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Evaluation of the Four-Hour Micro-ID Technique for Direct Identification of Oxidase-Negative, Gram-Negative Rods from Blood Cultures

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A 4-h Micro-ID technique for direct identification of oxidase-negative gram-negative rods from positive blood cultures was compared to subculture and species identification of single colonies by API 20E and Micro-ID, using standardized inocula. A total of 127 patients (220 positive cultures) were studied. Isolates included 96 *Escherichia coli*, 46 *Klebsiella pneumoniae*, 7 *Klebsiella oxytoca*, 8 *Enterobacter aerogenes*, 17 *Enterobacter cloacae*, 19 *Serratia marcescens*, 2 *Serratia liquefaciens*, 8 *Proteus mirabilis*, 1 *Salmonella* species, 1 *Morganella morganii*, 6 *Haemophilus influenzae*, 2 *Haemophilus parainfluenzae*, 3 *Bacteroides fragilis*, 3 *Acinetobacter calcoaceticus* biotype *anitratus*, and 1 *Pseudomonas maltophilia*. In 90% of the cultures, identification by Micro-ID was identical to that obtained after subculture; if the 15 non-enterobacterial isolates were excluded, the corresponding figure was 96.6%. Enterobacteria identified incorrectly by direct Micro-ID were three *S. marcescens* (two identified as *S. liquefaciens*, one as *Hafnia alvei*), two *S. liquefaciens* (both identified as *E. cloacae*), and two *K. pneumoniae* (one identified as *Klebsiella ozaenae*, the other as *Serratia rubidaea*). None of the 15 non-enterobacterial cultures were correctly identified by Micro-ID (non-identifiable, or classified as *Providencia/Yersinia/Klebsiella* species). Although biochemical discrepancies between direct and final Micro-ID tests occurred in 41% of the enterobacterial cultures, this did not seriously interfere with identification. Direct species identification of *Enterobacteriaceae* from blood cultures by direct Micro-ID is accurate and easily performed and identified organisms within 4 h compared to at least 24 h by most other methods; the direct Micro-ID technique would be rendered even more valuable by the additional capability of identifying non-enterobacterial gram-negative isolates.

The clinical importance of bacteremia necessitates the earliest possible isolation, species identification, and antimicrobial sensitivity testing of organisms from positive blood cultures (8, 10, 11, 14). Methods previously described for rapid identification of bacteria from such cultures have utilized pellets derived from positive culture bottles (10, 14) and direct inoculation of API 20E (4; M. F. Sierra, E. J. C. Goldstein, G. F. Pringle, and S. Landesman, Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, C134, p. 332) and Minitek systems (6), respectively. Bacteria from blood culture sediments were identified in 4 h or less (8, 10, 14), in contrast to direct inoculation of API 20E and Minitek (BBL Microbiology Systems, Cockeysville, Md.), respectively, where at least overnight incubation was required (4, 6). Currently, gram-negative bacteria of the family *Enterobacteriaceae* comprise a large proportion of positive blood cultures from hospitalized patients (11, 12). In tests utilizing sedimented positive cultures, enterobacterial identification has been by conventional methods (10, 14), Pathotec strips (10), and the Micro-ID system (8). Utilization of conventional blood culture bottles in most rapid identification studies has permitted aspiration and sedimentation of positive broth culture aliquots without admixture of erythrocytes.

The BACTEC blood culture system does not allow for separation of erythrocytes and culture broth during incubation. Laboratories using this technique require a rapid method for identification of *Enterobacteriaceae* (and other bacteria) from agitated positive blood culture bottles. Kocka and Morello (11) have described a method whereby blood from positive bottles is differentially sedimented to remove erythrocytes and *Enterobacteriaceae* in the pellet presumptively identified by the Inolex Enteric I

card method. We have adapted this differential sedimentation technique for rapid identification of *Enterobacteriaceae* and oxidase-negative non-*Enterobacteriaceae* from positive BACTEC bottles: in our modification of the above system, we have utilized the Micro-ID system, a well-recognized rapid method of enterobacterial species identification based on the effect of pre-formed bacterial enzymes on a series of impregnated filter disks in a packaged strip (1–3, 5, 7). Results of direct inoculation tests are compared with those obtained after subculture and testing of single colonies with both the Micro-ID and API 20E (13) methods.

