JOURNAL OF CLINICAL MICROBIOLOGY, July 1990, p. 1628–1634
0095-1137/90/071628-07$02.00/0
Copyright © 1990, American Society for Microbiology
Vol. 28, No. 7

Evaluation of autoSCAN-W/A Automated Microbiology System for the Identification of Non-Glucose-Fermenting Gram-Negative Bacilli

FRED C. TENOVER,[1,2]* T. SALLY MIZUKI,[1] AND LARRY G. CARLSON[1]

Veterans Affairs Medical Center, Seattle, Washington 98108,[1] and Department of Laboratory Medicine, University of Washington, Seattle, Washington 98195[2]*

Received 15 February 1990/Accepted 24 April 1990

We evaluated the ability of the autoSCAN-W/A (MicroScan Division, Baxter Healthcare Corporation, West Sacramento, Calif.), in conjunction with the dried colorimetric Neg ID type 2 panel (DCP) and new rapid fluorometric Neg ID panel (RFP), to identify non-glucose-fermenting gram-negative bacilli by challenging the system with 310 previously identified reference strains. Of these 310 isolates, 286 organisms were in the DCP data base and 269 were in the RFP data base. Use of the DCP panels resulted in 118 (41.3%) correct and 64 (22.4%) incorrect first choice identifications at ≥85% probability, 61 (21.3%) low-probability identifications, and 43 (15.0%) reports of unidentified organisms. The RFP system reported 135 (50.1%) correct and 25 (9.3%) incorrect identifications at ≥85% probability and 109 (40.5%) low-probability identifications. Unidentified isolates (DCP system only) and isolates producing low-probability first choice identifications (both systems) required supplementary biochemical testing. Over half (37 of 64 [57.8%]) of the DCP misidentifications were due to four commonly isolated, saccharolytic organisms (*Alcaligenes xylosoxidans* subsp. *xylosoxidans*, *Pseudomonas putida*, *Pseudomonas fluorescens*, and *Xanthomonas maltophilia*), while 7 of 25 (28%) of misidentifications in the RFP system were due to *P. fluorescens*. Of note, the RFP system identified non-glucose-fermenting gram-negative bacilli within 2 h of panel inoculation, allowing additional conventional biochemical tests to be set up the same day on low-probability isolates, whereas only 13.5% of the DCPs could be read at 18 h, with the remainder requiring 42 h of incubation before reading. When organism identifications were recalculated with the updated RFP data base and revised software, only 8.1% of all 310 isolates were misidentified at ≥85% probability while 77.1% of the isolates were now correctly reported at this same high probability.

Definitive identification of non-glucose-fermenting gram-negative bacilli (NFGNB) by conventional biochemical testing requires a variety of infrequently used media and reagents and a considerable amount of technical expertise (4, 5, 9). The advent of automated and semiautomated procedures for identification of this group of organisms (1–3, 6–8) has enabled a larger number of laboratories to undertake the identification of such organisms. However, this group of organisms is often neglected in evaluations of new automated systems. Thus, the accuracy of the identifications produced by these automated systems deserves further scrutiny.

As part of a continuing study to evaluate the ability of automated systems to identify the NFGNB, we tested 310 reference isolates in the autoSCAN-W/A Automated Microbiology system (MicroScan Division, Baxter Healthcare Corporation, West Sacramento, Calif.) by using the dried colorimetric Neg ID type 2 panel (DCP) and the new rapid fluorometric Neg ID panel (RFP) (L. A. McHugh, M. T. Clifford, B. A. Basille, and J. A. Washington, Abstr. Annu. Meet. Am. Soc. Microbiol. 1989, C266, p. 437). This paper reports the results of the comparison of these two automated methods and conventional biochemical testing.

MATERIALS AND METHODS

Microorganisms. The 310 NFGNB tested were selected from reference isolates that had been identified by exhaustive conventional biochemical testing during an earlier study at the Seattle Veterans Affairs Medical Center (7). Each isolate was subcultured two consecutive times on sheep blood agar plates to assure purity. On day 3, each isolate was subcultured on a sheep blood agar plate and a MacConkey agar plate and incubated for 18 to 24 h prior to testing. All incubations were carried out at 35°C.

