Evaluation of the API 20E System for the Identification of Gram-Negative Nonfermenters from Animal Origin

J.A. Devenish and D.A. Barnum*

ABSTRACT

The API 20E system was evaluated on isolates from animals of aerobic nonfermentative and cytochrome oxidase positive Gram-negative rods. An accuracy of identification of 80% (214/268 isolates) was achieved for those organisms included in the 1976-1977 API profile index. Members of the genera *Pseudomonas* and *Acinetobacter* were identified with 100% accuracy. Organisms not included in the API profile gave either an unacceptable profile number or were incorrectly identified as *Moraxella* spp. When the inoculum size was increased there was better identification.

RÉSUMÉ

Cette étude consistait à vérifier l'efficacité du système API 20E pour l'identification de souches animales de bâtonnets gram-négatifs aérobies, non fermentatifs et positifs à la cytochrome-oxydase. Le système permit d'identifier correctement 80%, i.e. 214 des 268 souches incluses dans l'index du profil de l'API, pour 1976-77. L'exactitude de l'identification des espèces des genres *Pseudomonas* et *Acinetobacter* atteignit 100%. Les organismes non inclus dans le profil de l'API donnèrent un numéro de profil inacceptable, ou on les identifia incorrectement comme *Moraxella* spp. Une augmentation de la quantité de l'inoculum se traduisit par une identification plus exacte.

INTRODUCTION

Commercially prepared rapid biochemical identification systems have been made available to diagnostic bacteriology laboratories in the past ten years (1, 4, 7). One of these systems, the API 20E (Analytab Products Ltd., St. Laurent, Quebec), was first developed to identify members of the family *Enterobacteriaceae*. It has been found to be a fast, reliable and accurate method for this purpose (2, 7, 14, 15, 18). As the Gram-negative nonfermentative rods may also be significant pathogens, their identification has received attention by several authors (5, 9, 11, 12). Computerization and the large number of possible profile numbers available in the data bank (2^{21}) have helped expand the API 20E system's identification to include many of this group of bacteria. Some evaluations of the API 20E system with Gram-negative nonfermentative rods have indicated questionable identifications (3, 6, 8, 10, 13, 16).

The purpose of this paper was to evaluate the API 20E system for the identification of aerobic nonfermentative Gram-negative rods, considered of clinical significance and isolated from diseased animals at the Ontario Veterinary College and the Veterinary Services Branch veterinary diagnostic bacteriology laboratories in Guelph.

MATERIALS AND METHODS

SOURCE AND PROCESSING OF BACTERIAL ISOLATES

All bacterial isolates were aerobic nonfermentative Gram-negative rods obtained from clinical samples of animal origin submitted to the Veterinary Services Branch (VSB) bacteriology laboratory at the Ontario Veterinary College (OVC). The isolates were obtained on either blood or MacConkey agar and a single colony was streaked onto a blood agar plate for overnight incubation at 37°C. An isolated colony was used to inoculate the API 20E strip. The identification of the organism was confirmed on routine laboratory medium using the scheme developed at the Center for Disease Control, Atlanta, Georgia by Weaver *et al* (17) for the unusual pathogenic Gram-negative bacteria.

PROCESSING OF THE API 20E STRIPS

The API 20E strip is a miniaturized system containing 20 biochemical tests (Table I). They were stored at 4°C until inoculated, then incubated at 37°C for 20 h and read according to the manufacturer's instructions. All cultures were oxidase tested both by adding the reagent to the colonies and by using the API method. All 20 biochemical reactions (plus oxidase)

*Department of Veterinary Microbiology and Immunology, Ontario Veterinary College, University of Guelph, Guelph, Ontario N1G 2W1. Present address of senior author: Central Laboratories Branch, Ontario Ministry of Health, P.O. Box 9000, Terminal A, Toronto, Ontario M5W 1R5.

Submitted April 14, 1981.

TABLE I. API 20E Biochemical Tests and their Abbreviations

Test Sequence	Biochemical Test	Test Abbreviation
1	O-Nitrophenyl-B-D-galactosidase	ONPG
2	Arginine dihydrolase	ADH
3	Lysine decarboxylase	LDC
4	Ornithine decarboxylase	ODC
5	Citrate utilization	CIT
6	Hydrogen Sulfide (H_2S) production	H_2S
7	Urease production	URE
8	Tryptophanedeaminase production	TDA
9	Indole production	IND
10	Acetoin production	VP
11	Gelatinase production	GEL
12	Glucose fermentation	GLU
13	Mannitol fermentation	MAN
14	Inositol fermentation	INO
15	Sorbitol fermentation	SOR
16	Rhamnose fermentation	RHA
17	Sucrose fermentation	SAC
18	Melibiose fermentation	MEL
19	Amygdaline fermentation	AMY
20	Arabinose fermentation	ARA

were recorded as a seven digit number. As O/F glucose medium, motility and MacConkey agar were inoculated as well, a nine digit number was obtained as described by the API analytical profile index. Bacterial identification was then determined by the profile number in the API profile index.

