Identification of *Enterobacteriaceae* by the API 20E system

B. HOLMES, W. R. WILLCOX, AND S. P. LAPAGE

From the National Collection of Type Cultures, Central Public Health Laboratory, Colindale, London NW9 5HT, UK

SUMMARY Since the introduction of the API 20E kit a number of identification schemes have been developed by the manufacturer for use with the kit. We evaluated the success of these various schemes in identifying 206 strains belonging to 34 taxa of the family *Enterobacteriaceae*. Many of the strains were atypical and only 94% could be identified by our own system of 50 conventional tests and a computer program. The most advanced identification scheme so far developed for the API 20E kit (the Analytical Profile Index and complementary Computer Service) allowed 88% of the 206 strains to be correctly identified, although 2% were incorrectly identified. The tests in the API 20E kit and 52 conventional tests were separately evaluated for their ability to discriminate between the 34 taxa considered in this study. Our results suggest that replacing some of the tests in the present API 20E kit might further improve its diagnostic performance.

Among the simplified biochemical test kits sold for the identification of bacteria is the API system. Different API kits have been designed for various groups of bacteria—for example, enterobacteria, lactobacilli, and anaerobes. These kits have in common the same form of construction. The individual tests consist of dehydrated chemicals in a set of plastic cupules (moulded to a strip of plastic) which are inoculated with a bacterial suspension. The development of this system of cupules has been described by Janin (1977). Three API kits are available for identifying enterobacteria—a screening kit of 10 tests (10S), a basic set of 20 tests (20E), and for further characterisation of an organism a kit of 50 tests (50E). The 20E kit contains all the tests of the 10S but only a few tests are in both the 20E and 50E.

The API 20E became available in the United Kingdom in 1971. The tests included in the kit have not been changed since nor, so far as is known, have their biochemical specifications (Doucet and Paule, 1971). The identification scheme provided by the manufacturer for use with the kit, however, has undergone considerable development. The original identification chart which gave the expected reactions for each taxon in a plus and minus form was replaced by schemes in which the results of an organism are converted to a numerical code, the 'profile' of the organism.

The first list of over 1000 profiles with their appropriate identities was known as the Profile Register. This was replaced by the Analytical Profile Index in which the entry for each profile is determined by a computer identification model. A computer program is also available through the API Computer Service to analyse individual profiles not in the index. The API 20E kit has been adapted for the identification of Gram-negative bacteria other than enterobacteria for which it was originally designed. In this report, however, we consider only identification of *Enterobacteriaceae*.

Several authors have evaluated the API 20E kit. Guillermet and Desbresles (1971) examined 522 strains of *Enterobacteriaceae* and 79 strains of *Moraxella* and *Pseudomonas* in the API 20E system and conventional tests. The results of the two methods agreed well, showing the usefulness of the API 20E system. Bartoli *et al.* (1972) found the API 20E system useful in identifying 671 strains of *Enterobacteriaceae*, especially *Klebsiella*, *Enterobacter*, *Proteus*, and *Providencia*, but they included additional conventional tests in their identification system.

Washington *et al.* (1971), Malmberg *et al.* (1972), Smith *et al.* (1972), Mitić *et al.* (1973), and Brooks *et al.* (1974) have also evaluated the API 20E kit, while others have examined it in parallel with one or more other kits for the identification of the *Enterobacteriaceae* (Bourgaux-Ramoisy *et al.*, 1973; Bisgaard *et al.*, 1974; Manning and Bordner, 1974; Nord *et al.*,

1974; Aquino and Dowell, 1975; Moussa, 1975; Smith, K. E., 1975; Smith, P. B., 1975; Willis and Cook, 1975; Hayek and Willis, 1976; Holmes *et al.*, 1977). All these authors report good agreement, in general, between the results of the API 20E system and parallel conventional tests. Figures, when given, range from 92% to 100% agreement. Most report identification rates ranging from 92% to 100% for *Enterobacteriaceae* with the API 20E kit. Washington *et al.* (1971), however, obtained an identification rate of only 88% initially but increased it to 93% after further tests with heavier inocula of organisms that failed to ferment glucose when first tested. Similarly, Brooks *et al.* (1974) obtained an initial identification rate of 88.2% with the API 20E system. This was increased to 98% by retesting cultures that gave equivocal results. Bisgaard *et al.* (1974) found only a 72% agreement rate between identification obtained with the API 20E kit and with conventional methods. In contrast, Gardner *et al.* (1972) could identify only some 25% of 99 clinical isolates of *Enterobacteriaceae* with the API 20E system, but they did not compare API 20E and corresponding conventional test results.

