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Clinical Evaluation of the MICRO-ID, API 20E, and Conventional Media Systems for Identification of *Enterobacteriaceae*

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MICRO-ID (General Diagnostics, Morris Plains, N.J.) is a new kit system designed for the identification of *Enterobacteriaceae* in 4 h. It consists of 15 biochemical tests of paper disks. Each test is in its own compartment in a molded plastic tray. Only one reagent need be added to the system (2 drops of 20% KOH, which is added to the Voges-Proskauer test). Based on the pattern of positive and negative biochemical test results, a five-digit octal code number is calculated. An identification is derived from a computer-generated identification manual. A study was conducted to compare three systems—the MICRO-ID 4-h and the API 20E (Analytab Products Inc., Plainview, N.Y.) 18- to 24-h systems 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 allow the laboratory to best use the data. The MICRO-ID compared quite favorably with conventional media. MICRO-ID yielded incorrect identifications with 1.5% of the isolates tested (API 20E, 4.7% misidentification rate). Half the MICRO-ID misidentifications occurred when the system identified a *Citrobacter diversus* as a lysine-negative *Escherichia coli*; all gave one octal number. A direct comparison of the MICRO-ID and API 20E was of limited value because percentage agreements were merely the sums of the errors of each. The ease of inoculation, the requirement for the addition of only one reagent, and the 4-h capability make the MICRO-ID system an extremely attractive development in the field of bacterial identification.

Reagent-impregnated paper strips have been used for many years for the rapid identification of *Enterobacteriaceae* (7). The MICRO-ID is a second-generation reagent-impregnated paper disk system containing 15 biochemical reactions in a molded plastic tray with a hinged cover (Fig. 1). The unit is designed for the 4-h identification of *Enterobacteriaceae* from a primary isolation medium. The biochemical tests, each in its own compartment, are: Voges-Proskauer, reduction of nitrate, deamination of phenylalanine, hydrogen sulfide production, indole production, decarboxylation of ornithine and lysine, malonate utilization, urea, esculin hydrolysis, *o*-nitrophenyl-β-D-galactopyranoside hydrolysis, and sugar fermentation tests for arabinose, adonitol, inositol, and sorbitol. Each compartment contains both the substrate and the indicator. MICRO-ID differs from other commercially available products in that only one reagent is added to the system (2 drops of 20% KOH to the Voges-Proskauer compartment) and results are available after 4 h of incubation. No reaction need be overlaid with oil, and each compartment receives 0.2 ml of inoculum equal to or greater than a 0.5 MacFarland turbidity standard.

Positive and negative reactions are divided into five groups of three tests each, and a five-digit number, or octal code, is generated from the test results. Accompanying the product is a computer-generated identification manual that provides the name of the most likely species; the LFR (likelihood fraction), a value that allows one to calculate how closely the test pattern observed with the unknown resembles the pattern most likely to be produced by each of the species; and another value, the PNOR (normalized probability), which allows one to determine the best choice of species by calculating how well each species is separated from the others. The manual also indicates the quality of an identification and specific additional tests, if re-

