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Rapid Identification of *Enterobacteriaceae* with the Micro-ID System Versus API 20E and Conventional Media

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The Micro-ID system for rapid (4 h) identification of *Enterobacteriaceae* was evaluated by testing 433 enteric bacilli and 9 other gram-negative bacilli. Each isolate was identified with conventional tubed media and was also tested in the Micro-ID and API 20E systems. The overall accuracy of both systems was 97%. Micro-ID tests for the Voges-Proskauer reaction, indole and H_2S production, and ornithine and lysine decarboxylase all demonstrated a 97 to 99% correlation with conventional methods. Only 86% of the Micro-ID urease tests agreed with Christenson urea agar. Two inoculum densities were tested in Micro-ID panels, with 157 stock cultures. Over 90% of the tests were unaffected by changes in inoculum density. Tests with four control strains suggested that the Micro-ID system was more reproducible when a light inoculum was used. The Micro-ID system was found to be a very convenient method for rapid, accurate, and precise identification of the *Enterobacteriaceae*.

Identification of the *Enterobacteriaceae* constitutes a fairly large proportion of the workload in clinical microbiology laboratories. For maximal accuracy and precision, a fairly large battery of biochemical, serological, and morphological characteristics must be determined. However, the number of biochemical tests that can be performed with standard methods must be limited because of the need for efficiency and economy. Furthermore, many standard methods require an incubation period of 2 or more days. Because of the need for rapidity, the conventional tests that are commonly used in clinical laboratories resemble the standard methods but are only incubated overnight, with some loss of accuracy.

A number of commercial "kits" are currently available for identifying the *Enterobacteriaceae*. These kits provide the opportunity to economically determine more biochemical characteristics than could be performed with conventional methods. To help interpret the results of these additional tests, identification manuals have been developed using an octal code to describe the pattern of reactions. The sensitivity and specificity of the individual tests in a kit are not necessarily the same as with standard or conventional methods, but the identification of the organism should be the same as with standard methods.

Analytab Products Inc. (API) provides one such kit, API 20E, which permits the determination of 20 different biochemical characteristics on one strip (5). With the API 20E, most enteric bacilli can be identified to the species level within 18 to 24 h after primary isolation; a few isolates require additional tests which can be completed after 1 or 2 additional days.

More rapid results can be achieved with the Micro-ID system, a new product recently marketed by General Diagnostics (Morris Plains, N.J.). The Micro-ID system uses the principle of the Pathotec strips, i.e., detection of enzyme activity by using substrates and reagents impregnated into filter paper strips (3). Micro-ID panels consist of plastic trays with filter paper disks set into individual compartments. Fifteen different characteristics can be determined after 4 h of incubation, with the addition of only one reagent (2 drops of 20% KOH). All other reagents are incorporated into disks in the test panel. A premarketing experimental version of this system has been described more completely by Aldridge et al. (1). The system they evaluated has been modified only slightly by altering the substrate in the urease test. In addition, a customized data base has been established using the results of tests with Micro-ID panels, as collected by several investigators. This should improve the identification manual used to interpret the five-digit octal code generated by this system.

The present study evaluates the Micro-ID system that is currently being marketed. A total

of 442 isolates were identified with conventional tubed media, with API 20E strips, and with the Micro-ID system. The identifications derived from the two commercial kits were compared to that determined with conventional media.

MATERIALS AND METHODS

Microorganisms. The 442 isolates tested by all three systems include three strains each of *Aeromonas hydrophilia*, *Pseudomonas maltophilia*, and *Acinetobacter calcoaceticus* subsp. *anitratus* and 433 *Enterobacteriaceae* (266 fresh clinical isolates and 167 stock cultures). The stock cultures were stored at −60°C in Trypticase soy broth (BBL Microbiology Systems) with 15% glycerol and were transferred to blood agar plates for at least 2 consecutive days before being tested. Repeated transfers were performed to reduce the possibility that the test strains were metabolically inactive when first recovered from storage.

