A Revised Probability Matrix for the Identification of Gram-negative, Aerobic, Rod-shaped, Fermentative Bacteria

By BARRY HOLMES,* CHRISTINE A. DAWSON AND
CLAIRE A. PINNING†

Computer Identification Laboratory, National Collection of Type Cultures, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT, UK

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The results of the identification of 933 strains of Gram-negative, aerobic, rod-shaped, fermentative bacteria (*Enterobacteriaceae*, *Pasteurellaceae*, *Vibrionaceae*) by a probabilistic method, in a computer, are given. The identification rate on the matrix was 89.2%. Many of the strains were atypical and had caused difficulty in identification in medical diagnostic laboratories. The results are given for each taxon by genus and species.

INTRODUCTION

A computer-assisted conditional probability method for the identification of enterobacteria was first described by Dybowski & Franklin (1968) and Lapage *et al.* (1970) used a similar scheme to successfully identify up to 80% of 279 freshly isolated strains. Later, Bascomb *et al.* (1973) published a matrix for the identification of Gram-negative rods of clinical importance and discussed its use in the identification of 1079 reference strains; the general aspects of probabilistic identification and the mathematical model used were described by Lapage *et al.* (1973) and Willcox *et al.* (1973), respectively. This latter matrix was then used as the basis for an identification service in our laboratory, the methods for which were reviewed by Willcox *et al.* (1980). In the operation of the identification service, the test results obtained for strains submitted for identification were accumulated by computer. These results were then sorted by taxon and printed in the form of summaries, as described by Holmes & Hill (1985). From these summarized results a revised matrix was derived for the fermentative organisms and, following evaluation, this matrix is now in current use for the routine identification service.

In this paper we present the results obtained in the identification of 933 strains of bacteria belonging to 110 taxa in the revised probability matrix.

METHODS

Overall procedure. A matrix was constructed which gave the probability of a strain of any given taxon yielding a positive result in each of the chosen tests (Table 1). Individual strains were then identified on the basis of these results.

Taxa. Of the taxa chosen for the matrix (Table 1), the majority gave a fermentative result in the oxidation/fermentation (O/F test) of Hugh & Leifson (1953). A few non-fermentative taxa were also included; these were taxa that produce acid from glucose in peptone/water/sugar media and that may possess other characteristics by which they may be confused with fermentative organisms.

Although the range of taxa was selected primarily to include those of known medical importance and those likely to occur in medical specimens, efforts were made to include as many recently described species as possible, so as to facilitate recognition of the latter should they occur in human clinical or veterinary material. Particular attention was paid to ensure inclusion of all *Enterobacteriaceae* taxa described in *Bergey's Manual of Systematic Bacteriology* (Brenner, 1984). The majority of the taxa are recognized species, genera or subgenera. Some are

† Present address: Gibco-Sensititre, Imberhorne Lane, East Grinstead, West Sussex RH19 1QX, UK.

without formal names, such as Group EF-4 (Tatum *et al.*, 1974), whilst others are recognized as distinct biochemical varieties of existing species, such as *Edwardsiella tarda* biogroup 1 (Grimont *et al.*, 1980; Farmer *et al.*, 1985a). Many of the taxa have been described by Holmes & Gross (1983).

Tests. The range of tests was largely as described by Bascomb *et al.* (1973), but with certain omissions and additions. Dirty coloured pigment was retained (but could easily be omitted because no strains of any taxon gave a positive result in this test) and gelatin liquefaction within 28 d (overall gelatinase production) was replaced with the more sensitive gelatin plate test that can be read after 5 d. Orange pigment production, casein digestion and production of extracellular DNAase were added. Acetate utilization, alkali production on Christensen's citrate and mucate fermentation were incorporated to differentiate primarily between *Escherichia coli* and *Shigella* species, and growth at 5 °C and at 42 °C were incorporated to aid discrimination between the *Klebsiella* species. For these last five tests, probabilities are not allotted in the matrix for all taxa. In practice, 65 test results were available. Fifty-six tests were set up of which one, pigmentation, if present, provided a choice of six possible colours and another, the O/F test, four possible results. Except for some taxa (in the case of the five tests mentioned above) the 65 test results were allotted probabilities for each taxon in the matrix (Table 1).

Methods. The media and methods used were as described by Bascomb *et al.* (1971) and those for the additional tests were as follows: overall gelatinase production was determined by method 3 of Cowan & Steel (1965); casein digestion was determined on a medium prepared from Oxoid skim milk powder 50 g, New Zealand agar 25 g and distilled water 1500 ml; production of extracellular DNAase was determined on Oxoid DNAase agar but with the modification of Schreier (1969); acetate utilization was determined in a medium similar to Simmons' citrate agar (except that 0.25%, w/v, sodium acetate was used in place of citrate); alkali production on Christensen's citrate was determined according to Cowan & Steel (1965) but with the omission of ferric ammonium citrate and of Na₂S₂O₃, and with New Zealand agar (11 g) in place of Japanese agar (20 g); mucate fermentation was determined by the methods described by Edwards & Ewing (1972); growth at 5 °C and at 42 °C was recorded from nutrient broth. Where appropriate, tests were read at 1, 2 and 5 d (including the additional tests described above, except for overall gelatinase production which was read at 5 d only). Except where otherwise required by the specification for the test, incubation was at the optimum growth temperature of the strain under examination, usually 37 °C, but occasionally 30 °C or room temperature (18–22 °C).

Coding of tests. The methods followed were as described by Bascomb *et al.* (1973) except that acid production from carbohydrates and gas from glucose were also recorded as four-state (+, ±, −, −), like the majority of tests, and the O/F test was also coded as four-state (oxidative, fermentative, alkaline and negative, when no change was effected to the medium).

Linkage of tests. This was as described by Bascomb *et al.* (1973) but with the plate test for gelatinase production in place of gelatin liquefaction within 28 d and with failure to produce β-galactosidase (ONPG test) linked with failure to produce acid from lactose.

Pigment production, with the six possible colours, was treated as a multistate test (Willcox *et al.*, 1973). The O/F test was treated in a non-standard way by the identification program. In calculating the identification scores, the first component, with the name Hugh & Leifson (Table 1), was treated as 'negative or alkaline' so the entries of the first three components for a taxon should add up to a nominal 100%. The fourth component, H & L (Hugh & Leifson) alkaline (Table 1) was treated as an independent test, so it could take any value less than or equal to the value of the first component. Possible entries would then be: 01, 01, 99, 01 (all strains fermentative); 99, 01, 01, 01, (all strains negative); 99, 01, 01, 99 (all strains alkaline); and 99, 01, 01, 25 (25% alkaline, 75% negative). In test selection and printed output, however, the O/F test was treated as a four-state test. On the identification reports the percent probability displayed was for a positive result in a particular test. Therefore the figure displayed for Hugh & Leifson for a negative result was the percent probability (%P) of Hugh & Leifson not negative and was calculated as follows:

$$\begin{aligned} \%P [\text{H \& L (negative or alkaline)}] - \%P [\text{H \& L (alkaline)}] &= \%P [\text{H \& L (negative)}] \\ \%P [\text{H \& L (not negative)}] &= 100\% - \%P [\text{H \& L (negative)}] \end{aligned}$$

Adjustments needed to be made before and after this calculation to allow for matrix figures of 1 and 99 being used in place of 0 and 100 respectively (see below).

Construction of matrix. The data for the various taxa used to compile the matrix were obtained only from strains tested in our laboratory, using standardized techniques, and the probability values allotted to each taxon represented the actual proportion of strains of each taxon found to be positive in a particular test. However, the methods for construction of the data base were otherwise similar to those adopted by Bascomb *et al.* (1973), including the setting of upper and lower limits for probabilities of 0.99 and 0.01 respectively. The matrix is given in Table 1.

Probabilistic identification. The methods were as described by Bascomb *et al.* (1973), but there was a considerable increase in cases where identification to a combined taxon (or composite group; see Holmes & Hill, 1985) was permitted. A combined taxon comprised two closely related taxa which had proved difficult to separate in the

construction of the matrix, as there were few or no constant (0.99/0.01) characters which would differentiate between them (see also results on individual taxa). The combined taxa are given in the footnote to Table 1. A strain was identified to the combined taxon if the two taxa appeared as first and second choice and the sum of their identification scores was greater than 0.999.

Strains. The results obtained using the revised matrix for the identification of 933 strains collected between 1965 and 1983 are reported. The aim was to include at least 10 strains of each taxon (if available), and the type strain (if there was one and if available) was always included. Many of the strains were from culture collections, and many others were designated strains from experts in the relevant group of bacteria, but for commonly encountered taxa, in particular, a number of recent clinical isolates were also included. Many of the reference strains from experts in the relevant group were also comparatively recent clinical isolates.

Identification procedure. We did not identify each strain independently by conventional means. Type strains, strains from culture collections and those from experts in the relevant groups were taken as correctly designated. The remaining strains used to evaluate the matrix were identified only by the computer method using the original matrix of Bascomb *et al.* (1973), although subject to final assessment by us. All strains were then assigned to taxa in the matrix. In several cases it was the same strains used to compile the matrix which were used to evaluate it (generally with the more recently described taxa of which few strains were readily available to us).

The strains were identified by computer using the revised matrix and the results were analysed. A strain was considered to be correctly identified where the computer identification agreed with the strain designation; a strain was incorrectly identified when the computer identification and strain designation did not agree. Strains for which the identification score did not reach the threshold identification level were considered not identified.

Excluded from this study were any strains which could not be identified on the original matrix; such intermediate or highly unusual strains are discussed by Lapage *et al.* (1973). Also, strains of taxa not in the matrix were not included.

Statistical evaluation. The quality of the matrix was tested by two statistical programs. Program OVERMAT (Sneath, 1980*b*) measures the overlap between pairs of taxa in the matrix. For each pair the extent and statistical significance of the overlap were determined. Groups which show unacceptably large overlap with others can thus be found. Program MOSTTYP (Sneath, 1980*a*) calculates the best identification score possible for a theoretically most typical member of the taxon (hypothetical median organism, or HMO).

RESULTS AND DISCUSSION

Identification of strains

The success of computer identification of the various taxa on the revised matrix is given in Table 2. The results show that a total of 89.2% of strains were identified correctly on the revised matrix; these included all the strains belonging to 68 (62%) of the 110 taxa, and 90% or more of the strains of a further 15 (14%) taxa.

Separate taxa

Names appearing below in quotation marks were not in the Approved Lists of Bacterial Names (Skerman *et al.*, 1980) and have not been validated subsequently. The nomenclature we have followed is in general accord with the Approved Lists, although where two (or more) names which are clearly synonyms exist on the Lists we point this out at the appropriate place, but we do not consider it appropriate to discuss here our reasons for preferring one name over another. However, if our reasons are published elsewhere then we give the appropriate reference. Our nomenclatural usage closely follows that given in *Bergey's Manual of Systematic Bacteriology* (Brenner, 1984).

Acinetobacter. Strains belonging to this genus are non-fermenters but *A. calcoaceticus* was included in the fermenter matrix because it gives a negative reaction in the oxidase test and produces acid from glucose and some other carbohydrates in peptone water sugars. Since it shares these characters with members of the *Enterobacteriaceae* it seemed prudent to include this species in the fermenter matrix. The species can be easily excluded from the *Enterobacteriaceae* by its oxidative reaction in the O/F test and its failure to reduce nitrate; but, not all laboratories carry out these tests routinely. All 10 strains of the species were correctly identified.

