

## Evaluation of the API 20E System for Identification of Nonfermentative Gram-Negative Bacteria

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The API 20E system for *Enterobacteriaceae*, recently broadened to include identification of nonfermentative gram-negative bacteria, was evaluated and compared with the conventional method for complete identification of 221 nonfermenters, which were well distributed into 48 species or biotypes and included organisms not listed in the API 20E data base. The results of 16 tests common to both systems were in close agreement. The API 20E system correctly identified 71 (43%) of the 165 organisms included in the API 20E data base. However, almost 90% of *Acinetobacter calcoaceticus*, three species of *Pseudomonas*, and *Bordetella bronchiseptica* were correctly identified to species.

The API 20E system is a plastic strip with microtubes containing dehydrated substrates, originally designed for the identification of *Enterobacteriaceae*. Later, API introduced the Profile Recognition System for numerical identification and then, using a computer-assisted program, developed the Analytical Profile Index, supplemented by the API Computer System Service. In 1976, the Analytical Profile Index for API 20E was expanded to include other fermentative and nonfermentative gram-negative bacteria. Five separate tests, not included in the strip, were then added to complete the system for identification of the nonfermenters.

Various investigators (1, 5, 10, 12) evaluated the API 20E system for the identification of *Enterobacteriaceae* and reported a high level of agreement with conventional methods in both biochemical reactions and identifications. The Analytical Profile Index (or Register) has also been mathematically evaluated as excellent (7). Two evaluations have been made of the complete API 20E system, including the five separate tests—fermentation of glucose (OFF), oxidation of glucose (OFO), motility, oxidase, and MacConkey—for identification of nonfermentative gram-negative bacteria (3, 6). Both reports found the system useful for identification of clinical isolates of *Pseudomonas* and *Acinetobacter*. We have now evaluated the system, which includes the API 20E and the five separate tests, for the complete identification (to species or biotypes) of 221 isolates of nonfermentative gram-negative bacteria as required by a reference laboratory.

### MATERIALS AND METHODS

**Bacteria.** Of the 221 isolates used in this study, 166 were from a culture collection maintained by the Di-

vision of Laboratories and Research, New York State Department of Health. All isolates originated from clinical specimens that had been submitted to our laboratory for identification or confirmation. Forty-seven of the cultures were received through the courtesy of Analytab Products Inc., Plainview, New York. Eight were kindly provided by G. L. Gilardi, Hospital for Joint Diseases and Medical Center, New York City. In our laboratory all cultures were either lyophilized or maintained on blood agar slants.

The organisms used in the evaluation were nonfermentative gram-negative rods, well distributed among 48 species and including 56 isolates not listed in the API 20E data base. No fermentative organisms were used.

**Conventional media and procedures.** The media were prepared by the Division's media section as described previously (9). The inoculated media were incubated for up to 5 days at 35 to 37°C. The organisms were identified by using generally accepted criteria (2, 4, 11, 13).

**API 20E system for nonfermenters.** The API 20E strip (this strip is the same one used for the identification of *Enterobacteriaceae*) contains 20 microtubes with substrates for the following 23 tests: *O*-nitrophenyl- $\beta$ -D-galactosidase (ONPG); arginine dihydrolase; lysine and ornithine decarboxylase; citrate utilization; hydrogen sulfide; urease; tryptophan deaminase; indole; Voges-Proskauer (acetoin); gelatin liquefaction; fermentation of the carbohydrates glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin, and arabinose; nitrate reduction and nitrogen gas production, tested in the glucose microtube; and catalase production, in any other carbohydrate microtube. The catalase test was not used in this study. A complete description of the strip is given in other reports (1, 5, 10, 12).

Additional media are required for the five separate tests not on the strip. Media for three of these tests are available from the manufacturer in snap-open ampules: API M for the motility test and API OF for both the glucose oxidation and glucose fermentation tests. Also available is the API oxidase test kit con-

taining oxidase reagent (a 1% solution of tetramethyl-*p*-phenylenediamine dihydrochloride) and plastic chambers with filter paper. Conventional MacConkey agar was used for the fifth separate test. In this study, methodology for the use of the system and interpretation of results were according to the manufacturer's recommendations.