Test reproducibility in the API 20E system has been studied by Butler *et al.* (1975), while Holmes *et al.* (1977) compared test reproducibility in the API 20E and two other kit systems. Both studies showed a high degree of test reproducibility in the API 20E kit.

Robertson and MacLowry (1974) developed a computer program for identifying bacteria on the results of API 20E tests. Using results supplied by the manufacturer they found that in 99.36% of 27 820 strains the identification shown in the API Profile Register was the same as that given by the program. Robertson and MacLowry (1975) produced a profile index for the 10S kit and found that only 4.1% of 37 476 isolates were identified to different taxa by the 10S index and the API 20E Profile Register.

Materials and methods

ORGANISMS

Two hundred and six strains of *Enterobacteriaceae* belonging to 34 taxa were used in this study (Table 1). The strains comprised 96 reference cultures maintained in the National Collection of Type Cultures (NCTC) and 110 atypical field strains which had been referred by diagnostic laboratories to the NCTC for computer-assisted identification. All 206 strains had been previously tested in the 50 conventional tests of Bascomb *et al.* (1971) and the identity of the reference strains was confirmed and that of the field isolates determined on the results of these 50 tests using the computer identification method of Lapage *et al.* (1973).

CONVENTIONAL TESTS

Out of the 50 conventional tests previously carried out on the strains 19 were the same as or could reasonably be equated with tests in the API 20E system—phenylalanine deamination with the API tryptophan deamination test, hydrogen-sulphide production on triple sugar iron (TSI) agar with the API H₂S test, and liquefaction of a nutrient gelatin stab after five days' incubation with the API gelatin liquefaction test. Acetoin production was determined in conventional media by the method of O'Meara (1931) but the reagents used for the API test were those of Barritt (1936) as recommended by the manufacturer.

Acid from amygdalin and melibiose had not been previously determined in conventional media for any of the strains included in this study, so these tests were inoculated at the same time as an API 20E kit for each strain. The conventional test media for amygdalin and melibiose fermentation were prepared by the method used for the other conventional carbohydrate fermentation media (Bascomb *et al.*, 1971).

API 20E SYSTEM

All 206 strains were examined in the API 20E system, the tests of which are given in Table 2. As well as the 20 basic tests, each of which has its own cupule, cytochrome-oxidase production and nitrate reduction can be determined in the kit by adding further reagents. In the identification of *Enterobacteriaceae* these two tests serve only to confirm that the isolate belongs to this family. The 20 basic tests were performed according to the manufacturer's instructions. In identifying the strains with the API schemes we used the conventional test results for cytochrome-oxidase production and nitrate reduction (all the strains reduced nitrate and failed to produce cytochrome-oxidase). For comparison with conventional results we determined nitrate reduction in 120 of the strains in the kit using the reagents recommended by the manufacturer and in the remaining 86 strains we substituted the reagents of Crosby (1967) in which the non-carcinogenic Cleve's acid replaces α -naphthylamine. Some of the 86 strains gave a negative result in the test for nitrate reduction using the substitute reagents, and these strains were inoculated into further API 20E kits and the test repeated with the reagents recommended by the manufacturer, one of which contains α -naphthylamine.