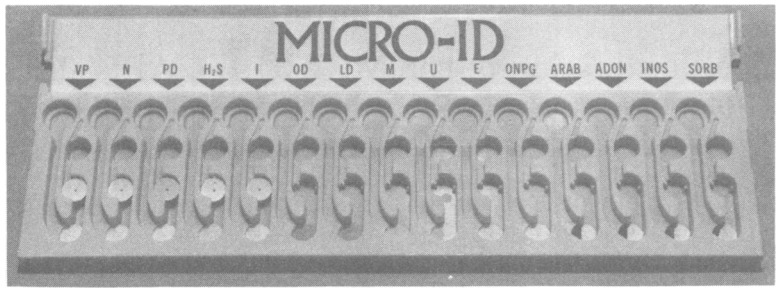

FIG. 1. *MICRO-ID contains 15 biochemical reactions on paper-impregnated disks. Each biochemical test is in its own compartment in a hard plastic tray. The first 5 tests on the left have a substrate disk and a detection disk; after incubation, the MICRO-ID is tilted to wet the upper disks. Only one reagent, 2 drops of 20% KOH to the Voges-Proskauer (VP) reaction, is added to the system. N, Nitrate reduction; PD, phenylalanine deamination; H_2S, H_2S production; I, indole production; OD, ornithine decarboxylation; LD, lysine decarboxylation; M, malonate utilization; U, urea; E, esculin hydrolysis; ONPG, o-nitrophenyl-β-D-galactopyranoside hydrolysis; ARAB, arabinose fermentation; ADON, adonitol fermentation; INOS, inositol fermentation; SORB, sorbitol fermentation.*

quired.

The API 20E system, made for the 18- to 24-h identification of enteric bacteria, is well described in the literature (6, 8) and has become a commonly used commercial system for the identification of *Enterobacteriaceae*. Analytab Products Inc. (Plainview, N.Y.) provided a computer-based identification manual that yields the likelihoods of identifications from numerically calculated biotype numbers. Both the MICRO-ID and the API manuals are based on data generated in the field.

A study was undertaken to compare three systems: the 24-h MICRO-ID, the 24-h API 20E, and a conventional media system based on the Center for Disease Control (CDC) biochemical series (3, 4).

(This work was presented in part at the 78th Annual Meeting of the American Society for Microbiology, Las Vegas, Nev. [S. C. Edberg, B. Atkinson, C. Chambers, H. Moore, C. Zorzon, and J. M. Singer, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, C167, p. 305].)

MATERIALS AND METHODS

Bacterial strains. A total of 768 fresh clinical isolates were obtained from the Microbiology and Immunology Laboratory, Montefiore Hospital and Medical Center, the Albert Einstein College of Medicine (New York, N.Y.). All clinical cultures were sequential isolates from the routine service laboratory except that colonies consistent with *Escherichia coli*, by morphology, were excluded after 100 strains were encountered in order to avoid a preponderance of this species in the data. Ten stock cultures were from the same source, and additional strains were from the City of New York Department of Health (courtesy of Y. Fauer).

Conventional identification. *Enterobacteriaceae* were identified according to procedures described by the CDC (4, 5). Conventional tests routinely used included: triple sugar iron agar, indole production, deoxyribonuclease, esculin hydrolysis, decarboxylation of ornithine and lysine, deamination of phenylalanine, hydrolysis of o-nitrophenyl-β-D-galactopyranoside, motility, utilization of citrate and malonate, fermentation of sorbitol, and production of cytochrome *c* oxidase. When necessary, appropriate serological and additional biochemical tests were performed. During the course of this investigation the taxonomic nomenclature of Edwards and Ewing (4, 5) rather than that of *Bergey's Manual of Determinative Bacteriology* (3) was used. Recently, changes in taxonomy have been recommended by the CDC (2). Whereas some of these changes were simply of nomenclature, others involved biochemical tests that were not used during the course of this study. Where the Edwards and Ewing taxonomy (4, 5) and the new CDC nomenclature (2) yield the same genus and species names based on the biochemical battery used in this study, we so indicate (Table 1).

MICRO-ID. For the MICRO-ID, directions supplied by the manufacturer were followed. All reactions were read after 4 h of incubation, and a five-digit octal number was calculated. Using the MICRO-ID computer identification manual (September 1978 edition), the isolate was given one of the following five designations: (i) identified to genus and species, (ii) identified to genus level only, (iii) two or more choices as possible identifications ("not separated"), (iv) octal code not in the computer identification manual, or (v) misidentified.

