JOURNAL OF CLINICAL MICROBIOLOGY, June 1978, p. 507-513
0095-1137/78/0007-0507$02.00/0

Vol. 7, No. 6
Printed in U.S.A.

Comparison of Micro-ID, API 20E, and Conventional Media Systems in Identification of *Enterobacteriaceae*

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Received for publication 9 September 1977

The Micro-ID, a new identification kit for *Enterobacteriaceae,* consists of 15 biochemical tests, with substrates and reagents impregnated in filter paper disks. A 0.2-ml amount of an organism suspension equal to a 0.5 McFarland standard is pipetted into each of the compartments. After 4 h of incubation and addition of potassium-hydroxide (KOH) to the Voges-Proskauer test, the color reactions are read according to the recommendations of the manufacturer. A five-digit octal code number is derived from each set of reactions from which an identification is derived by using a code book. In a single-blind, comparative study of the Micro-ID system with the API 20E system (Analytab Products Inc.) and conventional biochemical tube media, we found that the Micro-ID and the API 20E systems gave a 90% identification correlation when each was compared with the conventional tube media. A comparison of all three systems gave an 82% overall identification correlation. When the common tests of Micro-ID and API 20E were compared with conventional tube media, we found that the tests in the Micro-ID performed as well as or better than those of the API 20E. Certain groups of organisms, i.e., *Citrobacter, Enterobacter, Proteus, Salmonella,* and *Serratia* genera, were found to give low correlation on certain common tests. When using primary isolation MacConkey plates from the clinical laboratory, only 74% of the plates with *Enterobacteriaceae* had sufficient numbers of colonies of each enteric organism to produce the 0.5 McFarland inoculum density required. Problems concerning the misidentification of some organisms are discussed.

The majority of organisms identified in the clinical microbiology laboratory are members of the *Enterobacteriaceae.* Their role in the production of severe disease and in antibiotic resistance has become increasingly important; therefore, the rapidity and accuracy of identification of the enterics is a subject of much concern. In recent years, several "kit" methods (1, 2, 4, 7) have been made available which allow identification of enterics to the genus and species level within 24 h of isolation on primary media, and with at least two, the identification within a few hours (4, 5). In certain life-threatening infections a "same-day" identification is helpful, and the more rapid identification would be in line with the general availability of more rapid results in other clinical laboratory disciplines. A new biochemical identification kit, the Micro-ID (General Diagnostics, Morris Plains, N.J.), provides identification of enterics 4 h after primary isolation. The present communication reports a comparison of the Micro-ID system with two other systems widely used for enteric identification, the API 20E (Analytab Products Inc.) and conventional biochemical tube media.

MATERIALS AND METHODS

Organisms. A total of 373 organisms was tested by all three systems. Of these, 251 were consecutive clinical isolates, and 122 were stock cultures of uncommonly isolated pathogens which had been stored in sealed tubes of tryptic soy agar at room temperature. All organisms were assigned a number, and the identity of none of the organisms was known during testing.

Micro-ID system. All organisms tested, both clinical and stock strains, were taken from MacConkey agar plates incubated overnight. A suspension of each organism was made in isotonic saline to a turbidity of a 0.5 McFarland standard. Each Micro-ID strip (Fig. 1) was labeled with an appropriate identification number, and the upper well of each test chamber was inoculated with 0.2 ml of organism suspension. The top was closed, and the strip was set upright and gently tapped to force all of the inoculum to run into the substrate well. Each strip was placed in a plastic holder and incubated at 37°C for 4 h. The strip was then removed from the holder, the top was opened, and 2 drops (0.1 ml) of 20% KOH were added to the upper well of the Voges-Proskauer (VP) test. The strip was then set upright to allow the KOH to flow to the lower well. The strip was then rotated 90° and gently tapped to force the fluid of the first five wells onto the indicator disks, located in compartments adjacent to

