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Evaluation of Spectrum-10 System for Identification of Members of the Family *Enterobacteriaceae*

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A total of 378 isolates of the family *Enterobacteriaceae* were tested with conventional biochemical tests and with the Spectrum-10 identification system. Of these, 97.4% were correctly identified to the species level by using the seven-digit profile of Spectrum-10. The preliminary four-digit profile provided the correct species for 61.1% and the correct genus for 79.4% of the strains. Most misidentifications were observed with aberrant biotypes of *Citrobacter freundii*.

In clinical laboratories, the identification of isolates of the family *Enterobacteriaceae* and several other groups of bacteria is performed more and more with commercially prepared systems. These may be automated instruments, some of them providing identification within 5 h, but their use is limited because of the high initial purchase cost (2, 6). Many other systems consist of a tray with microtubes of media, either conventional or lyophilized, that can be read manually or in a semiautomated mode. The identification is generally obtained after an incubation period of 18 to 24 h, but recent improvements to some of these systems allow results to be obtained within 5 h (3). A new microtube-based system, Spectrum-10, was recently developed by Austin Biological Laboratories, Austin, Tex. It consists of 10 biochemical wells in each of two different molded polystyrene trays and is intended for the identification of organisms belonging to the family *Enterobacteriaceae*. The identification can be performed either by an enzymatic 4-h method or as a growth test, which is read after 18 to 24 h of incubation.

I evaluated the ability of the Spectrum-10 system to properly identify isolates of the family *Enterobacteriaceae* after 18 to 24 h of incubation. Several of the strains tested belonged to the less frequently encountered species.

The organisms were randomly selected strains of the family *Enterobacteriaceae* recently isolated from clinical specimens. In addition, several strains of rare occurrence stored in the culture collection at the state University of Ghent were tested as well. Before being tested, the strains were subcultured on a MacConkey agar plate. The conventional identification was done by the methods described by Kelly et al. (4). Special strains which could not be accurately identified by these methods were fully characterized by using additional tests described by Farmer et al. (1).

Tray one of Spectrum-10 contains the following biochemical tests: β-D-galactosidase; arginine, lysine, and ornithine decarboxylases; hydrogen sulfide production; urease; Voges-Proskauer; phenylalanine deaminase; indole production; and citrate utilization. The results from this tray can be converted into a four-digit profile number, which allows a presumptive identification. Tray two of Spectrum-10 consists of the following tests: malonate utilization and fermentation of rhamnose, adonitol, salicin, arabinose, inositol, sorbitol, sucrose, mannitol, and raffinose. The combined results of both trays are intended to allow a definite species identification (seven-digit profile number). The inoculum was prepared by suspending one colony in 3 ml of sterile water. One milliliter of this suspension was added to each tray, and the wells of lysine, arginine, ornithine, hydrogen sulfide, and urease were overlaid with mineral oil. After incubation for 18 to 24 h at 37°C, all tests, except for Voges-Proskauer, phenylalanine deaminase, and indole, were visually observed, and their color was read as suggested by the manufacturer. The Voges-Proskauer test was read 15 to 20 min after the addition of 40% KOH and 5% alpha-naphthol, phenylalanine deaminase was observed within 2 min of the addition of ferric chloride reagent, and indole formation was finally observed after the addition of Kovacs reagent. All reactions were recorded on a printed form and tabulated into both a four-digit profile number (tray one) and a seven-digit profile number (both trays). Profiles were interpreted with the aid of a computer code book. Six profile numbers were not in this book but nevertheless provided the accurate identification after the manufacturer was contacted for retrieval in the computer database. In the code book, identifications are classified according to the percentage of identification (%ID) (5) as follows: excellent (%ID, ≥99.9), very good (%ID, ≥99), good (%ID, ≥90), acceptable (%ID, ≥70) and low discrimination (%ID, <70, but with the sum of the two or three largest %IDs being greater than or equal to 70). When the identification was listed as the first in a range of low probabilities, it was considered as a correct result.

A total of 378 strains belonging to 36 different species were tested (Table 1). The seven-digit Spectrum-10 result agreed with the conventional identification for 368 strains (97.4%) at the species level and for 370 strains (97.9%) at the genus level. The four-digit result correctly identified 231 strains (61.1%) at the species level and 300 strains (79.4%) at the genus level. However, when identifications with low discrimination were excluded (which would require additional tests or the use of tray two), only 42.6% of the strains were correctly identified to the species level with the four-digit result.