API 20E. For the API 20E, directions for *Enterobacteriaceae* supplied by the manufacturer were followed except that before inoculation each isolate was tested for its ability to produce cytochrome *c* oxidase. The API strips were incubated for 20 to 24 h, and a biotype number was calculated. Using the API 20E profile index (April 1978 edition), each isolate was assigned one of the following five designations: (i) identified to genus and species, (ii) identified to the genus level only, (iii) two or more choices as possible identification ("not separated"), (iv) biotype number

TABLE 1. *Number of isolates and stock cultures studied in the evaluation*[a]

Name	No.	
	Clinical isolates	Stock cultures
Escherichia coli	157	0
Shigella species	4	0
Shigella sonnei	3	0
Edwardsiella tarda	0	1
Salmonella typhi	0	1
Salmonella enteritidis	20	3
Arizona hinshawii	0	2
Citrobacter freundii	34	1
Citrobacter diversus	26	0
Klebsiella pneumoniae	140	0
Klebsiella ozaenae	15	0
Enterobacter aerogenes	25	0
Enterobacter cloacae	53	0
Enterobacter hafniae	15	0
Enterobacter agglomerans	9	0
Serratia marcescens	29	0
Serratia liquefaciens	8	0
Proteus vulgaris	17	0
Proteus mirabilis	113	0
Proteus morganii	60	0
Proteus rettgeri	12	0
Providencia alcalifaciens	2	0
Providencia stuartii	26	0
Yersinia enterocolitica	0	2

[a] Conventional identifications were made according to Edwards and Ewing (4) and Ewing (5). According to recent CDC nomenclature (2) and based on the biochemical tests performed in this study, the following names are interchangeable: *K. pneumoniae,* indole positive (*K. oxytoca*), *E. hafniae* (*Hafnia alvei*), and *P. morganii* (*Morganella morganii*). One of the 12 *P. rettgeri* corresponded to *P. stuartii,* urea positive; the remaining 11 corresponded to *P. rettgeri. Enterobacter sakazakii* and *Enterobacter gergoviae,* if isolated, would be considered *E. aerogenes,* and *C. amalonaticus* would correspond to *C. freundii.*

not in the profile index, or (v) misidentified.

Procedure. After isolation on a primary plate, each isolate was restreaked once to insure purity and then identified by each of the three systems at the same time. The study was divided among bacteriology technologists. There was no correlation between errors in either system and the people who did the work. To closely parallel the actual clinical state, isolates were tested in each kit system once.

Evaluation. We chose to present the data from this study in a somewhat different form from that previously used in clinical microbiology. An in-house computer program (in collaboration with M. Feuer, Data Processing, Montefiore Hospital and Medical Center) was written so that the data derived from the three different identification systems could be easily compared. The resultant chart, which we call a comparison table, allows the display of all data in our study. The tables are constructed so that, in a uniform manner that does not require the reader to follow an often complex flow chart, system A can be compared with system B, A compared with C, and B compared with C. The strains identified from system A (the conventional system) are listed vertically, along with a column showing the total number of each species identified by system A. Horizontally, the names derived from system B (the MICRO-ID) are listed. The table is utilized in much the same way as a mileage chart to determine the distance between cities is used. For example, Table 2 shows that 157 *E. coli* isolates were identified by the conventional system; from the *E. coli* row on the horizontal axis it is seen that the MICRO-ID identified 154 (98.1%) of these as *E. coli* and 3 (1.9%) as "not separated." The dashes in the table refer to combinations that are not encountered and are used rather than zeroes to facilitate the user's orientation in space and to allow the eye to effortlessly travel horizontally and vertically on the table. To determine what the conventional biochemical series named an isolate when the MICRO-ID called it *E. coli,* one finds *E. coli* on the horizontal MICRO-ID axis and, following the column down the vertical axis, finds that of the 161 *E. coli* identified by MICRO-ID, 154 (95.7%) were *E. coli,* 1 was *Citrobacter freundii,* and 6 (3.7%) were *C. diversus* by the conventional system. The conduct of each of the strains studied can, therefore, be presented in one table. Likewise, Table 3 presents the results of system A (conventional) versus those of system C (API 20E), and Table 4 presents the results of system B (MICRO-ID) versus those of system C (API 20E). The comparison tables, therefore, allow a more direct visualization of areas of disagreement between multiple systems than do the traditional tables that vertically list results.