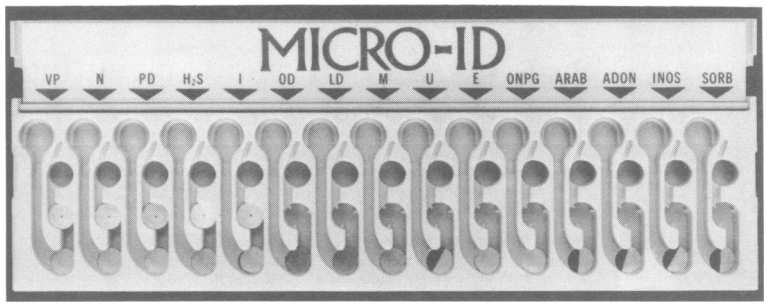

FIG. 1. *Micro-ID test strip. Note that the first five tests have two disks. The upper disk contains the indicator, and the lower disk contains the substrate. In the remaining tests, the substrate and indicator are combined in a single disk in the lower well.*

the substrate well and disks. The strip was again set upright, and the reactions were read according to their color as suggested by the manufacturer. All reactions were entered onto a recording chart, the five-digit octal number was calculated, and the identity of the organism was determined from the code book. (Each test contributes a binary [base 2] number; three binary digits [0's and 1's] form an octal [base 8] number equivalent to the integers 0 to 7; five octal integers form the unique organism identification code for the Micro-ID system, whereas seven octal integers are used in the API 20E system.) In certain cases, where there was an obvious discrepancy between biochemical tests and identity, organism identity was derived independently of the code book by using the results of the individual biochemical tests.

API 20E system. The API tests on all clinical isolates were performed in the Clinical Microbiology Laboratory at the University of Utah Medical Center from primary MacConkey plates. All stock cultures were tested in our research lab by an identical procedure with organisms stored on tryptic soy agar and streaked on MacConkey agar plates. A suspension of each organism was prepared by picking a single colony and suspending the organisms in distilled water. All 20 microwells were inoculated by filling the tube section and also the cupule sections of the citrate, VP, and gelatin tests. The cupule sections of the arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, and urease tests were filled with sterile mineral oil. A plastic lid was placed over the strip, and the strip was incubated at 37°C for 20 to 24 h. The cover was then removed, and 1 drop of 40% KOH and then 1 drop of 6% α-naphthol were added to the VP test; 1 drop of ferric chloride was added to the tryptophane deaminase test; and 1 drop of Kovac reagent was added to the indole test. All reactions were then read according to the recommendations of the manufacturer. The seven-digit octal number (see above) was calculated, and the organism identity was determined from the profile manual.

Conventional tube media. All organisms tested, both clinical and stock, were identically set up on a five-tube battery of conventional media. Triple sugar iron agar, lysine iron agar, motility-indole-ornithine agar, citrate agar, and phenylalanine-urea broth were routinely inoculated. The tubes were incubated at 37°C for 18 to 24 h. Reagents were added to indole, urease, and phenylalanine tests. Where necessary, additional tube media tests were performed to give complete identification. These included VP, adonitol, malonate, arabinose, acetate, deoxyribonuclease, and β-galactosidase. Deoxyribonuclease agar (Difco Laboratories) containing toluidine blue was inoculated and read after 24 h of incubation for deoxyribonucleic acid hydrolysis by the appearance of a pink halo around the bacterial growth. This is a modification of the method of Lachica et al. (5). β-Galactoside disks (Difco) were placed in 0.2 ml of organism suspension in saline and read after 4 and 24 h. The organisms were identified by examining the sets of biochemical reactions, using the charts of Edwards and Ewing (3).

Clinical evaluation. To assess the utility of the Micro-ID system in the clinical microbiology laboratory, duplicate MacConkey agar plates were inoculated with clinical specimens. One set of plates was then worked up for enterics with the Micro-ID system, whereas the other set of plates was processed with the API 20E identification system. The results of each system were then compared.

RESULTS

Overall identification correlation. The distribution of the clinical and stock isolates is shown in Table 1 by organism identification. In certain cases in which the Micro-ID gave a different identification than the other two systems, the identification manual misidentified the organisms even though the key biochemical reactions were consistent with the identification of the other two systems. Thus, in those cases a built-in "book-error" existed when the manual was used. Table 2 shows the overall correlation by identification of the isolates tested. Where Micro-ID is compared with the other systems, an adjusted value has been inserted to correct for misidentification of isolates. Considering the combined isolates, correcting "book-error" raised the correlation results 1 to 5%.

