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autoSCAN-4 System for Identification of Gram-Negative Bacilli

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A production model of the autoSCAN-4 system (American MicroScan, Inc., Mahwah, N.J.) was tested with not more than 11 strains each of 73 groups or species of gram-negative bacilli from various Centers for Disease Control culture collections. The strains included typical and atypical strains of enteric fermenters, nonenteric fermenters, and nonfermenters. The autoSCAN-4 system identified 95.3% of all 405 cultures accurately: 95.4% of 307 members of the family *Enterobacteriaceae*, 96.6% of 29 nonenteric fermenters, and 94.2% of 69 nonfermenters. Manual readings of the same trays provided essentially the same results, with a maximum change of only +1.6% identification accuracy of members of the *Enterobacteriaceae*. These data were obtained by all required additional tests, including serology and computer consultation when indicated. Only 19 of the cultures tested were misidentified. These were distributed randomly throughout the various groups and species except that *Edwardsiella tarda* was usually missed because of poor H_2S reactions in the test medium. Of six *Yersinia enterocolitica* isolates, two were not identified. Only one nonenteric fermenter, a *Pasteurella* sp., and four nonfermenters (three *Pseudomonas* sp. and one Centers for Disease Control group Ve-2) were misidentified.

So-called kits are the most frequently used systems for identification of pathogenic bacteria in clinical laboratories in the United States, and their popularity has led to the development of automated systems for performing the same tasks. Today a number of automated and semiautomated systems are available for use in the rapid identification and susceptibility testing of the organisms most commonly encountered in a clinical microbiology laboratory. One such system is MicroScan and its automated component, the autoSCAN-4 system, produced by American MicroScan, Inc., Mahwah, N.J., a division of American Hospital Supply Corporation.

This study was undertaken to determine the ability of the autoSCAN-4 system to identify commonly encountered gram-negative bacilli, including members of the family *Enterobacteriaceae*, non-*Enterobacteriaceae* fermenters, and nonfermenters. The system also has the capability of providing antimicrobial susceptibilities, but this will be discussed in a separate report. The apparently unique ability of this system to identify bacilli of a wide variety of taxa stimulated us to conduct this study with our collection of both typical and atypical cultures.

MATERIALS AND METHODS

Cultures tested. The 405 cultures tested included 307 members of the *Enterobacteriaceae*, 69 nonfermenters, and 29 nonenteric fermenters and consisted of not more than 11 strains each of 73 groups or species (Table 1). Cultures usually were maintained frozen at −60°C in rabbit blood except for some *Enterobacteriaceae* cultures which were stored at room temperature in nutrient agar stabs. All cultures were randomly coded by a third party, and their true identity was revealed only after all testing was completed. All cultures were obtained from culture collections maintained in various laboratories at the Centers for Disease Control, Atlanta, Ga. The cultures, which had been identified by classical biochemical and serologic techniques (2, 4), consisted of both typical and atypical phenotypes within the various taxa. After the study was completed, any discrepancies noted between autoSCAN-4 and conventional identifications were resolved by reidentification of the cultures by reference laboratory techniques (1, 3).

autoSCAN-4 system. The autoSCAN-4 system consists of a microtiter tray of substrates and tests for identification and determination of MICs, an automated tray reader (the autoSCAN-4), and an IBM PC XT computer. The tray contains the following 32 substrates and 2 controls for use in the identification process: glucose, sucrose, sorbitol, raffinose, rhamnose, arabinose, inositol, adonitol, melibiose, urea, H_2S, indole, lysine, arginine, ornithine, decarboxylase base (control), tryptophan, esculin, Voges-Proskauer, citrate, malonate, *o*-nitrophenyl-β-D-galactopyranoside, tartrate, acetamide, cetrimide, oxidation-fermentation base (control), oxidation-fermentation glucose, 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). Cultures to be tested were removed from storage, subcultured twice on Trypticase soy agar (BBL Microbiology Systems) containing 5% sheep blood, and incubated at 35°C overnight. The inoculum used for testing was prepared from the second subculture by suspending growth in 5 ml of Mueller-Hinton broth and adjusting to a turbidity equal to a 0.5 McFarland standard (approximately 10^8 CFU/ml). A 1:50 dilution of the test culture was made in sterile distilled water containing 0.02% Tween 80, and all identification wells were inoculated from this suspension. The tests for glucose, urease, lysine, arginine, and ornithine and the decarboxylase control wells were overlaid with mineral oil. A plastic sealing strip was placed over the wells containing citrate, malonate, acetamide, tartrate, *o*-nitrophenyl-β-D-galactopyranoside, cetrimide, oxidation-fermentation base and oxidation-

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TABLE 1. Cultures used to test autoSCAN-4 system

