

## Collaborative Clinical Evaluation of the Autobac IDX System for Identification of Gram-Negative Bacilli

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The Autobac IDX system (General Diagnostics, Warner-Lambert Co., Morris Plains, N.J.) for rapid, semiautomated identification of gram-negative bacilli was compared with the identification methods in routine use in four laboratories. The study included 1,515 organisms representing 30 species of enteric and nonenteric bacteria. Discrepancies between the results of the IDX system and routine methods were resolved by classical biochemical testing at a reference center. Overall, 98% of the organisms were correctly identified by the routine methods, and 93% were correctly identified by the IDX systems. After adjustment for frequency of clinical occurrence of the organisms tested, the IDX system performed with 95% accuracy. Results with the IDX system were available in 3 to 6 h. Results with the comparative methods were available in 4 to 48 h. A wide variety of organisms, including oxidase positive, oxidase negative, fermentative, and nonfermentative, were identified by a single system by using Autobac. Three or more systems were required to identify the 30 species by the comparative methods. Overall, the results indicate the Autobac IDX system is useful for the rapid identification of enteric and nonfermentative gram-negative bacilli.

A novel approach for automated bacterial identification based on differential growth inhibition was recently introduced (Autobac IDX; General Diagnostics, Warner-Lambert Co., Morris Plains, N.J.). The IDX system employs growth inhibitory compounds and computer analysis of the differential effects of these compounds to identify enteric and nonenteric gram-negative bacilli in 3 to 6 h (2, 4). A previous collaborative evaluation of this system revealed 95% accuracy for identification of reference strains and clinical isolates under experimental conditions (1). The present study was carried out to assess the performance of the IDX system for identification of clinical isolates in routine hospital practice and to evaluate the performance of the 18-chamber cuvette with 18 tests selected on the basis of the previous study and computer predictions. Organisms were identified in parallel by methods in routine use in four laboratories and by the IDX system. Identification discrepancies were arbitrated at the Centers for Disease Control, Atlanta, Ga. The results indicate an overall, weighted accuracy of 95% for the IDX system.

### MATERIALS AND METHODS

**Study design.** This evaluation of the IDX system was carried out in two phases: a precision study and a clinical evaluation. For the precision study, 30 bacterial strains selected from the Centers for Disease Control culture collection were evaluated. Included in this collection were two strains each of *Pseudomonas putida* and *Pseudomonas fluorescens* and single strains of *Acinetobacter calcoaceticus*, *Aeromonas* sp., *Alcaligenes* sp., *Salmonella* sp., *Citrobacter diversus*, *Edwardsiella tarda*, *Enterobacter aerogenes*, *Enterobacter agglomerans*, *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Flavobacterium*

sp., *Hafnia alvei*, *Moraxella* sp., *Morganella morganii*, *Proteus mirabilis*, *Providencia* sp., *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas cepacia*, *Pseudomonas stutzeri*, *Pseudomonas maltophilia*, *Serratia* sp., *Shigella* sp., *Yersinia enterocolitica*, and *Yersinia pseudotuberculosis*.

Each of these strains was frozen at  $-70^{\circ}\text{C}$  in sheep blood, and each of the four participating laboratories received 90 coded tubes representing three replicates of each strain. The frozen suspensions were thawed and subcultured onto sheep blood agar in each laboratory, and the organisms were identified by the IDX system and the comparative methods in use in each laboratory. The identification results were sent back to the Centers for Disease Control, where the code was broken, and the inter- and intralaboratory reproducibility was determined by the method of reproducibility indexes (1).

For the clinical evaluation, routine clinical isolates of gram-negative bacilli were identified by the methods in use in each of four laboratories and by the Autobac IDX system. Organisms were accepted into the study sequentially as they were encountered until preestablished quotas for each of 30 species were filled. Organisms identified differently by the in-laboratory versus IDX methods were forwarded to the Centers for Disease Control for definitive identification by classical biochemical tests (3).