Twenty-two of the initial tests were checked and recorded after 24 h of incubation and again at 48 h. At this point, reagents were added for the five remaining tests (tryptophan deaminase, indole, Voges-Proskauer, nitrate reduction, and nitrogen gas production), and those results were recorded. The Analytical Profile Index and (if necessary) the computer service were consulted for identification of the isolates. When indicated, supplemental tests recommended by the manufacturer for identification were done using conventional media. When sufficient reactions were clear at 24 h, identification was determined on those readings.

## RESULTS

Biochemical reactions of the 221 isolates in the API 20E system (excluding fermentative carbohydrate tests not used in the conventional method) were in close agreement with conventional method results (Table 1). The lowest agreement was in the citrate utilization and motility tests.

The level of identification expected to be attained by the API system for all 221 isolates was compared with the actual identification achieved (Table 2). Of the organisms included in the API data base (165 isolates), 43% were correctly identified to the level expected by the manufacturer, and only 29% were completely identified to species and biotype. The system misidentified 22.4% of the organisms included in the API 20E charts and 32.2% of those not listed.

Unidentified by the system were 24.8% of the organisms included and 26.8% of those not listed.

An analysis of the reactions and identification obtained with the API system for each of the 48 conventionally identified species used in this evaluation is given in Table 3. Of the 221 organisms tested, 119 (53.8%) required from one to

TABLE 1. Comparison of 16 biochemical reactions of 221 isolates, using the API 20E and conventional (C) tests<sup>a</sup>

Tests	No. in agreement		No. differing	
	API+, C+	API-, C-	API+, C-	API-, C+
ONPG	6	195	14	6
Arginine dihydrolase	16	195	2	8
Lysine decarboxylase	3	208	0	10
Ornithine decarboxylase	2	213	0	6
Citrate utilization	74	66	23	58
Hydrogen sulfide	2	219	0	0
Urease	17	168	0	36
Tryptophan deaminase <sup>b</sup>	0	195	1	25
Indole	3	210	0	8
Gelatin liquefaction	22	163	30	6
Oxidase	182	28	8	3
Nitrate to nitrate (NO <sub>3</sub> to NO <sub>2</sub> )	44	148	13	16
Nitrate to gas (NO <sub>3</sub> to gas)	31	162	9	19
Motility	93	68	25	35
Glucose, oxidative (OFO)	38	150	1	32
Glucose, fermentative (OFF)	0	217	0	4

<sup>a</sup> +, Positive reaction; -, negative reaction.

<sup>b</sup> Phenylalanine deaminase used in conventional method.

TABLE 2. Extent of identification of 221 isolates by the API system

API's expected level of identification	No. of isolates	API identification [no. (%)]				Complete identification (as required by our reference lab) [no. (%)]
		To expected level	Correct only to genus	Misidentified	Unidentified	
Genus <sup>a</sup>	64	23 (35.9)	— <sup>b</sup>	12 (18.8)	29 (45.3)	
Species <sup>c</sup>	87	47 (54.0)	8 (9.2)	22 (25.3)	10 (11.5)	47 (54.0)
Biotype <sup>c</sup>	14	1 (7.1)	8 (57.2)	3 (21.4)	2 (14.3)	1 (7.1)
Total	165	71 (43.0)	16 (9.7)	37 (22.4)	41 (24.8)	48 (29.1)
No expected identification (not included in API system)	56		23 (41.1)	18 (32.2)	15 (26.8)	0
Total	221	71 (32.1)	39 (17.6)	55 (24.9)	56 (25.3)	48 (21.7)

<sup>a</sup> Includes organisms designated "genus-like" when identified as that genus, e.g., *Alcaligenes*-like group IVe identified as *Alcaligenes* sp.

<sup>b</sup> For the 64 isolates in this category, "to genus only" was the expected level of identification.

<sup>c</sup> Unnamed organisms included in species and biotype whenever applicable.