All profile numbers not found in the profile index were referred by telephone to the API Profile Recognition System Computer Service. If the identification could not be made at this point, the isolate was forwarded to the laboratory of Analytab Products Limited in Montreal, for further tests.

RESULTS

A comparison of conventional versus API 20E identification for those organisms included in the API 20E profile index is presented in Table II. Two hundred and fourteen out of 268 isolates (80.2%) were correctly identified. There was 100% agreement for all organisms belonging to the genus Pseudomonas (both glucose oxidizers and nonoxidizers) and all *Acinetobacter calcoaceticus* strains. All other isolates were identified with less than 85% accuracy.

Fifteen *Pasteurella multocida* strains were incorrectly identified by the API 20E as CDC-Group II F or *Moraxella* spp. The failure to utilize either GLU, MAN, SOR or SAC in the strip was the reason for the incorrect identification. If one or more of these substrates had been utilized, the strains would have been identified as *P. multocida*. *Moraxella* spp. were correctly identified seven out of nine times but gave a dormant profile (no positive reactions except oxidase) even after 48 h incubation. Growth of the organism in the strip could not always be verified. Eighteen (37%) *Bordetella bronchiseptica* strains were incorrectly identified as *Pseudomonas* spp. 2 (nonoxidizers), producing only a positive nitrate test in the GLU cupule. In these cases, the organism could be retrieved from the VP or GEL cupules in large numbers. Conventionally, *B. bronchiseptica* is strongly urease positive, but in the API 20E strip react weakly and a 48 h incubation was required.

Aeromonas hydrophila strains were identified with 44.4% accuracy (12/27 isolates). The inability to reduce nitrate in the API 20E strip was the only factor responsible for an unacceptable nine digit profile number in five of the isolates. Likewise, *A. shigelloides* could be identified correctly only after five attempts in the API 20E strip, the problem being the inability to reduce nitrate in the first four attempts.

Table III shows the results obtained for organisms not included in the API profile register at that time, but which are isolated frequently from animal specimens. The profile numbers obtained for *P. aerogenes*, *P. haemolytica*, *Actinobacillus* spp., *Brucella* spp. and an *A. salmonocida* strain were all unacceptable ones. The *P. pneumotropica* was, like some *P. multocida* strains, identified incorrectly as *CDC Group II F*. Other *Pasteurella* spp., *CDC Group EF-4*, *Eikenella corrodens*, *Neisseria* spp. and *M. polymorpha* var. *oxidans* were obtained in low numbers from the diagnostic laboratory but all produced dormant profiles and were incorrectly

TABLE II. API 20E Identification of Nonenterobacteriaceae Gram-Negative Aerobic Rods Included in the Registry and Their Comparison to Conventional Methods

Conventional Identification	Number of Isolates	Agreement	
		API 20E	% Agreement
Pseudomonas aeruginosa	51	51	100
Pseudomonas putrefaciens	5	5	100
Pseudomonas fluorescens	3	3	100
Pseudomonas spp. (oxidizers)	6	6	100
Pseudomonas spp. (nonoxidizers)	6	6	100
Acinetobacter calcoaceticus	18	18	100
Pasteurella multocida	86	71	83
Moraxella spp.	9	7	78
Bordetella bronchiseptica	49	31	63
Aeromonas hydrophila	27	12	44
Aeromonas shigelloides	1	0	0
CDC Group II F	1	1	100
Alcaligenes spp.	3	2	67
Flavobacterium spp.	2	1	50
Vibrio parahaemolyticus	1	0	0
Total	268	214	80

TABLE III. API 20E Identification of 111 Bacterial Strains not Included in Profile Index

Conventional Identification	Number of Isolates	API 20E Identification[a]
Pasteurella aerogenes	15	Unacceptable profile numbers
Pasteurella haemolytica	35	Unacceptable profile numbers
Pasteurella pneumotropica	1	*CDC Group II F*
Pasteurella spp.	2	*Moraxella* spp.
Actinobacillus spp.	27	Unacceptable profile numbers
CDC Group F-4	7	*Moraxella* spp.
Brucella spp.	20	Unacceptable profile numbers
Mima polymorpha var. *oxidans*	1	*Moraxella* spp.
Eikenella corrodens (HB-1)	1	*Moraxella* spp.
Neisseria spp.	1	*Moraxella* spp.
Aeromonas salmonicida	1	Unacceptable profile number

[a]According to the 1976-77 Profile Register and data base

identified as *Moraxella* spp. using the API 20E strip.

