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Clinical Laboratory Experience with the Improved Enterotube

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The reactions elicited in this evaluation of the improved Enterotube indicate the efficacy of using this device for the differentiation of the members of the family *Enterobacteriaceae*.

The Enterotube was first described in 1969 (3) and represents a multitest system for the rapid identification of medically significant members of the family *Enterobacteriaceae*. Although the original report concluded that the particular approach employed was an advantage, the need to improve the system was recognized. This report constitutes the experience of an active clinical microbiology laboratory with the improved Enterotube.

MATERIALS AND METHODS

Organisms. The 586 representatives of the family *Enterobacteriaceae* employed in this study were comprised of isolates from clinical specimens and stock cultures kept in the file of this laboratory and originally supplied through the courtesy of the Center for Disease Control. All the bacteria from clinical material were isolated by methods described earlier (4). The stock cultures, maintained at -70 C, were inoculated into nutrient broth and then treated in the manner of a clinical specimen.

Conventional media. All microorganisms were inoculated on several media in conjunction with the Enterotube. This biochemical series consisted of triple sugar-iron agar (BBL), Simmons citrate agar (BBL), Christensens urea agar (BBL), tryptone broth (Difco), MR-VP broth (BBL), KCN broth (BBL), Malonate broth (BBL), and Moellers decarboxylase broth (BBL) containing either lysine, ornithine, or arginine as substrate. All media were prepared weekly according to the manufacurer's specifications and were stored at 4 C until used. Media were controlled with positive and negative reacting organisms within 24 h of their preparation. In addition, all examinations were accompanied by organisms that gave positive and negative reactions with each of the substrates used.

Modified Enterotubes. These tubes were obtained from Roche Diagnostics, Division of Hoffmann-LaRoche Inc. (Nutley, N.J.). The modified tube includes media for testing ornithine decarboxylase, a test for determining gas production and more sensitive media for measuring the production of urease, indole, H_2S, and lysine decarboxylase. The need for adding ferric chloride to the phenylalanine/dulcitol compartment has been eliminated by the incorporation of iron salt into this medium. In addition, the anaerobic milieu for the production of gases by fermentation of glucose and decarboxylation of lysine and ornithine is accomplished by the reinsertion of the inoculating needle into these compartments. The effect of the reinserted needle is complemented further by a wax overlay in these three compartments.

Inoculation of media. The manufacturer's instructions for inoculating the Enterotube were followed precisely. A well-isolated colony was picked directly with the Enterotube inoculating needle. All eight compartments were inoculated by withdrawing the needle through the tube. A tube containing 2 ml of Trypticase soy broth (BBL) was inoculated before reinsertion of the inoculating needle into the first three compartments of the Enterotube. The Trypticase soy broth was incubated at 35 C for 2 to 4 h, followed by the inoculation of the conventional media and an eosin methylene blue agar plate (BBL) as a check for the purity of the inoculation. Both the Enterotube and the conventional media were incubated at 35 C for 24 h before recording the reactions elicited. The conventional methods did not attempt to duplicate all of the reactions of the Enterotube system. The conventional method reflects the standard, initial approach to the identification of the family *Enterobacteriaceae* as practiced in this laboratory.

RESULTS

Table 1 summarizes the results obtained with the conventional method and the modified Enterotube, as well as the percentages compiled and reported by Ewing (1). In general, agreement between the two approaches was excellent. The reactions listed reflect the comparison of each culture, test by test. The higher percentage reported for either approach invariably included the bacteria giving a positive reaction in the other test system. The comparison of each test by each method to the percent reac-

TABLE 1. *Percentage of positive reactions obtained after 24 h of incubation*[a]

Bacteria	No. tested	Glucose			Gas			LDC			ODC			H_2S			Indole		
		C	R	E	C	R	E	C	R	E	C	R	E	C	R	E	C	R	E
Escherichia coli	52	100	100	100	100	100	91.1	86.5	86.5	88.7	75	76.9	64.2	0	0	0	98	98	98.6
Shigella sp	22	100	100	100	4.5	4.5	2.1	0	0	0	31.8	36.3	20.0	0	0	0	27.3	27.3	37.8
Salmonella sp.	34	100	100	100	88.2	97.0	91.9	100	100	94.6	94.1	94.1	92.7	91.2	91.2	91.6	0	0	1.1
Arizona sp.	25	100	100	100	80	96	99.3	96	96	100	96	96	100	96	96	98.7	0	0	2.0
Citrobacter freundii	10	100	100	100	100	100	90.9	0	0	0	20	20	17.2	100	90	81.6	0	10	6.7
C. diversus	2	100	100	100	100	100	97.3	0	0	0	100	100	100	0	0	0	100	100	100
Klebsiella pneumoniae	95	100	100	100	96.8	97.8	96.5	98.9	98.9	97.2	0	0	0	0	0	0	7.3	7.3	6.0
Enterobacter aerogenes	32	100	100	100	100	100	100	96.8	93.7	98.7	96.8	93.7	98.7	0	0	0	3.1	3.1	0
E. cloacae	46	100	100	100	100	100	100	0	0	0.5	93.4	91.3	96.0	0	0	0	2.2	2.2	0.5
E. hafniae	27	100	100	100	100	100	100	100	100	100	100	100	100	0	0	0	0	0	0
E. liquefaciens	25	100	100	100	88	96	94.1	84	84	82.4	84	84	98.5	0	0	0	4	8	0
Serratia marcescens	29	100	100	100	36.2	89.6	52.6	100	100	99.6	100	100	99.5	0	0	0	3.4	3.4	0.2
Proteus vulgaris	27	100	100	100	55.5	77.8	86.0	0	0	0	0	0	0	55.5	88.8	94.7	51.8	66.6	98.2
P. mirabilis	66	100	100	100	33.3	80.3	95.4	0	0	0	98.4	98.4	99.2	69.6	84.8	94.2	1.5	1.5	1.9
P. morganii	43	100	100	100	95.3	88.4	84.9	0	0	0	95.3	97.6	97.1	0	0	0	88.3	97.6	100
P. rettgeri	24	100	100	100	25	25	12.2	0	0	0	0	0	0	0	0	0	79.2	95.8	100
Providencia sp.	28	100	96.4	100	21.4	21.4	X	0	0	0	0	0	0	0	0	0	78.5	82.1	X

