Evaluation of API 20E System and Encise Enterotube for the Identification of Enterobacteriaceae of Animal Origin

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ABSTRACT

The API 20E System and the Encise Enterotube were evaluated for the identification of the Enterobacteriaceae isolated from clinical specimens of animal origin at a veterinary diagnostic laboratory. Compared to conventional tubed media, the API 20E System identified 235 of 240 isolates (97.9%) correctly. The Encise Enterotube correctly identified 229 of the 240 isolates (95.4%). Thus, both these identification systems could be used to replace conventional methods for identifying members of this family isolated from animal origin.

RÉSUMÉ

Cette expérience consistait à déterminer l'efficacité du système API 20E et de l'entérotube "Encise", pour l'identification des Enterobacteriaceae isolées de cas cliniques d'origine animale, dans un laboratoire de diagnostic vétérinaire. Au cours d'une étude comparative avec les milieux en tubes conventionnels, le système API 20E se révéla d'une efficacité de 97.9%, en permettant d'identifier correctement 235 souches d'entérobactéries, sur un total de 240; l'efficacité de l'entérotube "Encise" se situa par ailleurs à 95.4%, parce que cette technique ne permit d'identifier correctement que 229 de ces 240 souches. On pourrait par conséquent remplacer les méthodes conventionnelles par ces deux systèmes, pour l'identification des entérobactéries d'origine animale.

INTRODUCTION

It has been clear for many years that time-saving and inexpensive methods are desirable for the identification of bacteria in the diagnostic laboratory. The use of commercially prepared rapid biochemical microtechnique systems which utilize the standardized approach to the identification of bacteria of the family *Enterobacteriaceae*, as proposed by Edwards and Ewing (4), has partially fulfilled these requirements. Two microtechnique systems that are currently in use for this purpose are the API 20E System[1] and the Encise Enterotube[2]. These systems were initially found inadequate for bacterial identification (5, 6, 7, 11, 12, 20) but later improved with beneficial results (1, 3, 10, 14, 17, 18) and, more recently, they have been computerized. The latter has increased both the ease of handling and accuracy of identification (2, 9, 13, 16, 19). Work in evaluating these two systems, especially for the family *Enterobacteriaceae*, has been confined to human clinical isolates while no evaluations have been made of their use in the clinical veterinary laboratory.

The purpose of this study was to evaluate and compare the API 20E System and the Encise Enterotube against conventional methods for their ability to accurately identify members of the family *Enterobacteriaceae* from clinical samples of animal origin.

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[1]Analytab Products Limited, St. Laurent, Quebec.

[2]Roche Diagnostics, Hoffman-LaRoche Limited, Vaudreuil, Quebec.

MATERIALS AND METHODS

I. *API 20E strips* — The API 20E strip is a miniaturized system containing 20 biochemical tests (Table I). Each test was performed in a sterile plastic tube which contained the appropriate substrate, had a capacity of 0.12 mL and was affixed to an impermeable plastic backing. Upon delivery, all API 20E strips were kept refrigerated at 4°C until used.

II. *Encise Enterotubes* — The Encise Enterotube is a molded plastic tube containing eight compartments and 11 different biochemical reactions.

III. *Identification of bacterial isolates* — A total of 240 clinical isolates of the family *Enterobacteriaceae* (Table II) were obtained from the O.V.C. hospital diagnostic laboratory on Encise Enterotubes inoculated from the original plates. The organisms were subcultured onto MacConkey agar, then

TABLE I. Biochemical Tests and their Abbreviations as Used in the API 20E System

Test Sequence	Biochemical Test	Test Abbreviation
1	O–Nitrophenyl–β–D–galactosidase	ONPG
2	Arginine dihydrolase	ADH
3	Lysine decarboxylase	LDC
4	Ornithine decarboxylase	ODC
5	Citrate utilization	CIT
6	Hydrogen sulfide (H_2S) production	H_2S
7	Urease production	URE
8	Tryptophanedeaminase production	TDA
9	Indole production	IND
10	Acetoin production	VP
11	Gelatinase production	GEL
12	Glucose fermentation	GLU
13	Mannitol fermentation	MAN
14	Inositol fermentation	INO
15	Sorbitol fermentation	SOR
16	Rhamnose fermentation	RHA
17	Sucrose fermentation	SAC
18	Melibiose fermentation	MEL
19	Amygdaline fermentation	AMY
20	Arabinose fermentation	ARA