Conventional biochemical identification. Conventional biochemical identification by the methods of Gilardi (4, 5) and Rubin et al. (9) was performed on each isolate in parallel with testing by the automated systems. These tests determined the following: motility by the hanging-drop method; cytochrome oxidase production; growth on MacConkey agar; reduction of nitrate and nitrite; production of DNase, *o*-nitrophenyl-β-D-galactopyranoside, and urease; liquefaction of gelatin; presence of lysine decarboxylase and arginine dihydrolase; indole production; and activity in oxidation-fermentation basal medium containing 1% glucose, maltose, mannitol, or xylose.

Results of the 18 tests were coded as previously reported (7). Organisms yielding codes resulting in identification possibilities that conflicted with those determined in the previous study were tested further to determine the correct identification.

autoSCAN-W/A system. The autoSCAN-W/A system consists of an automated incubator-interpretation module and an IBM PS2/80 computer. After inoculation, panels were inserted into the incubation-interpretation module, where they were incubated at 35°C for 2 to 42 h, depending on the type of panel used and the length of time required to complete the biochemical and antibiotic tests. The incubator-interpretation module identifies the panel type by reading the user-generated bar code labels produced by the system printer during initial input of patient and panel information. At the appropriate time, reagents are automatically dispensed into

* Corresponding author.

certain wells of the panels and the panels are incubated for an additional period before the tests are read and interpreted by the system.

Colorimetric panels are read by guiding light from an interference filter into each of the 96 wells through optical fiber channels to light-sensitive photodiodes. Rapid panels are scanned by a system capable of reading the amount of fluorescence in each of the 96 wells and electrically quantifying these results. The entire system runs under the control of the MicroScan Data Management system (version 15.00H .W/A. 6.00F was installed for this study).

At the conclusion of the study, all organism identifications were recalculated by using an updated RFP data base and revised software.

DCP method. The DCP contained two control wells and the following substrates and antimicrobial agents: glucose, sucrose, sorbitol, raffinose, rhamnose, arabinose, inositol, adonitol, melibiose, urea, hydrogen sulfide, indole, lysine, arginine, ornithine, tryptophan deaminase, esculin, Voges-Proskauer, citrate, malonate, *o*-nitrophenyl-β-D-galactopyranoside, tartrate, acetamide, cetrimide, oxidation-fermentation glucose, oxidation-fermentation base, decarboxylase base, nitrate, penicillin G (4 μg/ml), kanamycin (4 μg/ml), colistin (4 μg/ml), nitrofurantoin (64 μg/ml), cephalothin (8 μg/ml), and tobramycin (4 μg/ml).

Sufficient inoculum was picked from an 18- to 24-h MacConkey agar plate to produce a turbidity equal to a 0.5 McFarland standard (approximately 10^8 CFU/ml) in 6.5 ml of 0.4% saline containing pluronic-D. The saline containing pluronic-D was vortexed for 2 to 3 s before 100 μl was pipetted into 25 ml of sterile water with pluronic-D, which was then poured into a seed tray. The MicroScan RENOK rehydrator-inoculator was used to inoculate the Neg ID type 2 tray from the seed tray. The glucose, urea, H_2S (oxidase-negative organisms only), lysine, arginine, ornithine, and decarboxylase base wells were covered with mineral oil. The panel was covered with a lid before being placed in the incubator-interpretation module. DCPs were incubated for a maximum of 42 h before the final reading.

