Comparison of the Microbact-12E and 24E systems and the API-20E system for the identification of *Enterobacteriaceae*

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SUMMARY

The Microbact-24E and the Microbact-12E systems are two new miniaturized identification systems for the identification of organisms belonging to the family *Enterobacteriaceae*. These two systems were compared to the API-20E system for the identification of 352 fresh clinical isolates of *Enterobacteriaceae*. All three systems were easy to use and came complete with computerized profile registers to assist with final identification of the isolates. The Microbact-24E identified 98%, the API-20E and MB-12E identifying 94.3% and 88.6% respectively. Where different identifications were obtained with the Microbact-24E and API-20E conventional biochemical tests, motility and serology were performed. The Microbact-24E system proved to be a very accurate and convenient means of identifying members of the family *Enterobacteriaceae*.

INTRODUCTION

The gram negative bacilli belonging to the family *Enterobacteriaceae* are among the most commonly isolated organisms in medical microbiology laboratories. Because of this, numerous conventional biochemical test media have been developed to enable both taxonomic studies and routine laboratory identifications to be carried out (Edwards & Ewing, 1972; Cowan & Steel, 1977). Conventional tube media used for organism identification are expensive to produce, require extensive storage space in refrigerators and incubators, and require staff with sufficient expertise to manufacture the often complex formulations required. Over recent years a number of miniaturized organism identification systems have become commercially available. Two new systems for the identification of *Enterobacteriaceae*, the Microbact-12E and Microbact-24E have been added to the range. These systems consist of dehydrated substrates contained in a microtitre tray to which saline suspensions of organisms to be tested are added. The systems are designed so that if part of the tray is used, the remaining rows of substrates may be used on following days. Both of these systems are supported by computer based profile registers to assist with organism identification. The Microbact-12E (MB-12E) is a 12 substrate system which is capable of identifying the majority of commonly encountered routine laboratory isolates to at least the genus level.

The second system, Microbact-24E (MB-24E) is a 24 substrate test panel which frequently permits identification to the species level, and is especially valuable for

Table 1. Organisms used in the evaluation of MB-1	12 <i>E</i> .		and.	API-20E
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Organism	Total no.
Escherichia coli	63
Shigella flexneri	10
Sh. sonnei	13
Edwardsiella tarda	1
Citrobacter freundii	4
C. diversus	7
Arizona hinshawii	13
Salmonella cholerae-suis	9
S. typhi	7
Salmonella sp.	29
Klebsiella pneumoniae	49
Enterobacter aerogenes	4
E. cloacae	17
E. agglomerans	1
Serratia marcescens	16
Yersinia enterocolitica	4
Y. pseudotuberculosis	1
Proteus vulgaris	7
P. mirabilis	65
P. rettgeri	8
P. morganii	13
Providencia alcalifaciens	7
P. stuartii	4
Total	352

identifying organisms with unusual biochemical reactions. These two systems provide the user with an accurate means of organism identification at a cost much less than that of the equivalent conventional media, and other comparable commercial test kit systems.

In this trial we have examined the ability of the two Microbact systems and the API-20E to identify clinical isolates from our routine laboratories. The API-20E was chosen to compare to the Microbact systems because of its wide acceptance in microbiology laboratories.

MATERIALS AND METHODS

Bacterial strains

A total of 352 clinical isolates belonging to the family *Enterobacteriaceae* (Table 1) were used to test the accuracy of the three systems. These organisms were originally isolated from routine urine, faeces and blood specimens submitted for routine bacteriological examination. The five yersinia examined were stock cultures. Each isolate was assigned an identity number for the trial, and the original laboratory identification was unknown during the trial. All isolates were stored on nutrient agar slopes at room temperature until examined.