Actinobacillus. Three species were included — *A. equuli*, *A. lignieresii* and *A. suis*. All three strains of the latter taxon were correctly identified. For *A. equuli* and *A. lignieresii*, however, only 7/10 and 8/10 strains respectively were correctly identified. For two of the three strains of *A. equuli* which were not identified, *A. equuli* was the highest scoring taxon, but identification was

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Character	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1. Motility (37 °C)	1	1	1	1	88	1	90	1	1	99	99	99	99	99	1	86	90	99	95	99	1	99
2. Motility (RT)	1	1	1	1	94	1	99	1	1	99	99	99	99	99	99	99	99	99	96	75	1	99
3. Growth (37 °C)	99	99	99	99	99	99	99	99	99	99	99	99	99	99	33	99	99	99	99	99	99	99
4. Growth (RT)	99	50	35	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99
5. Pigment negative	99	99	99	99	99	1	99	99	99	99	99	99	99	99	1	1	99	99	99	99	99	99
6. 'Dirty' pigment	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7. Brown pigment	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8. Violet pigment	1	1	1	1	1	1	1	1	1	1	1	1	1	1	99	99	1	1	1	1	1	1
9. Green pigment	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10. Yellow pigment	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11. Red pigment	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12. Orange pigment	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13. MacConkey growth	99	88	99	99	97	17	99	99	99	99	99	99	99	99	99	64	99	99	99	99	99	99
14. Catalase	99	99	75	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	67	99
15. Oxidase	1	99	20	99	99	99	1	99	99	1	1	1	1	1	99	69	1	1	1	1	1	1
16. Hugh & Leifson	1	1	25	1	1	1	1	13	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17. H & L oxidative	99	1	1	1	1	1	1	6	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18. H & L fermentative	1	99	75	99	99	99	99	81	99	99	99	99	99	99	99	99	99	99	99	99	99	99
19. H & L alkaline	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20. Nitrate reduction	4	99	95	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99
21. Simmon's citrate	84	1	1	1	44	1	50	1	1	1	99	99	99	1	33	43	99	99	96	1	1	1
22. Christensen's citr.	84	1	1	1	58	1	99	6	43	99	99	99	99	99	33	67	99	99	99	99	1	99
23. Urease	42	80	55	99	1	1	1	1	1	1	1	1	1	1	1	1	70	50	75	1	1	1
24. Gelatin stab	15	1	1	1	1	99	99	11	1	1	1	1	1	1	1	93	1	1	1	1	1	1
25. Gelatin plate	27	1	1	1	1	99	99	1	94	14	1	1	1	1	1	33	93	1	1	1	1	1
26. KCN	53	1	1	1	83	1	99	6	1	99	99	99	1	1	33	93	99	99	5	99	1	6
27. H ₂ S Paper	1	1	1	1	52	1	1	1	1	1	1	1	1	1	1	1	30	99	92	99	1	99
28. H ₂ S TSI	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	90	1	1	1	1	99
29. Gluconate	1	1	1	1	33	1	1	1	1	1	99	67	99	1	1	1	1	1	1	1	1	1
30. Malonate	50	1	1	1	1	1	90	1	1	99	99	99	99	1	1	1	1	1	94	63	1	1
31. ONPG	1	99	99	99	97	1	99	1	1	99	99	99	99	99	1	1	99	99	99	1	1	1
32. PPA	1	1	1	1	3	1	1	1	1	1	1	1	1	1	1	1	1	10	1	1	1	1
33. Arginine dihydrox.	1	1	1	1	93	99	1	99	1	99	99	99	99	99	1	99	99	80	98	1	1	1
34. Lysine decarboxase	1	1	1	1	4	83	10	1	1	1	1	1	1	1	1	1	1	1	1	99	99	99
35. Ornithine decarboxase	1	1	1	1	1	1	99	1	1	99	1	1	1	99	1	1	99	60	68	99	99	99
36. PWS glucose (acid)	99	99	95	99	99	99	99	99	71	99	99	99	99	99	99	86	99	99	99	99	99	99
37. PWS glucose (gas)	1	1	1	1	49	99	1	1	1	1	99	33	99	1	1	1	99	99	99	99	1	99
38. 0.4% Selenite F	1	22	10	1	62	25	1	1	1	67	99	99	99	1	1	1	99	99	97	1	1	17
39. Casein hydrolysis	4	1	1	1	82	1	1	1	1	1	1	1	1	1	99	99	1	1	1	1	1	1
40. DNAse	4	1	1	1	93	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
41. Adonitol PWS	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	99	1	1	1
42. Arabinose PWS	54	20	1	99	69	1	99	1	1	1	1	1	1	1	1	1	99	90	99	13	1	18
43. Cellobiose PWS	1	1	1	99	38	1	99	1	1	1	99	99	99	99	1	1	99	99	1	1	1	1
44. Dulcitol PWS	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	10	45	1	1	1
45. Glycerol PWS	1	11	1	1	97	99	70	1	1	67	33	67	99	1	1	1	30	90	99	99	99	50
46. Inositol PWS	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	4	1	1	1
47. Lactose PWS	1	99	10	99	29	1	99	1	1	1	67	67	99	1	1	1	99	99	85	1	1	1
48. Maltose PWS	1	99	95	99	99	99	99	1	1	99	99	99	99	99	99	14	99	99	99	99	99	99
49. Mannitol PWS	1	99	80	1	99	99	99	1	1	99	99	99	99	99	1	1	99	99	99	99	1	1
50. Raffinose PWS	1	99	10	99	1	1	90	1	1	1	1	33	1	99	1	1	1	40	1	1	1	1
51. Rhamnose PWS	1	1	1	1	10	1	99	1	1	1	1	1	1	1	1	1	99	99	98	1	1	1
52. Salicin PWS	1	1	1	1	99	55	67	99	1	99	99	99	99	99	1	1	90	10	91	99	1	1
53. Sorbitol PWS	1	10	1	1	12	1	10	1	1	1	1	67	99	99	1	1	99	99	99	1	1	1
54. Sucrose PWS	1	99	95	99	89	1	1	1	1	99	1	99	99	99	1	14	9	60	48	99	1	1
55. Trehalose PWS	1	80	1	99	99	8	99	1	1	99	99	99	99	99	67	71	99	99	99	99	1	1
56. Xylose PWS	67	99	85	99	1	1	99	1	1	99	1	99	99	99	1	1	99	90	99	1	1	1
57. Starch PWS	1	1	1	1	99	99	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
58. Methyl red (37 °C)	1	1	1	1	86	1	99	6	1	99	33	99	99	99	1	14	99	99	99	99	1	99
59. Methyl red (RT)	1	1	1	1	47	99	99	1	1	99	1	1	1	1	33	1	99	99	99	63	67	99
60. V-P (37 °C)	1	1	1	1	29	1	1	1	1	1	99	1	1	1	1	1	1	1	1	1	1	1
61. V-P (RT)	1	1	1	1	58	1	1	1	1	1	99	67	1	1	1	1	1	1	1	1	1	1
62. Indole	1	1	1	1	74	1	1	1	1	1	1	1	1	1	1	1	99	20	94	75	1	99
63. Acetate	0	1	1	1	83	0	99	33	50	99	99	99	99	99	0	0	0	0	1	1	1	0
64. Mucate	0	1	1	1	1	0	99	1	1	1	1	1	1	1	0	0	0	0	99	1	1	0
65. Growth (5 °C)	11	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
66. Growth (42 °C)	77	0	0	0	0	0	0	0	99	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 1. (continued)

Character	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44
	<i>Edwardsiella tarda</i>	<i>biogroup 1</i>																				
	*	<i>Enterobacter aerogenes</i>																				
	*	<i>Enterobacter amnigenus</i>																				
	*	<i>Enterobacter cloacae</i>																				
	*	<i>Enterobacter gergoviae</i>																				
		<i>Enterobacter intermedius</i>																				
	*	<i>Enterobacter sakazakii</i>																				
	*	<i>Erwinia herbicola</i>																				
		<i>Escherichia adecarboxylata</i>																				
		<i>Escherichia blattae</i>																				
	*	<i>Escherichia coli</i>																				
		<i>Escherichia fergusonii</i>																				
	*	<i>Escherichia hermannii</i>																				
	*	<i>Escherichia vulneris</i>																				
	*	<i>Hafnia alvei</i>																				
	*	<i>Klebsiella oxytoca</i>																				
	*	<i>Klebsiella pneumoniae</i> subsp. <i>aerogenes</i>																				
	*	<i>Klebsiella pneumoniae</i> subsp. <i>ozaenae</i>																				
	*	<i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i>																				
	*	<i>Klebsiella pneumoniae</i> subsp. <i>rhinoscleromatis</i>																				
		<i>Klebsiella terrigena</i>																				
	*	<i>Kluyvera</i> spp.																				
1. Motility (37 °C)	83	92	99	71	88	99	95	27	99	1	23	99	99	89	32	1	1	1	1	1	1	78
2. Motility (RT)	83	92	99	75	94	91	95	92	99	1	39	99	99	99	70	1	1	1	1	1	1	99
3. Growth (37 °C)	99	99	99	99	99	99	99	97	99	99	99	99	99	99	99	99	99	99	99	99	99	99
4. Growth (RT)	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99
5. Pigment negative	99	99	99	99	99	99	10	45	60	99	99	99	99	99	99	99	99	99	99	99	99	99
6. 'Dirty' pigment	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7. Brown pigment	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8. Violet pigment	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9. Green pigment	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10. Yellow pigment	1	1	1	1	1	1	90	55	40	1	1	1	1	1	1	1	1	1	1	1	1	1
11. Red pigment	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12. Orange pigment	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13. MacConkey growth	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99
14. Catalase	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99
15. Oxidase	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
16. Hugh & Leifson	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17. H & L oxidative	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18. H & L fermentative	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99
19. H & L alkaline	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20. Nitrate reduction	99	99	99	99	99	99	99	94	99	99	99	99	89	99	99	99	95	94	99	99	99	99
21. Simmon's citrate	1	99	80	93	99	99	64	1	50	1	33	1	16	99	87	31	99	1	99	87	99	99
22. Christensen's citr.	99	99	80	99	99	99	87	1	50	80	99	67	1	99	99	99	96	99	79	99	96	99
23. Urease	1	1	1	60	99	1	1	10	1	1	1	1	1	1	94	91	25	94	1	14	1	1
24. Gelatin stab	1	1	1	1	1	1	85	23	1	1	1	1	1	1	1	1	1	1	1	1	1	1
25. Gelatin plate	1	1	1	48	1	1	99	80	60	1	1	1	1	1	20	1	1	1	1	1	1	1
26. KCN	1	99	99	89	1	99	99	18	99	1	5	1	99	11	85	99	97	48	22	80	99	91
27. H ₂ S Paper	99	5	99	14	19	36	5	11	10	1	4	33	1	27	7	8	1	1	1	1	1	17
28. H ₂ S TSI	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
29. Gluconate	1	99	99	97	99	91	99	12	1	75	1	1	1	1	93	93	96	1	6	1	99	1
30. Malonate	1	99	99	87	99	99	25	78	99	99	1	1	1	89	61	95	86	4	99	97	99	96
31. ONPG	1	99	99	99	99	99	99	99	99	1	81	99	99	99	90	99	99	98	99	1	99	99
32. PPA	1	1	1	1	1	1	3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
33. Arginine dihydrox.	1	1	99	97	1	1	99	2	1	1	35	67	1	99	10	1	1	33	1	1	1	1
34. Lysine decarbox.	99	99	1	1	19	1	1	1	1	99	81	99	33	99	98	92	87	21	99	1	99	22
35. Ornithine decarbox.	99	99	99	96	99	91	85	1	1	99	72	99	99	1	99	1	2	1	1	1	1	96
36. PWS glucose (acid)	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99
37. PWS glucose (gas)	99	99	99	94	99	1	95	15	99	99	80	99	99	99	94	94	90	61	99	1	86	91
38. 0.4% Selenite	1	92	99	91	99	1	90	14	90	1	67	99	99	99	88	99	86	15	71	15	99	99
39. Casein hydrolysis	1	1	1	1	1	1	11	11	1	1	1	1	1	1	1	1	1	1	1	1	1	1
40. DNAase	1	1	1	1	1	1	90	6	10	1	1	1	1	2	1	1	3	1	1	1	1	1
41. Adonitol PWS	1	99	1	27	1	1	1	1	99	1	7	99	1	1	1	99	92	99	99	99	99	1
42. Arabinose PWS	99	99	99	99	99	99	99	93	99	99	99	99	99	99	95	99	99	92	99	99	99	99
43. Cellobiose PWS	1	99	99	99	99	99	57	99	1	5	99	99	99	45	99	99	99	99	99	99	99	99
44. Dulcitol PWS	1	8	1	12	1	99	10	7	99	1	64	1	99	1	64	23	1	99	1	29	9	9
45. Glycerol PWS	67	99	50	49	99	91	25	33	1	99	91	1	1	1	99	99	99	75	99	79	99	83
46. Inositol PWS	1	99	1	12	1	1	80	10	1	1	2	1	1	1	99	92	62	99	97	99	1	1
47. Lactose PWS	1	99	99	76	75	99	95	36	99	1	70	33	33	33	7	99	96	65	99	1	99	99
48. Maltose PWS	99	99	99	99	99	99	99	79	99	99	79	99	99	99	99	99	99	99	99	99	99	99
49. Mannitol PWS	99	99	99	99	99	99	99	99	1	96	99	99	99	99	99	99	99	99	99	99	99	99
50. Raffinose PWS	1	99	60	74	99	99	36	70	1	31	1	1	89	4	99	99	91	99	99	99	99	99
51. Rhamnose PWS	1	99	99	96	99	99	99	93	99	99	93	99	99	99	96	99	99	54	99	97	99	99
52. Salicin PWS	83	99	99	95	99	99	86	99	1	49	99	67	99	19	99	99	99	99	99	99	99	99
53. Sorbitol PWS	1	99	50	94	1	99	1	18	1	1	92	1	1	1	17	99	96	93	99	99	30	99
54. Sucrose PWS	99	99	50	96	99	91	99	88	70	1	35	1	33	11	2	99	99	31	99	94	99	99
55. Trehalose PWS	1	99	99	99	99	99	99	96	99	99	99	99	99	99	98	99	99	99	99	99	99	99
56. Xylose PWS	1	99	99	99	99	99	87	99	99	93	99	99	99	98	99	99	97	99	99	99	96	99
57. Starch PWS	1	15	1	2	1	1	1	1	7	1	1	1	1	1	75	77	29	94	27	1	9	9
58. Methyl red (37 °C)	99	31	99	30	94	99	5	82	99	99	98	99	99	99	83	70	50	98	99	99	99	99
59. Methyl red (RT)	99	1	1	2	1	1	15	31	99	75	96	67	99	89	4	17	21	93	99	99	14	99
60. V-P (37 °C)	1	46	1	80																		

