

Collaborative Evaluation of the Radiometer Sensititre AP80 for Identification of Gram-Negative Bacilli

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Received 2 March 1992/Accepted 1 February 1993

A multicenter trial of the Sensititre AP80 panel read on the Sensititre AutoReader (Radiometer America, Westlake, Ohio) for the automated identification of gram-negative bacilli was conducted with 1,023 clinical isolates (879 members of the family *Enterobacteriaceae* plus 144 nonenteric organisms). Assignment of taxa was based on the computer-assisted interpretation of the results of a series of reactions with fluorogenic enzyme substrates after 5 h of incubation, with an incubation interval of approximately 18 h used when indicated. Accuracy was determined initially by comparison with the results obtained with the API 20E or Rapid NFT system (Analytab Products, Plainview, N.Y.). Isolates showing discrepancies were identified by using conventional biochemical profiles. Identifications were available after 5 h of incubation for 918 isolates (90%). Agreements with reference results for members of the family *Enterobacteriaceae* were 95.3 and 92.5% at the genus and species levels, respectively, and for the nonmembers of the family *Enterobacteriaceae*, the agreements with reference results were 95.1 and 84.7%, respectively. The Sensititre AP80 panel was found to be simple and convenient to use, allowed for the testing of three isolates per panel, required minimal supplementary testing for completion of identification, performed in a reproducible fashion, and demonstrated an accuracy of same-day identification comparable to that reported for other automated systems. The AP80 panel appears well suited for routine use in the clinical microbiology laboratory as an automated means of identifying both members of the family *Enterobacteriaceae* and nonenteric gram-negative bacilli.

The identification of aerobic and facultatively anaerobic gram-negative bacilli makes up a large portion of the daily work load for the clinical microbiology laboratory and is considered a necessary and important effort for the specific diagnosis of infection and epidemiological tracking of nosocomial pathogens. This task can be carried out by using any one of a number of conventional or commercially available systems. Approaches range from the visual reading of multiple physiological tests in a tube macro- or microdilution format with incubation for 18 to 24 h, with subsequent interpretation of results done by using percentage charts or computerized taxonomic data bases, to the use of automated instruments that read and analyze reaction results in a matter of hours, print reports, and store historical data with the assistance of microcomputers. The theoretical advantages of speed, efficiency, labor reduction, objectivity in the reading of reactions, and data storage and data management capabilities plus the usefulness of such instruments for antimicrobial susceptibility testing explain the growing popularity of automated microbiology systems. The Sensititre AutoReader System (Radiometer America, Westlake, Ohio) has used fluorogenic enzyme substrates as sensitive markers for the growth of bacteria in an antibiotic susceptibility test environment, thus allowing automated MIC testing with either same-day or overnight incubation (4, 5, 8, 9). Formulation of a variety of reactions with fluorogenic enzyme substrates either to reflect conventional physiological test

results or to demonstrate the interaction of bacteria with unique substrates allowed for an identification product (AP80) in the Sensititre microtray format that could be read by the AutoReader instrument, which has been described previously (8). The AP80 panel permits the identification of aerobic and facultatively anaerobic gram-negative bacilli in as little as 5 h, with the option of additional overnight incubation if needed or desired. This report presents the results of a multicenter, collaborative evaluation of the AP80 panel for the identification of gram-negative bacilli in a clinical laboratory setting. The API 20E and the Rapid NFT systems (Analytab Products, Plainview, N.Y.) were used to provide reference identifications, with discrepancies being resolved by conventional biochemical testing.

MATERIALS AND METHODS

Source of isolates. A total of 1,023 isolates were obtained by culturing various foci of infection at the clinical microbiology laboratories at the University of Cincinnati Medical Center, Cincinnati, Ohio (354 isolates); Sinai Samaritan Medical Center, Milwaukee, Wis. (323 isolates); and the University of Connecticut School of Medicine, Farmington, Conn. (346 isolates). A combination of isolates was selected for use to simulate the frequency of occurrence of the types of organisms seen in daily practice in the clinical microbiology laboratory. Freshly isolated organisms represented the majority of isolates studied. Isolates from frozen stock cultures were used to provide a broad spectrum of taxa.