The performance of the Micro-ID system was

comparable to the API system when both were compared with conventional tube media. Both Micro-ID and API 20E, when compared with conventional tube media, gave a 90% identification correlation. The clinical isolates correlated much better than the stock cultures. This is not surprising in view of the "inactive" metabolic pathways of organisms which have been stored.

TABLE 1. *Organisms used in the study*

Organism	No. of clinical	No. of stock	Total
Escherichia coli	141	2	143
Klebsiella pneumoniae	39	0	39
Arizona species	0	5	5
C. freundii	9	11	20
C. diversus	3	6	9
Shigella species	0	13	13
P. mirabilis	23	0	23
P. rettgeri	6	7	13
P. morganii	3	8	11
P. vulgaris	2	5	7
Enterobacter agglomerans	1	5	6
E. aerogenes	1	4	5
E. cloacae	12	5	17
E. hafniae	3	0	3
Edwardsiella tarda	0	5	5
S. marcescens	2	9	11
S. liquefaciens	0	1	1
Providencia species	2	8	10
Salmonella enteritidis	3	18	21
S. cholerae-suis	0	2	2
S. typhi	0	2	2
Yersinia enterocolitica	0	5	5
Unable to name	1	1	2

Comparison of Micro-ID and API 20E showed an 83% correlation. A comparison of all three systems showed that for 82% of the time the isolates were named the same by all three systems. Again, the stock isolates performed less well than did the clinical isolates.

Common test correlation by system. A list of the biochemical tests in each of the three identification systems is shown in Table 3, indicating the common tests among the systems. Table 4 shows the percentage of each of the common tests that correlated in a comparison of the three systems. When a comparison of the Micro-ID and conventional tube media was made, all tests except lysine decarboxylase, urease, and adonitol showed a 95% or greater correlation among tests. A comparison of the common tests between API 20E and conventional tube media showed a 95% or greater correlation was achieved with only one-half (5/10) of the common tests. When Micro-ID and API 20E are compared, 5 of the 11 common tests, i.e., VP, urease, arabinose, inositol, and sorbitol, gave correlations below 95%.

Common test correlation by organism. Further analysis of the common tests in each comparison showed that certain groups of organisms were consistently responsible for lowering the percentage of correlation for some tests. It appears that members of the *Citrobacter, Enterobacter, Proteus, Salmonella,* and *Serratia* genera were most often responsible for the low correlation of the common tests. Most of the above organisms were stock isolates because many are infrequent isolates in the clinical microbiology laboratory. As previously mentioned, the stock cultures performed less well than fresh clinical isolates. However, certain fresh clinical isolates correlated very poorly in certain tests.

TABLE 2. *Correlation percentages of organism identification among the various systems*

Determination	% Combined isolates (no.)	% Clinical isolates (no.)	% Stock isolates (no.)
Micro-ID vs tube	85.3 (318/373)[a]	89.6 (225/251)	76.2 (93/122)
	90.1 (336/373)	93.2 (234/251)	83.6 (102/122)
API 20E vs tube	90.1 (336/373)	95.2 (239/251)	79.5 (97/122)
API 20E vs Micro-ID	81.5 (304/373)	86.9 (218/251)	70.5 (86/122)
	82.6 (308/373)	89.6 (225/251)	68.0 (83/122)
API 20E vs Micro-ID vs tube	79.1 (295/373)	86.5 (217/251)	64.8 (79/122)
	81.8 (305/373)	89.2 (224/251)	67.2 (82/122)

[a] During this study, it was found that the Micro-ID identification manual misinterpreted certain sets of biochemical reactions, resulting in erroneous identifications (see text). Therefore, in this table, whenever Micro-ID is used in a comparison, two sets of data are given. The upper set indicates the percent correlation when the Micro-ID manual was used, whereas the lower set indicates the percent correlation if the misidentifications of the Micro-ID manual were eliminated. We feel that this is valid because the manufacturer of Micro-ID indicates that organism identification may be derived independently of the identification manual by using percentage charts.

