of incubation. This was especially true with some of the *Proteus* and *Citrobacter* strains. In these situations, the color reaction in the conventional lysine-lactose-agar (Table 1) was taken as the determinant for the results. Of seven *Shigella*-Alkalescens-Dispar strains, two were positive and three were negative by both methods and two gave positive tests only in the Enterotubes. All 60

*Escherichia* strains showed negative results by the conventional agar method, but 3 atypical strains were positive in the Enterotubes. Although positive, the three reactions were weak and not clear-cut. Of 60 *K. pneumoniae* strains tested, 57 were negative by the conventional agar method, whereas 58 were negative by Enterotube testing. Of six *E. aerogenes* strains, five were negative by the conventional agar method compared with six of six by the Enterotube method. All 12 *E. hafniae* strains were negative by the conventional agar technique, but five were positive with the Enterotubes. Again, the reactions were weak and not clear-cut. The seven remaining negative *E. hafniae* strains were positive in conventional broth. Of the five *E. hafniae* strains that were positive with the Enterotubes, four were positive with the conventional broth. The results of the Enterotube tests could not be compared with the conventional broth of Moeller (10), since lactose fermenters usually gave negative results. Forty-six *E. coli*, 38 *K. pneumoniae*, and 6 of 6 *E. aerogenes* strains which were lysine decarboxylase-negative by the Enterotube were positive by the conventional broth test. Two atypical *Escherichia* strains (previously designated *P. coliforme* and *E. intermedia*) were also positive by the conventional broth test. There was agreement between the Enterotube system and conventional agar with 30 of 42 (71.4%) *Proteus* strains which were lysine decarboxylase-negative in the Enterotubes. All *P. mirabilis* strains were negative by conventional tests. Of 15 *P. mirabilis* strains whose tests were recorded as negative in the Enterotubes, 7 gave questionable reactions (violet or purple color) the results of which could not be interpreted. The 12 remaining strains were positive in the Enterotubes, but 9 of these strains gave weak (pale orange color in the medium) reactions. The results obtained in the conventional broth of Moeller (10) with *Proteus* strains correlated closely with that of the conventional agar method except for one *P. rettgeri* and two *P. morganii* strains. These strains were lysine decarboxylase-positive upon testing with the conventional broth medium but were negative by the other two test systems. One of three Providence cultures produced a weak reaction in the Enterotubes. There were two false-negative Enterotube tests with *Salmonella* strains comprising one *S. typhi* and the one *S. gallinarum* culture. Tests for lysine decarboxylase activity of the *Citrobacter* group showed 8 of 11 strains to be negative by both test systems. One strain that gave a weak reaction with the Enterotube gave a strong positive by the conventional broth method of Moeller (10). With two different strains, the results could not be

interpreted in the Enterotubes and the strains were considered negative. The two *Arizona* strains were positive by both methods.

**Citrate-agar.** When the results of the citrate tests with the two methods were compared (Table 1), it was found that the results of 237 of 242 species tested were in agreement. A few of the strains produced a delayed positive reaction with the Enterotube method. Three *P. rettgeri*, one *P. mirabilis*, and the single strain of *S. gallinarum* were positive in the conventional test but negative in the Enterotube.

A number of *Pseudomonas* and *Herellea* organisms were tested since they are frequently encountered in clinical material, and screening tests used for the *Enterobacteriaceae* with a few additions may be used for these bacteria. Also, colorless colonies produced by some of these bacteria cannot be delineated from non-lactose-fermenting enteric bacilli on eosin methylene blue or MacConkey agar and would be tested as a routine procedure.

Possible variation between different lots of Enterotubes was studied. Five batches of Enterotubes with different control numbers were obtained from the manufacturer. The results of repeated tests with *E. coli*, *K. pneumoniae*, *E. aerogenes*, *P. mirabilis*, and *Citrobacter* organisms with each batch were sufficiently constant to preclude errors attributed to different lots.