Preparation of isolates. Fresh isolates were subcultured from the primary plate onto MacConkey agar (MAC; Becton Dickinson Microbiology Systems, Cockeysville, Md.) and

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were incubated overnight at 35°C in an ambient atmosphere. Frozen isolates were subcultured onto trypticase soy agar plus 5% sheep blood (BAP; Becton Dickinson Microbiology Systems, Cockeysville, Md.), incubated for 18 h at 35°C in an ambient atmosphere, assessed for purity, and subcultured onto a MAC plate and incubated for 18 h at 35°C in an ambient atmosphere. Several identical colonies were then used to inoculate the identification systems.

Identification of isolates by the API 20E and Rapid NFT systems. The API 20E strip (API; Analytab Products) was inoculated, incubated for at least 18 h at 35°C, and read visually according to the manufacturer's instructions. Taxonomic designations were assigned by using the API 20E Analytical Profile Register (1) or, when necessary, through telephone access to the API identification service. Glucose-nonfermenting isolates not identified by the API 20E system were placed into the Rapid NFT system (Analytab Products), incubated for 48 h at 30°C, and read according to the manufacturer's instructions. Taxonomic designations were assigned by using the Rapid NFT Numerical Codebook (1a).

Identification of isolates by the Sensititre AP80 panel. The Sensititre system hardware consisted of an Auto Inoculator equipped with a nephelometer for inoculum standardization, an AutoReader, a DEC 350 computer, and a printer. The AP80 panel for the identification of gram-negative bacilli was composed of three identical sections of 32 reaction wells containing various fluorogenic substrates. Routinely, three isolates were tested per panel. Appropriate sections were inoculated by using the Auto Inoculator, with each well receiving 50 µl of the test isolate suspended in demineralized water adjusted to a turbidity equal to that of a 0.5 McFarland standard. Two designated wells in each section received a mineral oil overlay, and the plate was sealed with a plastic adhesive cover. The plastic adhesive cover contained perforations that allowed the exchange of air for selected wells and remained in place throughout the readings. The panels were incubated at 35°C in an ambient atmosphere and were read with the AutoReader module at 5 h. Additional incubation, if prompted by the 5-h reading report, was carried out at 35°C, and the panel received a final reading the next day (at approximately 18 h). The computer software (Sensititre version 1.2) analyzed the intensities of fluorescence from each reaction well and reported an organism identification along with a quality of identification according to numerical taxonomy principles from the computer's data base. If required for identification, the report lists the tests needed for identification of selected isolates. One or more of four additional tests could be requested: pigmentation, oxidase or indole production, or motility. Each test was performed according to the manufacturer's instructions, the results were entered via a keyboard, and a final identification was generated.

Experimental design. Each test organism was inoculated onto an API 20E strip. BAP and MAC purity plates were streaked from the API inoculum tube and incubated overnight. The growth from the MAC purity plate was used to prepare a suspension to match that of a 0.5 McFarland standard. This suspension served as the inoculum for the AP80 test panel. Because inoculation and reading of the API 20E system occurred before the 5-h reading of the AP80 panels, the possibility that the reading of API reactions would be biased by the Sensititre AP80 result was eliminated. This time sequence also permitted the investigators to control the mixture of isolates placed into the study in the interest of a balanced distribution, since in most cases the identification of the isolate was available at the time of inoculation of the AP80 system. All AP80 panels were read after 5 h and an identifi-

cation was noted. When the AP80 report provided a genus and species and a quality comment indicating "acceptable identification" or better, this taxon was recorded as the AP80 system identification. If a report indicated a genus-only identification, a "good likelihood but low selectivity" comment, or a "reincubate" comment, the panel was reincubated and read after approximately 18 h of total incubation. The 18-h report was recorded as the AP80 identification.