IDENTIFICATION USING API CHART

The 206 strains were identified on the results obtained with the API 20E kit using the identification chart supplied by the manufacturer (undated). There

Table 1 *Disagreements between API 20E and conventional test results for 34 taxa*

Taxon	No. of strains	No. of tests in which API and corresponding conventional media are compared	Disagreements between API and corresponding conventional test results	
			(%)	(No.)
<i>Citrobacter freundii</i>	9	189	14%	(27)
<i>Citrobacter koseri</i>	6	126	12%	(15)
<i>Edwardsiella tarda</i>	6	126	2%	(2)
<i>Enterobacter aerogenes</i>	5	105	9%	(9)
<i>Enterobacter cloacae</i>	10	210	11%	(24)
<i>Erwinia herbicola</i>	4	84	15%	(13)
<i>Escherichia adecarboxylata</i>	5	105	6%	(6)
<i>Escherichia coli</i>	14	294	11%	(31)
<i>Hafnia alvei</i>	10	210	10%	(20)
<i>Klebsiella aerogenes</i> and <i>K. oxytoca</i>	7	147	8%	(12)
<i>Klebsiella ozaenae</i>	4	84	12%	(10)
<i>Klebsiella pneumoniae</i>	7	147	2%	(3)
<i>Klebsiella rhinoscleromatis</i>	4	84	7%	(6)
<i>Proteus mirabilis</i>	8	168	5%	(9)
<i>Proteus morganii</i>	8	168	0%	(0)
<i>Proteus rettgeri</i>	8	168	12%	(20)
<i>Proteus vulgaris</i>	8	168	11%	(19)
<i>Providencia alcalifaciens</i>	8	168	11%	(19)
<i>Providencia stuartii</i>	5	105	8%	(8)
<i>Salmonella choleraesuis</i>	4	84	8%	(7)
<i>Salmonella ferlac</i>	4	84	11%	(9)
<i>Salmonella gallinarum</i>	4	84	7%	(6)
<i>Salmonella paratyphi</i> A	4	84	7%	(6)
<i>Salmonella pullorum</i>	4	84	12%	(10)
<i>Salmonella</i> subgenus I	4	84	4%	(3)
<i>Salmonella</i> subgenus II	4	84	7%	(6)
<i>Salmonella</i> subgenus III	6	126	10%	(12)
<i>Salmonella</i> subgenus IV	4	84	8%	(7)
<i>Salmonella typhi</i>	4	84	12%	(10)
<i>Serratia liquefaciens</i>	5	105	25%	(26)
<i>Serratia marcescens</i>	9	189	14%	(27)
<i>Serratia marinorubra</i>	4	84	24%	(20)
<i>Shigella sonnei</i>	4	84	6%	(5)
<i>Shigella</i> spp other than <i>S. sonnei</i>	6	126	10%	(12)

Table 2 *Comparison of results in 21 tests of the API 20E system and corresponding conventional media*

Test	Percentage disagreement between API and corresponding conventional test results	Numbers of pairs of API and conventional test results			
		API+ conv. +	API+ conv. -	API- conv. +	API- conv. -
β -galactosidase production (ONPG test)	11%	90	1	21	94
Arginine dihydrolase	22%	30	3	42	131
Lysine decarboxylase	8%	88	3	13	102
Ornithine decarboxylase	5%	110	1	10	85
Citrate utilisation	23%	77	4	43	82
H ₂ S production	15%	38	5	25	138
Urease production	9%	44	3	16	143
Deamination of tryptophan or phenylalanine	2%	44	5	0	157
Indole production	6%	61	4	8	133
Acetoin production at 37°C	9%	20	10	9	167
Gelatin liquefaction	10%	16	0	20	170
Acid from glucose	0%	206	0	0	0
Acid from mannitol	1%	157	0	3	46
Acid from inositol	8%	53	10	6	137
Acid from sorbitol	7%	100	2	12	92
Acid from rhamnose	6%	115	0	12	79
Acid from sucrose	12%	69	0	25	112
Acid from melibiose	13%	82	9	17	98
Acid from amygdalin	28%	21	56	2	127
Acid from arabinose	7%	125	4	11	66
Nitrate reduction	2%	202	0	4	0
Average	9.7%	Total 1748	120	299	2159

were no specific instructions on how to use the chart so we adopted the following procedure: a set of results was taken to match a taxon in the chart if the results differed with the entries for that taxon in not more than one test, allowing either result for $\pm$ and d entries. When the results matched only one taxon the strain was counted as correctly identified if the taxon corresponded to the identity of the strain and incorrectly identified if not. When the results did not match any of the taxa or matched two or more taxa the strain was counted as not identified. The chart indicated that the identification of certain taxa should be confirmed serologically and when a strain was incorrectly identified to one of these taxa it was counted as not identified. Although the serological examination was not carried out it was assumed that it would have refuted the incorrect identification.