For example, only 12.5% (1/8) of the clinical isolates of *Citrobacter freundii* correlated on the urease test when Micro-ID and tube media were compared. Additionally, only 13% (3/23) of the clinical isolates of *Proteus mirabilis* correlated on the citrate test when API 20E and tube media were compared. Thus, it appears that certain groups of organisms, whether fresh clinical isolates or stock cultures, pose problems in reproducibility from one identification system to another.

TABLE 3. *Biochemical tests in each identification system*

Determination	Micro-ID	API 20E	Conventional tube
H_2S production	+	+	+
Lysine decarboxylase	+	+	+
Indole production	+	+	+
Ornithine decarboxylase	+	+	+
Citrate	−	+	+
Phenylalanine deaminase	+	+[a]	+
Urease	+	+	+
VP	+	+	+
β-Galactosidase	+	+	+[b]
L-Arabinose	+	+	+[b]
i-Inositol	+	+	+[b]
D-Sorbitol	+	+	−
Nitrate reduction	+	−	−
Malonate	+	−	+[b]
Esculin hydrolysis	+	−	−
Adonitol	+	−	+[b]
Arginine dihydrolase	−	+	−
Gelatin	−	+	−
D-Glucose	−	+	+
D-Mannitol	−	+	−
L-Rhamnose	−	+	−
Sucrose	−	+	−
L-Melibiose	−	+	−
L-Amygdalin	−	+	−
Motility	−	−	+
Deoxyribonuclease	−	−	+[b]

[a] In the API 20E system, tryptophane deaminase was detected instead of phenylalanine deaminase.

[b] These tube media were only used where they were necessary for identification.

Identification book. The Micro-ID identification manual is a listing of the majority of the five-digit octal nubmers which key out to an organism identification. These computer indices are based on a probability of a certain set of reactions being more likely to occur for one organism than for another. Each octal code lists a first-, second-, third-, and fourth-choice identification, indications of atypical reactions, and special test instructions, as well as a likelihood and degree of identification. Isolates are placed into the various categories or degrees of identification based on the most probable identification, quality of fit to the first choice, and degree of separation from other possible identifications.

The Micro-ID data was basically generated from the percentage charts of Edwards and Ewing (3). One problem noted with the data base was that in computing the probabilities each test was weighted the same. This was the reason for most of the previously described book-errors. For example, a *Proteus* species which was H_2S negative, indole positive, ornithine negative, and inositol negative was called *P. morganii,* despite the negative ornithine. The identification was based on the negative inositol because only 2% of *P. rettgeri* are expected to be inositol negative, whereas 4% of the *P. morganii* can be ornithine negative. The sugar fermentation reactions can be very weak and sometimes very difficult to read, yet an incorrect reading can change an identification. Another source of book-error was the apparent omission of an appropriate octal code for *Salmonella paratyphi-A.* Each time

TABLE 4. *Comparison of the three systems by common test*

Determination	% Correlation (no. of isolates) with:		
	Micro-ID vs API 20E	Micro-ID vs tube	API 20E vs tube
VP	85 (318/373)	97 (56/58)	83 (48/58)
Phenylalanine deaminase	99 (368/373)	98 (367/373)	99 (370/373)
H_2S	97 (361/373)	99 (370/373)	96 (358/373)
Indole	97 (361/373)	98 (265/373)	97 (363/373)
Ornithine decarboxylase	97 (326/373)	96 (357/373)	95 (356/373)
Lysine decarboxylase	95 (352/373)	89 (333/373)	87 (324/373)
Urease	85 (316/373)	86 (321/373)	98 (364/373)
Malonate		100 (53/53)	
β-Galactosidase	97 (362/373)	95 (37/39)	92 (36/39)
Arabinose	91 (339/373)	100 (13/13)	39 (5/13)
Adonitol		92 (24/26)	
Inositol	89 (332/373)		
Sorbitol	89 (333/373)		
Citrate			86 (320/373)

this organism was encountered, it was called *Shigella,* although the set of reactions fit *S. paratyphi-A* very well.