## DISCUSSION

When the results with individual tests represented in both systems were compared (Table 1), no discrepancies were noted in the indole test, and only one was recorded for dextrose with *P. aeruginosa*. Satisfactory results were obtained with phenylalanine, dulcitol, lactose, the hydrogen sulfide test, and citrate. In 7 of 242 hydrogen sulfide tests, three of 242 phenylalanine, 22 of 242 urease, 15 of 242 dulcitol, 12 of 242 lactose, 27 of 217 lysine decarboxylase, and 5 of 242 citrate tests, the Enterotube results were contrary to those obtained with conventional methods. The results with the Enterotube urease test reported here (Table 1) differ somewhat from those reported by Grunberg et al. (8), who found a lower percentage of discrepancies with the Enterotubes. In the Enterotubes, most of the confusion seemed to result from weak or delayed reactions. This might be due, at least in part, to the small amount of agar present and a small inoculum which is deposited in a localized area. Although the results with *Proteus* strains were uniformly positive and the results with *Salmonella* and *Shigella* were uniformly negative, the results with *E. hafniae* and *Citrobacter* strains were not so reliable in excluding *Salmonella*, *Arizona*, and *Shigella*. The

positive results obtained by the conventional method with *E. hafniae* strains did not concur with the findings of Edwards and Ewing (4). With pseudomonads, organisms which are generally variable in urease activity, the results were generally negative with both test systems. However, we have an unusually high number of negative urease producers. The adaptive nature of *Pseudomonas* urease must always be considered when dealing with these organisms (3, 12, 13). There was some difficulty in reading fermentation reactions in dulcitol. This might be due to a low hydrogen ion concentration or leakage of acid components from the lactose compartment into the dulcitol compartment which resulted in a weak reaction.

Correlation of the agreement with individual tests in both test systems is presented in Table 2. Agreement was poorest with the urease and lysine decarboxylase tests, amounting to 90.9% with urease and 87.6% with lysine decarboxylase. Color-determining problems were encountered to some extent with the lysine decarboxylase test. The confusion in interpreting a violet or purple color reaction in the lysine-lactose-agar in the Enterotubes is apparently due to the diffusion of indicator from the citrate compartment into the lysine compartment, since the purple discoloration has been cited only with citrate-positive organisms. The violet or purple color, before it becomes more intense with age, can be confused with the pink or orange color produced by a positive reaction. In such instances, we relied on the results obtained with conventional tests. The Enterotubes also gave a lower correlation percentage with the conventional broth method of Moeller (10). An explanation for the poor results is that the manufacturer has incorporated lactose in the Enterotube agar to prevent a positive reaction with

TABLE 2. *Correlation of the agreement of enterotubes with conventional tests in 242 strains*

| Test procedure | No. of positives in agreement | No. of negatives in agreement | Total per cent in agreement |
|---|---|---|---|
| Hydrogen sulfide | 28 | 207 | 97.1 |
| Phenylalanine deaminase | 40 | 199 | 98.8 |
| Urease | 100 | 120 | 90.9 |
| Dulcitol | 46 | 184 | 95.0 |
| Lactose | 131 | 99 | 95.0 |
| Lysine decarboxylase[a] | 17 | 173 | 87.6 |
| Citrate | 157 | 80 | 97.9 |

[a] Does not include *Pseudomonas* or *Herellea* species.

lactose-fermenting organisms. This is because the method enhances the identification of certain *Salmonella* and is helpful in differentiating these organisms and *Arizona* strains from *Citrobacter*. Nonetheless, the exclusion of lactose from the lysine decarboxylase medium seems desirable to extend its range of usefulness to other members of the *Enterobacteriaceae* family isolated from those specimens in which salmonellae and shigellae are not detected. This would increase the accuracy of the test with rapid lactose-positive *Arizona* strains. Our strains were late lactose fermenters and both were lysine-positive.

The results of the Enterotube citrate tests were consistent when compared with Simmons citrate agar slants. These results disagree with those of Grunberg et al. (8), who found 27 of 38 (71%) of the Enterotube tests in agreement with the Simmons citrate test. This is considerably less than our 97.9% agreement rate (Table 2).

The urease test and dulcitol fermentation do not always produce consistent results with certain bacteria by conventional tests so that negative results from these tests must be carefully scrutinized. The lysine decarboxylase tests in the Enterotube system cannot be recommended as they are at present constituted because of a considerable number of discrepancies that occurred between the Enterotubes and those obtained by conventional methods. The violet-to-purple color that appeared with some of our organisms makes interpretation of the results difficult, if not impossible, to read.

From Table 1 it may be seen that a number of discrepancies exist between the Enterotube system and conventional methods. Quite often the Enterotube identifies strains as to genus rather than species. Occasionally aberrant forms are recognized which do not fit into the schema, but usually these are readily identified by further testing. It is obvious, of course, that the Enterotube test is not useful for nonfermentative gram-negative rods which do not belong to the enteric family. The addition of motility and gelatin studies when using Enterotubes would be most helpful in the differentiation of *Salmonella*, *Shigella*, *Klebsiella*, *Enterobacter*, and *Serratia*. Additional fermentation studies in arabinose, raffinose, xylose, and sodium malonate may be necessary to separate *Serratia* organisms from *E. liquefaciens*. The anaerogenic nature of these organisms is also a differential point. We do not agree with Grunberg et al. (8) that rapid lactose-positive members of the *Klebsiella-Aerobacter-Serratia* groups can be classified as *Klebsiella* on the basis of their reaction with lysine, dulcitol, and urease in the Enterotubes. Without other tests,

motility, gelatin, etc., these organisms probably should be identified only as to group. Although *P. aeruginosa* and *Herellea* strains were tested, the Enterotube system was not designed for the identification of these organisms.