Systems examined

Three micro-identification systems were examined, the Microbact-24E and Microbact-12E manufactured by Disposable Products Pty Ltd, South Australia

Test	Abbreviations
Lysine decarboxylase	Lys
Ornithine decarboxylase	Örn
Hydrogen sulphide production	H _z S
β-Galactosidase	ONPG
Indole production	Indole, Ind
Urea hydrolysis	Urease, Ure
Voges Proskauer reaction	VP
Citrate utilization	Citrate, Cit
Tryptophan deaminase	TDA
Gelatin liquefaction	Gelatin, Gel
Acid from:	
Glucose	Glue
Mannitol	Mann
Xylose	Xyl
Inositol	Ino
Sorbitol	Sor
Rhamnose	Rha
Sucrose	Suc
Lactose	Lac
Arabinose	Ara
Adonitol	Ado
Raffinose	Raff
Salicin	Sal
Dulcitol	Dul
Sorbitol	Sorb
Maltose	Malt
Malonate utilization	Malonate, Mal
Arginine dihydrolase	Arg
Esculin hydrolysis	Esculin
Deoxyribonuclease	DN'ase
Growth in Moeller's KCN medium	KCN

Key to tables of biochemical tests

and marketed in the United Kingdom by L.I.P. Services, Shipley, West Yorkshire and the API-20E manufactured by API Systems S.A., France and marketed in the United Kingdom by A.P.I. Laboratory Products Ltd, Basingstoke, Hampshire. The biochemical tests provided in each of the systems are listed in Table 2.

Inoculation and incubation

Before examination, each isolate was subcultured from the nutrient agar slope on to C.L.E.D. medium and incubated at 35 °C for 24 h. After the purity of the cultures was established an inoculum was prepared for each system as recommended by the manufacturer. Each system was prepared, inoculated, overlaid with oil where necessary and incubated according to the manufacturer's instructions. All three systems were incubated at 35 °C for 24 h before reading, and then incubated a further 24 h before discarding. Only those results recorded at 24 h were used to determine the final identification of the isolates since all three systems claim to provide an identification within 24 h.

API-20E	MB-12E	MB-24E
ONPG	Lysine	Lysine
Arginine	decarboxylase	decarboxylase
dihydrolase	Ornithine	Ornithine
Lysine	decarboxylase	decarboxylase
decarboxylase	H _s S	H ₂ S
Ornithine	Glucose	Glucose
decarboxylase	Mannitol	Mannitol
Citrate	Xylose	Xylose
H ₂ S	ONPG	ONPG
Urease	Indole	Indole
TDA	Urease	Urease
Indole	VP	VP
VP	Citrate	Citrate
Gelatin	TDA	TDA
Glucose		Gelatin
Mannitol		Malonate
Inositol		Inositol
Sorbitol		Sorbitol
Rhamnose		Rhamnose
Sucrose		Sucrose
Melibiose		Lactose
Amygdalin		Arabinose
Arabinose		Adonitol
		Raffinose
		Salicin
		Arginine
		dihydrolase

Table 2. Biochemical tests provided in API-20, MB-12E and MB-24Eidentification systems for the Enterobacteriaceae

INTERPRETATION OF RESULTS

Each of the three systems examined have a computer based Profile Register to assist with interpretation of the results. Both the MB-24E and API-20E systems use an octal coding system (Robertson & McLowry, 1975; McCulloch, 1977), which enables a set of biochemical reactions to be converted into a distinct numerical code number or profile number. The MB-12E uses a numerical code in which each of the 12 reactions is designated a distinct numerical value. The summation of the values for positive reactions gives a code number which relates to a series of reaction patterns rather than to a single discrete reaction pattern, i.e. more than one reaction pattern can produce the same code number. The test reaction pattern is then matched to the same pattern listed for the code number produced. The rationale behind such a system is that it does not replace a reaction pattern with a number, but guides the user to the general area of identification, leaving the final interpretation and identification to the operator.