Table 1. (continued)

Character	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
	<i>Morganella morganii</i>	<i>Neisseria dentrificans</i>	<i>Neisseria pharyngis</i>	<i>Obesumbacterium proteus</i> biogroup 1	<i>Obesumbacterium proteus</i> biogroup 2	<i>Pasteurella aerogenes</i>	<i>Pasteurella BL Group</i>	<i>Pasteurella gallinarum</i>	<i>Pasteurella haemolytica A</i>	<i>Pasteurella haemolytica T</i>	<i>Pasteurella multocida</i>	<i>Pasteurella multocida</i> (atypical)	<i>Pasteurella pneumotropica</i>	<i>Pasteurella testudinis</i>	<i>Pasteurella ureae</i>	<i>Plesiomonas shigelloides</i>	<i>Proteus mirabilis</i>	<i>Proteus myxofaciens</i>	<i>Proteus vulgaris</i> biogroup 3	<i>Proteus penneri</i>	<i>Proteus vulgaris</i> biogroup 2	<i>Providencia alcalifaciens/P. rustigianii</i>
1. Motility (37 °C)	85	1	1	1	1	1	1	1	1	1	1	1	1	1	1	83	86	99	50	75	75	36
2. Motility (RT)	91	1	1	1	1	1	1	1	1	1	1	1	1	1	1	83	89	99	67	75	82	82
3. Growth (37 °C)	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99
4. Growth (RT)	99	99	99	99	99	99	99	1	10	33	86	61	67	99	32	99	99	99	99	99	99	99
5. Pigment negative	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99
6. 'Dirty' pigment	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7. Brown pigment	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8. Violet pigment	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9. Green pigment	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10. Yellow pigment	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11. Red pigment	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12. Orange pigment	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13. MacConkey growth	98	68	50	99	99	99	99	99	84	75	38	1	49	99	5	99	98	99	99	99	99	99
14. Catalase	99	99	99	99	99	99	99	99	99	99	99	99	99	99	92	99	99	99	99	99	99	99
15. Oxidase	1	99	99	1	1	91	1	99	94	67	62	52	47	99	73	99	1	1	1	1	1	1
16. Hugh & Leifson	1	1	1	33	1	1	1	1	13	17	17	22	19	1	27	1	1	1	1	1	1	1
17. H & L oxidative	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18. H & L fermentative	99	99	99	67	99	99	99	99	87	83	83	78	81	99	73	99	99	99	99	99	99	99
19. H & L alkaline	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20. Nitrate reduction	98	99	1	99	99	91	1	99	99	99	98	99	98	99	99	99	99	99	99	99	99	98
21. Simmon's citrate	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	53	1	1	1	1	47	49
22. Christensen's citr.	52	1	1	1	1	1	1	1	1	1	1	1	3	1	1	99	99	17	50	88	99	99
23. Urease	97	1	1	1	1	99	99	1	8	1	1	49	1	68	1	95	99	99	99	94	1	1
24. Gelatin stab	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	92	99	99	99	82	1
25. Gelatin plate	1	1	1	1	1	1	1	1	1	1	1	2	99	1	6	94	99	83	99	99	94	1
26. KCN	97	1	1	1	1	1	1	1	1	19	4	2	99	1	1	99	1	99	99	99	99	99
27. H ₂ S Paper	93	1	1	1	99	91	1	50	3	8	6	4	28	99	1	17	99	99	99	99	99	66
28. H ₂ S TSI	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	98	1	33	25	69	9	
29. Gluconate	1	1	1	99	1	1	1	1	1	1	1	1	1	1	1	57	1	1	1	1	1	1
30. Malonate	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
31. ONPG	3	1	1	1	99	99	1	1	61	25	13	1	54	99	1	89	1	1	1	6	1	1
32. PPA	88	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	99	99	99	99	98	1
33. Arginine dihydrolysis	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	99	1	1	1	1	1	1
34. Lysine decarboxylase	2	1	1	67	99	1	1	1	1	2	1	1	1	1	1	99	1	1	1	1	1	1
35. Ornithine decarboxylase	99	1	1	1	99	46	1	99	7	25	90	96	61	1	1	99	96	1	1	1	1	1
36. PWS glucose (acid)	99	1	99	99	99	99	99	99	97	99	99	96	99	99	97	99	99	99	99	99	99	99
37. PWS glucose (gas)	99	1	1	1	1	99	1	1	1	1	1	2	1	1	1	97	99	99	99	99	67	1
38. 0.4% Selenite	17	1	1	1	1	73	1	47	8	39	1	1	99	1	18	89	99	50	75	99	64	1
39. Casein hydrolysis	1	1	1	1	1	1	1	1	1	1	10	1	1	1	1	41	1	1	1	33	1	1
40. DNAase	4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	57	99	99	99	99	33	1
41. Adonitol PWS	1	1	1	1	1	1	1	1	1	1	1	1	1	33	1	1	1	1	1	1	62	1
42. Arabinose PWS	1	1	1	67	1	73	99	1	7	8	6	1	2	67	1	1	1	1	1	1	1	1
43. Cellobiose PWS	1	1	1	1	1	1	1	1	1	50	1	1	1	1	1	1	1	1	1	1	1	1
44. Dulcitol PWS	1	1	1	1	1	1	1	1	1	1	3	1	1	1	1	1	1	1	1	1	1	1
45. Glycerol PWS	42	1	1	67	1	1	75	1	10	1	16	1	2	99	1	67	99	99	99	99	42	1
46. Inositol PWS	1	1	1	1	1	91	1	1	39	8	1	1	1	99	1	99	1	1	1	1	2	1
47. Lactose PWS	3	1	1	1	1	1	1	1	10	8	14	1	1	1	1	89	1	1	1	1	6	1
48. Maltose PWS	1	1	99	1	99	99	1	99	99	58	3	1	79	99	95	99	1	99	99	99	99	1
49. Mannitol PWS	1	1	1	67	1	1	99	1	99	99	92	1	1	99	99	1	1	1	1	1	1	1
50. Raffinose PWS	1	1	1	1	1	1	1	65	1	4	1	19	99	1	1	1	1	1	1	1	1	1
51. Rhamnose PWS	1	1	1	1	99	27	1	1	1	1	1	1	1	99	1	1	1	1	1	1	1	1
52. Salicin PWS	1	1	1	67	1	1	75	1	3	99	1	1	1	33	1	11	1	1	1	1	99	1
53. Sorbitol PWS	1	1	1	1	1	1	1	1	99	67	82	1	1	67	8	1	1	1	1	1	1	1
54. Sucrose PWS	1	1	99	1	1	99	1	99	99	99	99	96	93	99	92	1	4	99	99	99	80	1
55. Trehalose PWS	6	1	1	99	99	1	99	99	58	24	1	84	33	1	99	99	99	99	50	76	1	1
56. Xylose PWS	1	1	1	1	99	82	1	1	74	8	61	1	37	99	1	1	99	1	99	99	88	1
57. Starch PWS	1	1	1	1	1	1	1	1	13	1	1	1	1	1	1	1	1	1	1	1	1	1
58. Methyl red (37 °C)	90	1	1	1	1	1	1	1	1	1	1	1	1	1	1	99	82	99	99	75	99	89
59. Methyl red (RT)	92	1	1	99	99	1	1	1	1	1	1	2	99	1	99	35	1	99	75	88	94	1
60. V-P (37 °C)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	6	1	1	1	1	1	1
61. V-P (RT)	1	1	1	33	1	1	1	1	1	1	1	1	1	1	1	23	1	1	1	1	1	1
62. Indole	99	1	1	1	1	1	1	1	1	97	99	84	99	1	99	1	1	99	1	99	91	1
63. Acetate	1	1	0	0	0	0	1	0	1	1	6	1	1	1	1	88	83	0	50	0	71	99
64. Mucate	13	1	0	0	0	0	1	0	1	1	1	1	1	1	1	1	1	0	1	0	29	1
65. Growth (5 °C)	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0
66. Growth (42 °C)	0	0	0	0	0	0	0	0	0	0	99	0	0	0	0	99	0	0	0	0	0	0