Without additional tests some of the members of the *Enterobacteriaceae* family may be misidentified by the Enterotube test system. From the results obtained from the Enterotubes, one of two *Shigella* group A strains, the two group B strains, and the two Alkalescens-Dispar organisms were classified as atypical *Escherichia*. The *Shigella* group A strains gave negative lysine decarboxylase tests by the conventional method, but one gave a false-positive in the Enterotube. Acid with no gas production in fermentative carbohydrates, no reactions in lactose and sucrose, lysine decarboxylase, motility, and gelatin tests were conventional procedures, along with serological studies that were used to identify these strains. The two indole-positive *Klebsiella* were falsely identified as atypical *Escherichia*. Additional testing revealed their true identity. Three of four *E. cloacae* strains could not be differ-

TABLE 3. *Frequency of error in identification of strains by the Enterotube system*

| Organisms | No. of strains | No. of errors in identification | Per cent of error |
|---|---|---|---|
| *Shigella* group A....... | 2 | 1 | 50.0 |
| *Shigella* group B....... | 2 | 2 | 100 |
| *Shigella* group D...... | 1 | 0 | 0 |
| Alkalescens-Dispar.... | 2 | 2 | 100 |
| *Escherichia*[a].......... | 60 | 0 | 0 |
| *Klebsiella pneumoniae*.. | 60 | 2 | 3.3 |
| *Enterobacter aerogenes*............... | 6 | 0 | 0 |
| *E. cloacae*............ | 4 | 3 | 75.0 |
| *E. hafniae*............ | 12 | 0 | 0 |
| *Serratia marcescens*... | 4 | 4 | 100 |
| *Proteus mirabilis*....... | 27 | 0 | 0 |
| *P. rettgeri*............ | 10 | 3 | 30.0 |
| *P. morganii*........... | 4 | 0 | 0 |
| *P. vulgaris*............ | 1 | 0 | 0 |
| Providence group....... | 3 | 0 | 0 |
| *Salmonella paratyphi* B[b].................. | 2 | 0 | 0 |
| *S. typhi*.............. | 2 | 1 | 50.0 |
| *S. gallinarum*......... | 1 | 1 | 100 |
| *Salmonella* group E[b]... | 1 | 0 | 0 |
| *Citrobacter* group...... | 11 | 0 | 0 |
| *Arizona arizonae*....... | 2 | 0 | 0 |

[a] Includes *E. coli* and atypical *Escherichia*.
[b] Identified as to genus.

entiated from *E. aerogenes*. A negative lysine decarboxylase test, motility, and gelatinase activity were criteria used to identify *E. cloacae*. Without further study, the four nonchromogenic *Serratia* strains were incorrectly identified as *E. hafniae* in the Enterotubes. The *Serratia* strains were separated by rapid liquefaction of gelatin, inability to attack sodium malonate, and fermentative characteristics in carbohydrates with little or no gas production. Three of seven *P. rettgeri* strains could not be differentiated from *P. morganii* in the Enterotubes because of their failure to utilize citrate and were called *P. morganii*. Fermentation tests with mannitol, inositol, and sucrose and the ornithine decarboxylase reaction were conventional tests used to separate these two organisms. Of the two *Salmonella* lysine decarboxylase-negative strains, *S. typhi* was misidentified as a *Citrobacter* organism, whereas *S. gallinarum* could not be differentiated. Usually, without serological study and a more elaborate set of biochemical tests the *Salmonella* organisms could be identified only as to group in the Enterotubes as indicated by Grunberg et al. (8). These results are complied in Table 3 to show the frequency of error in identification of the above strains by the Enterotube system. This indicates that the use of the Enterotube test system may result in frequent misidentification of enteric gram-negative bacilli.

According to our studies, there are obvious deficiencies in the system. If these deficiencies were amended by the manufacturer, then the use of a combined chemical unit such as offered by the Enterotube is of value in rapid and simple biochemical testing. Such a method would have application in the busy diagnostic laboratory which does not prepare or stock differential media and reagents.

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