Evaluation of the Redesigned Enterotube—a System for the Identification of *Enterobacteriaceae*

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Received for publication 21 December 1972

The redesigned Enterotube has been evaluated with 414 unknown *Enterobacteriaceae* cultures from the stock culture collection of the Center for Disease Control. When the Enterotube was used as recommended by the manufacturer, an average of 96.4% of these cultures were correctly identified. Only two groups (*Salmonella* and *Edwardsiella*) were identified with less than 90% accuracy (89.2 and 87.5%, respectively). The Enterotube now provides a convenient, rapid, and accurate test system for the identification of typically reacting enteric bacteria.

In recent years, there has been a sharp increase in the development of test systems or kits for the rapid identification of members of the family *Enterobacteriaceae*. One of these is the Enterotube (manufactured by Roche Diagnostics); it has been available for several years and has been thoroughly described and evaluated in several reports. In 1969, Grunberg et al. (4) compared the Enterotube with the PathoTec (Warner-Chilcott Laboratories) reagent impregnated strip system. These investigators found the Enterotube to be useful only in placing organisms in the major groups of the family *Enterobacteriaceae*. In 1971 Martin et al. (5) compared the Enterotube with their conventional methods by using 89 clinical isolates and 11 stock cultures. They found that with the Enterotube they were able to correctly identify only 47% of the strains tested; an additional 38% were identified as members of the *Klebsiella-Enterobacter-Serratia* group. More recently, Elston and co-workers (2) evaluated the Enterotube, and they encountered some misidentification of the *Enterobacteriaceae* family.

In view of these and other reports, the manufacturer has recently redesigned the Enterotube. A test for ornithine decarboxylase has been added to one compartment, and lactose has been removed from the lysine decarboxylase compartment. The tests for phenylalanine deaminase and dulcitol fermentation have been combined in a single compartment. An iron salt has been added to this same compartment, thus eliminating the need to add ferric chloride reagent in testing for phenylalanine deaminase activity. To improve the decarboxylase reactions and to allow for the observance of gas production from the fermentation of dextrose, the manufacturer covered the compartments containing tests for dextrose fermentation, lysine decarboxylase, and ornithine decarboxylase with a sterile wax overlay. This report describes the results of our evaluation of this new Enterotube.

**MATERIALS AND METHODS**

The Enterotubes used were obtained from Roche Diagnostics, Nutley, N.J. They were used exactly as the manufacturer directed. Other media used were prepared in a central media kitchen from commercially available dehydrated media (Difco, BBL). Each batch of media was tested for reactions with a collection of stock cultures of known biochemical reactions. The tests or media used in the Enterotube and in our conventional procedures are as follows. The Enterotube consists of media for testing citrate utilization, urease, phenylalanine deaminase, acid from dulcitol, acid from lactose, H₂S production, indole, ornithine decarboxylase, lysine decarboxylase, and acid and gas from dextrose. Additional tests to determine acid production from the fermentation of raffinose and rhamnose are performed when necessary. The 17 tests used routinely in our conventional procedure are triple sugar iron agar, H₂S production, urease, indole, methyl red, acetoin, citrate utilization, lysine decarboxylase, ornithine decarboxylase, arginine dihydrolase, phenylalanine deaminase, motility, and acid from glucose, lactose, arabinose, raffinose, and rhamnose. The 10 additional tests that
are used when necessary are acid from dulcitol, inositol, sorbitol, adonitol, and mannotol, Jordan’s tartrate, deoxyribonuclease, mucate, sodium acetate, and malonate utilization. The media used for the conventional and extra tests were those recommended by Edwards and Ewing (1) and Ewing (3). In this evaluation, serology was not used either as an aid to identification or for confirmation of results, even though this is recommended by the manufacturer in their package insert. This also is a departure from our conventional procedures and was done only for the purpose of this evaluation.

