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Evaluation of the Four-Hour Rapid 20E System for Identification of Members of the Family *Enterobacteriaceae*

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A study was conducted to compare the API Rapid 20E 4-h system (API System S.A., France; commercially available in the U.S.A. under the name DMS Rapid E System; DMS Laboratories, Darts Mill, Flemington, N.J.), the API 20E 18- to 24-h system (Analytab Products, Plainview, N.Y.), and a conventional media system to measure the ability of each to identify members of the family *Enterobacteriaceae*. Comparison tables rather than simple percentage agreement tables were generated to define the particular strengths and weaknesses of each system and to allow the laboratory to best use the data. The Rapid 20E compared quite favorably with conventional media. It yielded correct identifications with 95.9% of the isolates tested (API 20E, 98% identification rate). In 2.5% of the isolates, the Rapid 20E gave only genus identifications, and in 1.4% the organisms did not correspond to any key in the code book and could not be identified by the manufacturer's computer service. The ease of inoculation and the 4-h capability make the Rapid 20E system an extremely attractive development in the field of bacterial identification.

Identification of *Enterobacteriaceae* isolates is a major function of clinical microbiology laboratories. Rapid identification of this bacterial group may be of clinical importance.

A new 20-test system, Rapid 20E, was developed by API System S.A., France for a 4-h identification of 36 *Enterobacteriaceae* species. In the United States, this system is commercially available under the name DMS Rapid E (DMS Laboratories, Darts Mill, Flemington, N.J.).

A data base was created from results obtained with stock cultures and clinical isolates. It enables the identification of 36 different species, with the help of a code book.

The purpose of the present study was to compare the Rapid 20E and API 20E systems with conventional biochemical methods for identifying clinical *Enterobacteriaceae* isolates.

MATERIALS AND METHODS

A total of 567 Lille hospital clinical isolates were examined by the three systems. They belonged to 24 species listed in Tables 1 and 2.

Before testing, all isolates were grown in brain heart infusion broth and then subcultured on a bromocresol purple agar plate to assure purity and viability.

Conventional biochemical tests. Conventional tests are listed in Table 3. The methods used were generally those described by Edwards and Ewing (5). A description of the tests follows.

Indole production was measured at 24 h by adding 0.5 ml of Kovacs reagent (10 g of *para*-dimethylaminobenzaldehyde, 50 ml of 12 N hydrochloric acid, 150 ml of isoamyl alcohol) to 24-h-old peptone water (20 g of peptone [Difco Laboratories, Detroit, Mich.], 5 g of sodium chloride, and 1.000 ml of water) cultures. The Voges-Proskauer test was done at 24 h by adding 1 ml of O'Meara reagent (40 g of potassium hydroxide, 0.3 g of creatine, 100 ml of water) to 1 ml of the culture (grown in methyl red Voges-Proskauer medium [Difco]) in a tube. Growth on citrate as the sole source of carbon and energy was tested on Simmons citrate agar (Institut Pasteur Production [IPP], Marnes La Coquette, France). Growth on malonate as energy source was determined in malonate broth (IPP). Hydrogen sulfide production (H_2S) was determined in triple sugar iron agar (Difco) with an additional 5 g of agar added per liter of medium. Urea hydrolysis was determined on Christensen urea agar (IPP). Phenylalanine "deamination" was tested by adding 0.1 ml of ferric chloride solution to a 24-h-old culture on phenylalanine agar (IPP). The Moeller method (5) was used to determine lysine and ornithine decarboxylases and arginine dihydrolase (IPP). Motility was determined at 36 and 22°C in motility test medium (IPP). Gelatin hydrolysis was determined at 22°C on nutrient gelatin (IPP). Acid production from carbohydrates, polyhydroxyl alcohols, and related compounds was tested in enteric fermentation base (Difco) to which 10 ml of Andrade indicator (0.2 g of acid fuchsin, 100 ml of water, 16 ml of 1 N NaOH) and 990 ml of water were added. The glucose fermentation tube contained a small glass insert tube (Durham tube) to detect gas production. Extracellular DNase was tested at 36°C on DNase test agar (BBL Microbiology Systems, Cockeysville, Md.) to which 0.05 g of toluidine blue was added per 1.000 ml. *o*-Nitrophenyl-β-D-galactopyranose (ONPG) was tested by the filter paper disk method (IPP).

Final identification was determined according to tables from Brenner et al. (2), and Edwards and Ewing (5). All cultures suspected of being *Salmonella* or *Shigella* species were confirmed serologically.

Rapid 20E. The Rapid 20E method consists of a strip of 20 microtubes containing dehydrated substrates to demonstrate the presence of enzymes or the fermentation of carbohydrates.

The strip was prepared by following the instructions given by the manufacturer. One well-isolated colony (or two with identical morphology if necessary) was homogenized in 1.25 ml of sterile saline to reach an opacity equal to a 0.5 MacFarland standard. The Rapid 20E strip was inoculated with a disposable pipette; 50 μl of suspension was distributed into each tube.