The interpretation of the results obtained with each of the three systems was made using the appropriate Profile Register. In each instance only the choice with the highest probability, i.e. first choice, was the identification considered. An exception to this rule was made with the identification of *Shigella flexneri*. In this case, an identification of either *Sh. boydii* or *Sh. flexneri* was accepted. The MB-24E

Identification system	Profile Register	Computer
Microbact-12E		
(Correct genus)	326(92·6 %)	338(96%)
(Correct species)	312(88.2%)	326(92.6%)
API-20E		
(Correct genus)	333(94·6 %)	345(98%)
(Correct species)	332(94·3 %)	343(97.4 %)
MB-24E		
(Correct species)	345(98 %)	352(100%)*

Table 3. Comparison of identification rates for the MB-12E, MB-24E andAPI-20E systems

* Where disagreement occurred between MB-12E, API-20E and MB-24E, the MB-24E reactions were confirmed by conventional tests giving an assumed 100% species identification.

Profile Register has been designed so that organisms appearing in the register have a probability of greater than $1:10^6$, based on the tables of positive reactions provided by the manufacturer and the MB-12E with probabilities of greater than $1:10^3$ (Dybowski, Franklin & Payne, 1963; Dybowski & Franklin, 1968; Lapage *et al.* 1970). Both Microbact systems have an on-line computer back up at the Institute of Medical and Veterinary Science which was consulted when reaction patterns were found which had probabilities less than the respective cut off points in the Profile Registers.

Five technicians of varying experience participated in the trial and all biochemical sets were inoculated, read and interpreted by any member of the group without any knowledge of the probable identification of the organism. No further information was used other than that provided by the manufacturers.

RESULTS

Table 3 shows the identification rates for the three systems examined. The MB-12E system was able to identify 312 (88.6%) of the isolates to the species level, and 326 (92.6%) of the isolates to the genus level. These figures are consistent with previous work performed by one of us (Mugg, 1979). When computer assistance was enlisted, 326 (92.6%) of the isolates were identified to the species level and 338 (96%) to the genus level.

The API-20E results were also interpreted on the basis of identification to both the genus and species level for the results obtained using the Profile Register and the computer service when it was consulted. The API-20E system was able to identify 333 (94.6%) of the isolates to the genus and 332 (94.3%) of the isolates to the species level using the Profile Register. When the Computer Service was consulted for the 12 isolates not on the Profile Register, the API-20E system was able to identify 345 (98%) of the cultures to the genus level and 343 (97.4%) to the species level. These rates of identification are consistent with the work performed by one of us (Mugg, 1979) and similar to those rates reported by other workers (Brooks, Jens & Sodeman, 1974; Aldridge *et al.* 1978; Goldschmidt & Fung,

Table 4. Misidentification by the MB-12E system and tests performed to confirm the identification

Organism	No.	Incorrect identification/reactions	Additional tests to confirm identification
Citrobacter freundii	1	C. diversus, Ind, H ₂ S	Ind, H _s S
Klebsiella pneumoniae	5	S. rubidaea, Ur, Xyl	DN'ase, Motility
	2	E. agglomerans, Lys, Ure	Motility, Ino, Ado
	1	E. aerogenes, Orn, Ure	Motility, Orn, Ure
Enterobacter aerogenes	1	E. hafniae, Cit	Ado, Sorb, Esculin
Serratia marcescens	14	E. aerogenes, Xyl	DN'ase, Raff
Proteus vulgaris	1	P. mirabilis, Orn, Cit	Suc, Malt, Orn, Cit
Providencia alcalifaciens	1	P. stuartii, Xyl	Gas from Glucose, Ado, Ino
P. stuartii	2	P. alcalifaciens, Mann, Xyl	Gas from Glucose, Ado, Ino

Table 5. Misidentification by the API-20E system and tests performed to confirmthe identification

Organism	No.	Incorrect identification/reactions	Additional tests to confirm identification
Escherichia coli	1	Sh. boydii, Ara, Rha	Motility, Serology
Shigella sonnei	1	Citrobacter freundii, Mel	Motility, Serology, KCN
•	1	Salmonella sp., ONPG	Motility, Serology
Arizona hinshawii	2	E. coli (H_2S+) , Ind, Cit	Serology, Cit, Mal
Salmonella sp.	2	A. hinshawii, Cit. H ₂ S	Mal, ONPG, Dul, Serology
Providencia alcalifaciens	1	P. stuartii, Ino	Gas from Glucose, Ino, Ado

1978) and reinforces the usefulness of the API-20E as a routine laboratory test system.