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MATERIALS AND METHODS

Blood culture. Blood cultures were drawn by a physician or technologist, with 3 to 5 ml of blood drawn directly into 30 ml each of 6B or 8B aerobic and 7B anaerobic BACTEC bottles (Johnston Laboratories, Cockeysville, Md.). The media contained in the above bottles are complex, consisting of a tryptic soy broth base with multiple additives, including ^{14}C-labeled substrates. Details of media components may be found in the manufacturer's package insert (Johnston Laboratories). For each venipuncture, each of the two bottles was inoculated. Bottles were incubated at 35°C with shaking and examined for growth visually as well as radiometrically (BACTEC model 460 culture system, Johnston Laboratories).

Inoculum preparation for direct Micro-ID testing. Bottles found to be visually or radiometrically positive were initially Gram stained: cultures containing gram-negative rods only were included in the study. Portions of 5 or 10 ml of blood from visually or radiometrically positive bottles, respectively, were sedimented for 10 min at 150 × *g*; supernatants were drawn off with sterile Pasteur pipettes and sedimented for 10 min at 1,000 × *g*. Pellets were tested for cytochrome oxidase activity by touching the edge with a sterile applicator stick, avoiding contact with erythrocytes as much as possible, and rubbing bacteria onto a Pathotec strip (General Diagnostics, Morris Plains, N.J.). Oxidase-negative pellets were processed further by suspension in 5 ml of saline with a cotton swab, so as to yield a suspension with turbidity equivalent to a 0.5 McFarland standard. Micro-ID strips (General Diagnostics) were inoculated and read after 4 h of incubation at 35°C, according to the manufacturer's instructions, utilizing a computer code book (September 1978 and July 1979 editions). With the exception of some previously designated *Proteus* strains being listed as *Providencia stuartii* (urease positive) in the latest edition, no identification differences were found in the two editions of the code book in our study. The manufacturer's computer facilities were consulted with regard to octal numbers not present in the code book.

Subculture and identification of isolated colonies. Positive blood cultures were subcultured to sheep blood agar plates (aerobic and anaerobic incubation), MacConkey/eosin methylene blue, and chocolate agar (aerobic incubation). After 16 to 18 h at 35°C, colonies were inoculated into Micro-ID (as above) and API 20E (Analytab Products, Inc., Plainview, N.Y.) systems, and identified with the aid of computer code books. *Enterobacteriaceae* whose identification differed in direct Micro-ID, final Micro-ID, or final API 20E tests were identified by the following conventional tests: reactions in triple sugar iron agar, hydrogen sulfide-indole-motility agar, urease, phenylalanine deaminase (PD), lysine decarboxylase, ornithine decarboxylase, arginine dihydrolase, citrate, methyl red, Voges-Proskauer, arabinose, inositol, and rhamnose (the latter three with cystine Trypticase agar base). Cultures yielding oxidase-negative nonfermenters, *Haemophilus* species, and gram-negative anaerobic rods were included in the study. Nonfermenters were identified by API 20E; *Haemophilus* species were identified by Gram staining, colonial morphology, and requirement of X and V factors; and anaerobes were identified by Gram staining, colonial morphology, and growth on bile-containing media and sensitivity to a 1-mg kanamycin disk.

RESULTS

Blood cultures from 127 patients (220 isolates) yielded a single species of gram-negative rods. The spectrum of organisms isolated is listed in Table 1. As can be seen, *Escherichia coli* was the commonest isolate (96 strains), followed by *Klebsiella pneumoniae* (46), *Serratia marcescens* (19), *Enterobacter cloacae* (17), *Enterobacter aerogenes* (8), *Proteus mirabilis* (8), *Klebsiella oxytoca* (7), *Haemophilus influenzae* (6), *Bacteroides fragilis* (3), *Acinetobacter calcoaceticus* biotype *anitratus* (3), *Haemophilus*

TABLE 1. *Gram-negative rods isolated from blood cultures*

Organism	No. of isolates	% Total isolates
Escherichia coli	96	43.6
Klebsiella pneumoniae	46	20.9
Klebsiella oxytoca	7	3.2
Enterobacter cloacae	17	7.7
Enterobacter aerogenes	8	3.6
Serratia marcescens	19	8.6
Serratia liquefaciens	2	0.9
Proteus mirabilis	8	3.6
Morganella morganii	1	0.5
Salmonella group D	1	0.5
Haemophilus influenzae	6	2.7
Haemophilus parainfluenzae	2	0.9
Bacteroides fragilis	3	1.4
Acinetobacter calcoaceticus biotype *anitratus*	3	1.4
Pseudomonas maltophilia	1	0.5

parainfluenzae (2), *Serratia liquefaciens* (2), *Morganella morganii* (1), *Salmonella* species (1), and *Pseudomonas maltophilia* (1).