The strip was incubated for 4 h at 37°C; the system cannot be used after 5 h even if preserved at 4°C. After incubation,

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TABLE 1. Comparison of Rapid 20E and conventional identifications of isolates

Species as determined by conventional methods	Total no. of strains	Species as determined by Rapid 20E (no.)																													
		E. coli	*C. freundii*	*C. diversus*	*C. amalonaticus*	*E. cloacae*	*E. aerogenes*	*E. agglomerans*	*E. sakazakii*	*H. alvei*	*K. pneumoniae*	*K. oxytoca*	*K. ozaenae*	*K. rhinoscleromatis*	*S. marcescens*	*S. liquefaciens*	*S. rubidaea*	*S. odorifera*	*P. mirabilis*	*P. vulgaris*	*M. morganii*	*P. stuartii*	*P. alcalifaciens*	*P. rettgeri*	*Salmonella* sp.	*Shigella* sp.	*Y. enterocolitica*	Additional tests[a]	Computer[b]	No Key[c]
Escherichia coli	30	30																													
Citrobacter freundii	30		27		1																								2	
C. diversus	30			30																											
C. amalonaticus	0																														
Enterobacter cloacae	30					30																									
E. aerogenes	30						30																								
E. agglomerans	16					2		8																							6
E. sakazakii	9								9																						
Hafnia alvei	30									26																				3	1
Klebsiella pneumoniae	30										30																				
K. oxytoca	30											30																			
K. ozaenae	10										1		6																	2	
K. rhinoscleromatis	7													6																	1
Serratia marcescens	30														30																
S. liquefaciens	19														6	8		2										3			
S. rubidaea	2																2														
S. odorifera	0																														
Proteus mirabilis	30																		30												
P. vulgaris	30																			30											
Morganella morganii	30																				30										
Providencia stuartii	25																					23	2								
P. alcalifaciens	9																						9								
P. rettgeri	22																							22							
Salmonella sp.	30																								30						
Shigella sp.	28																									27		1			
Yersinia enterocolitica	30									1[d]															1[d]	1[d]	25	27	2		

[a] Isolates which were correctly identified by using additional tests indicated in the code book.
[b] Isolates correctly identified with the aid of the manufacturer's computer service.
[c] The profile did not correspond to any key in the code book.
[d] There were three possible choices for the identification of this *Y. enterocolitica* isolate: *H. alvei*, *Salmonella* sp., and *Shigella* sp.

indole and acetoïn (Voges-Proskauer reaction) reagents were added.

The tests were read according to the instructions of the manufacturer, and the identification was determined with the aid of the code book (data base, 3,000 biochemical profiles). Unlisted profiles were interpreted by referring to the manufacturer's computer (data base, 15,000 biochemical profiles).

API 20E. The API 20E strips were inoculated according to instructions of the manufacturer and incubated overnight at 37°C. Reagents were added as required, and the color changes were read visually. The results were interpreted by referring to the API code book. The API 20E strips manufactured in France are identical to those available in the United States.

Evaluation. Like Edberg et al. (4), McCracken et al. (8), and Durosoir (3), we chose to present the data from this study in a somewhat different form (Tables 1 and 2) from that previously used in clinical microbiology. The strains identified from the conventional system are listed vertically along with a column showing the total number of each species identified by the media in tubes. Horizontally, the names derived from the kit system (Rapid 20E or API 20E) are listed. Comparison tables, therefore, allow a more direct visualization of areas of disagreement between multiple systems than do the traditional tables that list results vertically.

RESULTS

The results obtained by the two commercial systems and conventional tests for the 567 *Enterobacteriaceae* strains are shown in Tables 1 (Rapid 20E) and 2 (API 20E).

After 4 h of incubation, the Rapid 20E strip (Table 1) agreed on the genus and species name of an isolate in 544 of 567 cases (95.9%). Among these strains, six (1%) needed additional tests and nine were identified by the manufacturer's computer service. In 14 cases (2.5%), the Rapid 20E provided only genus identifications. Eight organisms (1.4%) did not correspond to any key in the code book and could not be identified by the computer. The identification of one *Yersinia enterocolitica* (1/567) isolate showed three possible choices: *Hafnia*, *Shigella*, or *Salmonella* species. The Rapid 20E system has some difficulties in identifying *Enterobacter agglomerans* and *Serratia liquefaciens*. Six (out of 16 tested) *E. agglomerans* isolates did not correspond to any key in the code book. For 19 *S. liquefaciens* isolates studied, 8 were identified only to genus level.

