Evaluation of the Enterotube System for Identification of Members of the Family Enterobacteriaceae

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The Enterotube system was evaluated, in parallel with conventional bacteriological procedures for the identification of members of the family Enterobacteriaceae, by using bacterial strains from a variety of clinical specimens and from stock cultures. Excellent agreement between the two test systems was obtained with the following reactions: hydrogen sulfide, indole, Simmons' citrate, glucose, and lactose. Agreement was not as good (<85%) with the urea, phenylalanine deaminase, and dulcitol reactions. The Enterotube lysine decarboxylase test was unsatisfactory. The Enterotube method will correctly identify strains of the family Enterobacteriaceae approximately 50% of the time; if identification only as Klebsiella-Enterobacter-Serratia group is needed, the method will be correct 85% of the time. On the basis of this evaluation, the Enterotube system appears to be both simple and rapid for the presumptive identification of these bacteria. Because of the limited usefulness of the lysine decarboxylase test, the results obtained by this test system are less reliable than those obtained by conventional methods.

Increasing demands on the clinical laboratory for the prompt differentiation of members of the family Enterobacteriaceae have stimulated the development in recent years of a number of commercially available test systems (or kits) designed primarily for the rapid identification of these bacteria (1-3, 7, 8, 11, 12, 14, 15). In general, most of these test systems offer both ease of operation and rapid identification. Some of these commercial test systems, however, have not compared favorably with the more conventional testing systems because of an inadequate number or selection of tests to provide adequate identification of certain members of the family Enterobacteriaceae (11, 12, 14) or because the results obtained with these test systems were, in many instances, difficult to interpret.

In recent months, a multiple-test system, Enterotube, which combines nine biochemical tests described as useful in the identification of members of the family Enterobacteriaceae has been introduced. Although this test system has been evaluated by Grunberg et al. (8), their comparison was made with the PathoTec (Warner-Chilcott Laboratories) reagent-impregnated strip system. This report describes the results of our evaluation of the Enterotube test system in comparison with conventional methods used in this laboratory.

MATERIALS AND METHODS

With the exception of 11 stock cultures, the specimens used for this evaluation represented fresh clinical isolates that were selected at random (Tables 1 and 2). Methods for the isolation from clinical specimens have been described (5, 10).

Enterotubes were purchased on the open market and used according to the directions of the manufacturer (Roche Diagnostics, Nutley, N.J.). Briefly, in this prepared test system, a semicircular molded plastic tube is divided into eight compartments; each compartment contains a slant of one of the following biochemical test media: citrate agar, modified lysine-lactose agar, lactose agar, dulcitol agar, urea agar, phenylalanine agar, hydrogen sulfide-indole agar, and dextrose agar. The test reactions were read after overnight incubation (18 to 24 hr) at 37 C. When indicated, the tube was reincubated to detect possible delayed reactions.

The primary set of biochemical tests routinely used in this laboratory for each gram-negative colony consists of the following: triple sugar-iron (TSI) agar, lysine-iron agar (LIA), Christensen's urea agar, Simmons' citrate agar, peptone water, and ornithine decarboxylase (Moeller base) in 0.3% agar. Although nearly all of the strains were initially identified with this basic set, additional biochemical tests, such as those described by Martin (10) and Washington et al. (16), were incorporated routinely in the identification process for this study. The decarboxylase tests were modified by the addition of 0.3% agar (9) which obviates the need for the oil overlay and permits
determination of motility. The indole, methyl red, and Voges-Proskauer tests were modified by the method of Douglas and Washington (4). The nomenclature and biochemical reactions used in this study were based on the taxonomic system of Ewing (6).

RESULTS

Agreement between results of conventional and Enterotube tests was at least 84% with the hydrogen sulfide, citrate, indole, phenylalanine deaminase, glucose, and lactose reactions (Table 3). Moderate agreement was noted with the urea (60%) and dulcitol (80%) reactions. Agreement was poor with the lysine decarboxylase test. Moreover, as will be discussed below, several isolates known to be lysine decarboxylase-negative gave false-positive reactions on the Enterotube lysine medium at both 24 and 48 hr of incubation.

Of the 100 strains, the Enterotube method correctly identified 47. Thirty-eight of the 100 strains were identified as belonging to the Klebsiella-Enterobacter-Serratia group by the Enterotube method (15 Klebsiella pneumoniae, 6 Enterobacter aerogenes, 8 E. cloacae, 2 E. liquefaciens, and 7 Serratia marcescens). Of the remaining 15 strains, 7 were identified incorrectly by this method. Three Providencia species and the Pectobacterium carotovorum were identified as Enterobacter species by the Enterotube method, the Shigella flexneri was

| Table 1. Sources of isolates |
|----------------------------|
| Source                     | No. |
|----------------------------|
| Urine                      | 47  |
| Sputum                     | 17  |
| Throat                     | 4   |
| Stool                      | 3   |
| Foot                       | 2   |
| Bronchial washing          | 2   |
| Abdominal abscess          | 2   |
| Finger                     | 2   |
| Ear                        | 1   |
| Appendix                   | 1   |
| Lochia                     | 1   |
| Wound                      | 1   |
| Hip                        | 1   |
| Abdominal wound            | 1   |
| Kidney                     | 1   |
| Vagina                     | 1   |
| Site of intravenous catheter| 1  |
| Catheter tip               | 1   |
| Stock*                     | 11  |
| Total                      | 100 |

