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Comparison of Micro-ID and API 20E Systems for Identification of *Enterobacteriaceae*

DONNA J. BLAZEVIC,* DIANE L. MACKAY, AND NETTIE M. WARWOOD

Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, Minnesota 55455

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The Micro-ID 4-h identification system for *Enterobacteriaceae* was compared to the API 20E overnight method, using 230 fresh clinical isolates and 74 stock cultures. Agreement was 97.8% for the clinical isolates and 93.2% for the stock cultures. Eighty-seven percent of primary culture plates containing gram-negative rods yielded sufficient growth to perform the 4-h Micro-ID identification on the same day the organisms were isolated.

A new kit system, Micro-ID (General Diagnostics, Morris Plains, N.J.), provides for identification of organisms in the family *Enterobacteriaceae* within 4 h. Aldridge et al. (1) compared the original Micro-ID system with the API 20E overnight kit (Analytab Products, Inc., Plainview, N.Y.) and with a conventional system. They found an 83% correlation in identification between API and Micro-ID and an 82% correlation between the three systems. Since the time of that evaluation a new data base has been developed, based on Micro-ID reactions. We present here an evaluation of the new data base identification as compared to identification using API 20E. In addition, selected Micro-ID biochemical reactions were compared to conventional tests.

MATERIALS AND METHODS

Organisms. In the comparison of selected biochemical tests, 608 organisms of the *Enterobacteriaceae* were tested. Of these, 376 were fresh clinical isolates, and 232 were stock organisms that had been stored in Trypticase soy agar deeps in the dark at room temperature. The organisms used are shown in Table 1.

In the clinical evaluation of the new Micro-ID identification system, 304 organisms were tested; of these, 230 were consecutive fresh clinical isolates and 74 were stock cultures. These stocks were not the ones used in the biochemical study.

Prior to testing by any system, all stock cultures were subcultured two to three times on sheep blood agar.

Micro-ID. The Micro-ID system was supplied by General Diagnostics and consists of a molded styrene tray containing 15 chambers and a hinged lid. The first five chambers contain a paper substrate disk and a paper reagent disk in separate wells; the tests in these chambers are Voges-Proskauer, nitrate reduction, phenylalanine deaminase, H_2S, and indole. The remaining chambers contain single paper disks containing both substrate and detection reagent; these include tests for ornithine decarboxylase, lysine decarboxylase, malonate utilization, urease, esculin hydrolysis, *o*-nitrophenyl-β-D-galactopyranoside, and arabinose, adonitol, inositol, and sorbitol fermentation.

For inoculation of the Micro-ID system, only oxidase-negative organisms were used. Organisms were taken from 18- to 24-h cultures on either 5% sheep blood agar or MacConkey agar. Growth was emulsified in 3.5 ml of 0.85% NaCl to match a no. 0.5 McFarland standard. With stock cultures, the suspension was made to match a no. 2 McFarland standard. Each well of the Micro-ID tray was inoculated with 0.2 ml of the standardized suspension. After inoculation the trays were placed upright in a plastic holder (five trays per holder) and were incubated in a 35°C incubator for 4 h. After incubation, 2 drops of 20% KOH were added to the Voges-Proskauer test. The trays were then rotated 90° to allow the suspension in the first five wells to wet the corresponding reagent disks. The trays were then set upright, and the reactions were read according to the manufacturer's directions. The tests were divided into threes, and the first test in each group received a 4 for a positive result, the second test a 2, and the third test a 1. The totals for each group were recorded, and a five-digit octal number was obtained. In the clinical evaluation, the Micro-ID code book containing the octal numbers was consulted for identification of the organisms.

The API 20E strips were inoculated, incubated, and read according to the manufacturer's directions. The seven-digit octal number obtained was used to find the identity of the organisms from the API Profile Register. All clinical isolates used in the study were inoculated into the API 20E from the primary culture plate by technologists in the Clinical Microbiology Laboratory of the University of Minnesota Hospitals. For the clinical evaluation of the Micro-ID system, the same plates were used to inoculate the trays, if sufficient growth was available. If there was insufficient growth a subculture was made to a MacConkey agar plate.

Conventional tests were performed as previously described (4).

When there was a disagreement between the API and Micro-ID identification, the cultures were checked for purity and the two systems were repeated. If there was still a discrepancy, appropriate conventional tests were inoculated to resolve the difference.

RESULTS

Table 2 shows the percent agreement between Micro-ID and conventional tests. All tests agreed at 95% or greater, except for urease (90%) and arabinose fermentation (91%). *Klebsiella pneumoniae* isolates accounted for most of the urease discrepancies; with these organisms there was only a 34% agreement between the Micro-ID urease and Christensen urea agar. *Serratia* sp. were responsible for the arabinose disagreements; with these organisms the agreement between the Micro-ID and conventional arabinose was 67%.

Table 3 shows the comparison of identifications obtained with API 20E and Micro-ID. With the 230 clinical isolates there was a 97.8% agreement, and with the stock cultures the agreement was 93.2%. Total agreement between the two systems was 96.7%.

