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Evaluation of Autoscan-4 for Identification of Members of the Family *Enterobacteriaceae*

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A study was performed to compare the Autoscan-4 (MicroScan, Inc., Mahwah, N.J.) with conventional biochemical methods for identifying clinical isolates of the family *Enterobacteriaceae*. The Autoscan-4 yielded correct identification of 95.4% of the isolates at the species level and 98.4% at the genus level. Only one misidentification was observed. The identification of both common and less-common isolates of *Enterobacteriaceae* makes this system highly efficient.

Identification of members of the family *Enterobacteriaceae* is of major importance in clinical laboratories. Today numerous automated systems are commercially available. One of these systems is the American MicroScan identification system with its automated reader, the Autoscan-4, produced by MicroScan, Inc., Mahwah, N.J., a division of Baxter Corp. In the past year, a new generation of dry panels was made available, and identification of gram-negative bacilli could be performed according to the most recent taxonomic studies. To evaluate the improvements made to the Autoscan-4 system, this study compared this system with conventional biochemical methods for identifying 372 clinical isolates of *Enterobacteriaceae* from the Hospital of Lille, Lille, France.

The isolates tested belonged to the 26 species listed in Table 1. Before being tested, all isolates were grown in brain heart infusion broth and subcultured on nutrient agar plates to assure purity and viability. The methods used for conventional biochemical tests were those described by Ewing (3). Indole production was measured after 24 h by adding 0.5 ml of Kovacs reagent (10 g of *para*-dimethylaminobenzaldehyde, 50 ml of 12 N hydrochloric acid, 150 ml of isoamyl alcohol) to 24-h-old peptone water cultures (20 g of peptone [Difco Laboratories, Detroit, Mich.], 5 g of sodium chloride, 1,000 ml of water). The Voges-Proskauer test was done after 24 h by adding 1 ml of O'Meara reagent (40 g of potassium hydroxide, 0.3 g of creatine, 100 ml of water) to 1 ml of the culture (grown in methyl red–Voges-Proskauer medium [Difco]) in a tube. Growth on citrate as the sole source of carbon and energy was tested on Simmons citrate agar (Institut Pasteur Production [IPP], Marnes-la-coquette, France). Growth on malonate as an energy source was determined in malonate broth (IPP). Hydrogen sulfide production was determined in triple sugar iron agar (Difco) with an additional 5 g of agar added per liter of medium. Urea hydrolysis was determined on Christensen urea agar (IPP). Phenylalanine deamination was tested by adding 0.1 ml of ferric chloride solution to a 24-h-old culture on phenylalanine agar (IPP). The Moeller method (5) was used to determine lysine and ornithine decarboxylases and arginine dihydrolase (IPP). Motility was determined at 36 and 22°C in motility test medium (IPP). Gelatin hydrolysis was determined at 22°C on nutrient gelatin (IPP). Acid production from carbohydrates, polyhydroxyl alcohols, and related compounds was tested in enteric fermentation base (Difco) to which 10 ml of Andrade indicator (0.2 g of fuchsin, 100 ml of water, 16 ml of 1 N NaOH) and 990 ml of water were added. The glucose fermentation tube contained a small glass insert tube (Durham tube) to detect gas production. Extracellular DNase was tested at 36°C on DNase test agar (BBL Microbiology Systems, Cockeysville, Md.) to which 0.05 g of toluidine blue was added per 1,000 ml. *o*-Nitrophenyl-β-D-galactopyranose was tested by the filter paper disk method (IPP), and β-xylosidase was tested by the Richard technique (8). Final identification was determined according to tables from Brenner et al. (2) and Ewing (3). All cultures suspected of being *Salmonella* or *Shigella* species were confirmed serologically.

For the MicroScan system, the Neg Combo panels used in this study were a new generation of dry panels with a new formulation. They were designed to identify fermentative and nonfermentative organisms. The identification profiles for isolates of *Enterobacteriaceae* were determined with 23 tests. Rehydration was automatically performed with a Hydroscan calibrated to deliver 115 μl of sterile distilled water with 10 μl of Pluronics per well. Inoculation was done according to the recommendations of the manufacturer. Inoculated trays were incubated for 18 h at 35°C. Reagents for the Voges-Proskauer, indole, and tryptophan deaminase tests were added before reading. Automatic reading was performed by an Autoscan-4.