IDENTIFICATION USING API PROFILE REGISTER

The 206 strains were also identified using the API Profile Register (dated 1973, with update letter No. 1). If the profile number derived from the reactions of a strain was listed in the register a correct identification was counted when the indicated taxon agreed with the identity of the strain, an incorrect identification when it disagreed. If a profile was not in the register the API Selector provided with the register was used, following the instructions given. If the selector indicated that two or more taxa were equally probable the strain was counted as not identified since the system gave no guidance on how to complete the identification. Strains for which serological confirmation was recommended were treated as in identification using the API chart.

IDENTIFICATION USING API ANALYTICAL PROFILE INDEX

The 206 strains were also identified with the API Analytical Profile Index (dated 1976), which was used in the same way as the Profile Register. Profiles not in the index were submitted to the API Computer Service. For some profiles two or more taxa were given as possible identities, with the comment 'Good likelihood but low selectivity identification', and the user was referred to identification tables containing additional conventional tests. We did not do the additional tests, but if the system indicated the correct taxon as one of the possible identities this was counted as a correct identification since the index gave the information necessary to complete the identification. Profiles receiving the comments 'unacceptable profile' or 'very doubtful diagnosis' were counted as not identified. Strains for which serological confirmation was recommended were treated as in identification using the API chart.

IDENTIFICATION BY COMPUTER ON API 20E AND CONVENTIONAL RESULTS

Two probabilistic identification matrices were constructed for the 34 taxa of Table 1, one from the API 20E results of the 206 strains and one from the results of the 20 conventional tests corresponding to the API 20E tests on the same strains. The 206 strains were identified using these two matrices and the computer method of Lapage *et al.* (1973). When the 'identification score' of the highest scoring taxon exceeded a threshold 'identification level' the strain was identified to this taxon, otherwise the strain was counted as not identified. For the API 20E results the effect of varying the identification level from 0 to 0.999999 was investigated but for the conventional results the level was set at 0.999, the value used by Lapage *et al.* (1973).

TEST SELECTION AND EVALUATION BY COMPUTER

The identification matrix of Bascomb *et al.* (1973) was reduced to contain only the 34 taxa of Table 1. The matrix included 50 tests, and probability figures for melibiose and amygdalin conventional test media were derived from the results of the 206 strains of this study and these figures added to the matrix. Using the test selection method described by Willcox *et al.* (1973) a set of tests was selected from this matrix to differentiate each pair of taxa by at least two tests. The same matrix was also used to evaluate the discriminating power of the 20 conventional tests corresponding to the API 20E tests, and the matrix derived from the API 20E results of the 206 strains was used to evaluate the API 20E tests themselves.

Results

COMPARISON OF TEST RESULTS

Although the number of strains belonging to each taxon was not the same most taxa showed differences between 5% and 15% in the test results obtained with the API 20E and conventional tests (Table 1). Some taxa—*Edwardsiella tarda* (2%), *Klebsiella pneumoniae* (2%), *Proteus morganii* (0%), and *Salmonella* subgenus I (4%)—showed closer agreement (percentage differences in parentheses) while *Serratia liquefaciens* (25%) and *Serratia marnorubra* (24%) showed poorer agreement.

A comparison of 4326 test results obtained with both the API 20E system and corresponding conventional tests is shown in Table 2. Of the 4326 test results obtained with the API 20E system 419 (9.7%) disagreed with the results obtained for the corresponding conventional tests. In 299 (6.9%) cases the API test was less sensitive than the corres-

ponding conventional test and in 120 (2.8%) cases the API test was more sensitive. Disagreement between individual API and corresponding conventional test results was less than 15% in most tests. Tests for arginine dihydrolase, citrate utilisation, and amygdalin fermentation showed poorer correlation with 22%, 23%, and 28% disagreements respectively.

All the 120 strains tested for nitrate reduction in the API 20E kit using only the reagents recommended by the manufacturer reduced nitrate, but 10 of the 86 strains tested in the kit using the reagents of Crosby (1967) failed to reduce nitrate. When these 10 strains were retested in the kit using the recommended reagents six reduced nitrate but four still failed to do so.