Misidentification. All 373 organisms tested in the study were assigned a number and blindly identified by all three systems. No system was assumed correct. Table 5 lists which system and which organism was at fault when one of the three systems gave a different identification. Those numbers which are marked with an as-

TABLE 5. *Misidentification by each system*

System	Organism	Was called	No. of times	
			Clinical	Stock
Micro-ID	*C. diversus*	*C. freundii*	2	
	C. freundii	*E. coli*	1	
	C. freundii	*Y. enterocolitica*	1	1
	E. aerogenes	*E. cloacae*	1	
	E. agglomerans	DNKO[a]		1
	E. cloacae	*E. agglomerans*		1
	E. cloacae	DNKO	1	
	E. coli	DNKO	6	
	E. coli	*Shigella*	1	
	K. pneumoniae	DNKO	1	
	K. pneumoniae	*S. rubidaea*	1	
	P. rettgeri	*P. morganii*	5[b]	3[b]
	P. stuartii	*P. rettgeri*		2
	S. enteritidis	*S. cholerae-suis*		1
	S. enteritidis	*Shigella*	3[b]	1[b]
	S. marcescens	DNKO	1	2
	S. marcescens	*S. liquefaciens*[c]		6[b]
	Shigella	*C. freundii*		3
	Shigella	DNKO		4
	Shigella	*P. morganii*		1
	Y. enterocolitica	*C. freundii*		1
	Y. enterocolitica	DNKO		2
	Y. enterocolitica	*E. agglomerans*		1
API 20E	*Arizona*	*S. enteritidis*		1
	C. freundii	*C. diversus*		1
	C. freundii	DNKO		2
	C. freundii	*E. cloacae*		1
	C. freundii	*E. coli*	2	
	E. agglomerans	*C. freundii*		1
	E. aerogenes	*E. cloacae*	1	
	E. cloacae	*C. freundii*		1
	E. coli	*C. freundii*	1	
	E. hafniae	*S. enteritidis*	1	
	P. morganii	*P. mirabilis*	1	
	P. morganii	*P. vulgaris*	1	
	P. rettgeri	DNKO	1	1
	P. rettgeri	*P. vulgaris*	2	1
	P. stuartii	DNKO		1
	P. stuartii	*P. vulgaris*	1	
	P. vulgaris	DNKO		1
	S. enteritidis	DNKO		3
	S. marcescens	*S. liquefaciens*		7
	Shigella	*C. freundii*		1
	Shigella	DNKO		1
	Y. enterocolitica	*K. ozaenae*		2
Tube	*E. cloacae*	*E. aerogenes*	1	
	S. liquefaciens[b]	*S. marcescens*		5

[a] DNKO indicates that the organism did not key out in the profile number book.

[b] Book-errors in the Micro-ID system.

[c] This organism was probably *S. marcescens.* Arabinose was negative on both tube and Micro-ID, but Micro-ID called it *S. liquefaciens* anyway.

terisk are considered Micro-ID book-errors. We felt that the *Serratia liquefaciens* identification made by Micro-ID was incorrect (see footnote, Table 5). Due to book-error, Micro-ID called the organism *S. liquefaciens,* although the negative arabinose should have classified it as *S. marcescens,* which matched the tube identification. In relation to tube media identification, there is only one case, other than the four *Serratia* misindentifications due to Micro-ID book-error, where a tube identification did not match the other two systems. In this case, the lysine decarboxylase reaction was consistently negative with tube media and positive by the other two methods; hence, the tube method identified the organism as *E. cloacae.*

Primary plates. To evaluate the practical aspects of using Micro-ID in a clinical setting, 191 consecutive specimens were evaluated for same-day identification from primary MacConkey plates. Of these 191, 147 had no growth of *Enterobacteriaceae.* The remaining 44 cultures yielded 50 enterics. Sufficient growth to bring the inoculum turbidity to a 0.5 McFarland standard was seen on 37 organisms (74%). To facilitate a same-day identification, the remaining 13 organisms were subcultured to Trypticase soy broth and incubated at 37°C on a shaker for 4 h. The resulting growth was centrifuged, washed, and resuspended to a 0.5 McFarland standard. Of the 13, five grew well enough to inoculate a Micro-ID strip, whereas the remaining 8 organisms could not practically be worked up in a regular working day. These organisms which were too few in number to bring the inoculum to a proper turbidity were restreaked, allowing next-day identification.