RESULTS

Tables 2, 3, and 4 show comparisons of the three tested systems. The tables were designed to show the name of an isolate in one system versus the name(s) of the isolate in each other system. In this way all isolates used in the study were presented. Percentages of general agreement between the three systems were also calculated.

After 4 h of incubation, MICRO-ID and conventional procedures (Table 2) agreed on the genus and species name of an isolate in 719 of 778 cases (92.4%). In 26 cases (3.3%) MICRO-ID yielded a "not separated" answer (two or more choices possible). The correct genera and species were listed among the choices in 25 of these 26 cases. The MICRO-ID identification manual provided a series of biochemical reactions to separate the choices. Using the recommended tests, the correct genus and species names were derived in all 25 cases. In one case (0.1%) an octal number could not be found in the MICRO-ID identification manual. This isolate was correctly identified from an extended computer manual by calling the MICRO-ID customer service telephone number. Overall, therefore, the

TABLE 2. *Comparison of conventional and MICRO-ID identifications of isolates*[a]

CONVENTIONAL IDENTIFICATION	Total number of strains	MICRO-ID IDENTIFICATION: *E. coli*	*Shigella* sp	*S. sonnei*	*E. tarda*	*S. typhi*	*S. enteritidis*	*A. hinshawii*	*C. freundii*	*C. diversus*	*K. pneumoniae*	*K. ozaenae*	*E. aerogenes*	*E. cloacae*	*E. hafniae*	*E. agglomerans*	*S. marcescens*	*S. liquefaciens*	*P. vulgaris*	*P. mirabilis*	*P. morganii*	*P. rettgeri*	*P. alcalifaciens*	*P. stuartii*	*Y. enterocolitica*	*Citrobacter* sp	*Proteus* sp	*Providencia* sp	*Serratia* sp	Not in book	Not separated
E. coli	157	154																													3
Shigella sp	4		4																												
S. sonnei	3			3																											
E. tarda	1				1																										
S. typhi	1					1																									
S. enteritidis	23						23																								
A. hinshawii	2							2																							
C. freundii	35	1							33	1																					
C. diversus	26	6								13																					7
K. pneumoniae	140										138																			1	1
K. ozaenae	15										1	14																			
E. aerogenes	25												25																		
E. cloacae	53													44																	9
E. hafniae	15														15																
E. agglomerans	9															7															2
S. marcescens	29																26												1		2
S. liquefaciens	8																	7													1
P. vulgaris	17																		15							2					
P. mirabilis	113																		1	107						5					
P. morganii	60																			2	56					1					1
P. rettgeri	12																					8				4					
P. alcalifaciens	2																						1				1				
P. stuartii	26																							20				6			
Y. enterocolitica	2																								2						

[a] This and Tables 3 and 4 are used in much the same way as standard mileage charts are used for determining the distance between cities. For example, if one wished to determine the MICRO-ID efficacy for the identification of *K. pneumoniae,* one would determine, from the "conventional identification" column, that 140 strains were tested. Following a parallel line to the "MICRO-ID identification" column, one would find that the MICRO-ID identified 138 of these as *K. pneumoniae,* 1 as "not in book," and 1 as "not separated." —, Combination not encountered, or 0.

MICRO-ID system correctly identified to the genus and species level 743 of 778 isolates (95.5%). In 20 cases (2.6%) the MICRO-ID provided only genus identifications. In all 20 cases the correct genera were identified. MICRO-ID supplied incorrect identifications in 12 of 778 cases (1.5%). The major discrepancy between the conventional battery and the MICRO-ID system occurred when the MICRO-ID identified 6 *C. diversus* isolates as lysine-negative *E. coli* strains (Table 2). All 6 misidentifications yielded one octal number, 23031.