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	<i>Providencia rettgeri</i>	<i>Providencia stuartii</i>	<i>Pseudomonas pseudomallei</i>	<i>Rahnella aquatilis</i>	<i>Salmonella choleraesuis</i>	<i>Salmonella gallinarum</i>	<i>Salmonella paratyphi A</i>	<i>Salmonella pullorum</i>	<i>Salmonella subgenus II</i>	<i>Salmonella ferlac</i>	<i>Salmonella subgenus I</i>	<i>Salmonella subgenus III (= Arizona)</i>	<i>Salmonella subgenus IV</i>	<i>Salmonella typhi</i>	<i>Salmonella typhisuis</i>	<i>Serratia ficaria</i>	<i>Serratia fonticola</i>	<i>Serratia liquefaciens</i>	<i>Serratia marcescens</i>	<i>Serratia marinorubra</i>	<i>Serratia odorifera biovar I</i>	<i>Serratia odorifera biovar II</i>	
Character	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	
1. Motility (37 °C)	80	50	99	1	99	1	92	1	99	99	87	99	99	94	99	99	99	61	96	96	99	99	
2. Motility (RT)	99	41	99	99	99	1	99	1	99	99	89	99	94	99	99	99	99	99	98	99	99	99	
3. Growth (37 °C)	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	
4. Growth (RT)	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	
5. Pigment negative	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	89	24	99	99	
6. 'Dirty' pigment	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
7. Brown pigment	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
8. Violet pigment	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
9. Green pigment	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
10. Yellow pigment	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
11. Red pigment	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	11	76	1	1	1	
12. Orange pigment	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
13. MacConkey growth	96	99	99	90	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	
14. Catalase	99	96	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	
15. Oxidase	1	1	99	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
16. Hugh & Leifson	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
17. H & L oxidative	1	1	99	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
18. H & L fermentative	99	99	1	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	
19. H & L alkaline	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
20. Nitrate reduction	96	99	99	99	99	99	99	99	99	99	99	99	99	99	99	80	99	99	99	99	75	99	
21. Simmon's citrate	92	89	99	10	40	1	1	99	75	94	99	99	99	6	1	99	99	99	99	99	99	99	
22. Christensen's citr.	99	99	99	99	99	90	92	99	99	99	99	99	99	93	1	99	99	99	99	99	99	99	
23. Urease	99	10	33	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	26	1	1	1	
24. Gelatin stab	1	1	96	1	1	1	1	9	1	1	8	1	1	1	1	99	1	68	94	95	99	99	
25. Gelatin plate	1	2	99	1	1	1	1	82	50	1	67	80	1	1	99	1	92	97	99	99	99	99	
26. KCN	99	98	99	1	1	1	1	9	1	10	1	90	1	1	90	99	97	97	82	99	88	1	
27. H ₂ S Paper	96	68	1	1	36	90	33	83	99	99	96	99	99	94	1	92	6	32	1	25	1	1	
28. H ₂ S TSI	1	1	1	1	30	80	8	75	99	99	96	99	99	77	1	1	1	1	1	1	1	1	
29. Gluconate	1	1	1	50	1	1	1	1	1	1	1	1	1	1	1	99	92	82	90	99	99	99	
30. Malonate	1	1	13	99	1	1	1	1	91	1	1	99	1	1	1	1	99	1	3	82	1	1	
31. ONPG	3	5	1	99	1	1	1	18	99	1	92	10	12	1	99	99	97	96	99	99	99	99	
32. PPA	97	94	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
33. Arginine dihydrox.	10	1	99	70	99	9	99	17	99	99	96	99	99	99	1	8	12	1	1	1	1	1	
34. Lysine decarbox.	1	1	1	1	99	99	1	99	99	94	94	99	99	1	1	99	89	99	73	99	99	99	
35. Ornithine decarbox.	1	1	1	1	99	1	99	99	99	97	99	99	1	99	1	99	99	99	1	99	1	1	
36. PWS glucose (acid)	99	99	96	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	
37. PWS glucose (gas)	13	1	1	10	99	1	99	18	99	99	94	99	99	1	1	85	55	34	1	1	1	1	
38. 0.4% Selenite F	92	33	1	80	55	80	99	99	99	99	95	99	99	99	1	10	69	44	89	86	75	88	
39. Casein hydrolysis	1	1	99	1	1	1	1	1	1	1	1	1	1	1	1	99	1	53	92	17	75	99	
40. DNase	75	75	6	1	33	33	1	17	60	1	27	33	1	1	20	70	1	21	93	27	25	1	
41. Adonitol PWS	99	1	12	1	1	1	1	1	1	1	1	1	1	1	1	10	99	1	73	99	75	99	
42. Arabinose PWS	7	2	17	99	1	91	99	99	99	99	97	99	99	1	99	99	99	99	1	99	99	99	
43. Cellobiose PWS	1	3	41	99	1	1	1	46	1	30	1	70	1	1	99	54	33	14	99	99	99	99	
44. Dicalitol PWS	1	2	41	99	27	91	99	1	99	99	97	1	1	24	60	1	99	1	1	1	1	1	
45. Glycerol PWS	68	97	83	99	27	70	83	1	18	25	28	8	20	77	40	99	99	99	99	86	99	99	
46. Inositol PWS	99	99	65	1	1	1	1	18	1	44	1	1	1	1	80	93	97	94	96	99	99	99	
47. Lactose PWS	3	5	29	99	1	1	1	1	99	1	33	1	6	1	10	99	14	2	99	50	99	99	
48. Maltose PWS	1	1	41	99	91	80	99	1	99	99	96	99	99	99	20	99	99	99	99	99	99	99	
49. Mannitol PWS	99	5	47	99	99	99	99	99	99	96	96	99	99	1	99	99	99	99	99	99	99	99	
50. Raffinose PWS	1	10	12	99	1	1	1	1	1	2	1	1	1	1	80	99	89	1	99	99	1	1	
51. Rhamnose PWS	48	1	1	99	99	60	99	83	99	99	96	99	99	1	80	50	85	1	1	99	99	99	
52. Salicin PWS	80	1	24	99	1	1	1	1	1	1	1	1	50	1	1	99	99	99	99	96	99	99	
53. Sorbitol PWS	7	1	47	99	99	27	99	8	99	25	94	99	99	99	20	99	99	99	99	1	99	99	
54. Sucrose PWS	38	80	6	99	1	1	1	1	1	1	1	1	1	1	99	31	99	99	99	99	1	1	
55. Trehalose PWS	1	99	65	99	1	99	99	92	99	99	97	99	99	99	99	99	99	99	99	99	99	99	
56. Xylose PWS	4	13	24	99	99	60	1	42	99	99	96	99	99	99	99	77	99	7	99	99	99	99	
57. Starch PWS	1	1	18	10	1	1	1	1	1	1	1	8	1	1	1	1	1	2	1	41	99	99	
58. Methyl red (37 °C)	99	91	1	70	99	99	99	99	99	99	99	99	99	99	99	99	99	94	11	41	99	99	
59. Methyl red (RT)	99	85	1	1	99	99	99	92	99	99	99	99	99	99	99	20	99	11	5	1	1	1	
60. V-P (37 °C)	1	1	1	70	1	1	1	1	1	1	1	1	1	1	1	1	12	89	59	1	1	1	
61. V-P (RT)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	20	1	74	96	77	1	1	1	
62. Indole	97	89	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
63. Acetate	75	83	0	99	99	50	50	25	99	0	99	99	99	1	99	99	99	99	99	99	99	0	
64. Mucate	1	1	0	99	33	99	1	99	0	97	99	1	57	1	90	1	13	9	50	1	0	0	
65. Growth (5 °C)	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
66. Growth (42 °C)	0	0	99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

	<i>Serratia plymuthica</i>											<i>Shigella sonnei</i>											<i>Shigella</i> spp. (not <i>sonnei</i>)											<i>Tatumella ptyseos</i>											<i>Vibrio alginolyticus</i>											<i>Vibrio anguillarum</i>											<i>Vibrio cholerae</i>											<i>Vibrio fluvialis</i> (= <i>V. fluvialis</i> biovar I)											<i>Vibrio furnissii</i> (= <i>V. fluvialis</i> biovar II)											<i>Vibrio metschnikovii</i>											<i>Vibrio mimicus</i>											<i>Vibrio parahaemolyticus</i>											<i>Vibrio vulnificus</i>											<i>Xenorhabdus luminescens</i>											<i>Xenorhabdus nematophilus</i>											<i>Yersinia enterocolitica</i>											<i>Yersinia frederiksenii</i>											<i>Yersinia intermedia</i>											<i>Yersinia kristensenii</i>											<i>Yersinia pestis</i>											<i>Yersinia pseudotuberculosis</i>											<i>Yersinia ruckeri</i>																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																												
Character	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	 	92</

Table 1. (continued)

Each entry is the estimated percentage probability that a strain of the relevant taxon will give a positive result in the test concerned. RT, room temperature or incubator at 22 or 30 °C; TSI, triple sugar/iron medium; PWS, peptone/water/sugar medium. Identification to a composite group is permitted between the following pairs of taxa: 8, 9; 12, 13; 17, 18; 39, 41; 53, 54; 55, 56; 63, 64; 63, 65; 72, 80; 75, 76; 75, 77; 75, 78; 96, 97; 104, 107; 105, 106; 108, 109.

Taxa marked with asterisks (*) are recommended for retention in a reduced matrix. The tests of greatest value for differentiating these taxa are, in order of differentiating value, as follows (with some of these tests the results are automatically available for another test, which is given in parentheses; these additional tests can thus be included in the reduced matrix): 51, 31, 54, 35, 59 (61), 26, 25, 62, 2 (4), 37 (36), 52, 53, 30 (32), 56, 34, 49, 33, 23, 27, 50, 55, 29, 44, 47, 43, 58 (60), 46, 24, 42, 28, 38, 41 and 57.

prevented by the scores for *Pasteurella haemolytica* A. For the remaining strain, *A. equuli* was neither the highest nor second highest scoring taxon, so perhaps this strain was incorrectly designated as *A. equuli*. For the two strains of *A. lignieresii* which were not identified, *A. lignieresii* was the highest scoring taxon, but identification was prevented by the scores for *A. equuli* or *Pasteurella ureae*.

A. actinomycetemcomitans and *A. capsulatus* were not included in the matrix, as strains of the former do not grow aerobically on nutrient agar and strains of the latter were unavailable for study.

Aeromonas. Two species were included — *A. hydrophila* and *A. salmonicida*. We include strains of '*A. formicans*' in *A. hydrophila*, and eight out of 10 strains of this species were correctly identified. *A. hydrophila* had the highest identification score in both of the remaining strains, but identification at the level of 0.999 was prevented by the score for *Vibrio cholerae* in one strain and the score for *Vibrio fluvialis* biovar 2 (= *V. furnissii*) in the other. All strains of *A. salmonicida* were identified successfully. *A. punctata*, although appearing on the Approved Lists was not included in the matrix as strains were unavailable for study.