Those isolates sent to the API reference laboratory for comparison of results because they could not be, or were incorrectly, identified are summarized in Table IV. There was disagreement between the two laboratories in the conventional identification of 14 out of 78 isolates sent. Eight of 14 strains of *Actinobacillus* spp. which grew well on MacConkey agar were placed with those *Pasteurella* species which do not grow on MacConkey. *Vibrio parahaemolyticus* was identified as *P. multocida* even though this organism was positive for motility and *E. corrodens* was identified as *CDC-Group EF-4* by the API reference laboratory even though glucose utilization was negative and the organism produced heavily pitted colonies in the blood agar medium.

Profile numbers obtained for the same isolate between the two laboratories were consistent in 22 cases and involved *P. multocida* and *Alcaligenes* spp. In 34 other cases, the conventional identifications obtained by the two laboratories were the same. This was observed for nine *P. aerogenes*, nine *P. haemolytica* and 13 *Actinobacillus* spp., but the profile numbers secured in all these cases had been unacceptable for identification. For *P. aerogenes*, the two main discrepancies in the API 20E strip were ONPG and nitrate which these researchers obtained as negative but which were positive from the API reference laboratory. While seven strains of both *P. haemolytica* and *Actinobacillus* spp. grew well in our laboratory in MacConkey agar, the API laboratory failed to substantiate these findings.

Two important bacteria inconsistent in both profile numbers and final identification, using the API 20E, between the two laboratories were *B. bronchiseptica* and *A. hydrophila*. While the authors found *B. bronchiseptica* isolates to be CIT, URE and occasionally VP negative, and consequently incorrectly identified, the API reference laboratory obtained positive reactions with a satisfactory identification for ten of those 11 isolates. The only inconsistent result of *A. hydrophila* was nitrate reduction in the GLU cupule. While our laboratory obtained negative results, the API laboratory reported positive nitrate reduction results for the same isolates.

Although the 1976-1977 profile index was used to assess the accu-

TABLE IV. Comparison of Finding on Selected Gram-Negative Rods in Two Laboratories

OVC[a] Conventional Identification	OVC API 20E Identification Result	API Laboratory Conventional Identification	The API Laboratory API 20E Identification Result
P. aerogenes (12)[b]	Unacceptable (12)	*P. aerogenes* (12)	Unacceptable (12)
P. multocida (12)	CDC gp II F (9)	*P. multocida* (9)	CDC gp II F (9)
	Moraxella ssp (2)	*P. multocida* (2)	CDC gp II F (1)
			Moraxella ssp. (1)
	Unacceptable (1)	*P. multocida* (1)	Unacceptable (1)
P. haemolytica (11)	Unacceptable (11)	*Pasteurella* ssp. (1)	Unacceptable (1)
		P. haemolytica (8)	Unacceptable (8)
		P. ureae (1)	*Moraxella* ssp. (1)
		P. gallinarum (1)	*Moraxella* ssp. (1)
P. pneumotropica (1)	CDC gp II F	*P. pneumotropica* (1)	DCD gp II F (1)
Pasteurella ssp. (2)	Unacceptable (1)	*P. gallinarum* (1)	Unacceptable
	Moraxella ssp. (1)	*Pasteurella* ssp. (1)	*Moraxella* ssp. (1)
B. bronchiseptica (11)	*Pseudomonas* ssp. 2 (6)	*B. bronchiseptica* (10)	*B. bronchiseptica* (10)
	Alcaligenes ssp. (5)	CDC gp IV C-2 (1)	CDC gp IV C-2 (1)
A. hydrophila (9)	Unacceptable (8)	*A. hydrophila* (9)	*A. hydrophila* (9)
Actinobacillus ssp. (14)	Unacceptable (11)	*Actinobacillus* ssp. (6)	Unacceptable (11)
	Pasteurella ssp. (1)	*P. ureae* (5)	*Moraxella* ssp. (2)
	Moraxella ssp. (1)	*Pasteurella* ssp. (1)	*Pasteurella* ssp. (1)
	Pseudomonas ssp. 2 (1)	*P. gallinarum* (1)	
		Unidentified (1)	
Alcaligenes ssp. (2)	CDC gp IV C-2 (2)	*A. faecalis* (2)	CDC gp IV C-2 (2)
Flavobacterium ssp. (1)	Unacceptable (1)	*Flavobacterium* ssp. (1)	*Flavobacterium* ssp. (1)
CDC gp EF-4 (1)	*Moraxella* ssp. (1)	CDC gp EF-4 (1)	*Moraxella* ssp. (1)
Eikenella corrodens (1)	*Moraxella* ssp. (1)	CDC gp EF-4 (1)	*Moraxella* ssp. (1)
Moraxella bovis (1)	CDC gp II F (1)	*Moraxella lacunato* (1)	*Pseudomonas* ssp. (1)
Vibrio parahaemolytica (1)	Unacceptable (1)	*P. multocida* (1)	*P. multocida (1)*