After 20 to 24 h of incubation, the API 20E and conventional systems (Table 3) agreed on the genus and species name of an isolate in 706 of 778 cases (90.7%). In 29 cases (3.7%) the API 20E yielded biotype numbers that were not found in the profile index. All 29 biotype numbers were checked with the API computer services. Eleven isolates of the 29 were correctly identified to genus and species, 8 yielded one or more choices possible with low selectivity, and 10 were misidentified. Of these 8 cases in which one or more choices were listed as possible identifications, the correct choice was present 6 times. The computer service provided sufficient discriminating biochemical tests to differentiate these 6. Including the diagnoses provided by the API computer services, the API 20E correctly identified 721 of 778 isolates (92.7%). In 2 cases the API 20E yielded biotype numbers with one or more species as possible answers. In both cases the correct species was listed, together with a series of biochemical reactions to identify the correct choice. In 18 cases (2.3%) the API 20E provided correct genus identifications but no species identifications. In all 18 cases the correct genera were identified. Of these 18 cases, 12 occurred when the system called a *C. diversus* a *Citrobacter* species. In 23 of 778 cases (3.0%) the API provided incorrect identifications. Including the 12 misidentifications provided by the API computer services from the 29 isolates "not in book," the overall misidentification rate was 4.5%. Overall, therefore, the API 20E provided the correct genus and species identifications 723

TABLE 3. *Comparison of conventional and API 20E identifications of isolates*[a]

CONVENTIONAL IDENTIFICATION	Total number of strains	API 20E IDENTIFICATION: *E. coli*	*Shigella* sp	*S. sonnei*	*E. tarda*	*S. typhi*	*S. enteritidis*	*A. hinshawii*	*C. freundii*	*C. diversus*	*K. pneumoniae*	*K. ozaenae*	*E. aerogenes*	*E. cloacae*	*E. hafniae*	*E. agglomerans*	*S. marcescens*	*S. liquefaciens*	*P. vulgaris*	*P. mirabilis*	*P. morganii*	*P. rettgeri*	*P. alcalifaciens*	*P. stuartii*	*Y. enterocolitica*	*Citrobacter* sp	*Proteus* sp	*Providencia* sp	*Serratia* sp	Not in book	Not separated
E. coli	157	153	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	3	-
Shigella sp	4	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. sonnei	3	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
E. tarda	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. typhi	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. enteritidis	23	-	-	-	-	-	22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
A. hinshawii	2	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. freundii	35	-	-	-	-	-	-	-	32	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-
C. diversus	26	-	-	-	-	-	-	-	1	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12	-	-	-	6	-
K. pneumoniae	140	-	-	-	-	-	-	-	-	-	138	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-
K. ozaenae	15	-	-	-	-	-	-	-	-	-	4	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E. aerogenes	25	-	-	-	-	-	-	-	-	-	-	-	23	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
E. cloacae	53	-	-	-	-	-	-	-	-	-	-	-	-	48	-	-	-	-	-	-	-	-	-	-	1	-	-	-	4	-
E. hafniae	15	-	-	-	-	-	-	-	-	-	-	-	-	-	14	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
E. agglomerans	9	-	-	-	-	-	-	-	2	-	-	-	-	-	-	6	-	-	-	-	-	-	-	-	-	-	-	-	1	-
S. marcescens	29	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	23	1	-	-	-	-	-	-	-	-	-	-	3	-
S. liquefaciens	8	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	3	-	-	-	-	-	-	-	-	-	2	-	-
P. vulgaris	17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	13	-	-	-	-	-	-	1	-	-	3	-
P. mirabilis	113	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	109	3	-	-	-	-	1	-	-	-	-
P. morganii	60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	57	-	-	-	-	-	-	-	1	-
P. rettgeri	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	-	4	-	-	-	-	-	-
P. alcalifaciens	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-
P. stuartii	26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	26	-	-	-	-	-	-
Y. enterocolitica	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-

[a] See footnote to Table 2.

of 778 times (92.9%).