RFP method. The RFP tray contained the following substrates: urea, 4-methylumbelliferyl (MEU)-β-D-*N*, *N'*-diacetylchitobioside, MEU-α-L-arabinoside, MEU-α-D-galactopyranoside, MEU-β-D-galactopyranoside, MEU-β-D-glucopyranoside, MEU-β-D-glucuronide, MEU-*N*-acetyl-β-D-glucosaminide, MEU-phosphate (pH 6), MEU-phosphate (pH 7.5), L-pyroglutamic acid-7-amido-4-methylcoumarin (AMC), L-tyrosine-AMC, L-alanine-AMC, *N*-glutaryl-glycyl-arginine-AMC, L-arginine-AMC, *N*-carbobenzoxy-L-arginine-AMC, L-citrulline-AMC, L-glutamic acid-AMC, α-L-glutamic acid-AMC, glycine-AMC, L-lysine-AMC, L-phenylalanine-AMC, L-proline-AMC, arabinose, glucose, lactose, maltose, mannitol, melibiose, salicin, decarboxylase base, sucrose, trehalose, lysine decarboxylase, ornithine decarboxylase, and indole.

The saline containing pluronic-D that had been prepared during inoculation of the DCP was vortexed again for 2 to 3 s, the remaining 6.4 ml was poured into the bottom portion of a seed trough, and a tube of sterile water with pluronic-D was poured into the top portion of a seed trough. The MicroScan RENOK rehydrator-inoculator was used to inoculate RFPs. The lysine, ornithine, and decarboxylase base wells were covered with mineral oil before insertion into the incubation-interpretation module. RFPs were incubated for a maximum of 2 h before final identification was concluded.

Identification interpretation. The results of the biochemical and antimicrobial tests of the isolates were translated by the system computer and software into an 8 (DCP)- or 15 (RFP)-digit biotype code. The system then used this code to produce a report listing the organism(s) contained in its data base that could produce this constellation of results and associated isolation probability figures. Individual identifications could be species, genus, or group specific. According to the recommendation of the manufacturer, a first choice isolation probability of <85% or first choice identification to only the group or genus level necessitated supplementary conventional testing to confirm identification.

Data analysis. Identification of NFGNB by the autoSCAN-W/A was evaluated by comparing the results of the two test systems with those obtained by parallel confirmatory conventional biochemical identification. A summary of the results of the two systems was accomplished by placing each identification into one of five categories as follows: (i) correct, a first choice identification of ≥85% probability, compatible with the standard biochemical identification; (ii) minor error, a first choice identification of <85% probability, with the compatible identification listed as any one of the choices; (iii) unidentified, reported as unidentified or as a very rare biotype (DCP system only); (iv) major error, a first choice identification of <85% probability and a compatible identification not listed as one of the choices; and (v) very major error, a first choice identification of ≥85% probability, not compatible with the standard biochemical identification.

Quality control. Quality control tests were run at regular intervals on the MicroScan W/A and with the introduction of each new lot of panels. The DCPs required only two quality control organisms, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27833, to be tested weekly, whereas the RFPs required the following five organisms: *Pseudomonas putrefaciens* AmMs 201 (from the American MicroScan collection), *Acinetobacter calcoaceticus* subsp. *anitratus* AmMs 202, *Aeromonas hydrophila* AmMs 199, *Klebsiella oxytoca* AmMs 101, and *E. coli* ATCC 25922.

RESULTS

A total of 310 reference isolates were evaluated in this study (Table 1). All isolates were tested simultaneously by using the DCPs and RFPs in conjunction with the autoSCAN-W/A instrument and by standard biochemical methods.

The results of the DCP tests for the organisms whose biochemical patterns were in the data base are shown in Table 2. Of 286 organisms, 41.3% were identified correctly, with a confidence level of ≥85%, while 22.4% were misidentified with the same high degree of confidence (very major errors). The DCPs demonstrated problems in identifying relatively common nonfermenters such as *Pseudomonas fluorescens*, *P. putida*, and *Xanthomonas maltophilia*, as well as members of the *Bordetella-Alcaligenes* group. Fifteen percent of the organisms were reported as unidentified. If one considers that additional biochemical tests would be required any time the system failed to present an identification with a confidence of ≥85%, then 36.3% of the time (17.8% plus 15.0% plus 3.5%, for minor error, unidentified, and major error categories, respectively), the DCPs would require additional testing but the operator would not receive this information until the full 42-h incubation time had elapsed. Table 3 shows the results for the organisms tested that were not in the DCP data base. Of the 24 organisms in this category, 9 consistently did not grow well, 4 were identified only as *Pseudomonas* species, which in fact were correct identifications, and the remaining 11 were misidentified, some at a high confidence level. Many of the organ-