Bacterial cultures, previously submitted to the Center for Disease Control for identification, were provided by the Center’s Enteric Bacteriology Laboratory of the Enterobacteriology Unit. The cultures were supplied for this study as coded unknowns on plain agar slants. One technician streaked each unknown onto a MacConkey agar plate and the following day inoculated the Enterotube using the following technique. Both caps were removed, and a single, well-isolated colony from the MacConkey Agar plate was touched with the tip of the inoculating needle. The wire was slightly twisted and then was drawn through the eight compartments of the Enterotube. The needle was then reinserted through the dextrose, lysine, and ornithine compartments and broken off. A plastic strip covering the side of the lactose, phenylalanine deaminase-dulcitol, urea, and citrate compartments was removed, and the tubes were incubated in an upright position at 35°C for 18 to 24 hr. After incubation, all reactions were observed, and an identification was made according to the manufacturer’s directions. A second technician transferred each culture to the media used in our conventional procedure. Conventional tests were incubated the recommended period of time before results were recorded and an identification was made by our regular schema. After this identification, all results were submitted to a third party, who compiled and compared them. Any discrepancies in test results were resolved by repeating the tests. When repeated testing did not resolve differences, the Enteric Bacteriology Laboratory was consulted as a reference laboratory.

RESULTS AND DISCUSSION

Our data have been analyzed in two ways. In one, individual tests results from the Enterotube were compared with the corresponding test results of our conventional procedure. Table 1 contains this comparison. This table also contains the results of a previous study we conducted using the original Enterotube. Acid from dextrose, H₂S production, ornithine decarboxylase, acid from lactose, lysine decarboxylase, acid from dulcitol, indole production, and phenylalanine deaminase showed better than 95% agreement in 414 parallel tests with the redesigned Enterotube. For urea hydrolysis and gas from dextrose, the agreement was slightly less than 90% for the two methods. The agreement for citrate utilization was slightly under 80%. The overall agreement was 95.1%.

With the exception of phenylalanine deaminase and citrate determinations, there was better agreement between the new Enterotube system and our conventional procedure than between the original Enterotube system and our procedure. The decreased agreement of the phenylalanine deaminase determinations was due to false-negative reactions obtained primarily with Proteus rettgeri. Of the 16 cultures of P. rettgeri tested, 12 were phenylalanine deaminase negative in the Enterotube. False-negative citrate utilization reactions in the Enterotube caused most of the lack of agreement, and these reactions were predominantly with Enterobacter, especially Enterobacter hafniae, Citrobacter, and Klebsiella. Although most of these organisms were weakly positive by our conventional citrate test, the results with the Enterotube system were still recorded as discrepancies. A few (11) false-positive citrate utilization results also occurred with the Enterotube, primarily in the genus Proteus. However, the manufacturer does state that rare false-positive citrate may occur with Proteus morganii. The 50 discrepancies in the urease test were predominantly due to the failure of the Enterotube to detect the weak positive reactions obtained conventionally with Citrobacter, Serratia, and Enterobacter, espe-

| Tests          | Original* Enterotube | % Agreement | No. Agreement | No. Disagreement | % Agreement |
|---------------|----------------------|-------------|---------------|------------------|-------------|
| Dextrose-Acid | 100                  | 414         | 0             | 100              |
| H₂S          | 97.1                 | 413         | 1             | 99.8             |
| Ornithine    |                      | 413         | 1             | 99.8             |
| Lactose      | 87.1                 | 411         | 3             | 99.3             |
| Lysine       | 83.4                 | 411         | 3             | 99.3             |
| Dulcitol     | 97.1                 | 410         | 4             | 99.0             |
| Indole       | 94.2                 | 410         | 4             | 99.0             |
| PPA*         | 97.9                 | 398         | 16            | 96.1             |
| Urea         | 87.5                 | 364         | 50            | 87.9             |
| Dextrose-gas |                      | 357         | 57            | 86.2             |
| Citrate      | 95.0                 | 330         | 84            | 79.9             |
| Total        | 4,331                | 223         |               | Avg. 95.1       |