TABLE II. Evaluation of Isolates of the Family *Enterobacteriaceae* Obtained from the Encise Enterotube: Correlation of API 20E, Enterotube and Conventional Media

Conventional Identification	Number	Agreement			
		API 20E		Encise Enterotube	
		Number	%	Number	%
Escherichia coli	38	37	97.4	35	92.1
Salmonella spp.	96	96	100	95	98.9
Arizona hinshawii	7	7	100	6	85.7
Klebsiella pneumoniae	24	24	100	23	95.8
Enterobacter aerogenes	3	2	66.7	2	66.7
Enterobacter agglomerans	6	6	100	6	100
Enterobacter cloacae	13	11	84.6	13	100
Enterobacter hafniae	2	2	100	2	100
Proteus mirabilis	16	16	100	16	100
Proteus morganii	8	8	100	7	87.5
Proteus rettgeri	1	1	100	1	100
Proteus vulgaris	1	1	100	1	100
Providencia alcalifaciens	1	1	100	1	100
Providencia stuartii	2	2	100	2	100
Citrobacter diversus	8	8	100	6	75
Cirobacter freundii	6	6	100	6	100
Serratia marcescens	5	5	100	4	80
Yersinia enterocolitica	3	2	66.7	3	100
Total	240	235	97.9[a]	229	95.4[a]

[a]Percent of the total

identified using a sequential table developed from King's classification for Gram-negative, MacConkey positive, cytochrome-oxidase negative fermenters (21). Single colonies were used to inoculate an API strip. All bacteria were processed and identified in the systems according to the instructions of both manufacturers. All API profile numbers obtained from the test organisms which could not be found in the API profile index were phoned into the API Profile Recognition System Computer Service in Montreal for identification. If identification still could not be made or if there was disagreement in identification, the individual isolate was placed onto a blood agar slope and sent to the manufacturer's laboratory in Montreal so that its identification could be established by their diagnostic method.

RESULTS

Table II shows the genera and species identified by the API 20E strip and the Encise Enterotube, compared to the conventional methods. Of the 240 isolates, 235 were correctly identified by the API 20E strip (an accuracy of identification of 97.9% at the species level). One of the five organisms was *Ent. cloacae* which was identified by the API 20E strip as *Klebsiella pneumoniae*. However, this organism was motile and so was confirmed as *Ent. cloacae*. Another was an *Ent. cloacae* identified in API as *Ent. agglomerans*. However, by conventional biochemical tests, the organism was arginine dihydrolase positive and inositol negative favouring an *Ent. cloacae* identification. The third organism was an *Escherichia coli* identified as a *Citrobacter freundii*. The API profile number plus one other additional test for adonitol identified this isolate as *C. freundii*. The conventional biochemical tests showed this organism to be negative for citrate, urea, H_2S, and malonate but positive for indole production, thus favouring *E. coli* identification. An *Ent. aerogenes* was identified as *Ent. cloacae* in the API 20E strip. The API reference identified this organism as *Ent. aerogenes* using the API 20E strip as they obtained a negative reaction in ADH while the authors found it to be ADH positive. Finally, a *Yersinia enterocolitica* strain which could not be identified as such after three inoculations in the API 20E strip was identified correctly at the API laboratory using the API 20E strip.

The results shown in Table II indicated that the Encise Enterotube correctly identified 229 out of the total 240 clinical isolates, an overall accuracy of identification of 95.5%. Three of the organisms were incorrect as species but in the correct genera. There were a *C. diversus, Ent. aerogenes* and a *Proteus morganii* identified respectively as *C. freundii, Ent. cloacae* and *P. mirabilis*. A *Salmonella* spp. was incorrectly identified as an *Arizona hinshawii* and vice versa. Three *E. coli* isolates were incorrectly identified as a *Shigella* spp., an *Ent. hafniae* and a *Serratia marcescens*. A *C. diversus* was misidentified as a *Proteus* spp., a *S. marcescens* as an *E. coli* and a *K. pneumoniae* as an *E. coli* and a *K. pneumoniae* as an *Ent. agglomerans*.