TABLE 1. Bacterial isolates

Group	Genus	Species or species group	No. tested	Data base[a]
Fluorescent	*Pseudomonas*	*P. aeruginosa*	35	DCP, RFP
		P. fluorescens	17	DCP, RFP
		P. putida	26	DCP
Pseudomallei	*Pseudomonas*	*P. cepacia*	21	DCP, RFP
		P. pickettii	3	DCP, RFP
Stutzeri	*Pseudomonas*	*P. stutzeri*	21	DCP, RFP
		P. mendocina	1	
Acidovorans	*Pseudomonas*	*P. acidovorans*	9	DCP, RFP
		P. testosteroni	3	
Diminuta	*Pseudomonas*	*P. diminuta*	3	RFP
Xanthomonas	*Xanthomonas*	*X. maltophilia*	34	DCP, RFP
Alcaligenes	*Pseudomonas*	*P. alcaligenes*	5	
		P. pseudoalcaligenes	5	
Other taxa	*Pseudomonas*	*P. putrefaciens*	3	DCP, RFP
	Chryseomonas	*C. luteola*[b]	2	DCP, RFP
	Flavimonas	*F. oryzihabitans*[c]	1	DCP, RFP
	Oligella	*O. ureolytica*[d]	4	RFP
	Oligella	*O. urethralis*[e]	1	DCP, RFP
	Achromobacter	Group Vd-1	3	DCP, RFP
Bordetella	*Bordetella*	*B. bronchicanis*	6	DCP, RFP
Alcaligenes	*Alcaligenes*	*A. xylosoxidans* subsp. *denitrificans*	8	DCP, RFP
		A. xylosoxidans subsp. *xylosoxidans*	12	DCP, RFP
		A. odorans	30	DCP, RFP
		A. faecalis	5	DCP, RFP
		CDC group IVc-2	5	DCP, RFP
Agrobacterium	*Agrobacterium*	*A. tumefaciens*	1	
Flavobacterium	*Flavobacterium*	*F. meningosepticum*	2	DCP, RFP
		F. odoratum	10	DCP, RFP
		CDC group IIf	1	DCP, RFP
Acinetobacter	*Acinetobacter*	*A. alcaligenes*	4	DCP, RFP
		A. lwoffii	3	DCP, RFP
		A. calcoaceticus	12	DCP, RFP
		A. hemolyticus	4	DCP, RFP
Moraxella	*Moraxella*	*M. nonliquefaciens*	4	DCP, RFP
		M. osloensis	4	DCP, RFP
		CDC group M-5	2	RFP

[a] Total numbers of isolates from the DCP and RFP data bases were 286 and 269, respectively.
[b] CDC group Ve-1.
[c] CDC group Ve-2.
[d] CDC group IVe.
[e] *Moraxella urethralis*.

isms in this latter group were *Pseudomonas* species that were identified as *Alcaligenes* species. The results of the RFPs are presented in Table 4. For the 269 organisms whose biochemical patterns were contained in the RFP data base, 50.1% of the time the RFP gave the correct identification at ≥85% confidence in 2 h. Only 9.3% of the time were incorrect answers given with this same high confidence level. The major problem appeared to be the identification of *P. fluorescens*. It should be noted that *P. putida* was not included in the data base.

Table 5 shows the identifications given by the MicroScan W/A for the remaining organisms not listed in the data base. Twenty-six *P. putida* strains were identified as a variety of different organisms, as this species is not included in the RFP data base. It is important to note that the RFP data base did not have a category for unidentifiable organisms, thus forcing uncertain identification of some isolates.