Organism	No. of cultures tested	Taxa
Enterobacteriaceae		
Citrobacter spp.	28	3
Edwardsiella spp.	6	1
Enterobacter spp.	40	5
Escherichia spp.	28	5
Hafnia spp.	10	1
Klebsiella spp.	31	4
Kluyvera spp.	1	1
Morganella spp.	10	1
Proteus spp.	29	3
Providencia spp.	26	3
Salmonella spp.	37	6
Serratia spp.	30	4
Shigella spp.	22	4
Yersinia spp.	9	3
Nonenteric fermenters		
Aeromonas spp.	2	1
Chromobacterium spp.	4	1
Flavobacterium spp.	5	3
Pasteurella spp.	9	3
Vibrio spp.	9	2
Nonfermenters		
Acinetobacter spp.	15	3
Alcaligenes spp.	2	1
Bordetella spp.	3	1
Moraxella spp.	6	1
Pseudomonas spp.	36	9
CDC[a] group IIF	1	1
CDC group IVc-2	3	1
CDC group Ve-1	2	1
CDC group Ve-2	1	1

[a] CDC, Centers for Disease Control.

fermentation glucose tests. Trays were stacked at 35°C in a non-CO_2 incubator for 18 h, after which reagents for the indole, VP, and tryptophan deaminase tests were added as per the instructions of the manufacturer. After the prescribed time for reactions had elapsed, the tray was placed in the autoSCAN-4 reader, and results were recorded.

Additional biochemical and serologic tests were used only when indicated by the autoSCAN-4 computer or instruction manual. A few cultures were identified only by consulting the more complete data bank maintained by the manufacturer.

Each reaction tray was also read manually, and results were compared with those obtained with the automated reader. As above, we used additional biochemical and serologic tests, as well as the complete data bank, only when we were so instructed by the identification manual.

RESULTS

Results obtained by manually reading the MicroScan trays and those recorded by the automated reader, the autoSCAN-4, were nearly identical, so only the results obtained with the automated system are presented hereafter.

Of the 405 cultures tested, the autoSCAN-4 system correctly identified 386 (95.4%) to the species level and 388 (95.8%) to the genus level. The ease with which this was accomplished varied somewhat, depending on the type of organism tested (Table 2). Additional biochemical tests were required for as few as 5.2% (members of the *Enterobacteriaceae*) to as many as 51.8% (non-*Enterobacteriaceae* fermenters) of the test cultures. The complete data bank was consulted only rarely, i.e., when the autoSCAN-4 answer was "very rare biotype." This occurred with only four strains of members of the *Enterobacteriaceae* and three nonfermenters. Serology was required for 35 cultures of members of the *Enterobacteriaceae*, primarily *Salmonella* spp. and *Shigella* spp. Eighteen cultures of nonfermenters required additional biochemical tests for identification, three required complete data bank consultation, and three required a combination of these aids.

The correct identifications were tabulated on the basis of the identification probability attained (Table 3). Overall, 60.4% of the correct identifications achieved 99.9% probability, 15.5% were identified at the 99.8% to 95.0% level, 5.7% were identified at the 94.9% to 85.0% level, and 18.4% were correctly identified at levels below 85.0% probability.

Only 19 (4.7%) of the 405 cultures tested were misidentified (Table 4). Misidentifications were fairly randomly distributed among the test cultures, except that three *Edwardsiella tarda* cultures were missed because weakly positive H_2S tests were recorded as negative, and two *Yersinia enterocolitica* cultures could not be differentiated from other biochemically similar organisms. Two cultures of *Salmonella* spp. were correctly identified to the genus level, but their specific epithet was incorrect. A more serious problem was encountered with a culture of *Shigella dysenteriae* that could not be identified even to the genus level. Of the other types of organisms tested, one culture each of three *Pseudomonas* species was misidentified.

DISCUSSION

The autoSCAN-4 is an extremely accurate and convenient system to use for the identification of the vast majority of

TABLE 2. Cumulative correct identification[a]

Organism (no. tested)	autoSCAN-4 reader only	autoSCAN-4 reader plus: Biochemical tests[b]	Data bank[c]	Biochemical tests and data bank	Serology	Biochemical tests and serology
Enterobacteriaceae (307)	72.3	77.5	78.8	79.1	90.5	95.3
Non-*Enterobacteriaceae*						
Fermenters (29)	44.8	96.6				
Nonfermenters (69)	59.4	85.5	89.8	94.2		

[a] Results are percent cumulative correct identification.
[b] Additional biochemical tests were required.
[c] Complete data bank was consulted.

TABLE 3. Cultures identified correctly by autoSCAN-4 system at various probabilities

Organism	No. of cultures tested	No. of cultures identified correctly				
		Total	Relative probability			
			99.9%	99.8–95.0%	94.9–85.0%	<85.0%
Enterobacteriaceae	307	293	200	43	15	35
Non-*Enterobacteriaceae*						
Fermenters	29	28	7	2	4	15
Nonfermenters	69	65	26	15	3	21

gram-negative bacilli encountered in the clinical microbiology laboratory. In our tests of slightly over 400 stock cultures, which included oxidase-positive and oxidase-negative strains and fermenters and nonfermenters, an overall accuracy of identification to the species level of 95.3% was attained, without adjustment of the data for distribution normally seen in clinical laboratories.