Conventional tubed media. All isolates were tested with a battery of eight different tubed media: triple sugar iron agar (for H_2S production), lysine iron agar, ornithine decarboxylase (Difco decarboxylase broth with 0.3% agar and 0.5% ornithine), MR-VP broth (1.0 ml for Voges-Proskauer tests), tryptone broth (for indole production), Simmons citrate agar, Difco G-I motility medium, and Christensen urea agar. All tubed media were inoculated with an actively growing tryptone broth culture, and the results were recorded after 18 to 24 h of incubation at 35°C. *Yersinia enterocolitica* was also tested at 25°C. When necessary, additional tests were performed to establish an identification, i.e., fermentation of various carbohydrates, gelatin liquification, serological confirmation of *Salmonella* or *Shigella* serogroups, etc.

API 20E. The strips were inoculated according to the instructions of the manufacturer. The results of the 20 biochemical tests plus an oxidase test were translated into a seven-digit octal code which, in turn, was interpreted by referring to the API code book (revised October, 1977). The species was recorded if the code book listed an excellent, very good, or acceptable identification for that octal code. The isolate was identified to the genus only, if the generic identification was listed as excellent, very good, or acceptable, and if additional tests were required to distinguish between two or more species. The results were recorded as "low selectivity" if no definite identification could be made, i.e., two or more possible identifications were listed but could not be separated without additional tests. If the octal code was not found in the identification manual, the results were recorded as "does not key out" and no effort was made to search the larger data base available through API.

To compare the identifications obtained with the API system to those derived from the Micro-ID system, *Proteus rettgeri* and urea-positive *Providencia stuartii* were considered to be the same. Also, *Salmonella arizonae* was considered the same as *Arizona hinshawii*. The two code books differ in their classifications of these two groups of microorganisms.

Micro-ID. The Micro-ID panels used in this study represent samples from three different lots (no. OA505, OA578, and OA580). The test panels were all stored at 4 to 8°C and were used well before the stated expiration date. Tests with four control strains detected no significant differences in the performance of panels from different lots and no change in performance during storage of the panels.

The inoculum for each Micro-ID panel was standardized by preparing a saline suspension of colonies from an 18- to 24-h blood agar plate and then adjusting the turbidity to match that of a MacFarland no. 0.5 standard. Most stock cultures were tested with two separate inocula; one was adjusted to match a MacFarland no. 0.5 standard, and the other was adjusted to match a MacFarland no. 2 standard. Approximately 0.2 ml of inoculum was delivered to each of the 15 compartments in the test panel, and the tray was then placed upright in an incubation rack, moistening the substrate disks. After 4 h at 35°C, 2 drops of 20% KOH were added to the V-P test well and the tray was tipped to moisten the five reagent disks. The results of all 15 tests were recorded as instructed by the manufacturer and were then translated into a five-digit octal code. The interpretation of each octal code was based on the identification manual (edition no. 09178, 1978) that was generated after the completion of this study. The identification was accepted if it was listed as being excellent, extremely good, very good, good, or acceptable. Low selectivity was recorded if two or more identifications were listed and if additional tests would be required to select the appropriate choice.

Quality control. Four quality-control strains were selected for this study: *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Proteus vulgaris*, and *Pseudomonas aeruginosa*. Each control strain was tested on nine different occasions during the 3-month study. Each trial included three tests, one API 20E strip, and two Micro-ID panels (one for each inoculum density).

RESULTS

Micro-ID versus conventional tests. The battery of conventional media included six determinations that were also included in the Micro-ID panels (Table 1). Tests for H_2S production and lysine decarboxylase were essentially the same in the two systems, i.e., about 98% agreement with equal numbers of false-negative

TABLE 1. *Conventional tubed media versus Micro-ID test results*

Biochemical test	Agreement (%)	Percent discrepant (tubed media/Micro-ID)	
		−/+	+/−
Voges-Proskauer	97.4	2.1	0.5
Hydrogen sulfide	97.7	1.2	1.2
Indole	98.4	0	1.6
Ornithine decarboxylase	97.4	1.6	0.9
Lysine decarboxylase	98.6	0.7	0.7
Urease	86.2	3.3	10.5

and false-positive results. Five strains were indole positive in conventional media but negative in the Micro-ID system; four of those five were indole positive in API 20E strips. Voges-Proskauer and ornithine decarboxylase tests were in agreement with nearly 98% of the strains, but the majority of discrepancies were positive in Micro-ID and negative in conventional media. This probably represents false-negative tests with the conventional media, since they were only incubated overnight.