Growth was determined by turbidimetry, and color changes resulting from biochemical reactions were determined by colorimetry. Six different wavelengths were used to interpret the color changes, and the 96 wells of the panel were read simultaneously; the optical reading process was completed in 3 s. Interpretation of the reading was done by a IBM PC XT microcomputer. For each biochemical test, the computer selected and interpreted readings done at one or more wavelengths according to the color change expected. The results of these reactions were translated into a eight-digit biotype number. From the biotype number and the data base in memory, the computer displayed one or more identifications with their relative probabilities and, if necessary, suggested supplementary testing to aid in discrimination. The probability values used were as follows: 95

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TABLE 1. Identification of isolates by Autoscan-4 and conventional methods

Species as determined by conventional methods	Total no. of strains	No. of strains correctly identified by Autoscan-4
Escherichia coli	31	31
Citrobacter freundii	24	21[a]
C. diversus	7	7
C. amalonaticus	9	9
Enterobacter cloacae	30	29[a]
E. aerogenes	8	8
E. agglomerans	7	6[b]
E. sakazakii	5	5
E. gergoviae	5	5
Enteric Group 41 (*Leclercia adecarboxylata*)	1	1
Hafnia alvei	14	14
Klebsiella pneumoniae	22	22
K. oxytoca	21	21
Serratia marescens	30	30
S. liquefaciens	15	14[a]
Proteus mirabilis	14	14
P. vulgaris	27	27
Morganella morganii	8	8
Providencia stuartii	12	12
P. alcalifaciens	9	9
P. rettgeri	8	8
P. rustigianii	3	3
Salmonella sp.	29	29
Shigella sp.	15	15
Yesinia enterocolitica	6	6
Y. frederiksenii	12	1[a]

[a] Species identification probability as determined by Autoscan-4 was less than 85% for the remaining isolates, and additional tests were performed (Table 2).

[b] One isolate incorrectly identified as *L. adecarboxylata*.

TABLE 2. Identifications made by Autoscan-4 with a probability below 85.0%

Identification by conventional tests (no. of strains)	Autoscan-4 results	*P* (%)
Citrobacter freundii (1)	*C. freundii*	76.4
	Enterobacter agglomerans	16.6
	E. cloacae	5.0
	Escherichia coli Lys^- Orn^-	2.0
C. freundii (1)	*C. freundii*	63.1
	Y. frederiksenii	23.5
	E. agglomerans	13.4
C. freundii (1)	*C. freundii*	64.2
	Salmonella sp.	35.0
	Salmonella arizonae	0.5
	S. paratyphi A	0.3
Enterobacter cloacae (1)	*E. cloacae*	82.7
	E. amnigenus 1	9.0
	E. amnigenus 2	8.3
Serratia liquefaciens (1)	*S. liquefaciens*	81.0
	S. odorifera 1	19.0
Yersinia frederiksenii (6)	*Y. enterocolitica*	74.3
	Y. frederiksenii	17.7
	Y. intermedia	8.0
Y. frederiksenii (5)	*Y. frederiksenii*	79.5
	Y. enterocolitica	13.7
	Y. intermedia	6.8

to 99.9%, identification most probable; 85 to 95%, identification very probable; 75 to 85%, identification probable; 60 to 75%, low selectivity; and <60%, questionable. Results were printed in patient reports, recorded on hard disk, and used for epidemiology.

Quality control strains were used in both the Autoscan-4 system and conventional methods. These were *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13882, and *Proteus vulgaris* ATCC 13315. The same isolates used for quality control were tested on three separate occasions to ascertain the reproducibility of results from the Autoscan-4 system. The results of this study are presented as in a previous evaluation (6) (Table 1).

The Autoscan-4 agreed with the conventional method in the identification of 355 of the 372 isolates (95.4%) at the species level and for 366 isolates (98.4%) at the genus level (Table 1). Among the 17 isolates not identified at the species level, 16 needed additional tests (Table 2). In 13 of these cases, good agreement was obtained at the genus level. One strain of *Enterobacter cloacae* and one of *Serratia liquefaciens* had to be differentiated from *Enterobacter amnigenus* and from *Serratia odorifera* 1, respectively. These two strains corresponded to only 1 of the 30 *E. cloacae* and 1 of the 15 *S. liquefaciens* isolates.