After completion of the study, the RFP data base and software were revised. Table 6 presents the identifications that would be expected if the same biochemical patterns were used in conjunction with the new software. A total of 239 (77.1%) isolates would have been correctly identified to the species or species group, while the number of misidentifications would have been reduced from 44 to 25. Of note,

TABLE 2. Results of identification with DCPs

Organism(s)	No. (%) of identifications (n = 286)				
	Correct	Minor error	Unidentified	Major error	Very major error
Pseudomonas aeruginosa	20 (57.1)	5 (14.3)	6 (17.1)	2 (5.7)	2 (5.7)
P. fluorescens	0	5 (29.4)	2 (11.8)	0	10 (58.8)
P. putida	2 (7.7)	10 (38.5)	3 (11.5)	2 (7.7)	9 (34.6)
P. cepacia	13 (61.9)	0	5 (23.8)	0	3 (14.3)
P. pickettii	1 (33.3)	1 (33.3)	1 (33.3)	0	0
P. stutzeri	15 (71.4)	5 (23.8)	0	0	1 (4.8)
P. acidovorans	5 (55.6)	2 (22.2)	0	0	2 (22.2)
Xanthomonas maltophilia	10 (29.4)	3 (8.8)	11 (32.4)	0	10 (29.4)
P. putrefaciens	2 (66.7)	1 (33.3)	0	0	0
Chryseomonas luteola	0	0	1 (50.0)	0	1 (50.0)
Flavimonas oryzihabitans	0	0	0	1 (100)	0
Oligella urethralis	0	0	0	0	1 (100)
Achromobacter group Vd-1	1 (33.3)	1 (33.1)	0	0	1 (33.3)
Bordetella bronchicanis	4 (66.7)	0	0	1 (16.7)	1 (16.7)
Alcaligenes xylosoxidans subsp. *denitrificans*	1 (12.5)	3 (37.5)	2 (25.0)	0	2 (25.0)
A. xylosoxidans subsp. *xylosoxidans*	0	1 (8.3)	2 (16.7)	1 (8.3)	8 (66.7)
A. odorans	23 (76.7)	6 (20.0)	1 (3.3)	0	0
A. faecalis	2 (40.0)	1 (20.0)	0	1 (20.0)	1 (20.0)
Alcaligenes CDC group IVc-2	1 (20.0)	1 (20.0)	0	0	3 (60.0)
Flavobacterium meningosepticum	0	1 (50.0)	0	1 (50.0)	0
F. odoratum	4 (40.0)	3 (30.0)	0	0	3 (30.0)
Flavobacterium CDC group IIf	1 (100)	0	0	0	0
Acinetobacter alcaligenes	0	0	4 (100)	0	0
A. lwoffii	2 (66.7)	0	1 (33.3)	0	0
A. calcoaceticus	6 (50.0)	2 (16.7)	4 (33.3)	0	0
A. hemolyticus	4 (100)	0	0	0	0
Moraxella nonliquefaciens	1 (25)	0	0	0	3 (75.0)
M. osloensis	0	0	0	1 (25.0)	3 (75.0)
Total	118 (41.3)	51 (17.8)	43 (15.0)	10 (3.5)	64 (22.4)

P. putida has been added to the data base, as has a possible-rare-biotype reporting category.

The time required to perform routine maintenance of the autoSCAN-W/A and to set up 10 DCP unknowns with two DCP quality control panels and 10 RFPs with five RFP quality control panels was recorded 2 weeks after the beginning of the study and again 2 months later. Initially, maintenance and setup required 34 and 55 min, respectively. After 2 months of working with the autoSCAN-W/A, these times were reduced to 18 min for maintenance and 40 min for setup, an overall savings of 31 min/day.

DISCUSSION

Two different gram-negative identification panels, the DCP and the RFP, were tested by using the autoSCAN-W/A

TABLE 3. Identification of 24 isolates not in MicroScan DCP data base

Biochemical identification of organism(s)	No. identified by MicroScan DCP						
	Pseudomonas putrefaciens	*Pseudomonas* species	*Bordetella bronchicanis*	*Alcaligenes xylosoxidans*	*Alcaligenes* species	*Moraxella* species	Insufficient growth
Pseudomonas mendocina					1		
P. testosteroni				1	1	1	
P. diminuta		2					1
P. alcaligenes		2	1				2
P. pseudoalcaligenes	2				3		
Oligella ureolytica							4
Agrobacterium tumefaciens						1	
Moraxella CDC group M-5							2