Buttiauxella. The only species, *B. agrestis* (Ferragut *et al.*, 1981), was included and all strains identified successfully. The success in identifying strains of this taxon was unexpected, as the genus *Kluyvera* (see below) was also included in the matrix and phenotypically the two taxa are very similar (Gavini *et al.*, 1983).

CDC Group EF-4. This unnamed taxon, first described by Tatum *et al.* (1974), is recovered predominantly from human animal bite wounds and has also been commonly recovered from the oral cavity or respiratory tract of dogs; the organism appears to cause respiratory infections in animals debilitated or under stress (Holmes & Ahmed, 1981). The taxon is divided into two biovars, one arginine-positive and generally gelatin-positive, the other arginine-negative and generally gelatin-negative, and they may represent separate species (Holmes & Ahmed, 1981). All strains of the arginine-positive biovar were identified correctly, five of the 12 as a composite group with CDC Group EF-4 arginine-negative biovar. Of the 10 strains of the latter taxon, only three were identified correctly — one as a composite group with CDC Group EF-4 arginine-positive biovar. In six of the seven remaining strains, CDC Group EF-4 arginine-negative biovar had the highest identification score, but identification at a level of 0.999 (either alone, or as a composite group with CDC Group EF-4 arginine-positive biovar) was prevented almost exclusively by the score for *Neisseria denitrificans*. For the final strain *N. denitrificans* achieved the highest identification score and CDC Group EF-4 arginine-negative biovar the second highest.

Cedecea. Five taxa were included, the three named species *C. davisae*, *C. lapagei* and *C. neteri* (formerly *Cedecea* species 4) together with *Cedecea* species 3 and *Cedecea* species 5 (see Grimont *et al.*, 1981; Farmer *et al.*, 1982). All strains of each taxon identified successfully, although one of *Cedecea* species 3 reached identification level only as a composite group with *C. neteri*.

Chromobacterium. Two species were included — *C. fluviatile* (Moss *et al.*, 1978) and *C. violaceum*. All strains of both species were correctly identified. *C. fluviatile* is an aquatic species.

Table 2. *Identification of fermentative bacteria*

Taxon no.	Taxon	No. of strains	Strains correctly identified by computer	
			No.	%
1. <i>Acinetobacter calcoaceticus</i>		10	10	100
2. <i>Actinobacillus equuli</i>		10	7	70
3. <i>Actinobacillus lignieresii</i>		10	8	80
4. <i>Actinobacillus suis</i>		3	3	100
5. <i>Aeromonas hydrophila</i>		10	8	80
6. <i>Aeromonas salmonicida</i>		10	10	100
7. <i>Buttiauxella agrestis</i>		10	10	100
8. CDC Group EF-4 arginine +ve biovar		12	12	100
9. CDC Group EF-4 arginine -ve biovar		10	3	30
10. <i>Cedecea davisae</i>		3	3	100
11. <i>Cedecea lapagei</i>		3	3	100
12. <i>Cedecea</i> species 3		3	3	100
13. <i>Cedecea neteri</i>		1	1	100
14. <i>Cedecea</i> species 5		1	1	100
15. <i>Chromobacterium fluviatile</i>		3	3	100
16. <i>Chromobacterium violaceum</i>		10	10	100
17. <i>Citrobacter amalonaticus</i>		11	11	100
18. <i>Citrobacter freundii</i>		12	11	92
19. <i>Citrobacter koseri</i>		10	10	100
20. <i>Edwardsiella hoshinae</i>		8	7	87.5
21. <i>Edwardsiella ictaluri</i>		3	3	100
22. <i>Edwardsiella tarda</i>		10	10	100
23. <i>Edwardsiella tarda</i> biogroup 1		6	6	100
24. <i>Enterobacter aerogenes</i>		10	9	90
25. <i>Enterobacter amnigenus</i>		10	10	100
26. <i>Enterobacter cloacae</i>		15	8	53
27. <i>Enterobacter gergoviae</i>		10	10	100
28. <i>Enterobacter intermedium</i>		10	10	100
29. <i>Enterobacter sakazakii</i>		10	10	100
30. <i>Erwinia herbicola</i>		10	10	100
31. <i>Escherichia adecarboxylata</i>		10	10	100
32. <i>Escherichia blattae</i>		4	4	100
33. <i>Escherichia coli</i>		15	10	66.7
34. <i>Escherichia fergusonii</i>		3	3	100
35. <i>Escherichia hermannii</i>		3	3	100
36. <i>Escherichia vulneris</i>		9	9	100
37. <i>Hafnia alvei</i>		10	9	90
38. <i>Klebsiella oxytoca</i>		11	3	27.3
39. <i>Klebsiella pneumoniae</i> subsp. <i>aerogenes</i>		12	5	41.7
40. <i>Klebsiella pneumoniae</i> subsp. <i>ozaenae</i>		12	10	83.3
41. <i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i>		11	10	91
42. <i>Klebsiella pneumoniae</i> subsp. <i>rhinoscleromatis</i>		11	11	100
43. <i>Klebsiella terrigena</i>		7	6	85.7
44. <i>Kluyvera</i> spp.		10	10	100
45. <i>Morganella morganii</i>		11	11	100
46. <i>Neisseria denitrificans</i>		3	0	0
47. ' <i>Neisseria pharyngis</i> '		6	5	83.3
48. <i>Obesumbacterium proteus</i> biogroup 1		3	3	100
49. <i>Obesumbacterium proteus</i> biogroup 2		3	3	100
50. <i>Pasteurella aerogenes</i>		11	10	90.9
51. <i>Pasteurella</i> BL Group		8	8	100
52. <i>Pasteurella gallinarum</i>		2	2	100
53. <i>Pasteurella haemolytica</i> A		10	6	60
54. <i>Pasteurella haemolytica</i> T		10	9	90
55. <i>Pasteurella multocida</i>		18	13	72.2
56. <i>Pasteurella multocida</i> (atypical)		10	7	70

Table 2. (continued)

Taxon no.	Taxon	No. of strains	Strains correctly identified by computer	
			No.	%
57.	<i>Pasteurella pneumotropica</i>	11	10	90.9
58.	<i>Pasteurella testudinis</i>	3	3	100
59.	<i>Pasteurella ureae</i>	10	9	90
60.	<i>Plesiomonas shigelloides</i>	10	10	100
61.	<i>Proteus mirabilis</i>	10	10	100
62.	<i>Proteus myxofaciens</i>	1	1	100
63.	<i>Proteus vulgaris</i> biogroup 3	6	4	66.7
64.	<i>Proteus penneri</i>	4	4	100
65.	<i>Proteus vulgaris</i> biogroup 2	10	10	100
66.	<i>Providencia alcalifaciens</i> / <i>P. rustigianii</i>	10	10	100
67.	<i>Providencia rettgeri</i>	10	10	100
68.	<i>Providencia stuartii</i>	10	10	100
69.	<i>Pseudomonas pseudomallei</i>	9	9	100
70.	<i>Rahnella aquatilis</i>	10	10	100
71.	<i>Salmonella choleraesuis</i>	10	9	90
72.	' <i>Salmonella gallinarum</i> '	10	10	100
73.	' <i>Salmonella paratyphi</i> A'	10	10	100
74.	' <i>Salmonella pullorum</i> '	10	10	100
75.	<i>Salmonella</i> subgenus II	9	7	77.8
76.	' <i>Salmonella ferlac</i> '	4	2	50
77.	<i>Salmonella</i> subgenus I	14	11	78.6
78.	<i>Salmonella</i> subgenus III (= <i>Arizona</i>)	10	10	100
79.	<i>Salmonella</i> subgenus IV	10	9	90
80.	<i>Salmonella typhi</i>	10	10	100
81.	' <i>Salmonella typhisuis</i> '	6	6	100
82.	<i>Serratia ficaria</i>	10	8	80
83.	<i>Serratia fonticola</i>	10	10	100
84.	<i>Serratia liquefaciens</i>	10	10	100
85.	<i>Serratia marcescens</i>	11	11	100
86.	<i>Serratia marinorubra</i>	10	9	90
87.	<i>Serratia odorifera</i> biovar I	4	4	100
88.	<i>Serratia odorifera</i> biovar II	8	8	100
89.	<i>Serratia plymuthica</i>	10	7	70
90.	<i>Shigella sonnei</i>	10	7	70
91.	<i>Shigella</i> spp. (not <i>sonnei</i>)	11	10	90.9
92.	<i>Tatumella pyseos</i>	5	5	100
93.	<i>Vibrio alginolyticus</i>	11	11	100
94.	<i>Vibrio anguillarum</i>	3	3	100
95.	<i>Vibrio cholerae</i>	11	10	90.9
96.	<i>Vibrio fluvialis</i> (= <i>V. fluvialis</i> biovar I)	11	11	100
97.	<i>Vibrio furnissii</i> (= <i>V. fluvialis</i> biovar II)	11	8	72.7*
98.	<i>Vibrio metschnikovii</i>	9	8	88.9
99.	<i>Vibrio mimicus</i>	3	3	100
100.	<i>Vibrio parahaemolyticus</i>	11	11	100
101.	<i>Vibrio vulnificus</i>	8	8	100
102.	<i>Xenorhabdus luminescens</i>	3	3	100
103.	<i>Xenorhabdus nematophilus</i>	3	3	100
104.	<i>Yersinia enterocolitica</i>	13	11	84.6
105.	<i>Yersinia frederiksenii</i>	10	8	80
106.	<i>Yersinia intermedia</i>	10	10	100
107.	<i>Yersinia kristensenii</i>	10	10	100
108.	<i>Yersinia pestis</i>	10	9	90
109.	<i>Yersinia pseudotuberculosis</i>	10	9	90
110.	<i>Yersinia ruckeri</i>	3	3	100
Total		933	832	89.2

* 1/11 strains incorrectly identified.

Citrobacter. Three species were included — *C. amalonaticus*, *C. freundii* and *C. koseri*. *C. diversus* and *Levinea malonatica* are generally recognized as being synonymous with *C. koseri* and we prefer to use the name *C. koseri* for this organism (for reasons, see Holmes *et al.*, 1974), although the name *C. diversus* is more commonly used in the USA. *C. amalonaticus* appeared on the Approved Lists as *Levinea amalonatica* but the species was subsequently transferred to *Citrobacter* by Farmer (1981).

C. amalonaticus and *C. freundii* are not easily distinguished and the probability figures in the matrix for these two species were based on relatively few strains (see Holmes & Hill, 1985). All 11 strains of *C. amalonaticus* were identified correctly although for three it was as a composite group with *C. freundii*. Of the 12 strains of *C. freundii*, 11 were identified correctly, two as a composite group with *C. amalonaticus*. For the remaining strain, *C. freundii* achieved the highest identification score, but identification at the level of 0.999 was prevented by the score for *Kluyvera* spp. All 10 strains of *C. koseri* were identified correctly.

Edwardsiella. Four taxa were included — *E. hoshinae*, *E. ictaluri* and *E. tarda* together with *E. tarda* biogroup 1, a distinct biovar from snakes (Grimont *et al.*, 1980; Farmer *et al.*, 1985*a*). Two species appeared on the Approved Lists: *E. anguillimortifera* and *E. tarda*. Since both species have the same type strain, they are synonymous and until the nomenclatural question is settled we use the more familiar name *E. tarda*. *E. hoshinae* (Grimont *et al.*, 1980) was isolated from birds, reptiles and water and *E. ictaluri* (Hawke *et al.*, 1981) was isolated from catfish, where it causes septicaemia. All strains of all four taxa were identified correctly with the exception of one strain of *E. hoshinae*, where the score for *E. tarda* biogroup 1 prevented identification at the level of 0.999.