**Autobac IDX system.** Each isolate to be identified was subcultured onto blood and MacConkey agar plates. The following day, growth, lactose fermentation, and bile precipitation were recorded from subcultures on the MacConkey agar plate; and spot oxidase, spot indole, and swarming growth tests were performed and the results recorded from subcultures on the blood agar plate (3). This information was entered into the Autobac computer. To inoculate a cuvette, one or more isolated colonies were suspended in phosphate-buffered saline (pH 7.0), and the suspension was adjusted to a standard turbidity ( $1.5 \times 10^7$  to  $3.0 \times 10^7$  CFU/ml) with the

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TABLE 1. Overall performance of IDX system

Institution	No. tested <sup>a</sup>	No. correct <sup>b</sup>	% <sup>c</sup>	Weighted % <sup>d</sup>
1	367	334	91.0	92.5
2	392	353	90.1	92.4
3	395	365	92.4	96.4
4	361	352	97.5	98.1

<sup>a</sup> Number of organisms identified by Autobac IDX and reference methods at each institution.

<sup>b</sup> Number of organisms correctly identified by Autobac IDX at each institution.

<sup>c</sup> For the four institutions, the average total percent was 92.7.

<sup>d</sup> Percent accuracy adjusted for relative frequency of occurrence of species tested. For the four institutions, the average total weighted percent was 95.3.

Autobac IDX photometer (General Diagnostics). The standardized inoculum (3 ml) was diluted into Autobac Low Thymidine Eugonic Broth (26.5 ml). Autobac IDX gram-negative identification disks were dispensed into an 18-

chamber cuvette, and the inoculum was dispensed into the cuvette by the instructions of the manufacturer. The cuvettes were incubated at 36°C with rotary agitation at 220 rpm, and photometer readings were taken at 3 h and then at 30-min intervals up to 6 h if the growth was insufficient at 3 h. The readings were automatically entered into the computer, along with manually entered pretest results, and identifications with relative probability values were reported for each isolate. Organism identifications with relative probability values of  $\geq 0.80$  were accepted without further testing; those with values  $< 0.80$  were subjected to post-testing by the instructions of the manufacturer.

**Comparative methods.** Each isolate identified by the IDX system was also tested by the identification method in routine use in each laboratory. The methods used at the four institutions (laboratories) were as follows. Laboratory 1 used API 20E (Analytab Products, Plainview, N.Y.) for identification of enteric bacteria and Oxi-Ferm (Flow Laboratories, Bethesda, Md.) and classical biochemical tests for identification of nonfermenters; laboratory 2 used the Auto-

TABLE 2. Performance of Autobac IDX system for identification of gram-negative bacilli

Species, identified	No. of species identified by Autobac when													
	<i>A. calcoaceticus</i>	<i>Aeromonas</i> sp.	<i>Alcaligenes</i> sp.	<i>Salmonella/Arizona</i> sp.	<i>C. diversus</i>	<i>C. freundii</i>	<i>Edwardsiella tarda</i>	<i>Enterobacter aerogenes</i>	<i>Enterobacter agglomerans</i>	<i>Enterobacter cloacae</i>	<i>Escherichia coli</i>	<i>Flavobacterium</i> sp.	<i>H. alvei</i>	<i>K. pneumoniae</i>
<i>A. calcoaceticus</i>	31									1				
<i>Aeromonas</i> sp.		25												
<i>Alcaligenes</i> sp.			14											
<i>Salmonella/Arizona</i> sp.				27										
<i>C. diversus</i>					30						1			1
<i>C. freundii</i>			1	2		51			1	7	1		1	2
<i>Edwardsiella</i> sp.							7				2			
<i>Enterobacter aerogenes</i>							45						2	1
<i>Enterobacter agglomerans</i>				1				25		3	1			2
<i>Enterobacter cloacae</i>				2		3			64		1			
<i>Escherichia coli</i>					1	2					238			3
<i>Flavobacterium</i> sp.												20		
<i>H. alvei</i>				1		3							35	
<i>K. pneumoniae</i>				1			1			1				150
<i>Klebsiella</i> sp.									1	1				
<i>Moraxella</i> sp.														
<i>Morganella morganii</i>														
<i>Proteus mirabilis</i>														
<i>Providencia</i> sp.	4									1				
<i>Proteus vulgaris</i>														
<i>Pseudomonas aeruginosa</i>			1											
<i>Pseudomonas cepacia</i>														
<i>Pseudomonas maltophilia</i>														
<i>Pseudomonas putida/fluorescens</i>			1											
<i>Pseudomonas stutzeri</i>														
<i>Pseudomonas</i> sp.			2									1		
<i>Serratia</i> sp.														
<i>Shigella</i> sp.														
<i>Y. enterocolitica</i>														
<i>Y. pseudotuberculosis</i>														
Total	35	25	19	34	31	59	8	45	27	80	244	21	38	159
No. correct	31	25	14	27	30	51	7	45	25	64	238	20	35	150
% agreement	89	100	74	79	97	86	88	100	93	80	98	95	92	94