Quality control isolates. As recommended by the manufacturer, five quality control isolates—*Escherichia coli* ATCC 4157, *Morganella morganii* ATCC 25830, *Pseudomonas aeruginosa* ATCC 10145, *Klebsiella oxytoca* ATCC 8724, and *Proteus vulgaris* ATCC 6896—were used with the AP80 system throughout the study. One or more isolates were included with each test run. Acceptable study data were predicated on the successful identification of the quality control isolates.

Reproducibility. The Centers for Disease Control (CDC) laboratory provided each of the three study sites with coded slants of 10 isolates (*Acinetobacter baumannii*, *Citrobacter diversus*, *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella oxytoca*, *Proteus mirabilis*, *Providencia rettgeri*, *Xanthomonas maltophilia*, *Serratia marcescens*, *Yersinia enterocolitica*) whose identities were known only to CDC. Each of these 10 isolates was identified by using the AP80 panel on 5 separate days at each site.

Data analyses. The API 20E and Rapid NFT systems provided the reference identifications for each isolate. If the taxonomic designation at the species level of the API 20E or Rapid NFT result matched that of the AP80 result, that designation was accepted as the identity of the organism. When results of AP80 panel readings did not agree at the species level with the results of the API 20E or Rapid NFT system, the isolate was sent to the CDC laboratory for arbitration. At CDC, conventional biochemical reactions were performed and organism identification was made by means of a computerized taxonomic data base. Study isolates were therefore sent to the CDC for one of the following reasons: (i) the results for the AP80 panel did not agree with those for the API systems, (ii) the API 20E or Rapid NFT system identified the organism to the genus level only and the AP80 panel yielded an identification to the species level, (iii) the AP80 panel identification was reported as "good likelihood but low selectivity" or "no probable ID," or (iv) the API systems failed to provide an identification. The CDC laboratory provided the reference identification in such instances.

The AP80 panel identification results were expressed as correct at the genus level, correct at the species level, incorrect, and "no identification possible." Reproducibility was determined by analysis of the results from multiple runs of the coded unknown isolates supplied by CDC.

RESULTS

A total of 1,023 isolates consisting of 20 genera and 40 species were examined by the three collaborating laboratories during the study. Of the 1,023 isolates tested, an identification with the designation of "acceptable" or better was offered for 918 (90%) isolates after 5 h. The other 105 isolates required further incubation to approximately 18 h either because of an initial "genus only," "low selectivity," or a "reincubate" report comment. (Among the members of the family *Enterobacteriaceae*, 93% of the results were available at 5 h and among the nonmembers of the family *Enterobacteriaceae*, 69% of the results were available at 5

