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Clinical Laboratory Evaluation of the Further Improved Enterotube and Encise II

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The most recent improvements in the Enterotube coupled with Encise II permitted identification of routinely and abnormally reacting members of the family *Enterobacteriaceae*.

Commercially produced systems approaches to the identification of clinically significant members of the family *Enterobacteriaceae* are gaining increasing acceptance in the clinical microbiology laboratory. As a consequence, continuing refinement of existing systems is required. This is actually proceeding along two major avenues; namely, the refinement and extension of biochemical reactions, as well as the broadening of the data base on which percentages of reactions are based. Recently Roche Diagnostics has improved the phenylalanine deaminase detection of the Enterotube and has introduced Encise II, a large data base which can be approached by a binomial computer system or a four digit number reference, both of which are available in an accompanying guide. The novelty of the computerized data base rests with keeping the number of biochemical tests for the recognition of the majority of *Enterobacteriaceae* to a minimum. Unusual reactions are referrable to the Encise II system where the likelihoods of identity are bolstered by a few additional tests aimed at confirming the identity. This report summarizes the clinical evaluation of the improvement in the detection of phenylalanine deaminase and the guidance provided with Encise II.

Bacteria. A total of 424 members of the family *Enterobacteriaceae* were tested. Of these, 100 represented the challenge bacteria described previously (3) which were selected originally to test the limits of any biochemical system designed to identify genera and species of the family. The remaining microorganisms in the study were isolated recently from clinically significant pathological specimens. The various species included are listed in Table 1.

Methods. The bacteria were isolated as described earlier (2) and in keeping with various caveats (4). The routine methods of this laboratory (3) were used for identification while Enterotube inoculation proceeded according to the instructions of the manufacturer and as described in earlier publications (1, 5, 7, 9). Results with the Enterotube were read and translated into the four digit identification number (ID) according to the manufacturer's instructions, followed invariably by consultation with Encise II when identity of the bacteria could not be established with the reactions of Enterotube.

No problems were encountered with the correct identification of 324 bacteria isolated from clinical sources. The guidance provided by Encise II was used in 30 instances, and satisfactory results were obtained quickly. All results were determined easily. No incorrect reactions were noted with the phenylalanine deaminase reagent in any instance. As stated in the package insert and reported earlier (7), the citrate

TABLE 1. *Enterobacteriaceae studied*[a]

Genus and species	No.
Arizona sp.	28
Citrobacter freundii	9
C. diversus	14
Enterobacter aerogenes	51
E. agglomerans	15
E. cloacae	55
E. hafniae	25
Escherichia coli	65
Klebsiella ozaenae	2
K. pneumoniae	26
Proteus mirabilis	2
P. morganii	2
P. rettgeri	7
Providencia alkalifaciens	4
P. stuartii	12
Salmonella sp.	30
Serratia liquefaciens	27
S. marcescens	43
Shigella sp.	8

[a] Two *E. coli* were indole negative, 11 produced H_2S; eight *C. freundii* did not produce H_2S; six salmonellae were H_2S negative, whereas four fermented lactose.

TABLE 2. *Organisms not identified by Enterotube and Encise II*[a]

Bacterium	Incidence	Enterotube/Encise II	Aberrant reactions
Enterobacter cloacae	1	*Salmonella, E. aerogenes*	Lys +
E. cloacae	3	*Serratia liquefaciens, Klebsiella ozaenae, Citrobacter freundii*	Gas −
E. agglomerans	2	*Escherichia coli, Citrobacter freundii*	Dul +
E. aerogenes	1	*S. liquefaciens, Salmonella, E. cloacae, C. freundii*	Lys −
Escherichia coli	1	Unidentifiable	Cit +
Klebsiella pneumoniae	1	Aerogenic *E. agglomerans, E. cloacae, C. freundii, Serratia rubidaea, K. ozaenae, Proteus rettgeri*	Lys −
Salmonella sp (H_2S-)	1	*E. coli*	Ind +
Salmonella sp.	4	*E. coli* (H_2S+)	Lac +
Serratia marcescens	1	*S. liquefaciens, K. ozaenae, Salmonella, C. freundii*	Lys −
Providencia stuartii	1	*P. alcalifaciens,* aerogenic *E. agglomerans, P. vulgaris*	Gas +

[a] Total: 16/424 (3.75%). Lys, Lysine decarboxylase; Gas, gas from glucose; Dul, dulcitol; Cit, citrate utilization; Ind, indole production; Lac, lactose fermentation.

reactions of the tribe *Proteae* may be disregarded without impairing the reliability of the species identification in the genera which comprised the tribe. The "challenge" bacteria were identified in the same manner. Inability to arrive at a proper identification occurred 16 of 424 times or at the 3.75% level. These bacteria and their reaction failures are listed in Table 2. In all instances only 1 of 11 reactions was involved.

The advantages of the commercial systems approach have been listed in several papers (3, 4, 7, 9). This investigation confirms earlier statements. The ability of a few well chosen pivotal reactions to separate *Enterobacteriaceae* of medical significance into genera and species is demonstrated even when the organisms studied consist in part of variants or biotypes encountered only rarely in clinical specimens. The concern of several manufacturers to provide a very broad data base is most encouraging. As stated before (H. D. Isenberg, in press), the reactions utilized are derived from experience with clinical laboratory isolates. There is little, if any, knowledge of the biochemical and physiological expressions of these bacteria when they occur in nonpathological states in man or in their natural habitats. This ignorance extends as well to the possible selective actions exerted by extra-intestinal tissues of the host with the potential of favoring certain species or biotypes. As appreciation of these factors increases, the real incidence of biochemical variation for taxonomic placement of *Enterobacteriaceae* should become apparent.

The widespread application of computer technology to problems of taxonomy and nomenclature has projected a distorted view of the diagnostic efforts of the clinical microbiology laboratory. There is a growing conviction that 20 or 30 test results approach Adansonian taxonomic requirements. With a properly programmed computer approach entailing a memory for diagnostic and other reactions of each species and biotype, these tests of diagnostic significance may well lead to identification in a carefully constructed mathematical matrix. However, the diagnostic nature of the actual tests cannot be ignored. Therefore, the number of such tests is irrelevant as long as the proper diagnostic properties are elicited and used to establish an intimation of identity in a phenetic sense (8). This investigation demonstrates that the 11 tests incorporated into the Enterotube can accomplish this end. When used with Encise II, the bacteria which defy classification with these tests can be investigated further with a modicum of additional characteristics. Frequently, the likelihood of identity also incorporated in Encise II may indicate that the isolate is of no medical significance in this specific instance and obviates the need for further investigations. Our findings confirm the excellent performance of the Enterotube, enhanced when used in conjunction with the guidance provided by Encise II.

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