The MICRO-ID and the API 20E agreed on the genus and species names of isolates in 667 of 778 cases (85.7%) (Table 4). This figure has great practical importance for the practicing clinical microbiologist, because it demonstrates the potential danger of comparing one commercial system directly with another without a proven reference system available to act as the arbitrator. An isolate misidentified, identified to genus only, or not separated (one or more choices as possible identification) could not be expected to agree with the genus and species name derived from the second system. The 14% disagreement is close to the sum of the genus and species disagreements derived from each system.

DISCUSSION

Both the MICRO-ID and the API 20E identified members of the family *Enterobacteriaceae* with a high degree of accuracy (Tables 2 and 3). We chose to analyze the data in a form which not only presents the identification of all strains used in the study but also does not require the additional explicative tables. These tables can easily be used to evaluate one product as compared with another. Whereas percent agreement figures are general reflections of the overall accuracy of a system, they do not adequately reflect the particular strengths and weaknesses of each system tested and often require additional explanatory materials. Comparison tables present results which clearly show all the data and do not require lengthy commentary. With this information a laboratory can optimally utilize a given system and, with a knowledge of the areas of discrepancy between the multiple systems, will exercise more caution in reporting results from these areas.

Any clinical evaluation can be flavored by the mix of isolates used, the personnel assigned, and the method of data analysis. We chose to analyze the data in the form presented because the 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 weakness of each system. For example, if there had been an overrepresentation of *C. diversus,* the MICRO-ID percentage of agreement would have suffered; if there had been an overrepresentation of *Serratia liquefaciens* and/or *C. diversus,* the API 20E percentage of agreement with the conventional method would have suffered. Based on colonial morphology, some selections

TABLE 4. *Comparison of MICRO-ID and API 20E identifications of isolates*[a]

API IDENTIFICATION	Total number of strains	E. coli	Shigella sp	S. sonnei	E. tarda	S. typhi	S. enteritidis	A. hinshawii	C. freundii	C. diversus	K. pneumoniae	K. ozaenae	E. aerogenes	E. cloacae	E. hafniae	E. agglomerans	S. marcescens	S. liquefaciens	P. vulgaris	P. mirabilis	P. morganii	P. rettgeri	P. alcalifaciens	P. stuartii	Y. enterocolitica	Citrobacter sp	Proteus sp	Providencia sp	Serratia sp	Not in book	Not separated
	Total number of strains	161	4	3	1	1	23	2	33	14	139	14	25	44	15	7	26	7	16	109	56	8	1	20	2	0	12	7	1	1	26
	E. coli	153	150	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
	Shigella sp	4	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	S. sonnei	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	E. tarda	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	S. typhi	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	S. enteritidis	22	-	-	-	-	-	22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	A. hinshawii	2	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	C. freundii	35	2	-	-	-	-	-	-	30	1	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	C. diversus	7	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	K. pneumoniae	142	-	-	-	-	-	-	-	-	-	137	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
	K. ozaenae	11	-	-	-	-	-	-	-	-	-	-	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	E. aerogenes	27	-	-	-	-	-	-	-	-	-	-	-	23	-	-	1	2	-	-	-	-	-	-	-	-	-	-	-	-	1
	E. cloacae	50	-	-	-	-	-	-	-	-	-	-	-	1	39	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	9
	E. hafniae	14	-	-	-	-	-	-	-	-	-	-	-	-	-	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	E. agglomerans	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	2
	S. marcescens	23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20	-	-	-	-	-	-	-	-	-	-	-	1	2
	S. liquefaciens	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	3	-	-	-	-	-	-	-	-	-	-	-	-
	P. vulgaris	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	13	-	1	-	-	-	-	-	-	-	-	-	-
	P. mirabilis	110	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	104	1	-	-	-	-	-	4	-	-	-	-
	P. morganii	60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	53	-	-	-	-	-	2	-	-	-	1
	P. rettgeri	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7	-	-	-	-	1	-	-	-	-
	P. alcalifaciens	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1	-	-	-
	P. stuartii	30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20	-	-	3	6	-	-	-
	Y. enterocolitica	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-
	Citrobacter sp	14	3	-	-	-	-	-	-	6	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	0	-	-	-	-	4
	Proteus sp	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1	-	-	-	-
	Providencia sp	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	-	-	-
	Serratia sp	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	0	-	-
	Not in book	29	6	-	-	-	1	-	3	3	2	-	1	4	1	1	3	-	2	-	1	-	-	-	-	-	1	-	-	0	-
	Not separated	2	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0