IDENTIFICATION RATES

Identification rates for the 206 strains employed in this study, using various identification systems, are summarised in Table 3. Of the API 20E systems the most recent, the Analytical Profile Index and Computer Service, was clearly the most successful in identifying these strains: 181 (88%) were correctly identified although 40 (19%) of these required additional conventional tests, 20 (10%) could not be identified, and 5 (2%) were incorrectly identified. The incorrect identifications with this system were a strain of *Klebsiella aerogenes* and *K. oxytoca* identified as *K. ozaenae*, a strain of *Providencia stuartii* identified as *P. alcalifaciens*, a strain of *Enterobacter aerogenes* identified as *K. pneumoniae*, a strain of *Salmonella ferlac* identified as *Hafnia alvei*, and a strain of *S. pullorum* identified as *H. alvei*.

The Figure shows the identification performance of the computer method of Lapage *et al.* (1973) at different identification levels with the matrix compiled in this study for the API 20E tests. At a level of 0.999 only 39% of the strains were identified with this matrix, while 53% were identified with the matrix compiled for the 20 conventional tests corres-

ponding to the API tests, no strains were misidentified at this level on either matrix. Using the results of 50 conventional tests and the matrix of Bascomb *et al.* (1973) 94% of the strains were identified by this method and none were misidentified.

TEST SELECTION AND EVALUATION

The results of the computer test selection and evaluation are summarised in Table 4. From the 52 conventional tests of the modified matrix of Bascomb *et al.* (1973) a set of 19 tests with a total separation value of 1121 was selected. For 34 taxa a separation value of 1122 is required before all pairs of taxa are separated by at least two tests but there was only one test in the matrix which separated *Salmonella* subgenus II from *Salmonella* subgenus III, so the value of 1121 is the maximum obtainable with this matrix. Using the same matrix the 20 conventional tests equivalent to the API 20E tests were evaluated. The total separation value of these tests was 1084. Eight pairs of taxa were not separated by any tests and 22 pairs were separated by one test only. The API 20E tests were evaluated using the API matrix compiled in this study. The total separation value was 1001. Thirty-three pairs of taxa were not separated by any tests and 55 pairs were separated by one test only.

Discussion

The overall rate of disagreement between the API 20E tests and the corresponding conventional tests was 9.7%. In a previous study, using almost the same range of taxa (Holmes *et al.*, 1977), we found 7% disagreements. The difference in the results of the two studies is statistically significant (χ^2 test, $p < 0.05$). In the earlier study, however, the results used in comparing the two systems were the majority results of three repeated tests on each strain, so the variability in test results known to occur within a

Table 3 Identification of 206 strains by different systems

Identification system	% and No. of strains correctly identified	% and No. of strains not identified	% and No. of strains incorrectly identified
API identification chart	33% (68)	62% (127)	5% (11)
API Profile Register alone	56% (115)	38% (79)	6% (12)
API Profile Register and Selector	66% (135)	21% (44)	13% (27)
API Analytical Profile Index alone	74% (153)*	24% (49)	2% (4)
API Analytical Profile Index and Computer Service	88% (181)†	10% (20)	2% (5)
Computer program with API matrix compiled in this study (identification level of 0.999)	39% (81)	61% (125)	0% (0)
Computer program with conventional test matrix compiled in this study (identification level of 0.999)	53% (109)	47% (97)	0% (0)

*63% (130) identified on API 20E results alone, 11% (23) required additional conventional tests.

†68% (141) identified on API 20E results alone, 19% (40) required additional conventional tests.

Figure Proportions of 206 strains identified by the computer method of Lapage *et al.* (1973) with the API 20E matrix compiled in this study at different identification levels: (a) percentage of strains correctly identified; (b) percentage of strains incorrectly identified; (c) percentage of strains incorrectly identified if serological confirmation is required for certain taxa.

system (Sneath and Johnson, 1972) did not affect the rate of disagreement. Correcting the results of the present study for test variability, using the formulae of Sneath and Johnson (1972) and assuming probabilities of erroneous test results of 1% for conventional tests and 2% for API 20E tests (Holmes *et al.*, 1977), gives a rate of disagreement of 7%, as found in the previous study. We agree with Robertson and MacLowry (1975) and Janin (1977), however, that in considering different identification systems it is not the comparability of individual test results but the equivalence of the final identifications which is important.