Enterobacter. Six species were included — *E. aerogenes*, *E. amnigenus* (Izard *et al.*, 1981*b*), *E. cloacae*, *E. gergoviae* (Brenner *et al.*, 1980*b*), *E. intermedium* (Izard *et al.*, 1980) and *E. sakazakii* (Farmer *et al.*, 1980). *E. amnigenus* and *E. intermedium* occur in soil and water, and all the remaining species occur in human clinical specimens. The name *Enterobacter agglomerans* is commonly used in the USA for the organism that we have included in the revised matrix under the name *Erwinia herbicola*.

All strains of *E. amnigenus*, *E. gergoviae*, *E. intermedium* and *E. sakazakii* identified correctly on the revised matrix, whereas for *E. aerogenes* and *E. cloacae* the identification rate was 90% and 53% respectively. There are two probable reasons for the poor identification of strains of *E. cloacae*, (i) the inclusion of several additional, phenotypically similar, species in the revised matrix and (ii) the rather broader definition of *E. cloacae* in the revised matrix because many of the strains used to compile the matrix figures were atypical. For the one strain of *E. aerogenes* that failed to identify, this taxon had the highest identification score, but identification at the level of 0.999 was prevented by the score for *Klebsiella pneumoniae* subsp. *aerogenes*. *E. cloacae* had the highest identification score for each of the seven strains of this species that were not identified, but identification at the level of 0.999 was prevented by the scores for *Cedecea lapagei*, *Cedecea* species 3, *Enterobacter gergoviae* (two strains), *Erwinia herbicola* (two strains) and *Rahnella aquatilis*.

Erwinia. Only *E. herbicola* was included, and all strains were identified correctly. Several other *Erwinia* species were not included in the matrix mainly because they are not known to occur in human clinical specimens and partly because further elaboration is required of the classification of the *Enterobacter agglomerans*/*Erwinia* complex.

Escherichia. Six species were included — *E. adecarboxylata*, *E. blattae*, *E. coli*, *E. fergusonii*, *E. hermannii* and *E. vulneris*. Except for *E. blattae*, from the hind-gut of cockroaches (Burgess *et al.*, 1973), all the other species occur in human clinical specimens, including the more recently described *E. hermannii* (Brenner *et al.*, 1982*a*) and *E. vulneris* (Brenner *et al.*, 1982*b*), both of which are predominantly associated with wounds, as well as *E. fergusonii* (Farmer *et al.*, 1985*b*) which is predominantly from faeces.

With the exception of *E. coli*, all strains of the other five species were identified successfully on the revised matrix. For *E. coli* only 10 of the 15 strains were correctly identified. For four of the five remaining strains *E. coli* had the highest identification score, but identification at the level

of 0.999 was prevented by the scores for *Klebsiella pneumoniae* subsp. *ozaenae* (two strains), *Shigella sonnei* and *Shigella* spp. (not *sonnei*). For the remaining strain, *Shigella* spp. (not *sonnei*) achieved the highest identification score, but *E. coli* had the second highest score. The distinction between *E. coli* and *Shigella* is one of convenience (Brenner *et al.*, 1973). Since the two taxa are not phenotypically distinct, intermediate strains are bound to occur and such strains will always prove difficult to identify to one or the other taxa. In the revised matrix, anaerogenic, non-motile, lactose-negative strains, formerly included in the '*Escherichia*: *Alkaescens-Dispar*' group are included in *E. coli*, which is taxonomically their correct place. However, since the latter strains show a particularly close biochemical similarity to *Shigella* strains, we added to the matrix certain additional tests (listed in Methods) to aid in the differentiation of *E. coli* from *Shigella* species. This strategy was not altogether successful, as the identification rate for *E. coli* on the revised matrix was only 66.7%.

Hafnia. The only species of this genus, *H. alvei*, was included and all but one strain were correctly identified. For the strain which did not identify, *H. alvei* had the highest identification score, but identification at the level of 0.999 was prevented by the score for *Escherichia blattae*.

Klebsiella. Six taxa were included — *K. oxytoca*, *K. pneumoniae* subsp. *aerogenes* (= '*K. aerogenes*'), *K. pneumoniae* subsp. *ozaenae* (= *K. ozaenae*), *K. pneumoniae* subsp. *pneumoniae* (= *K. pneumoniae*), *K. pneumoniae* subsp. *rhinoscleromatis* (= *K. rhinoscleromatis*) and *K. terrigena*. All taxa are recovered from human clinical material except for *K. terrigena*, which comes from soil and water (Izard *et al.*, 1981*a*). The reasons for the nomenclature adopted are described by Holmes & Gross (1983). The only other *Klebsiella* species appearing on the Approved Lists is *K. mobilis*. This is synonymous with *Enterobacter aerogenes* (they have the same type strain) and the species is included in the revised matrix under the latter name.

Although strains of both taxa were included in the same cluster by numerical taxonomy (Bascomb *et al.*, 1971) it was felt inappropriate to include '*K. aerogenes*' and *K. oxytoca* in the revised matrix as a single taxon, or even allow identification to a composite group between them, as these two taxa are so unrelated that they should, despite their close phenotypic similarity, probably be placed in separate genera (Jain *et al.*, 1974). *K. terrigena* was indistinguishable from '*K. aerogenes*' on the tests in the matrix, so growth at 5 °C and at 42 °C were added to the matrix to assist in differentiating these two taxa. Because of this problem of attempting to differentiate between phenotypically indistinct species, the unsatisfactory identification rate for klebsiellas on the revised matrix was not unexpected.

The identification rate was high for *K. pneumoniae* subsp. *ozaenae* (10/12 = 83.3%), *K. pneumoniae* subsp. *pneumoniae* (10/11 = 91%; one strain was identified as a composite group with *K. pneumoniae* subsp. *aerogenes*) and *K. pneumoniae* subsp. *rhinoscleromatis* (11/11 = 100%). For the two strains of *K. pneumoniae* subsp. *ozaenae* that were not identified, this taxon had the highest identification score, but identification at the level of 0.999 was prevented by the score for *K. pneumoniae* subsp. *aerogenes* in one strain and the score for *K. pneumoniae* subsp. *rhinoscleromatis* in the other. For the one strain of *K. pneumoniae* subsp. *pneumoniae* that was not identified, *K. pneumoniae* subsp. *aerogenes* had the highest identification score and *K. pneumoniae* subsp. *ozaenae* the second highest, so perhaps this strain was originally incorrectly designated as *K. pneumoniae* subsp. *pneumoniae*. For *K. terrigena*, 6/7 of the strains were identified correctly; for the remaining strain, *K. terrigena* had the highest identification score, but identification at the level of 0.999 was prevented, as expected, by the score for *K. pneumoniae* subsp. *aerogenes*. Also, as expected, the identification rate for *K. oxytoca* was poor; only 3/11 strains were identified correctly. For one of the eight remaining strains, *K. terrigena* had the highest identification score and *K. oxytoca* the second highest score. For the other seven strains, *K. oxytoca* had the highest identification score, but identification at the level of 0.999 was prevented by the score for *K. pneumoniae* subsp. *aerogenes* in five strains and by the score for *K. pneumoniae* subsp. *pneumoniae* in the other two. Similarly, for *K. pneumoniae* subsp. *aerogenes*, only 5/12 (41.7%) strains were identified correctly (one as a composite group with *K. pneumoniae* subsp. *pneumoniae*). For each of seven remaining strains, *K. pneumoniae* subsp. *aerogenes* obtained the highest identification score, but identification at the level of 0.999 was prevented

by the scores for *E. gergoviae* (one strain), *K. pneumoniae* subsp. *ozaenae* (one strain) and, as expected, *K. oxytoca* (five strains). If we had included '*K. aerogenes*' and *K. oxytoca* in the revised matrix as a single taxon and allowed that taxon to identify to a composite group with *K. pneumoniae* subsp. *pneumoniae*, then the identification rate for strains of '*K. aerogenes*' and of *K. oxytoca* would probably have been 20/23 (87%; including three strains identified as a composite group with *K. pneumoniae* subsp. *pneumoniae*).

Two *Klebsiella* species have been described since the revision of the matrix was completed, *K. planticola* from aquatic and botanical environments (Bagley *et al.*, 1981) and *K. trevisanii* from water and soil (Ferragut *et al.*, 1983). It seems likely that these two species may prove to be synonymous.

Kluyvera. The most common source for *Kluyvera* strains is sputum, where they are probably not clinically significant (Farmer *et al.*, 1981). The two species, *K. ascorbata* and *K. cryocrescens*, are not easily separated on routine conventional tests and they were therefore combined in a single taxon in the matrix as *Kluyvera* spp. All strains of this genus were identified correctly.

Morganella. The only species, *M. morganii*, was included and all strains were identified correctly.

Neisseria. Two species, *N. denitrificans* and '*N. pharyngis*', were included in the revised matrix because strains examined yielded a fermentative reaction in the O/F test. Strains of '*N. pharyngis*' also produced acid in peptone/water/sugar medium from glucose, maltose and sucrose. A third species, *N. mucosa*, also produces acid from these carbohydrates, but until the relationship between *N. mucosa* and '*N. pharyngis*' is clarified we have included only '*N. pharyngis*' in the revised matrix.

No strains of *N. denitrificans* identified correctly. For all three strains, *N. denitrificans* achieved the highest identification score, but identification at the level of 0.999 was prevented in each case by the scores for CDC Group EF-4 arginine-negative biovar. Since these two taxa are so unreactive in the tests in the matrix it is not surprising that they interfere in each other's identification. Five out of six strains of '*N. pharyngis*' were identified correctly. For the remaining strain, '*N. pharyngis*' had the highest identification score, but identification at the level of 0.999 was prevented by the score for *Actinobacillus lignieresii*.

Obesumbacterium. The only species, *O. proteus*, was included; the species is currently divided into two biogroups which are not closely related to each other, despite their phenotypic similarity (see Table 1). Strains of biogroup 1 are synonymous with *H. alvei* (Brenner, 1981) and may be regarded as strains of this species which have become adapted to the brewery environment. Strains of biogroup 2 should be placed in a separate genus from strains of biogroup 1. All strains of both biogroups were correctly identified.

Pasteurella. Ten taxa were included in the revised matrix, seven of which were named species. They comprised *P. aerogenes*, the *Pasteurella* bovine lymphangitis (BL) group of Jayaraman & Sethumadavan (1974), *P. gallinarum*, *P. haemolytica* (divided into two biovars A and T), *P. multocida* (divided into typical strains and atypical strains, the latter commonly recovered from human animal bite wounds and which ferment only glucose and sucrose), *P. pneumotropica*, *P. testudinis* (a parasite of desert tortoises; Snipes & Biberstein, 1982) and *P. ureae*. *P. gallicida* was not included in the revised matrix as it is an earlier synonym, but less well-known, of *P. multocida* (they have the same type strain). All strains of the *Pasteurella* BL group, *P. gallinarum* and *P. testudinis* were correctly identified. We must, however, record some reservations regarding *P. gallinarum*, as our two reference strains of this species (NCTC 11187 and 11188) were not originally obtained from a recognized culture collection. The strains differ in certain reactions from the original description given by Hall *et al.* (1955), perhaps due to differences in test methods or to some error in the designation of the strains.