MICRO-ID IDENTIFICATION

[a] See footnote to Table 2.

of specimens were made to prevent overrepresentation by the most commonly encountered species. Since more than 85% of strains isolated in our routine laboratory are *E. coli, Klebsiella pneumoniae,* or *Proteus mirabilis,* the overall percentage of agreement of any kit with conventional procedures will be significantly affected if the kit showed a particular ability to identify or misidentify any of these species. Comparison tables allow a rapid means of determining where a system errs while permitting the user to calculate percent agreements for each species.

It was felt that fresh clinical isolates would more closely mimic the performance of a system in the clinical setting than would stock cultures. Bacteria after storage or multiple transfers in vitro may undergo dissociation and lose R-factors. Because the MICRO-ID measures constitutive enzymes, it is possible that on storage some or all members of the *Enterobacteriaceae* may turn down or turn off enzyme systems, resulting in negative or weak reactions, whereas fresh isolates may produce a positive reaction. In its instructions to users, the manufacturer of MICRO-ID recommends that the inoculum density for stock cultures be four times that of fresh isolates.

To evaluate commercial systems on the most equitable basis while at the same time maintaining conditions as close to actual usage as possible, the personnel chosen for the study were experienced laboratory technologists who performed the evaluation in parallel with their normal workload. To insure a steady, unhurried generation of data, no technologist performed more than five evaluation cultures per day.

Table 4 shows that the comparison of a new test procedure with other than a standard reference procedure carries with it an inherent error. Errors in the comparison approximate the sums of the errors of each of the systems tested. The low general rate of agreement (85%) between the MICRO-ID and the API 20E is a demonstration of this concept. Each test system establishes its own data base founded on its own biochemical formulations, test series chosen, and means to handle the data. Because the biochemical test formulations may not be the same in all test kits, and no kit uses medium formulations exactly the same as those used by the CDC, a direct comparison of biochemical tests in one system with those of any other system, including a conventional one, is nonproductive. If, for example, a hypothetical kit were able to detect

H_2S production by *Enterobacter cloacae* but not other *Enterobacter* species, the kit could use this characteristic to differentiate *E. cloacae* even though the H_2S reaction is considered false-positive compared with the conventional test. This hypothetical example demonstrates the need each identification system has for its own data base. Computer-generated identification manuals, which usually accompany kits, provide selections based on patterns of positive and negative results derived from the kits, not from conventional tests. The manual may also provide a measure of probability, as in the case of the API 20E and MICRO-ID, and a likelihood fraction, as with MICRO-ID, both of which assist the user in "fine-tuning" the answer. Errors in identification can be solely a function of the computer-generated manual, even with a correct biochemical test series. The identification of an isolate should, of course, be correct, but errors of identification will be unique to the test system. Therefore, each system tends to have its strengths and weaknesses, and, when comparing one against the other directly, a low order of agreement is achieved because the errors of both are summed.

In our hands, the new MICRO-ID system is accurate and, in terms of the common criteria of acceptance, easily exceeds the 95% agreement level with conventional methodology. It appears that changes in the computer identification manual have improved the level of identification from the first edition of the manual (1). The ease of inoculation, the requirement for the addition of only one reagent, and the 4-h capability make it an extremely attractive development in the field of bacterial identification.

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