In assessing the identification rates achieved in this study it is important to remember that the rates cannot be applied directly to the routine diagnostic laboratory situation. We examined more taxa than the routine laboratory is likely to encounter, the strains chosen do not reflect their distribution in the clinical material that would be seen in a routine laboratory, and more than half the strains we examined were atypical isolates which had been sent to us by routine laboratories because the strains had proved difficult to identify by conventional means. The identification rate for these 206 strains on the results of 50 conventional tests using the probability matrix of Bascomb *et al.* (1973) was 94%. For the API Analytical Profile Index and Computer Service

to identify 88% of these strains on the results of the 21 API 20E kit tests, with a few additional conventional tests in some cases, is thus an excellent achievement.

The identification rate of 88% given above is slightly lower than the range of 92% to 100% obtained by other authors. Not all authors state precisely how they identified their strains on the results of the API 20E kit. Our results show a continuous improvement in the performance of the successive API identification schemes, so the results of different studies will be comparable only if they used the same scheme. The identification rate of 25% quoted by Gardner *et al.* (1972) may well have been obtained with the identification chart supplied by the manufacturer. We also obtained a low identification rate (33%) with the chart. Furthermore, some authors fail to specify the exact method that they used to determine by conventional means the identity of the strains used to test the API system. To evaluate a system as highly developed as the API 20E Analytical Profile Index and Computer Service a very reliable conventional identification method must be used, otherwise when the systems disagree the API identification may well be the correct one.

The following have reported misidentifications with the API 20E system (number of misidentifications followed by percentage): Hayek and Willis (1976) 2/245, 1%; Malmberg *et al.* (1972) 1/95, 1%; Brooks *et al.* (1974) 8/408, 2%; Aquino and Dowell (1975) 4%; Smith *et al.* (1972) 13/366, 4%; Washington *et al.* (1971) 8/128, 6%; and Bisgaard *et al.* (1974) 29/105, 28%. If, however, one follows our rule of counting as not identified strains for which an identification was given that would not be confirmed serologically then the misidentification rate for Smith *et al.* (1972) is reduced to 2% (7/366) and that for Bisgaard *et al.* to 11% (11/105). The misidentification rate of 2% (5/206) obtained in the present study is within the range of rates obtained by most authors. Holmes *et al.* (1977) used a set of 30 strains covering almost the same range of taxa as ours in a comparison of three kits and showed that misidentification rates could be comparatively high (up to 29%) for systems which did not employ computer-based identification schemes. Their results with the API 20E Analytical Profile Index and API Computer Service (92% correct identifications, no misidentifications) do not differ significantly from the results of the present study (χ^2 test, $P > 0.10$).

The improvement we found in performance of the successive API identification schemes was probably due to a combination of factors. Additional taxa have been included, the user is now referred to additional conventional tests when the kit test results for a particular strain do not allow a reliable

Table 4 *Conventional tests selected by computer to separate the 34 taxa*

Test	Separation value	Test	Separation value
KCN tolerance	288	Acid from PWS dulcitol	7
β -galactosidase production (ONPG)	255	Urease production	6
Lysine decarboxylase	200	Acid from PWS adonitol	5
Ornithine decarboxylase	123	Hydrogen-sulphide production (lead acetate paper)	3
Gelatinase production (plate method)	77	Acid from PWS lactose	3
Acid from PWS inositol	56	Acid from PWS rhamnose	3
Indole production	35	Gluconate oxidation	2
Motility at room temperature*	26	Acid from PWS raffinose	1
Acid from PWS arabinose	18	Acid from PWS trehalose	1
Malonate utilisation	12		
	Subtotal 1090†		Grand total 1121‡

PWS = peptone water sugar.

*18-22°C.

†Separation value of the 20 conventional tests equivalent to those included in the API 20E system = 1084.

‡Total separation value required to separate all 34 taxa by at least two tests = 1122.

identification, and the Computer Service is available for the more atypical isolates.