Aldridge et al. (1) previously reported an 85% agreement between urea agar and the earlier Micro-ID urea test. In our study, the two urease tests were in agreement with 86% of the strains. The weak urease activity of organisms such as *K. pneumoniae* was often seen on Christensen urea agar but not on Micro-ID panels. In contrast, the urea test on API strips agreed with conventional tests with 91.5% of our strains. The Micro-ID urease tests were read as positive if there was any evidence of an orange or red color in the liquid. With 126 strains, the urea solution turned a definite orange or red color, and 271 were clearly negative. Weak reactions were observed with 26 strains, including 17 *K. pneumoniae*, 2 *Citrobacter diversus*, 3 *Citrobacter freundii*, 3 *E. cloacae*, and 1 *Serratia marcescens*.

If the 26 weak urease reactions had been considered negative, the agreement with the agar medium would have been reduced to 83%. The identification of 21 of the 26 strains would not have been changed if the Micro-ID urease tests were considered negative. However, two of the *C. freundii* were misidentified as *Y. enterocolitica* because of a weakly positive urea tests; they would have been properly identified if the urea had been recorded as negative. Also, two *C. diversus* isolates could not be keyed out when the urea was read as positive but would have been correctly identified if the urea were negative. On the other hand, *K. pneumoniae* gave a weakly positive urea test and was properly identified; if the urease test were read as negative it would have been misidentified as *Enterobacter* species. We concluded that, with the Micro-ID system, urea tests should not be considered positive unless the test suspension turns a definite orange or red color. The strong urease activity of *Proteus* spp. was consistently detected with all three test systems.

Phenylalanine deaminase tests on the Micro-ID panels were positive with 90% of the *Proteus-Providencia* group and negative with all other enteric bacilli. Correlation between the phenylalanine test in the Micro-ID panels and tryptophane deaminase tests in the API 20E strips was excellent. Nine of 105 *Proteus* spp. were phenylalanine negative but tryptophane deaminase positive, and one *P. vulgaris* isolate was phenylalanine positive but tryptophane deaminase negative. All 10 discrepant strains were identified correctly in spite of the negative amino acid deaminase tests.

Nitrate reduction tests in the Micro-ID panels were positive with 92.6% of the enteric bacilli, with all three *A. hydrophilia* and two of three *P. maltophilia*. Negative nitrate reduction tests were observed with 14 of 51 *Proteus mirabilis*, 11 of 25 *Proteus morganii*, 3 of 15 *P. vulgaris*, 1 of 49 *S. marcescens*, 1 of 5 *Enterobacter agglomerans*, 1 of 51 *K. pneumoniae*, 1 of 3 *P. maltophilia*, and all 3 *Acinetobacter* spp.

Identification of microorganisms. The final identifications obtained with the two commercial systems were compared with those obtained with conventional methods (Table 2). In addition to the 433 *Enterobacteriaceae* listed in Table 2, 9 other gram-negative bacilli were also tested. Included were three strains of *A. hydrophilia*; two were identified correctly by the API system and one could not be keyed out in the API manual. Because *Aeromonas* sp. is oxidase positive, it should not be tested in the Micro-ID system. However, if the oxidase test is omitted or incorrectly performed, *Aeromonas* sp. might be tested occasionally in this system. Two of our strains would have been identified as *E. agglomerans* and one appeared to be *Escherichia coli*. This observation simply emphasizes the error that can occur if instructions are not followed, i.e., oxidase-positive microorganisms should not be tested.