TABLE 1. Summary of AP80 panel identification results

Isolate	No. (%) of isolates				
	Total	Correct genus	Correct species	Incorrect genus	No identification
<i>Enterobacteriaceae</i>					
<i>Citrobacter</i> sp.	1	1	1	0	0
<i>Citrobacter amalonaticus</i>	2	2	2	0	0
<i>Citrobacter diversus</i>	41	40	39	0	1
<i>Citrobacter freundii</i>	48	38	37	10	0
Enteric group 58	2	0	0	2	0
<i>Enterobacter</i> sp.	1	1	1	0	0
<i>Enterobacter aerogenes</i>	55	52	46	2	1
<i>Enterobacter amnigenus</i>	2	0	0	2	0
<i>Enterobacter cloacae</i>	81	74	73	6	1
<i>Enterobacter taylorae</i>	1	0	0	1	0
<i>Escherichia coli</i>	188	182	182	6	0
<i>Escherichia fergusonii</i>	2	1	0	1	0
<i>Hafnia alvei</i>	28	26	26	1	1
<i>Klebsiella ornithinolytica</i>	1	1	0	0	0
<i>Klebsiella oxytoca</i>	20	20	18	0	0
<i>Klebsiella ozaenae</i>	1	1	0	0	0
<i>Klebsiella pneumoniae</i>	92	92	85	0	0
<i>Morganella morganii</i>	34	34	34	0	0
<i>Pantoea agglomerans</i>	14	12	11	2	0
<i>Pasteurella multocida</i>	1	1	1	0	0
<i>Proteus mirabilis</i>	90	90	90	0	0
<i>Proteus penneri</i>	3	3	2	0	0
<i>Proteus vulgaris</i>	15	15	14	0	0
<i>Providencia alcalifaciens</i>	2	2	2	0	0
<i>Providencia rettgeri</i>	6	6	6	0	0
<i>Providencia stuartii</i>	14	14	14	0	0
<i>Salmonella</i> sp.	40	38	38	2	0
<i>Salmonella typhi</i>	2	2	2	0	0
<i>Serratia</i> sp.	1	1	1	0	0
<i>Serratia liquefaciens</i>	2	2	2	0	0
<i>Serratia marcescens</i>	59	59	59	0	0
<i>Shigella flexneri</i>	3	3	3	0	0
<i>Shigella sonnei</i>	22	20	20	2	0
<i>Yersinia enterocolitica</i>	5	5	4	0	0
Subtotal	879	838 (95.3)	813 (92.5)	37 (4.2)	4 (0.5)
<i>Non-Enterobacteriaceae</i>					
<i>Acinetobacter baumannii</i>	30	29	28	1	0
<i>Acinetobacter lwoffii</i>	3	3	3	0	0
<i>Aeromonas caviae</i>	1	0	0	1	0
<i>Aeromonas hydrophila</i>	5	4	0	1	0
<i>Bordetella bronchiseptica</i>	2	2	2	0	0
<i>Pleisiomonas shigelloides</i>	3	3	3	0	0
<i>Pseudomonas aeruginosa</i>	68	68	61	0	0
<i>Pseudomonas fluorescens</i>	3	1	0	2	0
<i>Pseudomonas putida</i>	1	1	0	0	0
<i>Pseudomonas stutzeri</i>	3	2	1	0	1
<i>Xanthomonas maltophilia</i>	25	24	24	1	0
Subtotal	144	137 (95.1)	122 (84.7)	6 (4.2)	1 (0.7)
Combined totals	1,023	975 (95.3)	935 (91.4)	43 (4.2)	5 (0.5)

h.) Among the organisms more frequently requiring 18 h of incubation for identification were *Acinetobacter* sp., *Shigella sonnei*, and *Xanthomonas maltophilia*.

The identification results of the AP80 panel compared with the reference identifications are presented in Table 1. Of the 879 isolates of members of the family *Enterobacteriaceae* tested, 838 (95.3%) results were correct at the genus level and 813 (92.5%) were correct at the species level. There were 37 incorrect genus level responses (4.2%) and 4 (0.5%) isolates for which no identification was offered (1 each of *Citrobacter diversus*, *Enterobacter aerogenes*, *Enterobacter cloacae*, and

Hafnia alvei). In 22 instances (2.5%), the correct genus but the incorrect species was given, while in 3 cases (0.3%), a partial identification (correct genus but no species designation) was offered by the AP80 panel, as detailed in Table 2. Table 3 lists those 37 isolates of members of the family *Enterobacteriaceae* for which an incorrect genus-level result was given by the AP80 panel. Among such organisms misidentified at the genus level, *Citrobacter freundii*, *Enterobacter cloacae*, and *Escherichia coli* made up over half of the isolates, perhaps reflecting the relatively frequent appearance of these taxa among the study isolates (Table 1).