The API 20E system required some extra tests to make a final species identification, particularly in the case of *S. marcescens* and *C. freundii*. Three strains of *S. marcescens* were given an identification of *Serratia* spp. and required pigment production and/or results from inositol to differentiate for correct identification to species. Similarly, two strains of *C. freundii* were given identification of *Citrobacter* spp. and a positive test for adonitol was required to separate *C. freundii* from *C. diversus*.

In this study, two strains of *A. hinshawii*, one strain of *Salmonella* spp., one strain of *K. pneumoniae* and a *Y. enterocolitica* failed to show nitrate reduction in the API 20E strip but were nitrate reducers by conventional methods. However, this inconsistency did not alter the final identification of these isolates since it is not required for the identification of the family *Enterobacteriaceae*. These strains were found to be weakly nitrate positive at the Montreal API reference laboratory.

DISCUSSION

The results indicate that both the API 20E and the Encise Enterotube are suitable systems for the identification of the family *Enterobacteriaceae*. The overall accuracy of identification for the API 20E strip was found to be 97.9% (235/240

isolates) and that of the Encise Enterotube, 95.4% (229/240 isolates). Previous researchers (8, 22) have reported similar results when comparing these two systems for human clinical isolates. It is recognized that only five strains or less were examined with eight of the 18 bacterial species studied. Therefore the percentage of accurate identifications could change with the testing of more strains.

With the Encise Enterotube when discrepancies in identification with the conventional methods did arise the differences were distinct, for example, in the identification of a *C. diversus* as a *Proteus* spp. or *S. marcescens* as an *E. coli.*

Interpretation of reactions in the Enterotube was sometimes a problem, particularly with the phenylalanine-dulcitol compartment. We found the colour comparison reactions in the instruction manual inadequate and not in accordance with actual reaction colours observed in the Enterotube. It is recommended that these be changed to avoid an incorrect reading of tests and thus a possible incorrect identification.

The few discrepancies in identification observed for the API 20E strip were more difficult to evaluate, especially when there was not agreement on the same organism between our laboratory and the API reference laboratory in Montreal. The API reference laboratory did not indicate use of conventional tubed media for their confirmatory process but rather placed the same organism in their API 50E strip. The *E. coli* identified as *C. freundii* exemplified this point. We were unable to determine why one *Y. enterocolitica* strain was not identified on the API 20E strip although the result given by the reference laboratory was satisfactory.

We are in agreement with Washington *et al* (20) and Brooks and coworkers (1) that the three minutes required to prepare and inoculate each API 20E strip is a disadvantage, especially if it is compared to the Encise Enterotube which takes only 30 seconds. However, the API 20E system with its 21 reactions, or 2^{21} possible profiles, has a much better probability of identifying atypical strains than does the Encise Enterotube which has only 11 biochemical reactions and thus only 2^{11} possible profiles. The API 20E system has a much longer storage life and is less expensive than the Encise Enterotube. In discussing the total cost of identifying bacteria, we find the actual cost saving of using either of these two systems over conventional methods somewhat dubious. Robertson *et al* (15) analyzed the total cost (materials and labour) for the identification of members of the family *Enterobacteriaceae* using conventional tubed media and the API 20E strip. They reported that the cost of the API 20E strip was $3.02, while a seven tube (10 test) set-up of conventional media cost $3.60. Where the expertise of a well-trained diagnostic bacteriologist is present, however, it is doubtful whether 21 or even ten biochemical tests are necessary to accurately identify many organisms. The availability of a well trained microbiologist is also recommended by the manufacturer. Since only a few tests are required to identify most of the *Enterobacteriaceae* the actual reduction of costs in using any rapid identification system in a laboratory where the proper facilities exist for preparing, storing and using tubed media under an experienced microbiologist can be argued. In such an environment, however, either the API 20E system or the Encise Enterotube could be used when aberrant patterns of reactions are produced by an organism. Both these systems with their updated data bases could be used with confidence in sorting out the ambiguous patterns of reactions with a high probability of achieving an accurate identificaton. These systems could also be advantageous when used where the proper facilities do not exist for identifying organisms.

In conclusion, both the API 20E system and the Encise Enterotube were found to be accurate in identifying members of the *Enterobacteriaceae* from animal origin with greater than 95% confidence. With their computer-updated models both systems are continually being revised and improved and could be used in lieu of conventional methods to identify these organisms.

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