TABLE 4. Results of identification with RFPs

Organism(s)	No. (%) of identifications (n = 269)			
	Correct	Minor error	Major error	Very major error
Pseudomonas aeruginosa	26 (74.3)	6 (17.1)	2 (5.7)	1 (2.9)
P. fluorescens	0	10 (58.8)	0	7 (41.2)
P. cepacia	15 (71.4)	2 (9.5)	2 (9.5)	2 (9.5)
P. pickettii	0	1 (33.3)	0	2 (66.7)
P. stutzeri	21 (100)	0	0	0
P. acidovorans	1 (11.1)	5 (55.6)	0	3 (33.3)
P. diminuta	1 (33.3)	1 (33.3)	0	1 (33.3)
Xanthomonas maltophilia	33 (97.1)	0	1 (2.9)	0
P. putrefaciens	3 (100)	0	0	0
Chryseomonas luteola	2 (100)	0	0	0
Flavimonas oryzihabitans	0	1 (100)	0	0
Oligella ureolytica	1 (25.0)	0	1 (25.0)	2 (50.0)
O. urethralis	1 (100)	0	0	0
Achromobacter group Vd-1	2 (66.7)	0	0	1 (33.3)
Bordetella bronchicanis	1 (16.7)	1 (16.7)	4 (66.7)	0
Alcaligenes xylosoxidans subsp. *denitrificans*	0	5 (62.5)	2 (25.0)	1 (12.5)
A. xylosoxidans subsp. *xylosoxidans*	3 (25.0)	9 (75.0)	0	0
A. odorans	4 (13.3)	25 (83.3)	0	1 (3.3)
A. faecalis	0	3 (60.0)	2 (40.0)	0
Alcaligenes CDC group IVc-2	0	1 (20.0)	2 (40.0)	2 (40.0)
Flavobacterium meningosepticum	0	1 (50.0)	1 (50.0)	0
F. odoratum	10 (100)	0	0	0
Flavobacterium CDC group IIf	0	0	0	1 (100)
Acinetobacter alcaligenes	0	4 (100)	0	0
A. lwoffii	0	2 (66.7)	1 (33.3)	0
A. calcoaceticus	7 (58.3)	4 (33.3)	1 (8.3)	0
A. hemolyticus	3 (75.0)	1 (25.0)	0	0
Moraxella nonliquefaciens	1 (25.0)	3 (75.0)	0	0
M. osloensis	0	3 (75.0)	1 (25.0)	0
Moraxella CDC group M-5	0	1 (50.0)	0	1 (50.0)
Total	135 (50.1)	89 (33.1)	20 (7.4)	25 (9.3)

to determine their accuracy in identifying NFGNB. A total of 310 reference isolates were chosen to challenge the system. A number of these isolates were collected during a recent evaluation of the Automated Microbial system (Vitek Systems, Inc., Hazelwood, Mo.) (7) and had undergone a limited number of passages. Since the biochemical patterns of these isolates did not change after storage, we are confident that the exclusive use of reference strains in lieu of

TABLE 5. Identification of 41 isolates not in MicroScan RFP data base

Biochemical identification of organism	No. identified by MicroScan RFP										
	Pseudomonas aeruginosa	*P. fluorescens*	*P. pickettii*	*P. stutzeri*	*P. acidovorans*	*P. diminuta*	*Flavimonas oryzihabitans*	*Oligella urethralis*	*Achromobacter* group Vd-1	*Alcaligenes xylosoxidans*	*Alcaligenes* species
Pseudomonas putida		9		5	6		1	3	1	1	
P. mendocina	1										
P. testosteroni					2			1			
P. alcaligenes			2		1	2					
P. pseudoalcaligenes		1		1	2						1
Agrobacterium tumefaciens									1		

TABLE 6. Results of identification with RFPs with upgraded data base and software