TABLE 2. Sensititre AP80 panel identifications in agreement with reference system identifications at the genus level only

Reference identification	No. of isolates	AP80 panel identification
<i>Enterobacteriaceae</i>		
<i>Citrobacter diversus</i>	1	<i>Citrobacter freundii</i>
<i>Citrobacter freundii</i>	1	<i>Citrobacter</i> sp.
<i>Enterobacter aerogenes</i>	5	<i>Enterobacter cloacae</i>
	1	<i>Enterobacter</i> sp.
<i>Enterobacter cloacae</i>	1	<i>Enterobacter</i> sp.
<i>Escherichia fergusonii</i>	1	<i>Escherichia coli</i>
<i>Klebsiella ornithinolytica</i>	1	<i>Klebsiella pneumoniae</i>
<i>Klebsiella oxytoca</i>	2	<i>Klebsiella pneumoniae</i>
<i>Klebsiella ozaenae</i>	1	<i>Klebsiella rhinoscleromatis</i>
<i>Klebsiella pneumoniae</i>	4	<i>Klebsiella ozaenae</i>
	3	<i>Klebsiella oxytoca</i>
<i>Pantoea agglomerans</i>	1	<i>Enterobacter cloacae</i>
<i>Proteus penneri</i>	1	<i>Proteus mirabilis</i>
<i>Proteus vulgaris</i>	1	<i>Proteus penneri</i>
<i>Yersinia enterocolitica</i>	1	<i>Yersinia frederiksenii</i>
Total	25	
Non- <i>Enterobacteriaceae</i>		
<i>Acinetobacter baumannii</i>	1	<i>Acinetobacter lwoffii</i>
<i>Aeromonas hydrophila</i>	3	<i>Aeromonas caviae</i>
	1	<i>Aeromonas sobria</i>
<i>Pseudomonas aeruginosa</i>	1	<i>Pseudomonas</i> sp.
	5	<i>Pseudomonas fluorescens</i>
	1	<i>Pseudomonas mendocina</i>
<i>Pseudomonas fluorescens</i>	1	<i>Pseudomonas</i> sp.
<i>Pseudomonas putida</i>	1	<i>Pseudomonas</i> sp.
<i>Pseudomonas stutzeri</i>	1	<i>Xanthomonas maltophilia</i>
Total	15	

Likewise, similar performance among the 144 nonmembers of the family *Enterobacteriaceae* was noted at the genus level, with 137 (95.1%) of AP80 panel identifications being correct. At the species level, 122 (84.7%) responses were correct, with 6 (4.2%) genus-level errors and only 1 (0.7%) isolate (*Pseudomonas stutzeri*) for which no identification was given (Table 1). In 13 (9.0%) instances, a correct genus assignment but an incorrect species assignment was made. Several isolates each of *Aeromonas hydrophila* and *Pseudomonas aeruginosa* received another species designation, and in two instances (1.4%), a correct genus but no species designation was given by the AP80 panel (Table 2). The distribution of the six nonmembers of the family *Enterobacteriaceae* for which genus-level errors occurred is given in Table 3.

Combined results for all 1,023 isolates in the study demonstrated that 975 (95.3%) received correct AP80 panel identifications at the genus level and 935 (91.4%) received correct AP80 panel identifications at the species level, while 43 (4.2%) received incorrect genus-level designations. The AP80 panel failed to offer an identification for only five (0.5%) of all isolates tested.

Ten isolates provided by CDC were tested on five consecutive runs at each of the three study centers to examine the reproducibility of the AP80 panel. Analysis of results for these organisms showed correct identification at the genus level among the 150 observations in 147 (98%) cases. One *Acinetobacter baumannii* result and one *Klebsiella oxytoca* result were reported as "no identification possible." Another *Acinetobacter baumannii* result was reported as "*Flavimonas oryzihabitans*." Correct identification at the species level was noted in 142 (95%) observations. Errors at the