TABLE 3. Results of the API 20E system identification of nonfermentative gram-negative bacteria

Organism	API's expected level of identification	No. of isolates tested	No. requiring supplemental tests (tests per isolate)	No. correctly identified		No. referred to computer	Incorrect identifications <sup>a</sup>	Incorrect reactions	
				Genus <sup>b</sup>	Species type			Initial tests	Supplemental tests
<i>Achromobacter xylosoxidans</i> IIIA	Species	10	5 (4), 5 (5)	0	0	1 Unidentified			Cetrimide -, gr. <sup>c</sup> at 42°C
<i>A. xylosoxidans</i> IIIB	Species	7	1 (1), 2 (2), 4 (4)	1	4	5 <i>Alcaligenes</i> sp. 4 <i>B. bronchiseptica</i> 5 <i>Alcaligenes</i> sp. 1 <i>Alcaligenes</i> sp.			Cetrimide - Cetrimide - Cetrimide -
<i>Achromobacter</i> sp. bio-type 1	Species	3	1 (4), 1 (5)	1	2	1 Unidentified	MAC - <sup>d</sup>		Acetamide +, gr. on SS -
<i>Achromobacter</i> sp. bio-type 2	Species	5	1 (2), 1 (5)	2	4	1 Unidentified 1 <i>A. xylosoxidans</i> 1 Unidentified	GEL +		Cetrimide -, gr. on SS -, flagella stain -
<i>Acinetobacter calcoaceticus</i> subsp. <i>anitratus</i>	Species (sub-species)	10	1 (1), 1 (2)	2	3	1 Unidentified 1 Unidentified 1 <i>A. calcoaceticus</i> subsp. <i>luoffi</i> 1 <i>A. calcoaceticus</i> subsp. <i>luoffi</i>	Unacceptable profile number OFO -		10% lactose -
<i>A. calcoaceticus</i> subsp. <i>luoffi</i>	Species (sub-species)	10		9	1	1 CDC group Ve-2 1 <i>Alcaligenes</i> sp.	MOT + NO <sub>2</sub> to gas +		
<i>Alcaligenes denitrificans</i>	Genus	5	4 (4), 1 (5)	1	0	2 Unidentified 1 Unidentified 1 Unidentified			Gr. at 42°C - Gr. at 42°C -, flagella stain - Gr. at 42°C -, flagella stain -, acetamide -
<i>A. faecalis</i>	Genus	5	1 (3), 4 (4)	4	1	1 <i>Pseudomonas</i> sp.			Gr. at 42°C -
<i>A. odorans</i>	Genus	4	4 (4)	1	2	1 Unidentified 1 Unidentified			Gr. at 42°C - Gr. at 42°C -, gr. on SS -
<i>Alcaligenes</i> -like group IVe	Not included	6	3 (4), 1 (5)	1	3	1 CDC group IVc-2 1 Unidentified 1 Unidentified			Gr. on SS - Acetamide -, flagella stain - Gr. at 42°C -, gr. on SS -, flagella stain -
<i>Bordetella bronchiseptica</i>	Species	7	1 (3), 1 (4)	7	1	1 Unidentified 1 CDC group IIf 1 <i>Moraxella</i> sp.			

TABLE 3—Continued

Organism	APIs expected level of identification	No. of isolates tested	No. requiring supplemental tests (tests per isolate)	No. correctly identified to:		No. referred to computer	Incorrect identifications <sup>a</sup>	Incorrect reactions	
				Genus <sup>b</sup>	Species type			Initial tests	Supplemental tests
<i>Eikenella corrodens</i>	Not included	5	1 (4), 1 (5)	0		1	1 Unidentified	LDC -, ODC -, NO <sub>3</sub> to NO <sub>2</sub> -, MOT +	
<i>Flavobacterium meningosepticum</i>	Genus	3		1		1	1 Unidentified	LDC -, ODC -, NO <sub>3</sub> to NO <sub>2</sub> -, MOT +	
<i>Flavobacterium</i> sp. bio-type IIb	Genus	3	2 (4)	0		1	1 Unidentified	LDC -, ODC -, NO <sub>3</sub> to NO <sub>2</sub> -, MAC +	
Group M-1	Not included	2		0		0	2 <i>Moraxella</i> sp.	LDC -, ODC -, NO <sub>3</sub> to NO <sub>2</sub> -,	
Group TM-1	Not included	1		0		0	1 <i>Moraxella</i> sp.	LDC -, ODC -, NO <sub>3</sub> to NO <sub>2</sub> -,	
Group II	Species	4	1 (3), 3 (5)	0	0	3	1 Unidentified	NO <sub>3</sub> to NO <sub>2</sub> -, MOT -, GEL +	
							1 <i>Alcaligenes</i> sp.	OFO -, MOT +	
							1 CDC group III	OFO -	
							1 Unidentified	GEL -	
							1 CDC group III	GEL -, MOT +	
							1 <i>Moraxella</i> sp.		
							2 <i>Moraxella</i> sp.		
							1 <i>Moraxella</i> sp.		
							1 Unidentified	MOT +	
							1 Unidentified	MOT +, ADH +	
							1 Unidentified	MOT +, NO <sub>3</sub> to GAS +	
							1 <i>P. putrefaciens</i>	MOT +	
							1 <i>Alcaligenes</i> sp.	MOT +	
							1 Unidentified		
							1 CDC group III-1	MOT +, ONPG +	
							1 Unidentified	MOT +	
							1 Unidentified	NO <sub>3</sub> to gas +	
							1 <i>B. bronchiseptica</i>		
							1 Unidentified	MOT +	
							1 CDC group IVc-2	MOT +	
							1 Unidentified	MOT +, CIT -	Gr. on SS +
							1 Unidentified	NO <sub>3</sub> to gas +	
							1 Unidentified	NO <sub>3</sub> to gas +, OXI -	
							1 <i>Alcaligenes</i> sp.	MOT +, NO <sub>3</sub> to gas +	
							5 CDC group III		