Microbic system EBC-Plus (Vitek Systems, Inc., Hazelwood, Mo.) and classical biochemical tests for identification of enteric bacteria and API 20E and classical biochemical tests for identification of nonfermenters; laboratory 3 used the AutoMicrobic system EBC-Plus and Micro-ID (General Diagnostics, Morris Plains, N.J.) for identification of enteric organisms and classical biochemical tests for identification of nonfermenters; and laboratory 4 used Micro-ID and classical biochemical tests for identification of enteric bacteria and classical biochemical tests for identification of nonfermenters. Discrepancies between the comparative method and the Autobac IDX results were arbitrated by classical biochemical tests (3), and the results of these tests were considered the correct identification.

**RESULTS**

**Precision study.** For the 30 strains tested in triplicate at the four institutions, the interlaboratory reproducibility indexes were 0.98 for the IDX system and 0.96 for the comparative

methods. The intralaboratory precision ranged from 0.83 to 0.95 for the IDX system and 0.95 to 1.00 for the comparative methods.

**Clinical evaluation.** Of the 1,515 organisms examined at the four institutions, 1,387 were given the same identification by the IDX system and comparative methods. The IDX system and comparative methods failed to agree on the identification of 128 organisms, and these were arbitrated by classical biochemical testing at the reference laboratory. Arbitration indicated that 17 of the organisms were correctly identified by the IDX system, and 97 were correctly identified by the comparative methods. Fourteen organisms were misidentified by both the IDX system and comparative methods. Overall, 1,484 of 1,515 organisms (98%) were correctly identified by the comparative methods, and 1,404 of 1,515 (92.7%) were correctly identified by the IDX system (Table 1). The IDX identification accuracy varied from 90.1 to 97.5% among the four institutions. When the percentages were adjusted according to the frequency of occurrence of the organisms in a clinical setting (1), the overall accuracy of

used to test the following species:

<i>Klebsiella</i> sp.	<i>Moraxella</i> sp.	<i>Morganella morganii</i>	<i>Proteus mirabilis</i>	<i>Providencia</i> sp.	<i>Proteus vulgaris</i>	<i>Pseudomonas aeruginosa</i>	<i>Pseudomonas cepacia</i>	<i>Pseudomonas maltophilia</i>	<i>Pseudomonas pseudoflava</i>	<i>Pseudomonas stutzeri</i>	<i>Pseudomonas</i> sp.	<i>Serratia</i> sp.	<i>Shigella</i> sp.	<i>Y. enterocolitica</i>	<i>Y. pseudotuberculosis</i>
							1								
											1	1			
													2		
1				1				1					2	2	1
											1		1		
								2							
7	13				2						2	1	1		2
		52			2										5
			134												
		1		45	1										
		1		1	22										1
						104									
						1	17	1							
								55							
									32						
										21					
										1					
		2		1							29			1	
												80			
													26		
														5	
															1
8	13	56	134	49	27	106	18	59	33	22	33	82	32	8	10
7	13	52	134	45	22	104	17	55	32	21	29	80	26	5	0
88	100	93	100	92	82	98	94	93	97	96	88	98	81	62	0

the IDX system was 95.3%, and it ranged from 92.4 to 98.1% at the different institutions.

Of the 30 species tested, 12 were identified by the IDX system with <90% accuracy overall (Table 2). These included *A. calcoaceticus* (11.4% misidentified as *Providencia* sp.), *Alcaligenes* sp. (26.3% misidentified as *Pseudomonas* sp. or *Citrobacter freundii*), *Salmonella/Arizona* sp. (20.6% misidentified as a variety of *Klebsiella* sp., *Enterobacter* sp., or *Citrobacter* sp.), *C. freundii* (13.6% misidentified as *Escherichia coli*, *Enterobacter cloacae*, or *H. alvei*), *Edwardsiella* sp. (12.5% misidentified as *K. pneumoniae*), *Enterobacter cloacae* (20% misidentified as *C. freundii* and a variety of other organisms), *Klebsiella* sp. (12.5% misidentified as *Enterobacter agglomerans*), *Proteus vulgaris* (18.5% misidentified as *Moraxella* sp., *Providencia* sp., or *Morganella morganii*), *Pseudomonas* sp. (12.1% misidentified as *Moraxella* sp., *Flavobacterium* sp., or *Alcaligenes* sp.), *Shigella* sp. (18.7% misidentified as *Klebsiella* sp., *Enterobacter* sp., or *Citrobacter* sp.), *Y. enterocolitica* (37.5% misidentified as *Enterobacter* sp. or *Serratia* sp.), and *Y. pseudotuberculosis* (100% misidentified as *Moraxella* sp. and a variety of other organisms). A total of 7 species were identified with 90 to 95% accuracy and 11 species were identified with 95 to 100% accuracy by the IDX system. Nonfermenters were identified with 94% accuracy overall, and fermentative organisms were identified with 92% accuracy overall.