Six oxidase-negative, nonenteric gram-negative bacilli were also tested. The API system failed to identify one *P. maltophilia* and one *Acinetobacter* sp.; the remaining four strains were properly identified. With the Micro-ID system, all three *Acinetobacter* sp. and one *P. maltophilia* gave octal codes that were not found in the identification manual. Two of the three *P. maltophilia* strains were considered to be *Shigella* sp. with the Micro-ID system. This observation emphasizes the need for serological confirmation of *Shigella* sp., as specified in the identification manual.

The interpretation of Micro-ID and API 20E test systems both agreed with that of the conventional tests with 97% of the enteric bacilli, including 5 to 7% which could only be identified to the generic level—additional tests were required to confirm the species. Low selectivity was recorded with 2% of the isolates; no identification could be recorded without further testing. Twelve isolates gave octal codes that were

TABLE 2. *Interpretation of conventional tests versus API 20E and Micro-ID systems for 433 strains*

Species identified by conventional methods	API 20E vs conventional tests (no. of strains)					Micro-ID vs conventional tests (no. of strains)				
	Complete agreement	Ident. genus only[a]	Low selectivity[a]	Not key out	Disagreement	Complete agreement	Ident. genus only[a]	Low selectivity[a]	Not key out	Disagreement
Escherichia coli	58					58				
Klebsiella ozaenae	1							1		
K. pneumoniae	48	1	1	1		49			1	1
Citrobacter diversus	2	11				8			2[b]	3
C. freundii	13	1	2	1		15				2[b]
Enterobacter cloacae	48	1		1		42	5	2		1
E. aerogenes	28				2	29				1
E. agglomerans	3		1	1		4	1			
Serratia rubidaea	2			1			1			2
S. marcescens	48			1		43	5	1		
Providencia stuartii	7					7				
P. alcalifaciens	1					1				
Proteus rettgeri	12		2			12		2		
P. morganii	24		1			24	1			
P. vulgaris	13			2		14	1			
P. mirabilis	50	1				51				
Edwardsiella tarda	4					4				
Arizona hinshawii	2					2				
Salmonella typhi	5					5				
S. enteritidis	1	8				8		1		
Shigella sp.[c]		8					8			
S. sonnei	5					5				
Yersinia enterocolitica	6			4		7				3
Total (percent)	381 (88)	31 (7)	7 (2)	12 (3)	2 (0.5)	388 (90)	22 (5)	7 (2)	3 (1)	13 (3)

[a] Additional tests would have been required to confirm species identification.

[b] Two *C. diversus* and two *C. freundii* were misidentified because of weakly positive urease tests; all four would have been identified correctly if urease were negative.

[c] Includes seven *S. dysenteriae* and one *S. flexneri*.

not found in the API identification manual. Only three did not key out in the Micro-ID code book, and two of those would have been identified correctly if a weakly positive urea test had been considered negative.

Fifteen strains were misidentified, 2 with API and 13 with Micro-ID. The API system identified two strains of *Enterobacter aerogenes* as *Serratia liquefaciens*; Micro-ID considered both strains to be *E. aerogenes*. The Micro-ID identification of 13 strains disagreed with those obtained with the two other test systems (Table 3). Two isolates gave atypical test patterns that could not be identified with confidence. They were considered to be *C. diversus* because conventional tests gave a positive citrate reaction and negative lysine decarboxylase test; one was urease positive and the other was urease negative. The Micro-ID system (which does not include a citrate test) identified both strains as lysine-negative *E. coli*. Three of 10 *Y. enterocolitica* strains were misidentified: two as *Shigella* sp. and one as *P. morganii*. Three strains of *Citrobacter* spp. were misidentified as *Y. enterocolitica* because of a positive urease test; all three gave negative urease tests when the inoculum was increased. Two other misidentifications resulted from false-negative or false-positive urease tests in the Micro-ID system. If the urea disks were considered to be positive only when a definite orange or red color appeared in the suspension, two misidentifications would have been avoided but one additional misidentification would have been added to the list (a second *K. pneumoniae* identifed as *Enterobacter* spp. because of a negative urease test).