* Two from American Society of Clinical Pathology; one from Center for Disease Control; eight from this laboratory.

| Table 2. Summary of isolates used |
|-----------------------------------|
| Isolate                           | No. |
|-----------------------------------|
| Escherichia coli                  | 20  |
| Shigella flexneri                 | 1   |
| S. sonnei                         | 1   |
| Edwardsiella tarda                | 1   |
| Salmonella typhi                  | 1   |
| S. enteritidis*                   | 2   |
| Arizona hinshawii                 | 2   |
| Citrobacter freundii              | 7   |
| Klebsiella pneumoniae             | 20  |
| Enterobacter cloacae              | 8   |
| E. aerogenes                      | 6   |
| E. hafniae                        | 2   |
| E. liquefaciens                   | 2   |
| Serratia marcescens               | 7   |
| Pectobacterium carotovorum        | 1   |
| Proteus mirabilis                 | 7   |
| P. morganii                       | 4   |
| P. rettgeri                       | 4   |
| Providence sp.                    | 4   |
| Total                             | 100 |

* Nomenclature based on the three-species concept (13).

identified as Escherichia coli, and two Proteus rettgeri were identified as P. morganii. Because of atypical biochemical reactions, the remaining eight strains could not be identified by the Enterotube method (three Citrobacter freundii, two E. hafniae, and three K. pneumoniae that were indole-positive).

DISCUSSION

With the exception of the lysine decarboxylase and urease test, the results obtained in this evaluation indicate good agreement between the two test systems. A relatively large number of lysine-negative strains gave positive reactions in the Enterotube method. Thus, of the 35 positive tests by the Enterotube method (at 24 and 48 hr), 13 were false-positive reactions (5 P. mirabilis, 2 P. rettgeri, 3 Providencia sp., 1 S. sonnei, 1 P. carotovorum, and 1 E. cloacae). In the Enterotube lactose test there occasionally was a reversion of the acidic reaction to alkalinity within 48 hr. Since the Enterotube lysine test also contains lactose, an alkaline reaction at 48 hr might represent delayed decarboxylation or reversal of the acidic pH caused by lactose fermentation. For example, of the 48 strains that were not readily interpretable (see Table 3), 7 strains produced an alkaline reaction in the Enterotube lysine test at 48 hr but also reversed the acidity in the lactose
test to alkaline at 48 hr. With 32 strains the lysine test remained negative after 48 hr without recovery of acidity to alkalinity in the lactose test. In nine strains, the lactose test reverted from acidity to alkalinity, but the lysine test remained negative after the same period of incubation.

Because of these false reactions, compared with the conventional test, there is a need for the exclusion of lactose from the lysine test medium currently used in the Enterotube system. Similar observations also were made (11, 14) with the PathoTec lysine test system. On the other hand, Grunberg et al. (8) found little or no difference in results between the Enterotube and the PathoTec lysine decarboxylase tests. Since both of these systems incorporate lactose in their lysine decarboxylase test, it is not surprising that no discrepancy was noted.

Grunberg et al. (8) reported discrepancies between results obtained on Simmons’ citrate agar and those obtained with the Enterotube system. But our results demonstrated close agreement between both these tests. Considering the position of the citrate test on the Enterotube—such that it receives the heaviest inoculation as the needle is withdrawn—this is indeed noteworthy. It has been stated that, for the Simmons’ citrate agar test to perform properly, a light inoculum should be used (4, 5, 10). Perhaps inoculum size on this medium is not as critical a factor as has been reported. Possible differences in the preparation of the two types of citrate media also may be a factor.

No doubt the greatest advantage in the use of the Enterotube system is that all tests are inoculated sequentially and rapidly from a single isolated colony. Our experience with this test system indicates that it is a simple and rapid method for the presumptive identification of members of the family Enterobacteriaceae. However, some qualification of this conclusion is needed, depending on the “level” of identification one is willing to accept. Our results showed that 47% of the strains evaluated were correctly identified by the Enterotube method, but an additional 38% were identified as members of the Klebsiella-Enterobacter-Serratia group. Therefore, if it is not necessary to separate the members of this large group, the Enterotube method will correctly identify members of the family Enterobacteriaceae approximately 85% of the time.

Regarding reliability, one definitely should keep in mind the problems with the Enterotube lysine decarboxylase test. As presently constituted, the Enterotube lysine decarboxylase test is useful mainly for non-lactose-fermenting organisms such as Salmonella and Shigella. Since the genera of the tribe Klebsielleae have been incriminated as etiological agents of nosocomial infections (Klebsiella, Enterobacter, and Serratia), it is of paramount importance that these bacteria be identified adequately. Toward this end, the use of lysine without lactose is strongly recommended. The combination of biochemical tests in the Enterotube system appears to be heavily oriented toward separation of Shigella and Salmonella from the other Enterobacteriaceae. Although the correct identification of these two genera, especially in stool cultures, is an essential requirement of any clinical bacteriology laboratory, consideration might be given to replacing the dulcitol and lactose tests with ornithine decarboxylase and arabinose media to permit separation of the genera of the tribe Klebsielleae in cultures of clinical specimens other than stool.

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