Organism(s)	No. (%) of identifications (n = 310)				
	Correct	Minor error	Major error	Very major error	Rare biotype
Pseudomonas aeruginosa	30 (85.7)	4 (11.4)	1 (2.9)	0	0
P. fluorescens + *P. putida*	33 (76.7)	9 (20.9)	0	1 (2.3)	0
P. cepacia[a]	17 (81.0)	1 (4.8)	1 (4.8)	2 (9.5)	0
P. pickettii	0	1 (33.3)	0	2 (66.7)	0
P. stutzeri + *P. mendocina*	17 (77.3)	5 (22.7)	0	0	0
P. acidovorans + *P. testosteroni*	9 (75.0)	0	0	3 (25.0)	0
P. diminuta	0	0	0	3 (100)	0
Xanthomonas maltophilia	33 (97.1)	0	0	1 (2.9)	0
P. alcaligenes + *P. pseudoalcaligenes*[a]	3 (30.0)	7 (70.0)	0	0	0
P. putrefaciens	3 (100)	0	0	0	0
Chryseomonas luteola	2 (100)	0	0	0	0
Flavimonas oryzihabitans	1 (100)	0	0	0	0
Oligella ureolytica	3 (75.0)	0	0	1 (25.0)	0
O. urethralis	1 (100)	0	0	0	0
Achromobacter group Vd-1	2 (66.7)	1 (33.3)	0	0	0
Bordetella bronchicanis	1 (16.7)	4 (66.7)	1 (16.7)	0	0
Alcaligenes xylosoxidans	9 (75.0)	1 (8.3)	0	2 (16.7)	0
Alcaligenes species	39 (90.7)	3 (7.0)	0	1 (2.3)	0
Alcaligenes CDC group IVc-2	2 (40.0)	0	0	3 (60.0)	0
Agrobacterium tumefaciens	0	0	0	1 (100)	0
Flavobacterium meningosepticum[a]	1 (50.0)	1 (50.0)	0	0	0
F. odoratum	10 (100)	0	0	0	0
Flavobacterium CDC group IIf	1 (100)	0	0	0	0
Acinetobacter alcaligenes + *A. lwoffii*	5 (71.4)	1 (14.3)	0	0	1 (14.3)
A. calcoaceticus	10 (83.3)	1 (8.3)	1 (8.3)	0	0
A. hemolyticus	3 (75.0)	0	0	1 (25.0)	0
Moraxella species[b]	4 (40.0)	1 (10.0)	1 (10.0)	4 (40.0)	0
Total	239 (77.1)	40 (12.9)	5 (1.6)	25 (8.1)	1 (0.3)

[a] Three isolates (one each of *P. cepacia*, *F. meningosepticum*, and *P. alcaligenes*) required a rapid indole test to confirm the identification.
[b] Includes CDC group M-5.

fresh clinical isolates did not significantly detract from the study. The distribution of isolates tested reflects that seen in our laboratory. Thus, there were limited numbers of several species available for testing, a limitation not uncommon in evaluations of NFGNB (6–9). The results were compared with those determined by standard biochemical identification schemes. We were particularly interested in the accuracy of the RFPs because the results are available in 2 h. Thus, if the organism cannot be identified, additional biochemical tests can be inoculated on the same day and little time is lost in identifying the organism. In this regard, the RFPs performed well. Slightly more than 50% of the data base organisms tested were identified correctly in 2 h, with confidence levels of ≥85%. Although 9.3% of the isolates in the data base were misidentified, this was most often due to the misidentification of the less common *Pseudomonas* species. The DCPs, on the other hand, did not perform well, resulting in misidentification of approximately 22% of the nonfermentative organisms in the data base, with a confidence level of ≥85%. This data base requires some revision before laboratories can have confidence in the results for NFGNB.