Effect of increased inoculum density. Micro-ID panels were challenged with two inoculum densities (MacFarland no. 2 and no. 0.5 turbidity standards) with 157 stock cultures. The majority of individual tests were not affected by changes in inoculum density; 90 to 100% of the different tests were in full agreement. With urea, ornithine, inositol, and adonitol disks, some tests were positive with the MacFarland no. 0.5 inoculum, but negative with the denser inoculum. However, the heavier inoculum gave more positive reactions with H_2S, *o*-nitrophenyl-β-D-galactopyranoside, and sorbitol disks. All 15 tests were the same with 105 of the 157 strains, and with 29 strains individual tests differed but the identification was not affected. Fourteen isolates were correctly identified to the generic level, but either the species could not be confirmed with

one inoculum density or the two inocula identified different species. Nine of 157 strains were identified to different genera with the two inocula. The interpretation of conventional tests better agreed with the Micro-ID system on 11 occasions when a heavy inoculum (MacFarland no. 2.0) was used and on 12 occasions when tested with a light inoculum (MacFarland no. 0.5).

Quality control of API 20E and Micro-ID. During this 3-month study, four control strains were tested periodically. On nine separate days, each organism was tested on one API 20E strip and on two Micro-ID panels, one of which was inoculated with a dense inoculum (MacFarland no. 2) while the other received a normal inoculum (MacFarland no. 0.5). Each API strip produced 20 different tests for each of four organisms, each tested nine times, thus producing 720 positive or negative results. Table 4 records the numbers of tests that deviated from that normally seen with each strain. Each Micro-ID panel produced 15 different test results, generating 540 test results for each inoculum density. When the inoculum was adjusted to match a MacFarland no. 0.5 standard, only 3 of 540 tests (0.5%) deviated from the expected. However, when the dense inoculum was used, 18 of 540

TABLE 3. *Isolates with significant disagreements between identifications achieved with Micro-ID and conventional media*

Identification derived from:		Probable reasons for discrepancies; comments[a]
Conventional tests and API 20E	Micro-ID	
E. cloacae	*E. agglomerans*	Micro-ID: VP and OD negative
S. rubidaea	*S. liquefaciens*	Micro-ID: OD and LD positive
S. rubidaea	*K. pneumoniae*	Micro-ID: sorbitol positive
C. diversus	*E. coli* (LD negative)	Identification uncertain; citrate positive, LD and urea negative
C. diversus	*E. coli* (LD negative)	Same as above, but urea agar positive and Micro-ID urea negative
C. freundii (H_2S negative)	*Y. enterocolitica*	Micro-ID: urea positive[b]; negative with heavy inoculum
C. freundii (H_2S negative)	*Y. enterocolitica*	Same as above[b]
C. diversus	*Y. enterocolitica*	Micro-ID: urea positive; negative with heavy inoculum
E. aerogenes	*K. pneumoniae*	Micro-ID: urea positive; negative with heavy inoculum
K. pneumoniae	*Enterobacter* sp.	Micro-ID: urea negative
Y. enterocolitica	*Shigella* sp.	Micro-ID: OD and urea negative
Y. enterocolitica	*Shigella cholerae-suis*	Micro-ID: urea negative
Y. enterocolitica	*P. morganii*	Indole and VP negative

[a] LD, Lysine decarboxylase; OD, ornithine decarboxylase; VP, Voges-Proskauer.

[b] Urease tests weakly positive; organisms would have been identified correctly if urease test was considered negative.

TABLE 4. *Quality control of Micro-ID (two inocula) and API 20E test systems*

Control microorganism	No. of tests[a] deviating from the expected		
	Micro-ID, two inocula[b]		API 20E
	MacF #2	MacF #0.5	
E. cloacae	8 (4 OD, 4 Esc)	1 (1 Esc)	1 (1 LD)
K. pneumoniae	3 (3 Ur)	0	3 (2 Ur, 1 Sorb)
P. vulgaris	1 (1 VP)	2 (2VP)	1 (1 OD)
P. aeruginosa	6 (4 LD, 2 Arab)	0	5 (3 OD, 2 Gluc)
Total deviating (percent)	18 (3.3)	3 (0.5)	10 (1.4)

[a] OD, Ornithine decarboxylase; LD, lysine decarboxylase; Esc, esculin hydrolysis; UR, urease tests; VP, Voges-Proskauer; Sorb, sorbitol; Arab, arabinose; Gluc, glucose. Each Micro-ID panel generated 15 test results × 9 trials × 4 microorganisms = 540 recorded results for each inoculum density. API 20E generated 20 tests per strip in the 9 trials for a total of 720 recorded results.

