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Evaluation of RapID onE System for Identification of 379 Strains in the Family *Enterobacteriaceae* and Oxidase-Negative, Gram-Negative Nonfermenters

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The ability of the RapID onE system (Innovative Diagnostic Systems, Inc., Norcross, Ga.) to identify 364 strains in the family *Enterobacteriaceae* and 15 oxidase-negative, gram-negative, nonfermentative rods was evaluated. Kits were inoculated with no. 2 McFarland standard suspensions, and reactions were interpreted after 4 h of incubation at 35°C. Overall, the method correctly identified (to the species level or to the genus level for salmonellas and non-*Shigella sonnei Shigella* species) 363 strains (95.8%) without additional tests. For four strains (1.0%), additional tests were required to delineate the correct identification from a range of two or more possibilities; these included one *Serratia liquefaciens* (*Serratia marcescens* or *Serratia liquefaciens*), one *Serratia rubidaea* (*Serratia rubidaea* or *Serratia odorifera*), one *Salmonella typhi* (*Leminorella richardii* or *Salmonella* sp.) and one *Yersinia enterocolitica* (*Yersinia frederiksenii*, *Yersinia intermedia*, or *Yersinia enterocolitica*). Twelve strains (3.2%) were misidentified or yielded codes with no identification; these comprised one *Citrobacter amalonaticus* (no identification), three *Enterobacter hormaechei* (not in the RapID onE database; two *Enterobacter amnigenus*, one *Enterobacter* sp.), one *Serratia liquefaciens* (*Enterobacter cloacae*), one *Serratia rubidaea* (no identification), four *Serratia fonticola* (not in RapID onE database; two *Enterobacter aerogenes*, one *Serratia marcescens*, one not identified), one *Proteus mirabilis* (*Proteus penneri*), and one *Proteus vulgaris* (*Providencia rustigianii*). If the seven strains not included in the database had been excluded, correct identification rates would have risen to 97.6% without additional tests and 98.7% with additional tests, with misidentification rates dropping to 1.3%. The RapID onE system is easy to set up and the results are easy to read, and the system provides an accurate, nonautomated commercially available method for the same-day identification of members of the family *Enterobacteriaceae* and oxidase-negative, gram-negative nonfermenters.

The necessity of identifying clinically significant members of the family *Enterobacteriaceae* has been complicated by the greatly increased number of genera and species as well as taxonomic changes in recent years (7). The need for microbiology laboratories to identify members of the family *Enterobacteriaceae* without the need for extended conventional testing has led to the development of many automated as well as nonautomated commercially available methods. Some of these also identify the more commonly encountered species of oxidase-positive and oxidase-negative, gram-negative nonfermenters (1–6, 8–14, 16–22, 24–27, 29–32).

Recently, Innovative Diagnostic Systems, Inc. (Norcross, Ga.), has applied the same principles used in its anaerobe, streptococcal, and nonfermenter identification methods (i.e., demonstration of preformed enzymes) to the 4-h identification of clinically significant members of the family *Enterobacteriaceae* and oxidase-negative, gram-negative nonfermenters (15, 28). In the study described here, we evaluated the ability of the RapID onE system to identify a spectrum of clinically isolated members of the family *Enterobacteriaceae* and oxidase-negative nonfermenters with and without the aid of supplementary conventional tests.

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MATERIALS AND METHODS

Bacteria. A total of 379 clinically isolated strains in the family *Enterobacteriaceae* and oxidase-negative, nonfermentative rods were tested (Table 1). These organisms were isolated at Hershey Medical Center or the University Hospitals of Cleveland or were kindly provided by G. Hall (Cleveland Clinic, Cleveland, Ohio), J. M. Miller (Centers for Disease Control and Prevention, Atlanta, Ga.), W. M. Janda (California State Department of Health, Berkeley, Calif.), or L. Utrup (SmithKline Beecham Laboratories, King of Prussia, Pa.). Definitive identification of all cultures was done by conventional methods (7); in all cases, when the species was included in the database, identification was also confirmed (with supplementary testing, if necessary) by the overnight API 20E system. All organisms yielded identical identifications by the conventional and the API systems. Strains were stored in double-strength litmus milk (Difco Laboratories, Detroit, Mich.) at −70°C until use. Cultures were transferred and subcultured twice onto MacConkey agar (BBL Microbiology Systems, Cockeysville, Md.) before inoculation of the test strips. Oxidase testing was performed with 1.0% tetramethyl-*p*-phenylenediamine dihydrochloride (Remel, Lenexa, Kans.). Incubation took place at 35°C. Cultures were checked for purity throughout the study by Gram staining and colonial morphology.