Bacteria	No. tested	Lactose			Dulcitol			PAD			Urease			Citrate		
		C	R	E	C	R	E	C	R	E	C	R	E	C	R	E
Escherichia coli	52	88.5	92.3	90.8	ND	21.2	49.5	ND	0	0	0	0	0	0	0	0.2
Shigella sp.	22	0	0	0.3	ND	9.0	5.4	ND	0	0	4.5	4.5	0	0	0	0
Salmonella sp.	34	0	0	0.8	ND	82.4	86.5	ND	0	0	0	0	0	97	97	80.1
Arizona sp.	25	0	24	61.3	ND	0	0	ND	0	0	0	0	0	96	92	98.7
Citrobacter freundii	10	40	70	39.4	ND	50	59.8	ND	0	0	90	90	69.4	100	100	90.4
C. diversus	2	0	0	32.7	ND	50	53.1	ND	0	0	100	100	77.9	100	100	99.1
Klebsiella pneumoniae	95	91.6	91.6	98.2	ND	30.5	31.5	ND	0	0	97.8	98.9	94.5	100	94.7	97.7
Enterobacter aerogenes	32	96.8	90.6	92.1	ND	15.6	4	ND	0	0	12.5	12.5	2.7	96.8	93.7	93.7
E. cloacae	46	76	80.4	93.5	ND	13	12.9	ND	0	0	78.2	73.9	64.7	95.6	93.4	99.5
E. hafniae	27	0	11.1	0	ND	7.4	1	ND	0	0	3.7	3.7	3	7.4	3.7	0
E. liquefaciens	25	32	44	1.5	ND	4	0	ND	0	0	20	16	4.4	84	76	91.2
Serratia marcescens	29	3.4	6.8	2.2	ND	0	0	ND	0	0	48.2	44.8	29.1	96.6	96.6	98.6
Proteus vulgaris	27	3.7	7.4	0	ND	0	0	ND	100	100	96.2	96.2	94.7	14.8	92.5	10.5
P. mirabilis	66	0	1.5	1.5	ND	0	0	ND	98.4	99.6	96.9	96.9	88.4	30.3	100	58.7
P. morganii	43	0	23.2	0	ND	0	0	ND	86	95.3	93	97.6	98.2	4.6	55.8	0
P. rettgeri	24	20.8	25	8.9	ND	0	0	ND	100	97.8	100	100	100	91.6	100	95.6
Providencia sp.	28	0	0	X	ND	0	0	ND	100	X	0	0	0	92.9	92.9	X

[a] Abbreviations: LDC, lysine decarboxylase; ODC, ornithine decarboxylase; PAD, phenylalanine deaminase; C, conventional method (see text); R, Enterotube; E, Ewing's tables; ND, not performed; X, different percentages applicable to different species.

tion tables compiled by Ewing underlines the very acceptable degree of agreement obtained. Obviously, such agreement in a single study with a limited number of the species represented cannot reflect completely the percentages reported in the monumental effort of Ewing. Nevertheless, the similarity in results is striking; when discrepancies of minor proportions were noted between the results and Ewing's figures, tests were repeated by both approaches. The small variations were accepted as reflecting the characteristics of the organisms included in the study. They were not considered as adversely affecting the reliability of the findings.

Included in this evaluation were a number of organisms, kept as stock cultures, which had aberrant reactions. These were used to challenge both approaches for their ability to still group the bacteria properly. Again, anomalous reactions on one or two of the test substrates were not considered to diminish the reliability of the results. These cultures were identified by both approaches with the same degree of accuracy.