TABLE 3—Continued

Organism	API's expected level of identification	No. of isolates tested	No. requiring supplemental tests (tests per isolate)	No. correctly identified to:		No. referred to computer	Incorrect identifications <sup>a</sup>	Incorrect reactions	
				Genus <sup>b</sup>	Species type			Initial tests	Supplemental tests
<i>Moraxella</i> -like group M-5	Not included	9		6		1	1 Unidentified 1 Unidentified 1 CDC group IVc-2 1 <i>Pseudomonas</i> sp.	GEL + MOT +, OFO + MOT + MOT +	
<i>Moraxella</i> -like group M-6	Not included	6	1 (4)	5		1			
<i>Pseudomonas acidovorans</i>	Genus	6	1 (3), 5 (4)	1		0	5 Unidentified		
<i>P. aeruginosa</i>	Species	9	2 (1), 1 (2) 1 (5)	9		8			
<i>P. alcaligenes</i>	Genus	3	1 (3), 2 (4)	2		2	1 Unidentified		Acetamide +
<i>P. cepacia</i>	Species	2	1 (1)	2		2			Acetamide +
<i>P. diminuta</i>	Genus	7	5 (4), 2 (5)	2		4	2 Unidentified 3 CDC group IVc-2 3 <i>Moraxella</i> sp.		Acetamide + Acetamide +
<i>P. fluorescens</i>	Species	3	2 (3), 1 (5)	0		0	1 Unidentified	OXI +	Deoxyribonuclease -
<i>P. maltophilia</i>	Species	8	6 (4), 2 (5)	7		4	7 Unidentified 1 Unidentified		Acetamide + Acetamide +, gr. at 42°C -
<i>P. pseudoalcaligenes</i>	Genus	9		1		1			
<i>P. putida</i>	Species	6	1 (3)	4	1	0	1 <i>P. fluorescens</i> group 1 <i>Moraxella</i> sp.	MOT -, ADH -, MAC -	
<i>P. putrefaciens</i>	Species	3		1	2	1	2 <i>P. fluorescens</i> group/ 1 <i>Pseudomonas</i> sp. 1 <sup>1</sup>	NO <sub>3</sub> to gas + (atypical strain)	
<i>P. stutzeri</i>	Genus	3	1 (4)	1	2	1	1 <i>Pseudomonas</i> sp. 2 <sup>2</sup> 2 <sup>2</sup>	MOT -	Gr. on SS -
<i>P. testosteroni</i>	Genus	7	5 (4), 2 (5)	3	3	1	3 Unidentified 1 CDC group IVc-2 1 <i>Pseudomonas</i> sp. 1 <sup>1</sup>		Acetamide + Acetamide + Acetamide +
<i>Pseudomonas</i> -like group III, biotype 1	Biotype	5	1 (4)	2		4	1 Unidentified 2 <i>Moraxella</i> sp. 1 <i>P. maltophilia</i> 1 <i>Pseudomonas</i> sp. 1 <sup>1</sup>	OFO - OFO - OFO - OFO -, NO <sub>3</sub> to gas +	