The analysis of incorrect results is summarized in Table 2. When the seven-digit profile was used, six strains of *Citrobacter freundii* were identified as *Salmonella* subgroup 5. All these strains were H_2S, arginine, and ornithine positive and yielded the same profile number. When the %ID (5) was calculated on the basis of the percentages in the database of

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TABLE 1. Identification of isolates of the family *Enterobacteriaceae* by the Spectrum-10 system after overnight incubation

Species as determined by conventional tests (*n*)	No. of isolates correctly identified by Spectrum-10			
	Seven-digit profile		Four-digit profile	
	Correct species	Low discrimination[a]	Correct species	Correct genus
Cedecea lapagei (1)	1		0	1
Citrobacter diversus (5)	5		0	4
C. freundii (28)	21		23	23
Enterobacter aerogenes (10)	10		9	9
E. agglomerans (6)	6		0	0
E. amnigenus biogroup 1 (1)	1	1	1	1
E. amnigenus 2 biogroup (1)	1		0	1
E. cloacae (28)	28	1	0	27
E. gergoviae (1)	1		1	1
E. sakazakii (1)	1	1	1	1
Escherichia coli (40)	39	4	38	38
Hafnia alvei (10)	10		7	7
Klebsiella oxytoca (19)	19		19	19
K. ozaenae (3)	3		0	1
K. pneumoniae (22)	22		20	20
Kluyvera spp. (1)	1	1	0	1
Morganella morganii (18)	18	1	17	17
Proteus mirabilis (22)	22		22	22
P. vulgaris (23)	23	1	20	20
Providencia alcalifaciens (2)	2		2	2
P. rettgeri (8)	8		8	8
P. rustigianii (3)	3	1	2	3
P. stuartii (12)	12	1	0	12
Salmonella arizonae (2)	2		0	2
Salmonella spp., other (29)	29	16	29	29
Serratia fonticola (1)	1		1	1
S. marcescens (35)	35	1	0	0
S. liquefaciens (1)	0		0	0
S. odorifera (2)	2		2	2
Shigella sonnei (7)	7	4	6	6
Shigella spp., other (5)	5		0	2
Yersinia enterocolitica (27)	27	1	1	16
Y. frederiksenii (1)	0		0	1
Y. intermedia (1)	1	1	1	1
Y. kristensenii (1)	1	1	0	1
Y. pseudotuberculosis (1)	1		1	1

[a] Isolates correctly identified to the species level but as the first identification in a range of low prababilities.

Farmer et al. (1) and using the Spectrum-10 test results (which all agreed with the conventional results), the values were 53.2% for *C. freundii* and 46.8% for *Salmonella* subgroup 5. The same calculation performed with the database percentages of Spectrum-10 would yield values of 8.6 and 91.4%, respectively. This biotype of *C. freundii* (which may be one of limited geographical distribution) happens to be statistically very close to the very rare *o*-nitrophenyl-β-D-galactopyranoside (ONPG)-positive *Salmonella* subgroup 5. Since the identification of *Salmonella* spp. should always include serological confirmation and in view of the very low frequency of this type of *Salmonella* spp., it is unlikely that the two species will be confused. One further strain of H_2S-positive, ONPG-negative *C. freundii* was identified by Spectrum-10 as *Salmonella* subgroup 1 with a probability of 65.1%. All Spectrum-10 reactions, except for lysine (negative), were typical for *Salmonella* spp. Thus, reliable differentiation of *Salmonella* spp. and aberrant biotypes of *C. freundii* requires additional tests.

TABLE 2. Errors in identification with the Spectrum-10 seven-digit profile

Correct identification (*n*)	Incorrect identification
Citrobacter freundii (6)	*Salmonella* subgroup 5
Citrobacter freundii (1)	*Salmonella* subgroup 1
Escherichia coli (1)	*Edwardsiella tarda*
Serratia liquefaciens(1)	*Serratia marcescens*
Yersinia frederiksenii (1)	*Yersinia enterocolitica*

One atypical strain of *Escherichia coli* (ONPG negative, H_2S positive, and sorbitol negative) was identified by Spectrum-10 as *Edwardsiella tarda* (%ID, 77.7). With conventional methodology, this strain needed extensive confirmatory testing for identification.

One strain of *Serratia liquefaciens* was identified as *Serratia marcescens* (%ID, 95.6). The strain proved to be arabinose positive, but the fermentation of raffinose was delayed with the conventional test. The high number of low-discrimination identifications with *Salmonella* strains was due to low discrimination between *Salmonella* subgroup 1 and *Salmonella* subgroup 4, which can only be differentiated reliably by the KCN test and dulcitol fermentation. These Spectrum-10 identifications can therefore be considered correct, since imperative serology would solve the problem.

The new system seemed to be capable of identifying less common isolates of the family *Enterobacteriaceae* such as *Cedecea lapagei*, *Enterobacter amnigenus*, *Serratia fonticola*, *Serratia odorifera*, *Kluyvera* spp., and *Providencia rustigianii*. Two of the newer *Yersinia* species tested were identified correctly, although with low discrimination (%ID, <70).

The four-digit profile number provided fairly good species or genus recognition of the common members of the family *Enterobacteriaceae*, with the exception of *Enterobacter agglomerans* and *S. marcescens*.

In conclusion, I have preliminary evidence that the Spectrum-10 system accurately identifies common and several uncommon members of the family *Enterobacteriaceae*. A limitation is the differentiation of certain atypical *C. freundii* isolates from *Salmonella* spp. The accuracy of the Spectrum-10 may be further improved by including more biochemical variants of such strains. In this respect, an additional study covering more strains of *Citrobacter* and *Salmonella*, as well as the less frequently encountered genera, seems necessary for the final evaluation of Spectrum-10. The system was very easy to use and relatively inexpensive.

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