RapID onE system. The RapID onE system consists of a test strip with 18 wells and 19 reactions (reactions in the last well occur before and after the addition of reagent). Strips were inoculated with suspensions prepared from MacConkey agar plates in RapID inoculation fluid (Innovative Diagnostic Sys-

tems, Inc.) adjusted to a turbidity of a no. 2 McFarland standard and incubated for 4 h at 35°C. The 18 reactions read without the addition of reagent were as follows: production of urease, arginine dihydrolase, ornithine decarboxylase, and lysine decarboxylase, tetrathionate utilization, hydrolysis of fatty acid ester, sugar aldehyde utilization, sorbitol utilization, hydrolysis of *p*-nitrophenyl-β-D-glucuronide, hydrolysis of *o*-nitrophenyl-β-D-galactoside, hydrolysis of *p*-nitrophenyl-β-D-glucoside, hydrolysis of *p*-nitrophenyl-β-D-xyloside, hydrolysis of *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide, malonate utilization, hydrolysis of proline β-naphthylamide, hydrolysis of γ-glutamyl-β-naphthylamide, hydrolysis of pyrrolidonyl-β-naphthylamide, and adonitol utilization. The last well of the strip is bifunctional, yielding adonitol utilization before and indole production within 2 min after the addition of the Innova spot indole reagent. Colors were interpreted according to the manufacturer's instructions, and a seven-digit computer code was constructed. Because of the absence of a code book at the time that the study was initiated, all codes were referred to the firm's database for interpretation. During the latter portion of the study, a code book was made available by the manufacturer. All identifications made with the preliminary database were checked against those obtained when a more extensive database became available. When discrepancies occurred, the identification obtained with the more extensive database was taken as correct. Identifications were classified as follows: (i) correct without extra tests, corresponding to excellent, very good, good, or implicit identifications, as listed in the database; (ii) probability overlap with low discrimination, necessitating additional testing to delineate the correct identification from two or more possibilities, as listed in the database (in such cases, all supplemental tests required by the database were performed by conventional tests); or (iii) misidentification. For the purposes of the present study, all RapID onE identifications of salmonellas and non-*Shigella sonnei Shigella* species identified to the genus level only were taken as correct. All isolates were tested blindly without prior knowledge of their identification. Additionally, all isolates yielding incorrect identifications by the RapID onE system yielded identical identifications on repeat testing.

RESULTS

In general, RapID onE reactions were easy to interpret. Results of organism identification with this system are presented in Table 1. A total of 363 of 379 strains (95.8%) were correctly identified to the species level (or to the genus level in the case of salmonellas and non-*Shigella sonnei Shigella* species), with 4 (1.0%) requiring additional tests to delineate the correct identification from two possibilities and 12 (3.2%) giving incorrect or no identifications.

The four strains requiring additional tests comprised one *Serratia liquefaciens* (*Serratia marcescens* or *Serratia liquefaciens*), one *Serratia rubidaea* (*Serratia rubidaea* or *Serratia odorifera*), one *Salmonella typhi* (*Leminorella richardii* or *Salmonella* sp.), and one *Yersinia enterocolitica* (*Yersinia frederiksenii*, *Yersinia intermedia*, or *Yersinia enterocolitica*). Additional tests comprised *Salmonella* serology and fermentation of arabinose, raffinose, rhamnose, and melibiose (Table 2).

The 12 strains which either were misidentified or yielded codes with no identification are listed in Table 3. One *Serratia liquefaciens* isolate was misidentified as *Enterobacter cloacae*, one *Proteus mirabilis* isolate was misidentified as *Proteus penneri*, and one *Proteus vulgaris* isolate was misidentified as *Providencia rustigianii*. The RapID onE system currently does not include *Enterobacter hormaechei* (two strains identified as