Eleven strains of *Yersinia frederiksenii* (six of which were initially identified as *Yersinia enterocolitica*) were difficult to distinguish from *Y. enterocolitica*. Only two characters (rhamnose acidification and esculin hydrolysis) were available to differentiate the two species. Additional tests, such as mucate utilization and presence of β-xylosidase, should be used instead of a test of salicin acidification, which was proposed. Identification of the three other strains for which additional tests were required (*Citrobacter freundii* isolates) revealed several possibilities from five different genera (Table 2). Two of them exhibited a positive sucrose reaction, and the other had a positive raffinose test and a negative β-galactosidase test. These characteristics were considered atypical reactions. In fact, they should be considered negative or near-negative tests, since their frequency was less than or equal to 0.25 but never equal to zero in the MicroScan array.

Only one misidentification (complete disagreement) was observed: one *Enterobacter agglomerans* strain was identified as Enteric Group 41 (syn. *Leclercia adecarboxylata*; 7, 9). Separation of these two species was mainly based on adonitol and indole tests in the MicroScan array. These tests did not clearly differentiate the two species. They were positive for the two strains identified as Enteric Group 41, with one being correctly identified and the other belonging to the species *E. agglomerans* according to conventional methods.

Classification of the *Erwinia herbicola-E. agglomerans* complex is being studied. A new definition of the *E. agglomerans* species sensu stricto has just been published (1). This will make its identification and differentiation easier. Reproducibility of the tests with the control-quality strains was 100% successful.

In summary, the performance of the Autoscan-4 is highly acceptable for the identification of commonly encountered members of *Enterobacteriaceae*, such as those tested in this study. Furthermore, identification of three *Providencia rustigianii* proved that the system was capable of identifying

less-common isolates of *Enterobacteriaceae*, such as *Enterobacter taylorae* (4) and *Koserellea trabulsii* (5).

Although an overnight incubation is required, we think that the accuracy of its identifications makes the system highly efficient in clinical laboratories.

LITERATURE CITED

1. **Beji, A., J. Mergaert, F. Gavini, D. Izard, K. Kersters, H. Leclerc, and J. De Ley.** 1988. Subjective synonymy of *Erwinia herbicola*, *Erwinia milletiae*, and *Enterobacter agglomerans* and redefinition of the taxon by genotypic and phenotypic data. Int. J. Syst. Bacteriol. **38**:77–88.
2. **Brenner, D. J., J. J. Farmer, F. W. Hickman, M. A. Asbury, and A. G. Steigerwalt.** 1977. Taxonomic and nomenclature changes in *Enterobacteriaceae*. U.S. Department of Health, Education, and Welfare publication no. (CDC) 78-8356. Center for Disease Control, Atlanta.
3. **Ewing, H. E.** 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Burgess Publishing Co., Minneapolis.
4. **Farmer, J. J., III, G. R. Fanning, B. R. Davis, C. M. O'Hara, C. Riddle, F. W. Hickman-Brenner, M. A. Asbury, V. A. Lowery III, and D. J. Brenner.** 1985. *Escherichia fergusonii* and *Enterobacter taylorae*, two new species of *Enterobacteriaceae* isolated from clinical specimens. J. Clin. Microbiol. **21**:77–81.
5. **Hickman-Brenner, F. W., G. P. Huntley-Carter, G. R. Fanning, D. J. Brenner, and J. J. Farmer III.** 1985. *Koserella trabulsii*, a new genus and species of *Enterobacteriaceae* formerly known as Enteric Group 45. J. Clin. Microbiol. **21**:39–42.
6. **Izard, D., M. O. Husson, P. Vincent, H. Leclerc, D. Monget, and J. M. Boeufgras.** 1984. Evaluation of the four-hour Rapid 20 E system for identification of members of the family *Enterobacteriaceae*. J. Clin. Microbiol. **20**:51–54.
7. **Izard, D., J. Mergaert, F. Gavini, A. Beji, K. Kersters, J. De Ley, and H. Leclerc.** 1985. Separation of *Escherichia adecarboxylata* from the "*Erwinia herbicola-Enterobacter agglomerans*" complex and from the other *Enterobacteriaceae* by nucleic acid and protein electrophoretic techniques. Ann. Inst. Pasteur (Paris) **136B**:151–168.
8. **Richard, C.** 1978. Techniques de recherche d'enzymes utiles au diagnostic des bactéries à Gram négatif. Ann. Biol. Clin. **36**:407–424.
9. **Tamura, K., R. Sakazaki, Y. Kosako, and E. Yoshizaki.** 1986. *Leclercia adecarboxylata* Gen. nov. Comb. nov., formerly known as *Escherichia adecarboxylata*. Curr. Microbiol. **13**:179–184.