DISCUSSION

In recent years several kits have become available for rapid biochemical identification for members of the *Enterobacteriaceae.* The Auxotab and Pathotec (4, 6) systems provide 3- and 4-h identifications, respectively. The Micro-ID system uses the same principle as the Pathotec system in that substrate/reagent-impregnated filter paper (disks) is set in individual compartments with 15 different biochemical tests per tray. This arrangement of tests is convenient, and the strip is easy to handle. Because the inoculum suspensions of the enterics tested, except for those from the clinical plates, were prepared from pure cultures on MacConkey agar, the inoculum density of a 0.5 McFarland standard presented no difficulty due to abundant growth. We found that the use of a sterile cotton-tipped applicator allows for a faster preparation of a homogeneous organism suspension than the use of a standard bacteriological inoculating loop. However, additional saline (approximately 1 ml) in the tube is required due to the loss of fluid when using a swab. When dealing with mixed cultures, as they appeared on primary plates from the clinical laboratory, the swab was not a practical tool, and the bacteriological loop was used for suspension preparation. As previously stated, about one-fourth of the primary plates which had enterics growing did not have sufficient growth to produce the necessary inoculum turbidity even though an inoculating loop was used. To facilitate a same-day identification of organisms with insufficient growth, colonies were inoculated into broth for 4 h. However, only 38% (5/13) of the broth cultures produced sufficient growth to be centrifuged, washed, and used as inoculum for a Micro-ID test strip. This additional step is very time consuming and does not appear to produce enough positive results to justify the time required, although it could be used in pressing situations. Alternatively, streaking a new MacConkey plate and incubating overnight almost always produces sufficient growth for inoculation.

In comparison with API 20E and conventional tube media, the Micro-ID performed almost as well in the identification of 373 members of the *Enterobacteriaceae.* We found that the octal code book had errors which gave the wrong organism name for certain sets of reactions. If the errors were corrected, the percentage of correlation by using the Micro-ID was raised up to 5%. In a comparison of common tests with Micro-ID versus tube media, and the API 20E versus tube media, we found that on the common tests Micro-ID performed as well as API 20E, and in the cases of the arabinose and VP tests the Micro-ID performed significantly better. However, a comparison of the Micro-ID versus API 20E with a larger number of organisms shows better correlation of the arabinose but little change in the VP. In a comparison of all three systems, greater than 85% correlation was found for all common tests except two, arabinose and VP.

An analysis of each of the common tests for each comparison shows that certain groups of organisms consistently did not correlate in certain reactions. For example, when Micro-ID and conventional tube media were compared for lysine decarboxylase correlation, problem organisms were found to be *K. pneumoniae* and *S. enteritidis.* Likewise, the same organisms did not correlate when API 20E and conventional tube media were compared.

The lack of correlation when Micro-ID was

used for comparison may stem from the sensitivity of the substrate/reagent concentration used in the tests. We found that occasionally the color reaction on some of the tests was neither positive nor negative but a color not described by the manufacturer. These reactions were noted in 7 of the 15 tests. Sorbitol and inositol had the greatest number of unusual colors, with 26 aberrant reactions each. Under these unusual circumstances, we had to subjectively decide if the reactions were closer to a positive or a negative reaction. It is possible that in some cases we misinterpreted the reaction. Thus, it may be that a very fine line exists in determining the optimum concentration of substrate/reagent for some organisms to give a distinct shift in reaction in 4 h. The addition of a color chart to the kit describing some of these reactions would be a great help.

In conclusion, we feel that if the inadequacies of the Micro-ID described in this report are eliminated, the Micro-ID system would be competitively accurate with other rapid microbiological identification systems.

ACKNOWLEDGMENT

This study was supported by General Diagnostics, Division of Warner-Lambert Company, Morris Plains, N.J.

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