TABLE 1. Identification of strains with the RapID onE system

Organism (no. of strains tested)	No. (%) of strains identified:		
	Correctly[a]	With probability overlap	Incorrectly[b]
Escherichia coli (44)	44	0	0
Escherichia vulneris (1)	1	0	0
Escherichia fergusonii (1)	1	0	0
Escherichia hermannii (2)	2	0	0
Klebsiella pneumoniae (20)	20	0	0
Klebsiella oxytoca (10)	10	0	0
Citrobacter freundii (12)	12	0	0
Citrobacter diversus (10)	10	0	0
Citrobacter amalonaticus (10)	9	0	1
Enterobacter cloacae (18)	18	0	0
Enterobacter sakazakii (3)	3	0	0
Enterobacter aerogenes (15)	15	0	0
Enterobacter agglomerans (7)	7	0	0
Enterobacter taylorae (5)	5	0	0
Enterobacter asburiae (4)	4	0	0
Enterobacter gergoviae (2)	2	0	0
Enterobacter hormaechei[c] (3)	0	0	3
Enterobacter amnigenus (3)	3	0	0
Serratia marcescens (20)	20	0	0
Serratia liquefaciens (9)	7	1	1
Serratia plymuthica (4)	4	0	0
Serratia odorifera (3)	3	0	0
Serratia rubidaea (4)	2	1	1
Serratia fonticola[c] (4)	0	0	4
Hafnia alvei (3)	3	0	0
Proteus mirabilis (17)	16	0	1
Proteus vulgaris (5)	4	0	1
Morganella morganii (10)	10	0	0
Providencia rettgeri (10)	10	0	0
Providencia stuartii (15)	15	0	0
Providencia alcalifaciens (4)	4	0	0
Providencia rustigianii (1)	1	0	0
Cedecea davisae (3)	3	0	0
Cedecea lapagei (1)	1	0	0
Leminorella richardii (1)	1	0	0
Moellerella wisconsensis (1)	1	0	0
Leclercia adecarboxylata (1)	1	0	0
Edwardsiella tarda (4)	4	0	0
Tatumella ptyseos (2)	2	0	0
Kluyvera cryocrescens (2)	2	0	0
Kluyvera ascorbata (2)	2	0	0
Salmonella spp.[d] (42)	41	1	0
Shigella spp.[e] (12)	12	0	0
Shigella sonnei (group D) (7)	7	0	0
Yersinia enterocolitica (6)	5	1	0
Yersinia pseudotuberculosis (1)	1	0	0
Xanthomonas maltophilia (7)	7	0	0
Acinetobacter calcoaceticus (8)	8	0	0
Total (379)	363 (95.8)	4 (1.0)	12 (3.2)

[a] Correct to the species level or to the genus level for salmonellas and non-*Shigella sonnei Shigella* species.

[b] Includes organisms yielding no codes.

[c] Not currently included in the RapID onE database.

[d] Includes 1 *Salmonella typhi*, two *Salmonella choleraesuis*, 1 *Salmonella paratyphi* type A, 2 *Salmonella paratyphi* type B, 12 *Salmonella agona*, 1 *Salmonella heidelberg*, 1 *Salmonella newport*, 4 *Salmonella typhimurium*, 5 *Salmonella* group D, 1 *Salmonella arizonae*, and 12 unclassified salmonellas (subgroup I).

[e] Includes two *Shigella dysenteriae* (group A), five *Shigella flexneri* (group B), and five *Shigella boydii* (group C).

TABLE 2. Organisms yielding low-probability identifications by the RapID onE system

Conventional identification[a]	RapID onE identification	Extra tests required
Serratia liquefaciens	*Serratia marcescens* or *Serratia liquefaciens*	Fermentation of arabinose and rhamnose
Serratia rubidaea	*Serratia rubidaea* or *Serratia odorifera*	Fermentation of rhamnose
Salmonella typhi	*Leminorella richardii* or *Salmonella* spp.	Serology
Yersinia enterocolitica	*Yersinia frederiksenii*, *Yersinia intermedia*, or *Yersinia enterocolitica*	Fermentation of raffinose, rhamnose, and melibiose

[a] One strain of each species was tested.

Enterobacter amnigenus, one strain identified as *Enterobacter* sp.) or *Serratia fonticola* (two strains misidentified as *Enterobacter aerogenes*, one strain misidentified as *Serratia marcescens*, one strain not identified). One strain each of *Citrobacter amalonaticus* and *Serratia rubidaea* yielded codes with no identifications.