*Results obtained in a previous study with a total of 624 tests.
*Phenylalanine deaminase.
cially *E. cloacae*. Difficulty in interpreting gas production from dextrose fermentation with the Enterotube led to an 86% agreement between the two systems. Much of this disagreement came from those species which produced small amounts of gas or no gas at all in our conventional tests, especially *Serratia*, *Enterobacter liquefaciens*, *Providencia* species, and *Proteus* species. The manufacturer does state that the gas production by *Serratia*, *Proteus*, and *Providencia* species may be slight and, therefore, gas production may not be evident in the Enterotube. Thirty-four such negative tests were recorded in our evaluation. However, 23 strains which produced no gas in our conventional tests were positive in the Enterotube. In such cases, a very small lifting of the wax overlay was often interpreted as positive.

The second type of data analysis concerns the accuracy of the Enterotube system in correctly identifying unknown enteric bacteria. Table 2 shows the accuracy with which the unknown organisms were correctly placed in one of the 17 genera or groups tested. As in Table 1, results obtained in an earlier evaluation of the original Enterotube are also listed. Results which were 100% correct were obtained with the new Enterotube for cultures of *Arizona*, *Citrobacter*, *Klebsiella*, *Serratia*, *Shigella*, and two species of *Proteus*. Results with *Providencia* species, *E. hafniae*, *E. cloacae*, *E. aerogenes*, and *Escherichia coli* were 95 to 100% correct, and *P. morganii*, *E. liquefaciens*, and *Proteus rettgeri* results were 90 to 95% correct. Only the cultures of *Salmonella* and *Edwardsiella* were identified with less than 90% accuracy. Of the 414 cultures tested, 399 or 96.4% were identified correctly by the new Enterotube.

The identification errors are listed in Table 3. These errors were caused both by aberrant reactions in the Enterotube and by atypical cultures. Of the 15 errors, nine were associated with atypical cultures and six by false-positive or negative reactions in the Enterotube. As previously stated, only *Salmonella* and *Edwardsiella* were identified with less than 90% accuracy. All three of the misidentified *Salmonella* cultures were dulcitol-negative strains. The Enterotube relies on dulcitol fermentation to differentiate *Salmonella* and *Arizona*. The manufacturer does state that all cultures identified as *Arizona* by the Enterotube should be serotyped with *Salmonella* polyvalent antisera to determine if this may be a dulcitol-negative *Salmonella* strain. Both of the *Edwardsiella* cultures missed were also atypical. An indole-negative strain was identified as *Arizona*, and an H$_2$S-negative strain was called *E. coli*.

The main problems encountered with the original Enterotube were the speciation of
Enterobacter and Proteus and the identification of Arizona, Citrobacter, Shigella, and Klebsiella. The redesigned Enterotube has largely resolved these problems. With the exception of Enterobacter and two species of Proteus, all of the problem organisms were identified with 100% accuracy by the new Enterotube. The removal of the lactose from the lysine decarboxylase test medium, and the addition of a test for ornithine decarboxylase now permit the differentiation of Klebsiella, Enterobacter, and Serratia and the speciation of Proteus. However, for the speciation of the Enterobacter-Serratia group, two additional sugar tests, rhamnose and raffinose, may be required as indicated by the manufacturer. The elimination of lactose from the lysine test medium also assures more accurate differentiation of Salmonella, Arizona, and Citrobacter.

The Enterotube, in its present form, appears to offer a wholly acceptable alternate to conventional systems for identifying Enterobacteriaceae. We found it to be easy to use, reliable in the identification of unknown cultures, accompanied by complete instructions, readily disposable, and easily stored. Obviously, the Enterotube must be used in accordance with the manufacturer's instructions and only on suspected cultures of Enterobacteriaceae. Even so, additional information such as colonial morphology on differential and selective media, and results of serological studies should also be considered in making an identification. Serologic tests are definitely indicated when an unknown is suspected of being a Salmonella, Shigella, or Arizona. Under these conditions, the user can expect a high degree of identification accuracy with the new Enterotube system.

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