Biochemical reactions in direct Micro-ID tests differed from those of final Micro-ID in a large proportion (41%) of enterobacterial cultures. Reactions which yielded the highest degree of variability included sorbitol, adonitol, inositol, urease, esculin, ornithine decarboxylase, PD, and *o*-nitrophenyl-β-D-galactopyranoside (ONPG) (Table 2). This disagreement did not, however, adversely affect direct and final Micro-ID species identification (by computer code book or manufacturer's computer facilities), which corresponded, and was the same as that obtained by API single-colony testing, in 96.6% (198/205) of enterobacterial cultures. When all 220 gram-negative isolates were included, correct identification by direct Micro-ID occurred in 90% of cultures. Seven enterobacterial cultures were misidentified by direct Micro-ID (Table 3): these organisms comprised three *S. marcescens* (two identified as *S. liquefaciens*, one as *Hafnia alvei*), two *S. liquefaciens* (both identified as *E. cloacae*), and two *K. pneumoniae* (one identified as *Klebsiella ozaenae*, the other as *Serratia rubidaea*). Three organisms were misidentified by final Micro-ID testing (Table 3): two *S. liquefaciens* strains were identified as *Enterobacter agglomerans*, and one *K. pneumoniae* was identified as *S. rubidaea*. In all seven strains, single-colony speciation by API 20E corresponded to that obtained in conventional tests (Table 3).

TABLE 2. *Correspondence between reactions for Enterobacteriaceae in direct and final Micro-ID tests*

Reaction	No. of isolates with:	
	Positive direct Micro-ID, negative final Micro-ID[a]	Negative direct Micro-ID, positive final Micro-ID[a]
Voges-Proskauer	—[b]	—
Nitrate	—	—
PD	6	1
Hydrogen sulfide	—	—
Indole	—	—
Ornithine decarboxylase	7	3
Lysine decarboxylase	1	2
Malonate	—	2
Urease	8	6
Esculin hydrolysis	2	8
ONPG	—	8
Arabinose	1	2
Adonitol	19	2
Inositol	10	5
Sorbitol	21	—

[a] Total number of isolates giving divergent reactions. Isolates not listed gave identical biochemical reactions in direct and final Micro-ID.

[b] —, No reactions which did not correspond.

All 15 non-enterobacterial isolates were misidentified by both direct and final Micro-ID: five *H. influenzae* strains were identified by both direct and final Micro-ID as *Providencia stuartii* (urease positive), and one strain was identified as *Yersinia enterocolitica/P. stuartii* (urease positive) by both methods. All *H. influenzae* strains were urease and nitrate positive, with various reactions in indole and sorbitol tests. Both *H. parainfluenzae* strains were identified as urease-positive *P. stuartii* with direct Micro-ID (nitrate, urease positive) and as *Yersinia pseudotuberculosis* by final Micro-ID (nitrate, urease, ONPG positive). Two *B. fragilis* strains were misidentified by both direct and final Micro-ID as *Y. pseudotuberculosis* (esculin, ONPG positive), and the remaining *B. fragilis* strain was misidentified as *K. ozaenae* by direct Micro-ID (esculin, ONPG, inositol, sorbitol positive) and as *Y. pseudotuberculosis* by final Micro-ID (esculin, ONPG, sorbitol positive). All three *A. calcoaceticus* strains yielded octal numbers (00010, 01000, 01010) which are not listed in the code book or manufacturer's computer facilities at this time. The one *P. maltophilia* strain was identified by both direct and final Micro-ID as urease-positive *P. stuartii* (nitrate, urease positive).

DISCUSSION

A striking feature of this study was the high percentage (41%) of octal codes yielded by direct Micro-ID testing which differed from those obtained by Micro-ID identification of isolated enterobacterial colonies. In the majority of cases, final Micro-ID species identification yielded more typical reactions. False-positive as well as false-negative reactions occurred in direct Micro-ID tests. The organisms and biochemicals which varied most often were *E. coli* (adonitol, inositol, sorbitol), *K. pneumoniae* (urease), *E. cloacae* (esculin, adonitol, inositol), and *S. marcescens* (adonitol, ONPG, sorbitol). The reason for these discrepancies is not clear, but may be related to small differences in inocula with the two Micro-ID techniques, the presence of blood traces in the direct Micro-ID inoculum, and other undefined compounds in patients' blood which could have influenced reactions in direct Micro-ID biochemical tests. In spite of the above biochemical differences, however, species identification by the two Micro-ID methods (as yielded by computer code book and the manufacturer's computer facilities) corresponded, and