TABLE 3. Sensititre AP80 panel identifications incorrect at the genus level

Reference identification	No. of isolates	AP80 panel identification
<i>Enterobacteriaceae</i>		
<i>Citrobacter freundii</i>	1	<i>Enterobacter aerogenes</i>
	1	<i>Enterobacter cloacae</i>
	7	<i>Escherichia coli</i>
	1	<i>Pantoea agglomerans</i>
Enteric group 58	2	<i>Enterobacter amnigenus</i>
<i>Enterobacter aerogenes</i>	1	<i>Klebsiella ozaenae</i>
	1	<i>Citrobacter diversus</i>
<i>Enterobacter amnigenus</i>	1	<i>Citrobacter amalonicus</i>
	1	<i>Citrobacter freundii</i>
<i>Enterobacter cloacae</i>	5	<i>Citrobacter amalonicus</i>
	1	<i>Citrobacter diversus</i>
<i>Enterobacter taylorae</i>	1	<i>Citrobacter freundii</i>
<i>Escherichia coli</i>	2	<i>Salmonella</i> subgenus 1
	1	<i>Salmonella</i> sp.
	1	<i>Shigella sonnei</i>
	1	<i>Shigella</i> sp.
<i>Escherichia fergusonii</i>	1	<i>Pantoea agglomerans</i>
<i>Hafnia alvei</i>	1	<i>Koserella trabulsi</i>
<i>Pantoea agglomerans</i>	1	<i>Citrobacter freundii</i>
	1	<i>Aerimorrella</i> sp.
<i>Salmonella</i> sp.	2	<i>Escherichia coli</i>
<i>Shigella sonnei</i>	1	<i>Salmonella</i> sp.
	1	<i>Escherichia coli</i>
Total	37	
Non- <i>Enterobacteriaceae</i>		
<i>Acinetobacter baumannii</i>	1	<i>Flavimonas oryzihabitans</i>
<i>Aeromonas caviae</i>	1	<i>Vibrio furnissii</i>
<i>Aeromonas hydrophila</i>	1	<i>Citrobacter freundii</i>
<i>Pseudomonas fluorescens</i>	2	<i>Flavimonas oryzihabitans</i>
<i>Xanthomonas maltophilia</i>	1	<i>Escherichia coli</i>
Total	6	

species level were largely attributable to one isolate. The *Klebsiella oxytoca* isolate was reported as "*Klebsiella ozaenae*" in four cases.

Additional tests were infrequently required for identification by the AP80 panel. Only 38 of the 1,023 isolates required a total of 62 individual tests (12 pigmentation and 15 motility observations and 10 oxidase and 25 indole production tests).

DISCUSSION

The AP80 panel is designed to permit the identification of gram-negative bacilli either within the same day (5 h) or after overnight incubation (approximately 18 h). The user may choose the appropriate data base within the system software to analyze and interpret the data for either incubation interval used. It was deemed appropriate in the present study to evaluate the characteristics of the AP80 panel as it would likely be used in a clinical setting; therefore, same-day results were accepted if they were available at the 5-h reading and isolates received further incubation (to 18 h) if it was so indicated by the 5-h report or if an incomplete identification (to the genus level only) was reported at 5 h. The choice of the API 20E and Rapid NFT systems for the initial characterization of organisms was based on the widespread acceptance of these identification products in clinical laboratories. The approach used the assumption that identical taxonomic identifications from the API systems and from the AP80 panel would, in the majority of instances, be correct and would thus be acceptable as reference identifi-

cations. Any deviation in agreement between the two API systems and the AP80 panel or incomplete designation by the API systems prompted submission of the isolate to the CDC laboratory for arbitration, and conventional biochemical reagents and the CDC computer-based identification profile for gram-negative bacilli were used. There are precedents in the literature for this study design (3, 6).