The unweighted identification accuracy varied from 90 to 97.5% at the four institutions in the study (Tables 1 and 3), and 361 to 395 organisms were examined. All 30 species were tested at three institutions, but 6 of the 30 were omitted

at one institution. Identification errors were comparable at the four institutions for most species, but some exceptions were noted (Table 3). Less than 75% identification accuracy was encountered for *Alcaligenes* sp., *Enterobacter cloacae*, *Proteus vulgaris*, and *Pseudomonas* sp. at institution 1. *Salmonella* sp., *C. freundii*, *Enterobacter cloacae*, and *Pseudomonas cepacia* were identified with <75% accuracy at institution 2. Similar results were encountered for *A. calcoaceticus*, *Edwardsiella tarda*, *H. alvei*, *Klebsiella* sp., and *Y. enterocolitica* at institution 3 and for *Shigella* sp. at institution 4. The Autobac system was unable to identify *Y. pseudotuberculosis* at any of the institutions.

## DISCUSSION

The Autobac IDX system presents a unique approach to the rapid identification of gram-negative bacilli based on differential growth inhibition by antibacterial compounds (2, 4). The IDX system uses a panel of 18 dyes, antibiotics, and other chemicals (Table 4) that differentially inhibit the growth of fermentative and nonfermentative gram-negative bacilli. The effects of these inhibitors, together with preliminary test results (oxidase, MacConkey agar reactions, spot indole, etc.), are used to identify isolates by quadratic discriminate analysis (4). A previous collaborative evaluation of the system compared its identification accuracy with that of classical biochemical tests, and excellent performance of the system was reported (1). The present study was carried out as a field trial of the IDX system comparing its performance against the methods in routine use in each of four laboratories. When discrepancies between results of the

TABLE 3. Performance of Autobac IDX at four institutions

Organism	Institution <sup>a</sup>			
	1	2	3	4
<i>A. calcoaceticus</i>	100 (11)	100 (10)	64 (11)	100 (3)
<i>Aeromonas</i> sp.	100 (5)	100 (4)	100 (10)	100 (6)
<i>Alcaligenes</i> sp.	50 (8)	100 (8)	50 (2)	100 (1)
<i>Salmonella/Arizona</i> sp.	89 (9)	75 (12)	90 (10)	33 (3)
<i>C. diversus</i>	100 (10)	88 (8)	100 (11)	100 (2)
<i>C. freundii</i>	100 (9)	69 (16)	80 (10)	96 (24)
<i>Edwardsiella tarda</i>	100 (2)	100 (2)	75 (4)	— (0)
<i>Enterobacter aerogenes</i>	100 (10)	100 (15)	100 (8)	100 (12)
<i>Enterobacter agglomerans</i>	89 (9)	86 (7)	100 (2)	100 (9)
<i>Enterobacter cloacae</i>	62 (13)	76 (21)	83 (18)	96 (28)
<i>Escherichia coli</i>	96 (50)	94 (52)	100 (54)	99 (88)
<i>Flavobacterium</i> sp.	100 (5)	89 (9)	100 (5)	100 (2)
<i>H. alvei</i>	93 (15)	100 (16)	60 (5)	100 (2)
<i>K. pneumoniae</i>	85 (34)	89 (35)	100 (40)	100 (50)
<i>Klebsiella</i> sp.	100 (1)	100 (4)	67 (3)	— (0)
<i>Moraxella</i> sp.	100 (3)	100 (1)	100 (6)	100 (3)
<i>Morganella morganii</i>	100 (15)	93 (15)	81 (16)	100 (10)
<i>Proteus mirabilis</i>	100 (36)	100 (36)	100 (35)	100 (27)
<i>Proteus vulgaris</i>	67 (9)	83 (6)	88 (8)	100 (4)
<i>Providencia</i> sp.	80 (10)	80 (10)	100 (15)	100 (14)
<i>Pseudomonas aeruginosa</i>	100 (26)	100 (26)	100 (26)	93 (28)
<i>Pseudomonas cepacia</i>	100 (9)	67 (3)	100 (1)	100 (5)
<i>Pseudomonas maltophilia</i>	100 (14)	94 (17)	90 (21)	86 (7)
<i>Pseudomonas fluorescens</i>	100 (8)	100 (10)	93 (15)	— (0)
<i>Pseudomonas stutzeri</i>	100 (10)	100 (1)	91 (11)	— (0)
<i>Pseudomonas</i> sp.	71 (7)	78 (9)	100 (14)	100 (3)
<i>Serratia</i> sp.	93 (15)	100 (21)	94 (18)	100 (28)
<i>Shigella</i> sp.	86 (7)	83 (12)	82 (11)	50 (2)
<i>Y. enterocolitica</i>	100 (2)	100 (2)	25 (4)	— (0)
<i>Y. pseudotuberculosis</i>	0 (5)	0 (4)	0 (1)	— (0)
Total	91 (367)	90 (392)	92 (395)	98 (361)