The computer identification method of Lapage *et al.* (1973) with the two identification matrices constructed in this study and using an identification level of 0.999 could identify a comparatively low proportion of the strains (39% with the matrix for API 20E tests, 53% with the matrix for the corresponding conventional tests), though no strains were misidentified. The computer evaluation of the discrimination provided by the tests in these two matrices showed that the API 20E tests had a separation value of 1001, lower than the value of 1084 for the corresponding conventional tests. This difference would account for the lower identification rate with the API 20E tests and must be because some of the API tests that do not show a high correlation with the corresponding conventional tests do not discriminate between the taxa used in our study as well as do their conventional counterparts.

The Figure shows that the identification performance of the method of Lapage *et al.* (1973) with the matrix for API 20E tests could be improved for these particular strains by decreasing the identification level. For example, at a level of 0.979% of the strains were correctly identified with no misidentifications provided serological confirmation was required for the identification of certain taxa. Discounting the use of additional conventional tests the performance of the method at a level of 0.9 is better than the performance of the API Profile Index and Computer Service, which identified 68% of the strains correctly and 2% incorrectly. However, the matrix used with the method of Lapage *et al.* (1973) was based on the same 206 strains that were used to test the method. The performance of the method would probably not be so good on a further series of strains.

Lapage *et al.* (1973) adopted an identification level

of 0.999 because their method was used in a reference laboratory and was intended to identify aberrant as well as typical strains with a low risk of misidentification. To achieve this reliability 30-40 test results are required for the identification of aberrant strains (Lapage *et al.*, 1970). The computer program used to construct the API Analytical Profile Index and incorporated in the Computer Service carries out a very similar calculation to that of Lapage *et al.* (1973) but the method used by the API program to decide whether a definite identification should be indicated has not been published (Willcox and Lapage, 1977a). The results of the present study suggest that the API computer model is designed to identify a high proportion of strains on their API 20E results with some risk of misidentifying the most aberrant strains. This is probably a suitable strategy for a scheme to be used in routine laboratories. As computer methods are used more widely for identifying bacteria the problems of assessing the performance required by the users of the methods and adjusting the methods to give this performance will become more apparent (Willcox and Lapage, 1977b).

Using a computer program to select from 52 conventional tests the tests which best discriminated between the 34 taxa a set of 19 tests was selected (Table 4). These 19 tests separated all pairs of taxa except one and the first 10 tests selected had the same theoretical value as the 20 conventional tests corresponding to the API 20E tests. According to the test selection model a system of 20 tests having a better diagnostic performance than the present API 20E should be possible, or alternatively the same performance as the API 20E could be obtained with a 10-test system. There are a number of qualifications to these conclusions, however. In evaluating the tests only the 34 taxa of Table 1 were considered,

whereas the API 20E system is now applicable to Gram-negative bacteria other than the *Enterobacteriaceae*. Some of the tests selected—for example, KCN tolerance or motility at room temperature—are probably unsuitable for including in a kit such as the API, though such tests carried out conventionally might make useful supplements to the kit. Finally, only conventional tests were evaluated. Obviously it would be more relevant to evaluate a range of tests already in kit form such as the 70 tests of the API 20E and 50E systems. Brooks *et al.* (1974) and Smith *et al.* (1972) have also suggested that the range of tests in the present API 20E kit should be altered and certain tests such as amygdalin be replaced by more familiar or more useful tests.

The manufacturer of the API system should consider the possibility of recommending the reagents of Crosby (1967) for determining nitrate reduction in place of those presently recommended, one of which contains the carcinogen α -naphthylamine. In our study nitrate reduction in 86 strains was determined in the API 20E kit using the reagents of Crosby (1967) and 10 gave a negative result. When these 10 strains were retested in API 20E kits using the reagents recommended by the manufacturer six gave a positive result but four were still negative. It seems therefore that with a slight modification of either the kit or the reagent the non-carcinogenic reagents of Crosby (1967) could be used to determine nitrate reduction in the API 20E kit. Negative results for strains of *Enterobacteriaceae* (5/128) in the test for nitrate reduction, using the reagents recommended by the manufacturer, have also been recorded by Washington *et al.* (1971).

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