The overall performance of the AP80 panel for identification of members of the family *Enterobacteriaceae* approaches that tentatively suggested in guidelines by Sherris and Ryan (7) for acceptable accuracy in automated bacterial identification procedures. Results for all organisms tested with the AP80 panel were >95% correct at the genus level and the correct identification at the species level was 92.5%, as shown in Table 1. Stratification of the data into members of the family *Enterobacteriaceae* and other organisms (predominantly glucose-non-fermenting gram-negative bacilli) indicated similar performance characteristics for each of the two groups at the genus level (>95%). Accuracy at the species level differed somewhat between the *Enterobacteriaceae* and non-*Enterobacteriaceae* (92 and 85%, respectively). Colonna et al. (1b) have reported on the performances of the AP80 panel and other instrumented systems for the identification of gram-negative bacilli. They found that the identification accuracy at the species level with the AP80 panel was 93% among the *Enterobacteriaceae* isolates tested; these results are similar to those reported here (Table 1), but among nonenteric organisms, Colonna et al. (1b) reported lesser accuracy at the species level (71%) than was noted in the present study (85%), perhaps because of differences in the isolates examined. The spectrum of nonenteric organisms in the present study was limited, and further studies of the less commonly encountered members of this group may be warranted. The distribution of the taxa identified by the AP80 panel showing disagreement with the reference identification (Tables 2 and 3) suggests little systematic or consistent error in AP80 panel identifications. The one exception may perhaps be the ability of the AP80 panel to correctly identify to the species level *Aeromonas hydrophila*. Three of five members of this taxon examined were called "*Aeromonas caviae*." It would be anticipated, as with any newer identification system, that as more organism biotypes are contributed, the data base would further mature, having a positive impact on both the spectrum and accuracy of organism identifications. Since the present study was completed, updates of the panel software addressing recent changes in nomenclature have been issued. The data presented here incorporate these most recent changes.

Multiple testing of a selected battery of isolates within each laboratory suggested excellent reproducibility characteristics for the AP80 panels at the genus level. Variation among the 150 observations was less than 5% at the species level and was largely due to a single *Klebsiella* isolate.

The majority of enteric pathogens were recognized by the AP80 panel in the present study; however, there were four instances in which an enteric pathogen of particular clinical significance was misidentified. Among 42 *Salmonella* isolates tested, 2 *Salmonella* isolates were reported as *Escherichia coli*, and among 25 *Shigella* isolates tested, 2 *Shigella* isolates were reported as a *Salmonella* sp. and *Escherichia coli*, respectively. All four of these occurrences were after 5 h of incubation. Upon reincubation, the two *Salmonella* isolates and one of the *Shigella* isolates were reported correctly by the AP80 panel, with the second *Shigella* isolate being reported as "no identification possible," a report that would prompt identification by an alter-

nate means. It might be prudent, when the suspicion of an enteric pathogen from a gastrointestinal specimen source exists, to continue incubation in the AP80 panel for the 18-h period. Of 188 *Escherichia coli* isolates, an identification of 2 *Salmonella* spp. and 2 *Shigella* spp. occurred. Such results would have had little clinical impact theoretically, because an AP80 software prompt suggested that serology be used, which is a routine practice for the confirmation of enteric pathogens in the laboratory setting.