Table 4 shows the organisms that accounted for the 10 disagreements between API and Micro-ID identifications. By additional conventional tests Micro-ID was correct in four of the disagreements, and API was correct in five of the disagreements. With the remaining disagreement neither of the systems was exactly correct; this organism was a stock culture.

Of 210 consecutive cultures that contained gram-negative, oxidase-negative rods, 183 (87.1%) contained sufficient isolated colonies to inoculate Micro-ID. There was no problem with mixtures.

TABLE 1. *Organisms used to compare Micro-ID biochemical reactions with conventional tests*

Organism	No. of strains:	
	Clinical	Stock
Citrobacter diversus	9	9
Citrobacter freundii	24	7
Edwardsiella tarda	0	9
Enterobacter aerogenes	44	7
Enterobacter cloacae	51	0
Enterobacter agglomerans	7	6
Escherichia coli	52	0
Klebsiella pneumoniae	51	0
Proteus mirabilis	52	0
Proteus morganii	21	13
Proteus rettgeri	12	15
Proteus vulgaris	17	0
Providencia stuartii	5	7
Salmonella arizonae	0	19
Salmonella enteritidis	0	34
Salmonella typhi	0	6
Serratia liquefaciens	2	6
Serratia marcescens	27	23
Serratia rubidaea	0	3
Shigella sonnei	0	22
Shigella sp.	2	37
Yersinia enterocolitica	0	6
Yersinia pseudotuberculosis	0	3

TABLE 2. *Comparison of Micro-ID biochemical reactions with conventional tests using 608 organisms*

Test	Percent agreement
Adonitol	95
Arabinose	91
H_2S	98
Indole	95
Lysine decarboxylase	99
Ornithine decarboxylase	99
ONPG[a]	99
Phenylalanine deaminase	99
Urease	90

[a] ONPG, *o*-Nitrophenyl-β-D-galactopyranoside.

DISCUSSION

In this study the identifications obtained with Micro-ID were compared to the API 20E because the latter system has been shown to have a high accuracy of identification when compared to the conventional system used at the Center for Disease Control (5). The 97.8% agreement we obtained with the clinical isolates is excellent and shows that the Micro-ID system provides a useful, accurate, and rapid method for the clinical microbiology laboratory. In the study of Aldridge et al. (1), the agreement between Micro-ID and API 20E was only 83%, and between Micro-ID and conventional tests agreement was only 82%. The low accuracy is undoubtedly the result of using the percentage charts of Edwards and Ewing (3) for the Micro-ID data base at that time. However, this problem has clearly been resolved with the development of a data base from extensive testing of known organisms on Micro-ID in various laboratories throughout the country.

The Micro-ID system is an outgrowth of the 4-h PathoTec system (2), but is clearly superior because of the ease of inoculation and the identification code book. Inoculation of Micro-ID is extremely simple, especially if an automatic micropipette is used. Wells do not have to be filled, and one need have no concern for avoiding bubbles. The trays themselves are rigid and thus very easy to handle. The hinged cover obviates the need for an outer chamber to be filled with water; there is no problem with drying of the tests in the 4-h incubation period. Only one

TABLE 3. *Comparison of Micro-ID and API 20E identification*

API identification	No. of strains:		Total strains tested	No. in agreement with Micro-ID
	Clinical	Stock		
Citrobacter diversus	2	8	10	10
Citrobacter freundii	7	1	8	7
Edwardsiella tarda	0	3	3	3
Enterobacter aerogenes	4	3	7	7
Enterobacter agglomerans	1	0	1	1
Enterobacter cloacae	16	0	16	15
Enterobacter hafnei (Hafnia alvei)	1	1	2	1
Enterobacter sp.	1	0	1	1
Escherichia coli	103	0	103	103
Klebsiella pneumoniae[a]	41	3	44	44
Pasteurella sp.	0	1	1	0
Proteus mirabilis	20	0	20	20
Proteus morganii	4	6	10	10
Proteus rettgeri	1	4	5	5
Proteus vulgaris	3	4	7	7
Providencia stuartii	0	3	3	2
Salmonella arizonae	0	6	6	5
Salmonella sp.	0	15	15	15
Serratia liquefaciens[b]	4	0	4	4
Serratia marcescens[b]	14	0	14	14
Serratia rubidaea[b]	0	3	3	0
Serratia sp.[b]	1	0	1	1
Shigella	0	13	13	12
Oxidase-negative nonfermenter	7	0	7	7
Total	230	74	304	294 (96.7%)

[a] Includes *Klebsiella oxytoca*.
[b] Called agreement if genus agreed.

additional reagent has to be added to the wells. In general, the reactions were very easy to read, although, as with any system, experience improves the ease of reading. With both the decarboxylase and the fermentation tests the colors should be either purple or yellow. Occasionally an in-between shade would be observed; in these cases, one could read the test as positive or negative and look up either number to be obtained. All the other reactions were clearly readable in 4 h. Extending the incubation time for 1 to 2 h would probably be acceptable, but longer periods would defeat the rapid nature of the test. Also, one could encounter contamination problems in lengthy incubation because the test does not require aseptic conditions.