For four of the remaining seven taxa, all but one strain of each were correctly identified, giving identification rates in the range 90 to 90.9%. For the one strain of *P. aerogenes* not correctly identified, this taxon achieved the highest identification score, but identification at the level of 0.999 was prevented by the score for '*N. pharyngis*'. One of the strains of *P. haemolytica* T

that correctly identified did so as a composite group with *P. haemolytica* A. For the one strain of *P. haemolytica* T not correctly identified, this taxon had the highest identification score, but identification at the level of 0.999 was prevented by the score for *P. ureae*, and for the one strain of *P. pneumotropica* not correctly identified, *P. gallinarum* prevented a score of 0.999. For the one strain of *P. ureae* not correctly identified, this taxon achieved the highest identification score but identification at the level of 0.999 was prevented by the score for '*Neisseria pharyngis*'.

Of the 10 strains of *P. haemolytica* A, six were correctly identified, none as a composite group with *P. haemolytica* T. For the four strains not correctly identified, *P. haemolytica* A achieved the highest identification score, but identification at the level of 0.999 was prevented by the scores for *P. ureae* in three strains and *Actinobacillus lignieresii* in the remaining strain.

Of the 18 strains of *P. multocida*, 13 were correctly identified, one as a composite group with *P. multocida* (atypical). For only one of the five remaining strains did *P. multocida* achieve the highest identification score, but identification at the level of 0.999 was prevented by the score for *P. pneumotropica* in this strain. For two further strains, *P. pneumotropica* achieved the highest identification score and *P. multocida* the second highest score. Further work would appear to be necessary on the differentiation of *P. multocida* and *P. pneumotropica*, especially as the distinction between these two species has become increasingly blurred with the receipt of so many atypical strains of these taxa. For a further strain, *P. multocida* (atypical) achieved the highest identification score and *P. multocida* the second highest score, but the sum of their scores was insufficient to permit identification to a composite group at the level of 0.999. For the final strain, *P. haemolytica* T had the highest identification score and *P. gallinarum* the second highest score, but this was an atypical lysine-positive strain of Frederiksen's *P. multocida* biotype 1 from a guinea pig.

Of the 10 strains of *P. multocida* (atypical), seven were correctly identified, five as a composite group with *P. multocida*. For each of the three remaining strains, *P. multocida* (atypical) achieved the highest identification score. For one of these three, *P. multocida* had the second highest score, but the sum of their scores was insufficient to allow identification as a composite group at a level of 0.999. For the other two strains identification at the level of 0.999 was prevented by the scores for *P. pneumotropica*.

Plesiomonas. The only species, *P. shigelloides*, was included and all strains were correctly identified.

Proteus. Four named species were included of which one was further subdivided into two biogroups. The five taxa comprised *P. mirabilis* and *P. myxofaciens* (a pathogen of gypsy moth larvae), whilst *P. vulgaris* was divided into three biogroups as proposed by Hickman *et al.* (1982*b*). These three biogroups are similar phenotypically but are distinct by DNA homology. The name *P. penneri* has been proposed for biogroup 1; no name has yet been proposed for biogroup 2 and as the type strain of *P. vulgaris* belongs to biogroup 3 then the name *P. vulgaris* will remain with that biogroup. Other *Proteus* species appearing on the Approved Lists are included in the matrix under other names. *P. inconstans* is a senior, though less well-known, synonym of *Providencia alcalifaciens* (they have the same type strain). *P. morganii* is included as *Morganella morganii* and *P. rettgeri* is included as *Providencia rettgeri*.

All strains of *P. mirabilis* were correctly identified. The only strain of *P. myxofaciens* was also correctly identified. Since *P. vulgaris* was divided into the three biogroups, it was expected that the identification rates would not prove entirely satisfactory. In particular it was expected that strains of biogroups 2 and 3 would not be easily differentiated, as the most useful conventional tests for separating them are aesculin hydrolysis and salicin fermentation and only the latter is included in the matrix. Several composite group linkages were allowed (Table 1) in an effort to reduce the problem. Following this strategy, all strains of *P. penneri* were correctly identified, two as a composite group with *P. vulgaris* biogroup 3. All strains of *P. vulgaris* biogroup 2 were also correctly identified, one as a composite group with *P. vulgaris* biogroup 3. Four out of six strains of *P. vulgaris* biogroup 3 were correctly identified, two as a composite group with *P. penneri*. For both the remaining strains, *P. vulgaris* biogroup 3 achieved the highest identification score. For one, *P. penneri* had the second highest score, whilst for the other *P.*

vulgaris biogroup 2 had the highest score, but in neither case was the sum of the scores sufficient to permit identification to a composite group at the level of 0.999.

Providencia. Three taxa were included — *P. alcalifaciens*, *P. rettgeri* and *P. stuartii*. Recently, *P. alcalifaciens* has been divided into two species (Hickman-Brenner *et al.*, 1983). Strains fermenting adonitol but not D-galactose are retained in *P. alcalifaciens*, whilst strains giving the converse results in these two tests are now placed in *P. rustigianii*. These findings became known to us too late for the two taxa to be separated in the matrix, and so there is a single taxon in the revised matrix based on a mixture of *P. alcalifaciens*/*P. rustigianii* strains. In any case, to have included the two species separately in the revised matrix would have presented problems, since only adonitol fermentation was included in the matrix. All strains of all three *Providencia* taxa included in the revised matrix were correctly identified.

Pseudomonas. Strains belonging to this genus are non-fermenters but *P. pseudomallei* is included in the matrix because strains of this species produce acid from glucose and some other carbohydrates in peptone/water/sugar media. Since *P. pseudomallei* shares this characteristic with fermentative organisms, it seemed prudent to include this clinically important species in the fermenter matrix. The species can be easily excluded from the fermenters by its oxidative reaction in the O/F test, but not all laboratories carry out this test routinely. All 10 strains of the species were correctly identified.

Rahnella. The only species, *R. aquatilis* (Izard *et al.*, 1979), was included and all 10 strains were correctly identified. This species has been isolated from water and non-polluted soil.

Salmonella. Eleven taxa were included — *S. choleraesuis*, '*S. ferlac*', '*S. gallinarum*', '*S. paratyphi* A', '*S. pullorum*', *Salmonella* subgenera I, II, III (= *S. arizonae*) and IV, *S. typhi* and '*S. typhisuis*'. The names *S. enteritidis* and *S. typhimurium* also appear on the Approved Lists. All the above named *Salmonella* 'species' belong in subgenus I, but they are entered separately in the matrix if they have particular biochemical characteristics which distinguish them from each other and from other serovars retained in subgenus I.

All strains of '*S. gallinarum*', '*S. paratyphi* A', '*S. pullorum*', *Salmonella* subgenus III, *S. typhi* and '*S. typhisuis*' were correctly identified. For four strains of *Salmonella* subgenus III identification was to a composite group with *Salmonella* subgenus II. For *S. choleraesuis* and *Salmonella* subgenus IV all but one strain of each were correctly identified, giving identification rates of 90% for both taxa. For the strain of *S. choleraesuis* not correctly identified, this taxon had the highest identification score, but identification at the level of 0.999 was prevented by the score for *Salmonella* subgenus I. Similarly, for the strain of *Salmonella* subgenus IV not correctly identified, this taxon had the highest identification score, but identification at the level of 0.999 was prevented by the score for *Salmonella* subgenus I.

Two of the four strains of '*S. ferlac*' were correctly identified, one as a composite group with *Salmonella* subgenus II. For the two remaining strains, '*S. ferlac*' achieved the highest identification score, but in each case identification at the level of 0.999 was prevented by the score for *Salmonella* subgenus I.

Of the 14 strains of *Salmonella* subgenus I, 11 were correctly identified, six as a composite group with *Salmonella* subgenus II. For two of the three remaining strains, *Salmonella* subgenus I had the highest identification score and *Salmonella* subgenus II the second highest, but for both cases the sum of their scores was insufficient to permit identification to a composite group. For the remaining strain, *Salmonella* subgenus I again achieved the highest identification score, but identification at the level of 0.999 was prevented by the score for *S. choleraesuis*.

Seven of the nine strains of *Salmonella* subgenus II were correctly identified, three as a composite group with *Salmonella* subgenus III. For both the remaining strains, *Salmonella* subgenus II achieved the highest identification score. For one, *Salmonella* subgenus I had the second highest score, but the sum of the scores was insufficient to permit identification to a composite group. For the second strain, identification at the level of 0.999 was prevented by the score for *Salmonella* subgenus IV.

Serratia. Eight taxa were included, comprising *S. ficaria*, *S. fonticola*, *S. liquefaciens*, *S. marcescens*, *S. marinorubra*, *S. odorifera* (divided into its two separate biovars) and *S. plymuthica*. *S. rubidaea* is a synonym of *S. marinorubra* (they have the same type strain). *S. proteamaculans* and the more recently described *S. grimesii* (Grimont *et al.*, 1982) are indistinguishable from *S. liquefaciens* in routine biochemical tests, but since both species will be encountered so rarely in human clinical specimens no attempt has been made to include them in the revised matrix. *S. ficaria* is associated with figs and fig wasps (Grimont *et al.*, 1979); *S. fonticola* comes from water (Gavini *et al.*, 1979), as does *S. plymuthica*. *S. marinorubra* is also associated with the aquatic environment but occasionally strains are found in human clinical material. *S. odorifera* was described from human clinical isolates but the organism is apparently rarely encountered in a clinical setting. The *Serratia* species of major clinical interest are thus *S. liquefaciens* and *S. marcescens*.

All strains of *S. fonticola*, *S. liquefaciens*, *S. marcescens* and *S. odorifera* biovars I and II were correctly identified. Eight of the ten strains of *S. ficaria* were correctly identified. For each of the two remaining strains, *S. ficaria* achieved the highest identification score, but identification at the level of 0.999 was prevented by the score for *S. plymuthica*. Nine of the ten strains of *S. marinorubra* were correctly identified. For the remaining strain, *S. marinorubra* achieved the highest identification score, but identification at the level of 0.999 was prevented by the score for *S. plymuthica*. Of the strains of *S. plymuthica*, only seven were correctly identified. For each of the three strains not identified, *S. plymuthica* achieved the highest identification score, but identification at the level of 0.999 was prevented by the scores for *E. herbicola* in one strain, *S. ficaria* in another and *S. liquefaciens* in the remaining strain.

Shigella. Two taxa were included — *S. sonnei* and *Shigella* spp. (not *sonnei*) (the latter containing *S. boydii*, *S. dysenteriae* and *S. flexneri*).

Of the 10 strains of *S. sonnei*, seven were correctly identified. For each of the three remaining strains, *S. sonnei* achieved the highest identification score, but identification at the level of 0.999 was prevented by the score for *Shigella* spp. (not *sonnei*) in two strains and by the score for *S. pullorum* in the remaining strain. For the one strain of *Shigella* spp. (not *sonnei*) that was not identified, this taxon achieved the highest identification score, but identification at the level of 0.999 was prevented by the score for *Pasteurella* BL group.

Tatumella. The only species, *T. ptyseos* (Hollis *et al.*, 1981), was included. Most isolates were recovered from human sputum specimens. All strains were correctly identified.