The API 20E strip agreed with the conventional method for 558 of the 567 isolates (98.4%) at genus and species level (complete agreement). For 27 of these (5%) complementary tests were necessary. Four were identified with the help of the computer. Five organisms (1%) could not be identified by the code book nor by the computer. Three misidentifications (complete disagreement) were observed: one *S. lique-*

TABLE 2. Comparison of API 20E and conventional identifications of isolates

Species as determined by conventional methods	Total no. of strains	Species as determined by API 20E (no.)																												
		E. coli	*C. freundii*	*C. diversus*	*C. amalonaticus*	*E. cloacae*	*E. aerogenes*	*E. agglomerans*	*E. sakazakii*	*H. alvei*	*K. pneumoniae*	*K. oxytoca*	*K. ozaenae*	*K. rhinoscleromatis*	*S. marcescens*	*S. liquefaciens*	*S. rubidaea*	*S. odorifera*	*P. mirabilis*	*P. vulgaris*	*M. morganii*	*P. stuartii*	*P. alcalifaciens*	*P. rettgeri*	*Salmonella* sp.	*Shigella* sp.	*Y. enterocolitica*	Additional tests[a]	Computer[b]	No Key[c]
Escherichia coli	30	30																												
Citrobacter freundii	30		30																											
C. diversus	30			30																										
C. amalonaticus	0																													
Enterobacter cloacae	30					30																								
E. aerogenes	30						30																							
E. agglomerans	16							16																						
E. sakazakii	9								7																				2	
Hafnia alvei	30									14																			16	
Klebsiella pneumoniae	30										30																			
K. oxytoca	30											30																		
K. ozaenae	10													6															4	
K. rhinoscleromatis	7													7																
Serratia marcescens	30														27														3	
S. liquefaciens	19						1									10													3	
S. rubidaea	2							1[d]					1[d]				1													
S. odorifera	0																													
Proteus mirabilis	30																		30											
P. vulgaris	30																			30										
Morganella morganii	30																				30									
Providencia stuartii	25																					24								1
P. alcalifaciens	9																						9							
P. rettgeri	22																							21					1	
Salmonella sp.	30																								30					
Shigella sp.	28																									27		1		
Yersinia enterocolitica	30																										30			

[a–c] See Table 1 footnotes *a* through *c*.

[d] There were two possible choices for the identification of this *S. rubidaea* isolate: *E. agglomerans* and *K. ozaenae*.

TABLE 3. Biochemical tests used in each identification system[a]

Test	Identification systems		
	Rapid 20E	API 20E	Conventional
Glucose acidification	+	+	+
Lactose acidification			+
Arabinose acidification	+	+	+
Rhamnose acidification	+	+	
Cellobiose acidification	+		
Melibiose acidification	+	+	+
Sucrose acidification	+	+	+
Trehalose acidification	+		
Raffinose acidification	+		
Xylose acidification	+		
Adonitol acidification	+		+
Glycerol acidification			+
Inositol acidification		+	+
Mannitol acidification		+	+
Sorbitol acidification		+	+
Amygdalin acidification		+	
Esculin acidification	+		
Salicin acidification			+
Lysine decarboxylation	+	+	+
Ornithine decarboxylation	+	+	+
Citrate utilization	+	+	+
Malonate utilization	+		+
Acetoïn production	+	+	+
Arginine dihydrolase		+	+
DNase			+
β-Galactosidase	+	+	+
Gas production from glucose			+
Gelatin hydrolysis		+	+
H_2S production		+	+
Indole production	+	+	+
Motility			+
Phenylalanine deaminase	+		+
Tryptophan deaminase		+	
Urea hydrolysis	+	+	+

[a] Explanations of the conventional tests are given in the text.

faciens strain was identified as *E. aerogenes*, one *Serratia rubidaea* presented a profile of *Klebsiella ozaenae* or *Enterobacter agglomerans*, and one *Shigella* organism was identified as *E. coli*. This latter misidentification was confirmed serologically.

In our study, we found that API 20E had problems with the identification of *H. alvei* and *S. liquefaciens*. Supplemental tests were necessary for identifying 53% of the *Hafnia*

strains. For *S. liquefaciens*, 5 strains out of 19 tested did not correspond to any key in the profile number book.

DISCUSSION

Our results demonstrate that the Rapid 20E strip produced a highly acceptable level of identification accuracy for *Enterobacteriaceae*. We chose to analyze the data in the form presented because percent agreement figures are very much a function of the mix of isolates used in the study and may not adequately reflect the strengths and weaknesses of a system.

Most of the profiles which did not correspond to any key in the Rapid 20E code book were clustered in two species: *E. agglomerans* and *S. liquefaciens*. This may be due to an insufficient data base.

In the present study we found the API 20E to have problems with the identification of *H. alvei*. This is probably due to the incubation temperature (37°C), which is not the best for this species (7).

From the data above it can be concluded that the Rapid 20E strip is capable of identifying the majority of *Enterobacteriaceae* species. The main advantage is an incubation time of 4 h. Miniaturization and sensitivity enable identification from a primary plate with one colony, versus three or four colonies with the Micro-ID system (1, 6) and MS-2 Abbott (7, 8). The main disadvantage is the inability of the user to obtain results after 5 h. This factor must be stressed, for clinical laboratories would have to adjust their work habits to attend to Rapid 20E so as not to lose results. Finally, we feel that a system like Rapid 20E, providing rapid identification results, is desirable from a bacteriological and clinical point of view.

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