[a]Ontario Veterinary College
[b]Number in parentheses indicates number of isolates involved

racy of the API 20E system, a revised profile index was available during the latter part of 1977. Using the newer index, accuracy of identification using the API 20E strip would have increased for both *P. multocida* and *A. hydrophila*. For *P. multocida*, nine of the 12 incorrect identifications were *CDC Group II F* having produced only a positive reaction in the IND cupule in the older profile number. In the new profile index, however, for the same profile number, an additional TSI slant must be inoculated in order to distinguish between *CDC Group II F* and *Pasteurella* spp. This would have increased the accuracy to 93% at a genus level. Similarly, for *A. hydrophila*, the nitrate test was the only reason a correct identification could not be made and this test is not necessary for identifying this organism with the API 20E system. A seven digit number as opposed to a nine digit one (which includes the nitrate test in the eighth digit) would have identified *A. hydrophila* in five cases in the old profile index and 15 cases in the newer profile index. Therefore, the accuracy of identification would have increased with the newer profile index to over 95%.

The newer profile index also included some organisms not included in the older profile index. These were *CDC Group EF-4*, *Brucella* spp. and *Eikenella corrodens*, which were all found unacceptable for identification using the older profile index. However, in the new profile index, only 11 of 20 *Brucella* spp. would have been identified correctly and the *CDC Group EF-4* and *E. corrodens* isolates would have continued to be incorrectly identified as *Moraxella* spp.

DISCUSSION

It is difficult to assess the true accuracy of the API 20E system as the profile index is constantly being improved. This occurred with *P. multocida* and *A. hydrophila* which had increased accuracy of identification in the newer profile index. The continual expansion of the system is exemplified by the addition of *Brucella* spp., *CDC Group EF-4* and *E. corrodens* to the newer profile index during the course of this research.

A few organisms such as *Actinobacillus* spp., *P. aerogenes* and *P. haemolytica* produced unacceptable profile numbers in the API 20E strip which were not cross identified with some other organism already included in the profile index. Thus these organisms could be added to an expanded profile index at a later date.

The per cent of the results positive for each organism on each test of the API 20E strip of the profile index did not always compare to the percentage obtained by the authors. For example, the nitrate test for *P. multocida* is listed as 82.4% positive for the newer profile index but nitrate reducing *P. multocida* with the API 20E strip was never encountered during the course of this research. This might indicate that there are strains of organisms from animal sources which have yet to be studied and included in the API profile index. Accuracy in identifying organisms from animal sources would increase if this were done.

Bordetella bronchiseptica and *A. hydrophila* were the two organisms most in disagreement between the two laboratories. It has been stated in the results that the nitrate test for *A. hydrophila* has become unnecessary for a correct identification, but it does point to a weakness in the API 20E strip. This could make a difference in differentiating other organisms in the API profile index where a nitrate test result is important.

Initially the API 20E strip was used to identify members of the family *Enterobacteriaceae* which grow quickly and produce large single colonies which provide a sufficient inoculum. However, many of the nonfermenters such as *B. bronchiseptica* are much more fastidious and produce small colonies even after 48 h growth on blood agar. This slower growth fails to produce a positive reaction. Preliminary studies showed that when the bacterial concentration of the inoculum is increased to MacFarland's nephelometer standard 0.5, *B. bronchiseptica* became CIT, URE and VP positive with subsequent correct identification. When a one or five colony inoculum of the same organism was used, an incorrect identification of *Pseudomonas* spp. 2 was made.

In evaluating the API 20E system for Gram-negative nonfermenters other workers (3, 6, 8, 10, 13, 16) have reported accuracy of identification ranging from 41 to 88.9% for isolates present in the API profile index. The work reported here gives an identification accuracy of 80.0% and is in general agreement with others that common clinical isolates of *P. aeruginosa* and *A. calcoaceticus* are identified very well using the API 20E. However, the much less commonly isolated nonfermenters from humans are found more frequently from animals and these were identified less satisfactorily.