[b] Micro-ID panels inoculated in duplicate; one inoculum was adjusted to match a MacFarland no. 2 (MacF #2) and the other a MacFarland no. 0.5 turbidity standard.

reactions (3.3%) were other than the expected. At the same time, API 20E strips yielded 10 (1.4%) reactions that deviated from the expected.

DISCUSSION

In our opinion, the Micro-ID system is satisfactory in many respects. The substrate and detection disks are packaged in a convenient panel which is clearly labeled and easy to handle. A satisfactory inoculum can be developed by suspending a few colonies in saline. Aldridge et al. (1) examined 191 consecutive specimens: 147 had no growth and 44 contained 50 enterics. They found that 37 of the 50 enteric bacilli produced enough isolated colonies on the primary plates to initiate the rapid identification system on the first day, and 13 isolates had to be subcultured for identification the following day. Consequently, the Micro-ID system can significantly reduce the time required to identify many enteric isolates in clinical specimens.

Our results with two inoculum densities indicate that little difficulty would occur as a result of minor variations in adjusting turbidity of the inoculum, but for the sake of standardization each inoculum should approximate the turbidity of a MacFarland no. 0.5 standard. If the inoculum exceeds this density, the results are not as well defined and tend to be less reproducible. Unsatisfactory results will also be obtained if the inoculum is too light.

A premarket experimental version of the Micro-ID system was evaluated by Aldridge et al. (1). Once an initial error in the earlier identification manual was corrected, the Micro-ID and API systems both demonstrated a 90% correlation with conventional methods. The Micro-ID system that we evaluated and the API system both provided a 97% correlation with conventional tests. We cannot conclude that the accuracy of the Micro-ID system has been improved, because the accuracy of the API 20E strips in the two studies changed in the same order of magnitude. The differences between the two studies might only represent differences in the culture collections that were studied. We can conclude that the Micro-ID correlation with conventional methods is excellent and equivalent to the API 20E system.

The Micro-ID system correctly identified 417 of 433 enteric bacilli and misidentified only 13 strains. Two of the strains that were misidentified gave atypical reactions that could not be interpreted with confidence. Three of the misidentified strains were reported to be *Y. enterocolitica*, a very rare clinical isolate which should be confirmed before being reported. On the other hand, 7 of 10 *Y. enterocolitica* isolates were properly identified. Two of the three *Y. enterocolitica* that were misidentified appeared to be *Shigella* sp., but they would not agglutinate with *Shigella* antisera. In addition, two of three *P. maltophilia* isolates would have been reported to be *Shigella* sp. with the Micro-ID system. All 13 *Shigella* included in this series were appropriately identified, but the need for confirming such an identification by serological procedures cannot be overemphasized.

Five misidentifications involved difficulties with the Micro-ID urease test. Redefinition of the color change which constitutes a positive urea test did not significantly alter the accuracy of the test system. However, we concluded that only definite orange, pink, or red color should be considered positive. The sensitivity and specificity of the Micro-ID urease test are not the same as those of conventional agar media or of the urea test in the API strip.

A review of our quality control data suggests that an excellent degree of reproducibility might be expected when the Micro-ID system is inoculated with a light inoculum (adjusted to a MacFarland no. 0.5 standard). However, more variability was observed when the inoculum was adjusted to a MacFarland no. 2 standard. The reproducibility of the API 20E system might have been improved if the inoculum density were better standardized (4), but in this study we elected to follow the manufacturers' instructions as carefully as possible in performing tests with both systems.

In summary, the Micro-ID system offers the clinical laboratory a convenient, simple method for rapid identification of the *Enterobacteriaceae*. The accuracy and precision of this system seem nearly comparable to those of the API 20E system; both are quite acceptable for use in clinical laboratories.

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