TABLE 3—Continued

Organism	API's expected level of identification	No. of isolates tested	No. requiring supplemental tests (tests per isolate)	No. correctly identified to:		No. referred to computer	Incorrect identifications <sup>a</sup>	Incorrect reactions	
				Genus <sup>b</sup>	Species type			Initial tests	Supplemental tests
<i>Pseudomonas</i> -like group Va, biotype 1	Not included	2	1 (4)	0		2	1 <i>Achromobacter</i> sp. 1 CDC group Ve-2 1 Unidentified	ONPG +, OFO - OXI - OFO -	
<i>Pseudomonas</i> -like group Va, biotype 2	Not included	1	1 (5)	0		1			
<i>Pseudomonas</i> -like group Ve, biotype 1	Biotype	2	1 (2), 1 (4)	2		2	1 <i>P. cepacia</i> 1 <i>Pseudomonas</i> sp.	OXI + (weak) OXI + (weak)	Gr. at 42°C - Gr. at 42°C -
<i>Pseudomonas</i> -like group Ve, biotype 2	Biotype	3	1 (1)	2		2	1 <i>A. calcoaceticus</i> subsp. <i>anitratius</i> 1 <i>P. maltophilia</i> 1 <i>P. putida</i>	MOT - GEL +, OFO - GEL +, OXI + NO <sub>2</sub> to NO <sub>3</sub> +	
<i>Campylobacter fetus</i> ( <i>Vibrio fetus</i> )	Not included	1		0		1	1 <i>A. calcoaceticus</i> subsp. <i>tuwoffi</i>	OXI -, MOT - NO <sub>3</sub> to NO <sub>2</sub> -	

<sup>a</sup> Incorrect identifications found by the system combined with the supplemental tests.

<sup>b</sup> Includes organisms designated "genus-like" when identified as that genus, e.g., group IVe identified as *Alcaligenes* sp.

<sup>c</sup> Gr., Growth.

<sup>d</sup> ADH, arginine dihydrolase; CIT, citrate; GEL, gelatin; LDC, lysine decarboxylase; MAC, growth on MacConkey agar; MOT, motility; NO<sub>3</sub> to NO<sub>2</sub>, reduction of nitrate to nitrite; NO<sub>2</sub> to gas, reduction of nitrate to nitrogen gas; ODC, ornithine decarboxylase; OXI, oxidase.

<sup>e</sup> 7/10 isolates correctly identified to variety, 9/10 correct to species.

<sup>f</sup> In the API 20E system, commonly isolated species of *Pseudomonas* are usually identified to species, but they may be identified by a group designation. All *Pseudomonas* in the system are categorized as members of three groups, two of which overlap. The less commonly isolated species are identified by their group designation only.

five supplemental tests. Eighty-one isolates generated profile numbers not found in the Analytical Profile Index and had to be referred to API's computer service, yet 63 of these were in categories included in the API 20E system. Of the 63, only 26 were correctly identified to the API system's expected level of identification (15 requiring supplemental tests), while 23 were misidentified (12 requiring supplemental tests) and 14 remained unidentified (13 requiring supplemental tests).

### DISCUSSION

We used 221 isolates which belong to 48 species and biotypes of nonfermentative gram-negative rods, 15 of which are not included in the API 20E system charts. The results of the initial API 20E biochemical tests (excluding the carbohydrate fermentation tests) showed close agreement with the conventional method results. This compares with the close agreement for the same common tests as reported by Smith et al. (10) in a study using only *Enterobacteriaceae*.

Despite this close agreement in biochemical reactions, only 110 (50%) of the 221 isolates were correctly identified to genus, and only 48 (22%) to species and biotype. However, the API 20E system does not claim to completely identify all nonfermenter isolates used in this study to genus, species, and biotype. When the expected level of identification was based on the Analytical Profile Index and reaction chart, 43% of the isolates included in the system were correctly identified.

All isolates of *Pseudomonas aeruginosa*, *P. cepacia*, and *Bordetella bronchiseptica* and most of the two subspecies of *Acinetobacter calcoaceticus* and *P. maltophilia* isolates were correctly identified to species by the API 20E system. Three of these organisms (*P. aeruginosa* and *Acinetobacter* species) are among those more commonly found in clinical specimens.

These findings suggest that the API 20E may be useful for the identification of the nonfermentative gram-negative bacteria more commonly

encountered in the clinical laboratory, but, as with the Oxi/Ferm tube system (8) the API system is not suitable for use by reference laboratories.

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