The collection of cultures tested was not representative of that encountered in the usual clinical laboratory but was selected to include strains that were both typical and atypical in their biochemical and antimicrobial susceptibility patterns. We do not consider this a liability in our study because we intended to test the extreme limits of performance of the autoSCAN-4 system.

The performance of the autoSCAN-4 system is highly acceptable for the identification not only of commonly encountered members of the *Enterobacteriaceae* but also for the much less common members of this family and, surprisingly, for most of the less frequently encountered bacilli of the genus *Pseudomonas* as well as other oxidase-positive organisms. More interesting is the ability of the autoSCAN-4 system to identify, with an unexpectedly high degree of accuracy, many of the members of that group we call the non-*Enterobacteriaceae* fermenters. These include bacteria that are far less frequently encountered than are the enterics or pseudomonads yet still can cause serious diseases and are responsible for extended morbidity in immunocompromised patients. Although we did not test a large population of these strains, the sample tested indicates excellent potential for identification.

The autoSCAN-4 system for identification has some faults, as does every other system currently available. For example, an answer from the system such as "very rare biotype" does not afford the user with any valuable information, because the obvious next question is, "A very rare biotype of what?" In addition, a biotype is a subspecific identification of an organism already identified to species, but no species has been named. We suggest that this answer be modified to a simple "unidentified" or "insufficient data to identify." Either answer would be more direct, but neither would be an admission of inadequacy by the manufacturer, since such answers are provided by reference laboratories with increasing frequency. Not all the biochemical test wells of the autoSCAN-4 tray perform equally well, as might be expected. In this study, the vast majority of tests used performed well within the parameters we expected, but two tests, H_2S production and arginine dihydrolase, proved troublesome. The H_2S test was responsible for five errors in identification, and the arginine test was responsible for four. For the most part, these errors were caused by the inability of the automated reader (autoSCAN-4) to recognize weakly positive reactions. The manufacturer is aware of these shortcomings and has taken steps to remedy them.

The autoSCAN-4 system usually does not utilize oxidase test results unless other data indicate the possibility of a nonfermenter. In most cases this should cause no problem but an exception is illustrated in Table 4, in which one *E. tarda* isolate was misidentified as a *Vibrio parahaemolyticus* isolate. This error was caused by several factors, including misreading two key reactions (H_2S and indole), the absence

TABLE 4. Misidentifications

Organism (no. of strains)	Incorrect identification	% Probability	Cause[a]
Escherichia coli, indole negative	*Salmonella* sp.	99.9	H_2S read as positive
Edwardsiella tarda	*Vibrio parahaemolyticus*	94.6	H_2S and indole read as negative
Edwardsiella tarda (3)	No ID[b] (3)		H_2S read as negative
Salmonella cholerae-suis	*Salmonella typhi*	77.9	Sucrose and decarboxylases read as negative
Salmonella arizonae	*Salmonella paratyphi* A	86.6	Malonate and decarboxylases read as negative
Citrobacter freundii	*Enterobacter agglomerans*	99.9	H_2S and several sugars read as negative
Citrobacter freundii	*Serratia rubidaea*	99.9	VP read as positive
Yersinia enterocolitica (2)	No ID (2)		Unable to differentiate between several organisms
Yersinia ruckeri	*Hafnia alvei*	99.9	VP read as positive
Enterobacter agglomerans	*Vibrio fluvialis*	99.9	Several tests read as negative
Shigella dysenteriae	No ID		Unable to differentiate between several organisms
Pasteurella sp.	No ID		Unable to differentiate between several organisms
Pseudomonas fluorescens	No ID		Unable to differentiate between several organisms
Pseudomonas stutzeri	No ID		Unable to differentiate between several organisms
Pseudomonas putrefaciens	No ID		Unable to differentiate between several organisms
CDC[c] Ve-2	*Acinetobacter anitratus*		Citrate read as negative

[a] Cause as determined by autoSCAN reader.
[b] ID, Identification.
[c] CDC, Centers for Disease Control.

of oxidase results, and the absence of other tests which could differentiate these two taxa (e.g., maltose, mannose, or mannitol). Further, the high probability calculated for the incorrect answer (94.6%) did not suggest a need for oxidase test results. In this particular case, oxidase results would have been helpful, but we feel that in the overwhelming majority of cases, oxidase results are not routinely needed, and the system has been programmed to request them when necessary. Thus, we do not consider this omission a major fault.

In summary, we found the MicroScan (and autoSCAN-4) system to be highly efficient, accurate, and reliable for identification of gram-negative bacteria. It cannot claim to be quick, since an overnight incubation period is required, but this is offset by accuracy of identification, even with unusual and difficult-to-identify cultures. The system does require freezer storage for the test trays, as do some other products, and this may be an inconvenience for some users. However, since this study was completed, the manufacturer has introduced dry trays in Canada and some European countries, so this problem may soon disappear. In our opinion, the MicroScan System, with the autoSCAN-4 reader, is an excellent addition to the automated products of value in the clinical microbiology laboratory.

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