Even though the Micro-ID system is easy to use and interpret, its accuracy is enhanced by having experienced technologists carrying out the testing. For example, there were three strains of *Serratia rubidaea* that were identified as *K. pneumoniae* by Micro-ID. These strains had a bright red pigment and thus should not be called *K. pneumoniae* by a microbiologist regardless of what the Micro-ID code number indicated.

The discrepancies between the Micro-ID urease and Christensen urea agar are not surprising. Christensen urea is a very sensitive indicator of urease production. That the disagreements occurred primarily with *K. pneumoniae* also is not unexpected; many of these strains produce a small amount of urease that is only detected by a very sensitive method. However, the urease disagreements did not result in any misidentification of *K. pneumoniae* strains; all 44 were accurately identified by Micro-ID using their new data base.

In addition to its rapidity, the Micro-ID system has an advantage over the API 20E kit in that it has a test for adonitol fermentation. This allows for accurate differentiation of H_2S-negative *Citrobacter freundii* from *Citrobacter diversus* without the need for additional testing. Also, with organisms within the *Serratia* genus, Micro-ID more often gives a species identification, whereas API 20E frequently identifies these organisms simply as *Serratia* sp.

A limitation of the Micro-ID system is that it is only suited to identification of *Enterobacteriaceae*. An oxidase test must be performed first, and only oxidase-negative organisms are to be inoculated. In addition to the *Enterobacteriaceae*, these could include the relatively common

TABLE 4. *Disagreements between API and Micro-ID*

Strain source	API		Micro-ID		Conventional
	Code no.	Identification choices	Code no.	Identification choices	
Stock	1104152	*C. freundii*[a] *S. sonnei* *E. coli*	21031	*S. sonnei*[a] *C. freundii* *E. coli* *Y. enterocolitica*	*S. sonnei*
Clinical	3205573	*E. cloacae* *E. agglomerans*	60271	*E. agglomerans*[a] *E. cloacae* *S. rubidaea* *K. pneumoniae*	*E. cloacae*
Stock	5104152	*H. alvei* *E. coli*	21030	*S. sonnei*[a] *C. freundii* *E. coli* *Y. enterocolitica*	*S. sonnei*
Stock	0074000	*P. stuartii*[a] *P. morganii* *P. rettgeri*	32100	*P. rettgeri*[a] *P. vulgaris* *P. morganii* *P. alcalifaciens*	*P. rettgeri*
Stock	5104552	*S. arizonae*[a] *E. coli*	20431	*E. coli* *K. ozaenae* *S. rubidaea* *E. agglomerans*	*E. coli*
Clinical	1207363	*S. rubidaea*[b] *E. agglomerans* (three isolates)	60277	*K. pneumoniae*[b] *E. agglomerans* *S. rubidaea* *E. cloacae*	*S. rubidaea*
Stock	0104102	*Shigella* sp.[a]	21011	*C. freundii*[a] *S. enteriditis* *S. sonnei* *Y. enterocolitica*	*S. sonnei*
Stock	104000017	Not in book	20071	Not in book	*S. liquefaciens*

[a] Further tests and/or serology were indicated in the identification book.
[b] All three isolates gave the same API and Micro-ID code numbers.

clinical isolates *Acinetobacter calcoaceticus* and *Pseudomonas maltophilia*. In this study we encountered seven such organisms; they were identified by API 20E, and no number for these was in the Micro-ID code book. In these cases one would have to then subculture the organism to an appropriate medium or system for the identification of nonfermenters. This would not delay identification, however, because the discovery is made on the same day the cultures are first read.

In this study we found that the Micro-ID could be used with 87.1% of primary plates that contained gram-negative rods. Aldridge et al. (1) could use it in 74% of clinical specimens on the first day. The applicability will vary with the types of specimens received. The usefulness of the system is not diminished, however, because it is a simple matter to pick up one colony for subculture and set up the Micro-ID the next day. The identification is not delayed as compared to an overnight identification system. Indeed, in 87% (or 74%) of cases, with Micro-ID the identification occurs 1 day earlier than with overnight systems.

LITERATURE CITED

1. **Aldridge, K. E., B. B. Gardner, S. J. Clark, and J. M. Matsen.** 1978. Comparison of Micro-ID, API 20E, and conventional media systems in identification of *Enterobacteriaceae*. J. Clin. Microbiol. **7**:507–513.
2. **Blazevic, D. J., P. C. Schreckenberger, and J. M. Matsen.** 1973. Evaluation of the PathoTec "Rapid I-D System." Appl. Microbiol. **26**:886–889.
3. **Edwards, P. R., and W. H. Ewing.** 1972. Identification of *Enterobacteriaceae*, 3rd ed. Burgess Publishing Co., Minneapolis.
4. **Matsen, J. M., and D. J. Blazevic.** 1969. Characterization of ornithine decarboxylase-positive, nonmotile strains of the *Klebsiella-Enterobacter* group. Appl. Microbiol. **18**:566–569.
5. **Smith, P. B., K. M. Tomfohrde, D. L. Rhoden, and A. Balows.** 1972. API system: a multitube micromethod for identification of *Enterobacteriaceae*. Appl. Microbiol. **24**:449–452.