TABLE 3. *Enterobacterial misidentification by direct and final Micro-ID*

Conventional identification	Direct Micro-ID identification	Final Micro-ID identification	Final API 20E identification	Biochemical(s) responsible	Octal no. with direct Micro-ID
S. marcescens	*S. liquefaciens*	*Serratia* species[a]	*Serratia* species[a]	Arabinose, adonitol	61457
S. marcescens	*S. liquefaciens*	*S. marcescens*	*S. marcescens*	Voges-Proskauer, ONPG, lysine decarboxylase, inositol	21041
S. marcescens	*H. alvei*	*S. marcescens*	*S. marcescens*	Inositol	61401
S. liquefaciens	*E. cloacae*	*E. agglomerans*	*S. liquefaciens*	Inositol, ornithine decarboxylase	61071
S. liquefaciens	*E. cloacae*	*E. agglomerans*	*S. liquefaciens*	Inositol, ornithine decarboxylase	61071
K. pneumoniae	*K. ozaenae*	*K. pneumoniae*	*K. pneumoniae*	Voges-Proskauer, urease, inositol	20471
K. pneumoniae	*S. rubidaea*	*S. rubidaea*	*K. pneumoniae*	Urease	60637

[a] Additionally, a raffinose-negative species was identified as *S. marcescens*.

was the same as that obtained by API techniques, in 96.6% of enterobacterial cultures. This figure is similar to that reported by Edberg et al. (8), who used conventional blood culture bottles and only one sedimentation step.

The PD test was the most difficult to interpret, in both direct and single-colony Micro-ID tests. In most positive cases, a light-green color in the inoculum fluid was seen without development of a green color in the disk. False-positive as well as false-negative reactions were seen, but these did not interfere with correct species identification.

We feel that species identification by conventional methods of enterobacteria whose identification corresponded in both Micro-ID methods, as well as in the API technique, was not essential in our study. Conventional biochemical tests were therefore only performed when species identification differed in any one of the above three tests. In problem enterobacterial identifications, the API 20E system invariably agreed with results of conventional tests, while direct and final Micro-ID corresponded with the latter in 0 and 57% of cases, respectively. Nevertheless, direct and final Micro-ID tests corresponded with API 20E in 96.6 and 98.5% of total enterobacterial isolates. The latter results confirm previous reports (1-3, 7) of the accuracy of both methods for single-colony enterobacterial identification. Although the accuracy of direct Micro-ID identification was lower than that of single-colony Micro-ID tests, results are still good enough to support use of this method in the clinical microbiology laboratory. Many enterobacterial strains were isolated more than once from the same patient and could essentially be considered to be the same strain. Because *E. coli* was the commonest isolate and posed no problems in identification, it is realized that results were weighted heavily in one direction. Nevertheless, we feel that our results of a wide cross section of commonly encountered enterobacterial isolates provide clear evidence for the usefulness of Micro-ID in rapid identification of *Enterobacteriaceae* from blood cultures.

In the course of this study, 15 non-enterobacterial isolates were encountered (Table 1), all of which yielded octal numbers which either were not listed in the computer manual or were misidentified as *Providencia/Yersinia/Klebsiella* species. A drawback of the present Micro-ID system as compared to API 20E or other currently available packaged systems is its lack of ability to identify oxidase-negative (and -positive) nonfermenters, which are occasionally misidentified as species of *Enterobacteriaceae*. Modification of the Micro-ID system so as to include identification of nonfermenters would be a distinct advantage. To this end, a 4-h test for glucose fermentation could be added in the future to the Micro-ID panel. In addition, the system may be modified for identification of other bacterial groups (*Haemophilus*, anaerobes); Edberg and colleagues have used this technique for the biotyping of *Haemophilus* species from clinical isolates (9).

In summary, the direct Micro-ID method permits rapid and accurate identification of *Enterobacteriaceae* from blood cultures. The method is easy to use and requires addition of only one reagent (20% KOH) before biochemicals are read. If the identity of organisms is correlated to current patterns of antimicrobial susceptibility, important presumptive information would be available to the clinician 4 h after the blood

culture becomes positive. If this rapid technique is coupled with a rapid method for antimicrobial sensitivity, the physician would receive an even more complete report. Present drawbacks of the Micro-ID system include lack of sensitivity of the PD test and inability to identify nonfermenters and other non-enterobacterial organism groups. If these problems could be solved, the method would become even more valuable.

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