The results reflected in Table 1 can best be considered in two parts. The various genera of the family *Enterobacteriaceae*, with the exception of the tribe *Proteeae*, revealed very close agreement between the conventional method, the improved Enterotube, and the definitive results of Ewing. Complete agreement was obtained with the capacity to ferment glucose. Gas production was uniformly reproducible. There is an exception in the gas production found by the conventional approach with *Arizona*. Here, only 80% of the 25 arizonae produced gas, whereas the improved Enterotube yielded 96% gas as compared to the 99.3% reported by Ewing. *Serratia marcescens* fermented glucose with the production of gas in 36.2% by the conventional method and 89.5% by the improved Enterotube, whereas the gas production for the large series of Ewing is at the level of 52.6%. The small discrepancies noted in indole production reflect the presence of anomalously reacting strains included. The ability to ferment lactose was not manifested by the conventional method in the *Arizona* representatives studied. Although the improved Enterotube yielded 24% positive lactose fermenters, it must be compared to the higher percentage reported by Ewing. Thus, it would appear that the improved Enterotube has a more sensitive mechanism for detection of lactose. Consideration of *Citrobacter freundii* shows a 70% fermentation of lactose within the first 24-h period, which is considerably more than the 40% obtained by the conventional method or the 39.4% reported by Ewing. This impression is also supported by experiences with *Enterobacter cloacae*. The result obtained with *E. liquefaciens* obviously represented strain differences and selection. The small discrepancies noted with urease fall into the same category, namely, the strains employed in this study apparently expressed this property somewhat in disproportion to the overall reactions recorded by Ewing. This same reason explains the slight variation noted with citrate. All other reactions require no comment.

The package insert accompanying the improved Enterotube states that the reaction on citrate by the tribe *Proteeae* is unreliable with this system and should not be used to delineate the species of the genus *Proteus*. The manufacturer advises that the reactions with the ornithine decarboxylase should be used for this purpose. The experience in this study bears out the suggestion of the manufacturer and indicates that the reactions of these organisms vis-a-vis citrate must be ignored. The reaction of *Proteus* with production of gas during the fermentation of glucose was not in agreement with the conventional method. The small amount of gas produced under these circumstances was readily detected by the overlay provided in the improved Enterotube. Similarly, the improved Enterotube was a much better detector of hydrogen sulfide production than the triple sugar-iron agar conventionally used. Although the detection of indole was somewhat lower with the improved Enterotube than the percentages reported by Ewing with *Proteus vulgaris*, this discrepancy was more marked with the conventional method. The conventional method was also inferior to the improved Enterotube with *P. morganii* and *P. rettgeri*. Again, greater sensitivity of lactose fermentation detection was experienced with the improved Enterotube. Thus, 23.2% of the 43 representatives of *P. morganii* fermented lactose in a 24-h period. With the same organism, a diminished production of phenylalanine deaminase was noted as well.

DISCUSSION

The need to quickly and accurately differentiate between various members of the family *Enterobacteriaceae* has become very apparent within the past decade. Bacteria in this family not only represent the traditional bacteria with the ability to incite human intestinal disease but include a number of species significant in nosocomially acquired complications. The im-

proved Enterotube has the ability to sequester significant reactions which lead to the identification of the various constituents of the family *Enterobacteriaceae*. It is necessary to follow some of the suggestions in the package insert for speciation, especially of *Enterobacter*. Confirmation of the differentiation, especially of salmonellae, shigellae, arizonae, and enteropathogenic escherichiae, by serological techniques is also suggested. Above all, the Enterotube is a simple single-tube system which brings to the unsophisticated laboatory, heretofore unaccustomed to identifying members of the family to the species level, the capacity to do so and to render a better service to the clinician.

The reactions elicited with the improved Enterotube compare favorably with those obtained with media prepared in the laboratory, quality controlled both at the time of preparation and during its performance. Thus, there is a great advantage to the use of this and similar devices. A greater degree of standardization of the salient reactions is possible with the system approach. The limitations of preparation and standardization, neglected or ignored in laboratories without adequate facilities, personnel, and experience, can be overcome with this type of device. In this study, the improved Enterotube reacted more closely to the findings reported in the percentage tables of Ewing than did the conventional method for the first approximation of the identity of members of the family. Attention to the instructions included by the manufacturer leads one to disregard the most discrepant reaction, namely the citrate utilization by the genus *Proteus*. All other reactions are within acceptable limits and underline the usefulness of the improved Enterotube as a laboratory guide to the members of the family *Enterobacteriaceae*.

The objections listed against the original Enterotube by several authors (2, 5, 6) have been overcome significantly by the improvements in the present device. Our study supports the opinions expressed by the group at the Center for Disease Control (K. M. Tomfohrde, D. L. Rhoden, P. B. Smith, and A. Balows, Bacteriol. Proc., p. 88, 1972) that very high overall correlation of tests can be achieved with the improved Enterotube. We echo as well the sentiment expressed in that abstract that the product has been greatly improved by the manufacturer and should now be a useful device in the clinical microbiology laboratory to aid in the identification of gram-negative enteric rods.

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