The MB-24E with its 24 substrates proved to be an accurate system for the identification of the family *Enterobacteriaceae*. Using the Profile Register to determine final identifications, 345 (98%) of the isolates were identified to the species level. The remaining 7 (2%) of the isolates were identified to the species level using the Computer Service.

When the substrate reactions agreed in all three systems under examination, there was complete agreement with conventional substrates. In all cases where the identification differed between the systems reference was made to the conventional substrate reactions to determine the correct identification (Tables 4 and 5). In all instances the conventional tests confirmed the MB-24E identification.

DISCUSSION

The MB-12E system failed to identify 36 of the isolates examined (Table 6); 31 of these isolates were either K. pneumoniae, E. aerogenes, E. cloacae or S. marcescens. The difficulty in achieving adequate differentiation of these species, particularly E. aerogenes and S. marcescens with the MB-12E has previously been reported (Mugg, 1979) and is still an inherent problem with systems containing a limited range of substrates (Brooks, Jens & Sodeman, 1974; Aldridge et al. 1978).

Organism	Agreement	Incorrect identification
Escherichia coli	63/63 (100%)	
Shigella flexneri	10/10 (100%)	
Sh. sonnei		
Edwardsiella tarda	1/1 (100%)	
Citrobacter freundii	3/4 (75 %)	1 ('. diversus
C. diversus	7/7 (100%)	
Arizona hinshawii	13/13 (100%)	
Salmonella cholerae-suis	9/9 (100%)	
S. typhi	7/7 (100%)	
Salmonella sp.	29/29 (100%)	
Klebsiella pneumoniae	40/49 (82%)	5 S. rubidaea
1		2 E. agglomerans
		1 E. aerogenes
		1 Not on Profile Register
Enterobacter aerogenes	3/4 (75%)	1 E. hafniae
E. cloacae	8/17 (47%)	8 E. aerogenes
		1 Not on Profile Register
E. agglomerans	1/1 (100%)	
Serratia marcescens	2/16 (12%)	14 E. aerogenes
Yersinia enterocolitica	4/4 (100%)	
Y. pseudotuberculosis	1/1 (100%)	
Proteus vulgaris	6/7 (86 %)	1 P. mirabilis
P. mirabilis	63/65 (97%)	2 Not on Profile Register
P. rettgeri	8/8 (100%)	· · · · · · · · · · · · · · · · · · ·
P. morganii	13/13 (100%)	
Providencia alcalifaciens	6/7 (86 %)	1 P. stuartii
P. stuartii	2/4 (50%)	2 P. alcalifaciens
Total	312	36 Incorrect identification 4 Not on Profile Register

Table 6. Identifications and misidentifications by the MB-12E system

The mis-identification of 14 strains of S. marcescens as E. aerogenes was in all cases due to positive xylose fermentation. Although eight strains of K. pneumoniae were identified as either S. rubidaea, E. agglomerans or E. aerogenes the second choice of identification was K. pneumoniae. In each case the probability of the first choice was low, and the probabilities of the first and second choices were close. A motility test as recommended in the Profile Register may have lead to a correct identification on the same day. All of the salmonella and shigella examined were identified to the genus level.

In a previous trial (Mugg, 1979) difficulty was encountered in identifying shigella due to negative glucose and mannitol tests. This was found to be due to the inability of the carbohydrate base to support the growth of these organisms. This has since been rectified and strong carbohydrate fermentation reactions now occur. If an isolate is negative in all substrates and if growth has occurred on the purity plate, an oxidase test and motility should be performed. If the organism is non-motile and oxidase negative serological agglutination tests should be performed to exclude shigella. These recommendations are intended to eliminate the possibility of missing a shigella.