Although the use of the API 20E strip was found to be inadequate in some areas of this study, improvements can be made so that the system will provide a useful identification method, especially for small laboratories.

ACKNOWLEDGMENTS

This study forms part of a thesis submitted to the Faculty of Graduate studies, University of Guelph, by the senior author, in partial fullfilment of the requirements for the M.Sc. degree. The project was financed in part by the Ontario Ministry of Agriculture and Food. The assistance of Analytab Products Ltd. in supplying materials and of the diagnostic laboratories of the Ontario Veterinary College and the Veterinary Services Branch in supplying cultures is acknowledged.

REFERENCES

1. **COX, N.A., F. McMANN and Y.C. FUNG.** Commercially available minikits for identification of Enterobacteri-

aceae: A review. J. Fd Prot. 40: 866-872. 1977.
2. **DEVENISH, J.A.** and **D.A. BARNUM.** Evaluation of API 20E System and Encise Enterotube for the identification of *Enterobacteriaceae* of animal origin. Can. J. comp. Med. 44: 315-319. 1980.
3. **DOWDA, H.** Evaluation of two rapid methods for identification of commonly encountered nonfermenting or oxidase-positive, Gram-negative rods. J. clin. Microbiol. 6: 605-609. 1977.
4. **FINKLEA, P.J., M.S. COLI** and **T.M. SODEMAN.** Clinical evaluation of the Minitek differential system for identification of Enterobacteriaceae. J. clin. Microbiol. 4: 400-405. 1976.
5. **GILARDI, G.L.** Nonfermentative Gram-negative bacteria encountered in clinical specimens. Antonie v. Leeuwenhoek 39: 229-242. 1973.
6. **HOFHERR, L., H. VOTAVA** and **D.J. BLAZEVIC.** Comparison of three methods for identifying nonfermenting Gram-negative rods. Can. J. Microbiol. 24: 1140-1144. 1978.
7. **NORD, C.E., A.A. LINDBERG** and **A. DAHLBACK.** Evaluation of five test-kits — API, Auxotab Enterotube PathoTec and R/B — for identification of Enterobacteriaceae. Med. Microbiol. Immun. 159: 211-220. 1974.
8. **OBERHOFER, T.R.** Comparison of the API 20E and Oxi-Ferm Systems in identification of nonfermentative and oxidase-positive fermentative bacteria. J. clin. Microbiol. 9: 220-226. 1979.
9. **OBERHOFER, T.R., J.W. ROWEN** and **G.F. CUNNINGHAM.** Characterization and identification of Gram-negative nonfermentative bacteria. J. clin. Microbiol. 5: 208-220. 1977.
10. **OTTO, L.A.** and **U. BLACKMAN.** Nonfermentative bacilli: Evaluation of three systems for identification. J. clin. Microbiol. 10: 147-154. 1979.
11. **PEDERSEN, M.M., E. MARSO** and **M.J. PICKETT.** Nonfermentative bacilli associated with man: III. Pathogenicity and antibiotic susceptibility. Am. J. clin. Pathol. 54: 178-192. 1970.
12. **PICKETT, M.J.** and **M.M. PEDERSEN.** Characterization of saccharolytic nonfermentative bacteria associated with man. Can. J. Microbiol. 16: 351-362. 1970.
13. **SHAYEGANI, M., P.S. MAUPIN** and **D.M. McGLYNN.** Evaluation of the API 20E system for identification of nonfermentative Gram-negative bacteria. J. clin. Microbiol. 7: 539-545. 1978.
14. **SMITH, P.B., K.M. TOMFOHRDE, D.L. RHODEN** and **A. BALLOWS.** API system: a multitube micromethod for identification of Enterobacteriaceae. Appl. Microbiol. 24: 449-452. 1972.
15. **SWANSON, E.C.** and **M.T. COLLINS.** Use of the API 20E system to identify veterinary Enterobacteriaceae. J. clin. Microbiol. 12: 10-14. 1980.
16. **WARWOOD, N.M., D.J. BLAZEVIC** and **L. HOFHERR.** Comparison of the API 20E and Corning N/F systems for identification of nonfermentative Gram-negative rods. J. clin. Microbiol. 10: 175-179. 1979.
17. **WEAVER, R.E., H.W. TATUM** and **D.G. HOLLIS.** The identification of unusual pathogenic Gram-negative bacteria (Elizabeth O. King). Atlanta, Georgia: Centre for Disease Control. 1972.
18. **WILLIS, G.** and **I.J.Y. COOK.** A comparative study of API, Encise and conventional methods. Med. Technologist 5: 3-5. 1975.