<sup>a</sup> Results listed as percent correctly identified (number tested).

TABLE 4. Panel of agents used in the Autobac IDX system

Agent	Disk mass (µg)
Acriflavine	30
Brilliant green	3
Cobalt chloride	375
Cycloserine	78
Cycloserine	240
3,5-Dibromosalicylic acid	750
Dodecylamine hydrochloride	18.7
Floxuridine	36
Malachite green	3
Methylene blue	255
Omadine disulfide	5.5
Sodium azide	75
Thallos acetate	150
Carbenicillin	40
Cephalothin	13.5
Colistin	13
Kanamycin	5.4
Novobiocin	48

routine laboratory method and the IDX system were encountered, they were resolved by classical biochemical testing at a reference center.

In the evaluation of the precision of the methods in the present study, the reproducibility of the IDX system and comparative methods was excellent. The second phase of the study evaluated the routine clinical performance of the systems, and the overall identification accuracy was 93% for the IDX system and 98% for the comparative methods. Although the IDX system performed with lower accuracy than the comparative methods, it should be noted that all 30 species were identified with a single system (IDX) compared with three or more systems needed for the comparative methods. Also, many of the comparative methods required longer incubation times than the 3- to 6-h IDX incubation time. Furthermore, this study examined a very challenging group of organisms, some of which occur rarely in clinical practice. When the results were weighted for the frequency of occurrence of the 30 species, the IDX identification accuracy was 92, 93, 96, and 98% at the four institutions, respectively. The overall weighted accuracy of 95% was comparable to that previously reported (1).

The variability of results among the four institutions is unexplained. Although the institution that reported 98% accuracy failed to test 6 of the 30 species, weighting of the results should have normalized the results with respect to the other institutions. Another possible explanation is that the Autobac instruments varied among the institutions, but they performed in a comparable manner in preliminary

precision and accuracy studies that were performed with control organisms distributed to each laboratory (data not shown). A third possibility is the variation in the characteristics of individual species encountered at the four institutions. This may be especially important because the original data base used in development of the IDX system was generated at institution 4, which recorded 98% accuracy in this study. *Acinetobacter* strains were selected to test this hypothesis. Clinical isolates of this organism were identified by the IDX system with 64% accuracy at institution 3 and with 100% accuracy at institution 4. *Acinetobacter* strains from institution 3 were reidentified by the IDX system at institution 4, and the identification accuracy was similar to that originally obtained at institution 3. This suggests that isolates of certain species encountered at one institution may be more difficult for the IDX system to identify than those at other institutions. Of the 12 species identified with <90% accuracy overall, 7 were due to problems at only one or two institutions. Only five species were identified by the IDX system, with <90% accuracy at a majority of the institutions. These species included *Salmonella*/*Arizona* sp., *Enterobacter cloacae*, *Proteus vulgaris*, *Shigella* sp., and *Y. pseudotuberculosis*.

The IDX system performed very well overall, and it offers a unique approach to gram-negative rod identification. No other commercial system offers the combination of identification of a broad variety of both fermentative and nonfermentative organisms and results in 3 to 6 h. Although the IDX system can benefit from some additional refinements, it merits consideration for clinical laboratory application.

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