The API-20E (Table 7) incorrectly identified eight isolates and 12 isolates gave profile numbers which were not listed on the Profile Register. When the API-20E

Organism	Agreement	Incorrect identification
Escherichia coli	62/63 (98%)	1 Sh. boydii Mot (-), Serol (-)
Shigella flexneri	10/10 (100%)	
Sh. sonnei	11/13 (85%)	1 C. freundii
	•	1 Salmonella sp.
Edwardsiella tarda	1/1 (100%)	•
Citrobacter freundii	3/4 (75%)	1 Not on Profile Register
C. diversus	5/7 (71%)	2 Not on Profile Register
Arizona hinshawii	10/13 (77%)	$2 E. coli (H_{*}S+)$
	, , , , , , , , , , , , , , , , , , , ,	1 Not on Profile Register
Salmonella cholera-suis	9/9 (100%)	0
S. typhi	7/7 (100%)	
Salmonella sp.	25/29 (86%)	2 A. hinshawii
•	, , , , , , , , , , , , , , , , , , , ,	2 Not on Profile Register
Klebsiella pneumoniae	48/49 (98%)	1 Not on Profile Register
Enterobacter aerogenes	4/4 (100%)	8
E. cloacae	17/17 (100%)	
E. agglomerans	1/1 (100%)	
Serratia marcescens	15/16 (94%)	1 Not on Profile Register
Yersinia enterocolitica	3/4 (75%)	1 Not on Profile Register
Y. pseudotuberculosis	1/1 (100%)	8
Proteus vulgaris	7/7 (100%)	
P. mirabilis	63/65 (97%)	2 Not on Profile Register
P. rettgeri	8/8 (100%)	0
P. morganii	12/13 (92%)	1 Not on Profile Register
Providencia alcalifaciens	6/7 (86%)	1 P. stuartii
P. stuartii	4/4 (100%)	
Total	332	8 Incorrect identification 12 Not on Profile Register

Table 7. Identifications and misidentifications by the API-20E system

was repeated on these isolates, identical results were obtained. Of the 12 isolates not listed in the Profile Register the API-20E computer service was able to identify 11 isolates to the species level (including two Salmonella sp. and one Arizona hinshawii) and the remaining isolate to the genus level. Of the eight incorrect identifications two Salmonella sp. were biochemically identified as A. hinshawii due to negative H.S and citrate reactions (correct species identification would have been made using serology) (Table 4). Two A. hinshawii were identified as Escherichia coli (H,S+) because of incorrect indole and citrate reactions. All of these reactions were confirmed as being incorrect by referring to conventional substrates and if the profile numbers were corrected using the reactions from these conventional media results, correct identifications would have been made. Difficulty was encountered with weak glucose fermentation in the API-20E system, especially with K. pneumoniae, and the lysine decarboxylases often exhibited weak positive colour changes. A high percentage of citrate reactions were negative at 24 h but positive at 48 h, particularly with the K. pneumoniae and Proteus sp. strains examined. Colour development with positive VP reactions was often delayed, taking up to 15 min for colour development to occur, even with fresh reagents. These problems with the API-20E system have previously been reported by one of us (Mugg, 1979) and by other workers (Lapage et al. 1970).

•	•	0
Organism	Agreement	Incorrect identification
Escherichia coli	62/63 (98%)	1 Not on Profile Register
Shigella flexneri	10/10 (100%)	Ŭ
Sh. sonnei	13/13 (100%)	
Edwardsiella tarda	1/1 (100%)	
Citrobacter freundii	4/4 (100%)	
C. diversus	6/7 (86%)	1 Not on Profile Register
Arizona hinshawii	11/13 (85%)	2 Not on Profile Register
Salmonella cholerae-suis	9/9 (100%)	U
S. typhi	7/7 (100%)	
Salmonella sp.	29/29(100%)	
Klebsiella pneumoniae	47/49 (96%)	2 Not on Profile Register
Enterobacter aerogenes	4/4 (100%)	8
E. cloacae	17/17 (100%)	
E. agglomerans	1/1 (100%)	
Serratia marcescens	15/16 (94%)	1 Not on Profile Register
Yersinia enterocolitica	4/4 (100%)	8
Y. pseudotuberculosis	1/1 (100%)	
Proteus vulgaris	7/7 (100%)	
P. mirabilis	65/65 (100%)	
P. rettgeri	8/8 (100%)	
P. morganii	13/13 (100%)	
Providencia alcalifaciens	7/7 (100%)	
P. stuartii	4/4 (100%)	
Total	345	7 Not on Profile Register