Critical evaluations of new identification systems necessarily stress their ability to identify a wide range of species in order to determine specific strengths and weaknesses in the system. In routine functioning in the clinical laboratory, however, the true identity of the isolate is unknown and predictive value is important. During the course of this study, 136 (75.6%) of the 180 strains identified by the RFP system with confidence levels of ≥85% were correctly reported. The number of erroneous results was substantially reduced by using simple screening methods. Screening *Pseudomonas stutzeri*, *Achromobacter* group Vd-1, *Alcaligenes odorans*, and *X. maltophilia* strains for consistent colonial morphology and odor and rejecting all *P. fluorescens* and *Oligella urethralis* identifications decreased the number of errors from 44 to 14 while reducing the number of correct identifications by only 1 (135 of 149 [90.6%]). We also noted that correction of the inability of the RFP system to identify *P. fluorescens* and inclusion of *P. putida* in the data base

would significantly lessen misidentification of nonfermentative organisms.

In fact, the updated data base and revised software, scheduled to be released in 1990, include *P. putida* in a *P. fluorescens-P. putida* group category (Table 6). The new data base also consolidates some other closely related species into species groups for reporting purposes, a practice used in other automated systems (6, 7). This modification of the data base and revision of software identification algorithms resulted in a significant increase in both the range of isolates the RFP system could identify and the number of correct identifications. With the new software and data base, 239 (77.1%) of the 310 organisms tested would be correctly identified to the species or species group level at ≥85% probability while the number of incorrect identifications would be reduced from 44 to 25 (8.1%). A means of reporting unidentified organisms was another important addition to the updated software. One *Acinetobacter lwoffii* isolate misidentified under the current RFP system would have been reported as a possible rare biotype (Table 6).

In a clinical laboratory, the predictive value of an identification system depends on an interplay between the relative frequency of isolation of a given species and the ability of the system to correctly identify those strains. To determine the relative frequency of isolation of NFGNB, we tested 150 consecutive gram-negative species isolated from patient specimens (Tenover et al., unpublished data). Thirty-six (24%) of the 150 strains were, in fact, NFGNB. Of these, the RFP system misidentified 3 (8.3%) and correctly identified 28 (77.8%) at a confidence level of 85%, a rate similar to that seen during the evaluation of reference isolates.

In summary, with the release of the new software and expanded organism data base for the RFPs and the use of a few simple screening criteria, the autoSCAN-W/A promises to be a very rapid and reasonably accurate system for the identification of NFGNB.

LITERATURE CITED

1. **Baker, C. N., S. A. Stocker, D. L. Rhoden, and C. Thornsberry.** 1986. Evaluation of the MicroScan Antimicrobial Susceptibility System with the autoSCAN-4 automated reader. J. Clin. Microbiol. **23**:143–148.
2. **Edberg, S. C., and M. K. Edberg.** 1982. Comparison of identification systems for Enterobacteriaceae. J. Clin. Lab. Auto. **2**:263–265.
3. **Gavini, F., M. O. Husson, D. Izard, A. Bernigaud, and B. Quiviger.** 1988. Evaluation of Autoscan-4 for identification of members of the family *Enterobacteriaceae*. J. Clin. Microbiol. **26**:1586–1588.
4. **Gilardi, G. L.** 1985. *Pseudomonas*, p. 350–372. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
5. **Gilardi, G. L.** 1989. Identification of glucose-nonfermenting gram negative rods. Department of Laboratories, North General Hospital, New York.
6. **Kampfer, P., and W. Dott.** 1989. Evaluation of the Titertek-NF system for identification of gram-negative nonfermentative and oxidase-positive fermentative bacteria. J. Clin. Microbiol. **27**: 1201–1205.
7. **Plorde, J. J., J. A. Gates, L. G. Carlson, and F. C. Tenover.** 1986. Critical evaluation of the AutoMicrobic system Gram-Negative Identification Card for identification of glucose-nonfermenting gram-negative rods. J. Clin. Microbiol. **23**:251–257.
8. **Rhoden, D. L., P. B. Smith, C. N. Baker, and B. Schable.** 1985. autoSCAN-4 system for identification of gram-negative bacilli. J. Clin. Microbiol. **22**:915–918.
9. **Rubin, S. J., P. A. Granato, and B. L. Wasilauskas.** 1985. Glucose-nonfermenting gram-negative bacteria, p. 330–349. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.