DISCUSSION

The RapID onE system was easy to set up and the results were easy to interpret. A useful feature of this method in comparison with other commercially available kits is the fact that only one reagent (for the detection of indole production) needs to be added. With the exception of *Enterobacter hormaechei* and *Serratia fonticola*, all species tested in the current study, comprising most members of the family *Enterobacteriaceae* and oxidase-negative nonfermenters encountered in a clinical setting, are included in the database. We included several strains of the more recondite species of the family *Enterobacteriaceae* in the current study in order to adequately challenge the system under investigation.

When results of previously published reports were compared with those obtained in the current study, the RapID onE method appeared to perform as well as if not better than commonly used commercial methods such as the overnight API 20E system in the identification of members of the family *Enterobacteriaceae*, including newly described taxa that are not included in the database of other commercially available systems (1–6, 8–14, 16–22, 24–27, 29–32) and that are difficult to differentiate even by conventional methodologies. However, we did not specifically compare the RapID onE system with the API 20E system, so the work presented here does not directly support the equivalence of the RapID onE system to the API 20E system or any other commercially available system. *Enterobacter hormaechei* is, as far as we are aware, not included in the database of any commercially available system, and strains are identified as miscellaneous *Enterobacter* spp. This species is biochemically similar to *Enterobacter taylorae* and can be differentiated from the latter by positive urease, sucrose, and dulcitol fermentations, α-methyl-D-glucoside, and negative esculin hydrolysis reactions (23). *Serratia fonticola* (7) is included in the database of some commercial systems, including the API 20E system. Both species will be included in the database of the RapID onE system as more strains become available and identification algorithms are built.

TABLE 3. Organisms misidentified or not identified by the RapID onE system

Identification by conventional method (no. of strains)	Identification by RapID onE system (code no.)
Citrobacter amalonaticus (1)	No identification (4035231)
Enterobacter hormaechei[a] (2)	*Enterobacter amnigenus*
Enterobacter hormaechei[a] (1)	*Enterobacter* sp.
Serratia liquefaciens (1)	*Enterobacter cloacae*
Serratia rubidaea (1)	No identification (6517570)
Serratia fonticola[a] (2)	*Enterobacter aerogenes*
Serratia fonticola[a] (1)	No identification (4333760)
Serratia fonticola[a] (1)	*Serratia marcescens*
Proteus mirabilis (1)	*Proteus penneri*
Proteus vulgaris (1)	*Providencia rustigianii*

[a] Not in the RapID onE database at present.

In a preliminary study of the RapID onE system by Schreckenberger and coworkers (28) of 152 strains representing 15 genera and 38 species of the family *Enterobacteriaceae* and oxidase-negative nonfermenters, 140 (92.1%) were correctly identified to the genus and species levels, 2 (1.3%) were correctly identified to the genus level only, 3 (1.9%) yielded questionable or low-probability identifications, and 7 (4.6%) were misidentified; of the latter, results for 4 of 7 strains were resolved, yielding correct identifications upon repeat testing.

All salmonellas are identified to the genus level only with the RapID onE system, with serology required for species identification. The overnight API 20E system correctly identified to the species level the one *Salmonella typhi*, two *Salmonella choleraesuis*, and one *Salmonella paratyphi* type A strains tested in the present study; all other salmonellas were identified to the genus level only. As is the case for other commercially available systems, all non-*Shigella sonnei Shigella* species were identified to the genus level only, with serology necessary for species identification.

Of the 12 misidentifications, 7 comprised two species (*Enterobacter hormaechei* and *Serratia fonticola*) not included in the database; an additional two strains yielded codes with no identifications. If the seven strains not included in the database (and very infrequently isolated from human clinical specimens) had been excluded, correct identification rates would have risen to 97.6% without additional tests and 98.7% with additional tests, with misidentification rates dropping to 1.3%. Both *Enterobacter hormaechei* and *Serratia fonticola* are, as far as we are aware, infrequently encountered in clinical specimens. However, many databases are not equipped to detect *Enterobacter hormaechei*, so the frequency of isolation of this species may be underrated.

In summary, the RapID onE method is a rapid and accurate method for the same-day identification of members of the family *Enterobacteriaceae* and oxidase-negative, gram-negative nonfermenters from clinical specimens. The RapID onE system is easy to set up, the results are easy to read, and the system yields accurate identification of strains without the need for expanded supplemental testing. Results of the study lead us to believe that RapID onE represents an accurate, same-day commercially available method for identification of members of the family *Enterobacteriaceae*. Future expansion of the database to include more species and additional codes will make the method even more accurate.

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