Table 8. Identifications and misidentifications by the MB-24E system

The MB-24E was able to identify all isolates which produced profile numbers listed in the Profile Register (Table 8) and when those isolates not on the Profile Register were entered on to the computer, identifications to the species level were obtained. Although the computer was in this instance able to provide identifications on those isolates which did not appear on the Profile Register, it must be remembered that these isolates have reaction patterns with very low probabilities, i.e. less than 1:10⁶. The degree of accuracy of identification at these levels, even with 24 tests and a result generated by the computer, is not known. Whenever the MB-24E identification differed from either the MB-12E or API-20E identification, reference was made to the conventional tests and the MB-24E was shown to have given the correct result. When these conventional tests were compared to the MB-24E test panel, 100% correlation between conventional and MB-24E results occurred. This is consistent with the original concept of Microbact tests being miniaturized conventional tests (Mugg, 1979). This close correlation with conventional substrates may allow reactions to be interpreted using accepted texts for organisms other than Enterobacteriaceae. All the technicians involved in the trial agreed that the reactions in both Microbact systems were easy to interpret and that the colour intensity of both positive and negative Microbact reactions was greater than those in the API system. The VP reactions showed strong colour development in less than 5 min with the majority of positive isolates, although the occasional isolate took slightly longer. Gentle agitation of the test panels after the addition of reagents assisted with mixing and colour development. Unlike the API-20E system the majority of citrate reactions were positive at 24 h, the

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remainder being positive at 48 h. If the instructions provided by the manufacturer were followed correctly, the lysine decarboxylase reactions presented few interpretation problems. In practice there are isolates which when tested in conventional lysine decarboxylase media produce reactions which cannot be interpreted as being positive or negative with any confidence. Equivocal results are also obtained in the Microbact systems with these isolates. When these isolates were encountered the lysine decarboxylase was interpreted as both positive and negative and the Profile Numbers for both reaction patterns consulted. If the identification obtained for the Profile Numbers differ, further biochemical tests should be performed to confirm the identification. This problem and a possible solution has been reported by other workers (Blazevic, Mackay & Warwood, 1979).

Although all three systems examined were convenient to use, the two Microbact systems were more easily handled by laboratory staff. The API-20E test panels required more effort to prepare. The task of separating and assembling the three components making up each test panel, filling the humidity chamber and . inoculating the cupules without producing air bubbles was time consuming. If the lid of the humidity chamber was not closed tightly the inoculum evaporated and the complete test had to be repeated adding a further 24 h and additional cost on to the handling of a specimen. The light weight, length and narrow base of the API-20E panels also proved to be a problem. When the test strips are laid out in an incubator which is frequently used, the chances of knocking and tipping the panels is high. These problems were not apparent with the Microbact test panels. Their larger base and greater weight made them a great deal more stable. If, however, a test panel was knocked or tipped, no spillage occurred because the Microbact test panels are completely sealed with a transparent tape. This tape also protects the panel from dehydration. If a number of isolates were being identified at any one particular time the Microbact systems enable considerable savings in incubator space, each MB-12E test panel accommodating up to eight isolates. Any unused test rows can be used on subsequent days, though a limit of no more than three incubations is recommended. The MB-24E test panel allows up to four isolates to be identified at one time, any unused test rows can be used later.

The MB-12E system is an inexpensive system which could find a place in most routine laboratories as a screening system for enteric pathogens and as an identification system for the gram negative organisms causing urinary tract and wound infections. The MB-24E and API-20E systems by virtue of their larger numbers of tests, offer greater accuracy of identification, than the 12 substrate MB-12E system. However, the MB-24E system by being simpler to use and less expensive than the API-20E, offers some advantages to laboratories concerned about the cost of pathology tests.

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