Other investigators have reported the accuracies of other automated bacterial identification systems to be in the same range as that for the AP80 system presented here. Colonna et al. (1b) found that 83% of members of the family *Enterobacteriaceae* were accurately identified with the autoSCAN-W/A (Baxter Healthcare Corp., Sacramento, Calif.), a fluorogenic substrate-based system with a 2-h incubation interval, and that 94.7% of members of the family *Enterobacteriaceae* were accurately identified with the Vitek AutoMicrobic System (bioMerieux Vitek, Inc., Hazelwood, Mo.), a colorimetric-based system with an incubation interval that ranges from 4 to 18 h. Pfaller et al. (6), using a study design similar to that reported here, also evaluated the performance of the autoSCAN-W/A system and the Vitek AutoMicrobic System for identification of gram-negative bacilli. The autoSCAN-W/A system correctly identified 87.7% of organisms tested to the species level, while the Vitek AutoMicrobic System correctly identified 92.7% of isolates tested to the species level. Accuracy could be improved for the autoSCAN-W/A and the Vitek systems in the report of Pfaller et al. (6) if answers with the designation "low probability" were accepted. In the present study, performance improved when AP80 panel results indicating "low selectivity" or identification to the genus level only were reincubated. York et al. (10) also reported the autoSCAN-W/A system to have 96% accuracy for members of the family *Enterobacteriaceae* if additional tests were carried out beyond the automated reading. For 63 of 366 isolates (17%), such tests were indicated (excluding spot indole and oxidase tests). York et al. (10) also pointed out that the API 20E system called for additional tests in 106 (29%) of instances to reach a species-level identification. Likewise, Pfaller et al. (6) and Debates et al. (2) reported that for 7.6 and 14% of isolates, respectively, reported as "low probability" by the autoSCAN-W/A system, supplementary testing, most requiring an additional overnight incubation period, was called for by the system. Likewise, in the present study, 57 of 1,023 isolates (5.6%) identified with the API 20E or Rapid NFT system required additional testing to obtain more information than was offered by the strip. However, the AP80 panel required additional testing for only 38 of these 1,023 isolates (3.7%). Results of any of the four possible supplementary tests prompted for use with the AP80 panel could be obtained immediately: pigmentation (observation), oxidase (spot test) or indole (performed on panel), or motility (hanging drop). Avoidance of an additional incubation day could therefore be achieved. It should be noted, however, that 10% of isolates (notably, a proportionately larger number of the non-*Enterobacteriaceae* group) required additional overnight incubation for complete identification. The total turnaround time for identification results from automated or manually read systems, such as the AP80 panel, the autoSCAN-W/A system, the Vitek AutoMicrobic System, or the API 20E system, depends on the nature of the organism tested, the need for supplementary tests, and the time required to obtain the results of such tests. Comparisons of results, turnaround times, and accu-

racies between identification systems from the literature must be interpreted cautiously and all variables must be taken into consideration.

Procedurally, as judged by the technical staff, the AP80 panel offered convenient automated inoculation and oil overlay of panels. The accommodation of three organisms per panel had positive work flow and economic implications. Although in the present study the colonies used for processing by the AP80 panel were picked from MAC plates, colonies may be picked from any nonselective agar, such as BAP, according to the manufacturer. The AP80 panel approach to same-day testing differs from that of most short incubation identification systems with respect to work flow considerations in that an organism for which there is a partially completed (genus-only), "low selectivity" result or an organism for which there are insufficient data for identification at 5 h can be reincubated overnight and read without the necessity of repeating the identification with an alternate product. The dual data base (5 or 18 h) allows flexibility in the choice on any given workday for the completion of identification either within the same workday or on the following day, even after the panels have been inoculated.

Unlike the autoSCAN-W/A or the Vitek system, the Sensititre automated system used in the present study lacked full automation and required operator attendance at the reading step. An incubator/tray transport module presently offered to the European market imparts fully automated incubation and reading of Sensititre panels in the walkaway mode.

In summary, the Sensititre AP80 panel allowed the testing of three organisms per panel, provided results in 5 h for the majority of isolates tested, was found to be simple and convenient to use, performed in a reproducible fashion, required minimal supplementary testing to complete identifications, and demonstrated accuracy comparable to those reported for other automated identification systems. The AP80 panel therefore is well suited for routine use in the clinical microbiology laboratory and is acceptable as an automated method for the identification of members of the family *Enterobacteriaceae* and nonenteric gram-negative bacilli.

ACKNOWLEDGMENTS

This study was supported in part by grants from Radiometer America.

We greatly appreciate the efforts of the technical staff of the participating microbiology laboratories at the University of Cincinnati Medical Center, the Mount Sinai Medical Center, and the North American Laboratory Group for collection of clinical isolates, and we thank Mary Anne McMillan of Cincinnati for secretarial support.

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