Vibrio. Nine species were included — *V. alginolyticus*, *V. anguillarum*, *V. cholerae* (including the so-called non-cholera vibrios belonging to serovars of *V. cholerae* other than O1), *V. fluvialis* (formerly *V. fluvialis* biovar I), *V. furnissii* (formerly *V. fluvialis* biovar II), *V. metschnikovii*, *V. mimicus*, *V. parahaemolyticus* and *V. vulnificus*. Other *Vibrio* species appearing on the Approved Lists were not included in the revised matrix because they are not known to occur in human clinical specimens. Several other *Vibrio* species have also been described comparatively recently, including some from human clinical specimens, such as *V. damsela* (Love *et al.*, 1981) and *V. hollisae* (Hickman *et al.*, 1982a), but they were not included in the matrix as reference cultures were unavailable for study. All the *Vibrio* species included in the matrix occur in human clinical material except *V. anguillarum*.

All strains of *V. alginolyticus*, *V. anguillarum*, *V. fluvialis*, *V. mimicus*, *V. parahaemolyticus* and *V. vulnificus* were correctly identified. In *V. fluvialis*, three of the strains were identified to a composite group with *V. furnissii*. In *V. cholerae* and *V. metschnikovii*, only one strain of each taxon was not identified, giving respective identification rates 90.9% and 88.9%. For the one strain of *V. cholerae* not identified, this taxon achieved the highest identification score, but identification at the level of 0.999 was prevented by the score for *V. furnissii*. For the one strain of *V. metschnikovii* not correctly identified, this taxon achieved the highest identification score, but identification at the level of 0.999 was prevented by the score for *V. alginolyticus*. Eight of the 11 strains of *V. furnissii* were correctly identified. Of the three remaining strains, one misidentified as *V. cholerae*; this was the only known misidentification observed in our evaluation of the

revised matrix. For a further strain, *V. cholerae* achieved the highest identification score and *V. furnissii* the second highest score. *V. fluvialis* and *V. furnissii* are not very distinct from each other, nor are *V. furnissii* and *V. cholerae*. The only consistent character separating *V. fluvialis* and *V. furnissii* is that strains of the former are said to be anaerogenic and strains of the latter aerogenic (Brenner *et al.*, 1983). However, all reference strains of both *V. fluvialis* and *V. furnissii* examined by us proved to be anaerogenic! For the remaining strain, *V. furnissii* achieved the highest identification score, but identification at the level of 0.999 was prevented by the score for *A. hydrophila*.

Xenorhabdus. The two species were included — *X. luminescens* and *X. nematophilus*. These organisms are of no clinical interest at present as they have so far been isolated only from certain nematodes and from the insect larvae they parasitize. All strains of both species were correctly identified.

Yersinia. Seven taxa were included — *Y. enterocolitica*, *Y. frederiksenii*, *Y. intermedia*, *Y. kristensenii*, *Y. pestis* (= *Y. pseudotuberculosis* subsp. *pestis*), *Y. pseudotuberculosis* (= *Y. pseudotuberculosis* subsp. *pseudotuberculosis*), and *Y. ruckeri*. *Y. philomiragia* also appears on the Approved Lists but it does not occur in human clinical specimens. Moreover, it is not a yersinia or a member of the *Enterobacteriaceae* (Ursing *et al.*, 1980*b*), and it is a fastidious organism. It is therefore not included in the matrix. All the *Yersinia* species in the matrix occur in human clinical specimens, except for *Y. ruckeri*, which causes the so-called red-mouth disease of salmonid fishes.

All strains of *Y. intermedia*, *Y. kristensenii* and *Y. ruckeri* were correctly identified. Three of the *Y. intermedia* strains identified to a composite group with *Y. frederiksenii* and one of the *Y. kristensenii* strains identified to a composite group with *Y. enterocolitica*. For each of *Y. pestis* and *Y. pseudotuberculosis*, only one strain could not be identified correctly. For the one strain of *Y. pestis* not identified, this taxon achieved the highest identification score, but identification at the level of 0.999 was prevented by the score for *Shigella* spp. (not *sonnei*). For the one strain of *Y. pseudotuberculosis* not identified, this taxon achieved the highest identification score and *Y. pestis* the second highest score, but the sum of their scores was insufficient for identification to a composite group. Of the 13 strains of *Y. enterocolitica*, 11 were correctly identified. For both the strains not identified, *Y. enterocolitica* achieved the highest identification score, but identification at the level of 0.999 was prevented by the scores for *Y. frederiksenii*. Of the 10 strains of *Y. frederiksenii*, eight were correctly identified, one as a composite group with *Y. intermedia*. For both the remaining strains, *Y. frederiksenii* achieved the highest identification score. For one of these two strains, *Y. intermedia* achieved the second highest score, but the sum of their scores was insufficient to allow identification to a composite group. For the other strain, identification at the level of 0.999 was prevented by the score for *Y. enterocolitica*.

Certain strains previously regarded as atypical *Y. enterocolitica* or *Y. enterocolitica*-like have been shown by DNA-DNA reassociation to constitute separate species (Brenner *et al.*, 1980*a*; Ursing *et al.*, 1980*a*; Bercovier *et al.*, 1980*b*). *Y. intermedia* differs from *Y. enterocolitica* and the other species previously included in *Y. enterocolitica* in fermenting D-melibiose, α -methyl-D-glucoside and D-raffinose (Bercovier *et al.*, 1980*a*). However, only the latter test is included in the matrix and a composite group had to be allowed between *Y. intermedia* and *Y. frederiksenii*. *Y. kristensenii* differs from *Y. enterocolitica* and the other species previously included in *Y. enterocolitica* in giving negative results in the Voges-Proskauer test and in sucrose fermentation. Since both these tests are in the matrix, few problems were encountered in the successful identification of strains of this species, although a composite group had to be allowed between *Y. kristensenii* and *Y. enterocolitica*. *Y. frederiksenii*, however, differs from *Y. enterocolitica* only in being able to ferment L-rhamnose, and from *Y. intermedia* (of the tests in the matrix) only in failure to ferment D-raffinose. Not surprisingly, these taxa therefore interfere in their identification, and as the program would not permit a composite group identification between *Y. enterocolitica* and *Y. frederiksenii* it is not surprising that the identification rate was lowest for these two *Yersinia* species.

Statistical evaluation

If the percentage overlap between two taxa in the matrix is large, an unknown belonging to one or other taxon will not score highly with either. An overlap of less than 1.0% is desirable (Sneath, 1980*b*). All pairs of taxa overlapped by less than this value, except for *Klebsiella oxytoca* and *K. pneumoniae* subsp. *aerogenes* (overlap 1.4%).

If the matrix is satisfactory, the most typical strain of a taxon should give a high identification score close to 1.000 against its own taxon (Sneath, 1980*a*). A much lower score should be given by the second best identity. Of the 110 taxa, six did not achieve a score of ≥ 0.999 for the HMO; these were *Enterobacter amnigenus* (0.998), *Klebsiella oxytoca* (0.997), *Klebsiella pneumoniae* subsp. *aerogenes* (0.992), *Neisseria denitrificans* (0.998), *Proteus penneri* (0.998) and *Salmonella* subgenus I (0.980).

GENERAL DISCUSSION

The probability matrix of Bascomb *et al.* (1973) yielded an identification rate of 90.3% for fermentative bacteria. In the revised matrix the number of taxa has been doubled and the number of tests only slightly increased; yet we obtained an overall 89.2% identification rate. Many of the 'new' taxa were previously included as atypical variants of existing species. For some of these 'new' species there are few conventional differences between them and the species in which they were originally placed; also, not all the useful distinguishing tests were included in the matrix. A decline in the identification rate on the revised matrix was expected, but as this was only 1% the revision was considered highly successful. In fact the identification rate on the revised matrix could probably have exceeded that on the original had we not chosen to reflect current taxonomic thinking and if we had continued the combination of phenotypically similar (but not closely related) taxa (such as *Klebsiella oxytoca* and *Klebsiella pneumoniae* subsp. *aerogenes*). Newer biochemical tests, and newer methods of performing established tests, have been described since the original matrix of Bascomb *et al.* (1973) was published. Since, however, we automatically accumulate the test results for every strain we test, we retain the original methods as closely as we can so that the results for each strain remain comparable, even when the strains are received over a period of several years. These traditional methods have stood the test of time, but further tests could well be included in the matrix to aid differentiation of the 'new' taxa (see above).

Farmer *et al.* (1985*a*) presented the reactions of 98 different taxa of the *Enterobacteriaceae* in 47 tests. The reactions were given in percentage form and so although not intended as a probability matrix, the information is readily available for the construction of one. However, problems would arise as the lack of 0.99/0.01 differences would prevent the separation of several pairs of taxa. Thus for a working probability matrix the number of identifiable taxa would have to be decreased, or the number of tests increased. Although the data base they present is smaller than the matrix described here, it is purely for the *Enterobacteriaceae*, and so for this family it covers a larger number of taxa than does our matrix. A probability matrix for the identification of vibrios and related taxa has been published by Dawson & Sneath (1985). Half the tests in their matrix comprised the API 20E system and the other half conventional tests. Although their identification rate figures are not directly comparable with ours, it was of interest to note that they also could not differentiate *V. fluvialis* and *V. furnissii* unequivocally on the basis of production of gas from glucose.

For Bascomb *et al.* (1973), the factors which determined the success of a probabilistic identification scheme were uncertain. They found no correlation between the successful identification of strains of a taxon and the number of biopatterns which strains of that taxon gave. They did, however, suggest that a successful identification rate could not be assumed (although may be obtained) where there were fewer than two 0.99/0.01 test differences between a particular taxon and the taxon most similar to it in the matrix. There appeared to be no way of determining in advance whether a probabilistic matrix would operate satisfactorily in practice and an empirical trial had to be performed. We have carried out such a trial of our matrix but we have also employed two programs for the statistical analysis of probability matrices. These

showed that almost all taxa in the matrix were suitably discrete and achieved a high identification score for their HMO. All showed the desired level of homogeneity and separation. The results of the statistical evaluation thus corresponded closely to the results of the empirical trial.

We have retained an identification threshold level of 0.999 in the revised matrix to reduce, as much as possible, the risk of misidentification. However, logically, as one increases the number of taxa (as we have done considerably with the revised matrix) the fewer strains one should encounter which do not correspond to one of the taxa in the data base, thereby reducing the overall risk of possible misidentification. Reducing the identification threshold level to 0.990 would have meant that in only one taxon (*Salmonella* subgenus I) would the most typical strain of the taxon have failed to reach identification level. Nevertheless, we have resisted the temptation to lower the identification level until such time as we have included in the matrix additional tests for the adequate differentiation of the 'new' taxa (see above).

In some cases a combined score for two taxa is needed to reach identification, e.g. *Klebsiella pneumoniae* subsp. *aerogenes* and *pneumoniae*, which are clearly closely related even if separable. The inclusion of tests to separate such groups, e.g. serological tests, might overload the matrix with tests inapplicable to most taxa. Moreover, for practical purposes, identification to a combined taxon may be sufficient.

It is also obvious from Table 2 that with the exception principally of *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumoniae* subsp. *aerogenes* and several of the *Pasteurellaceae*, a very high rate of identification was achieved. Reasons for the poor identification of some taxa are described under the results for separate taxa. Many of the strains of the other taxa which did not identify on our matrix were ones which varied from the expected test results in their taxon; such differences tend to be deliberately ignored by the microbiologist when making a decision on the identification of these strains. It would be useful if the logic of such decisions could be incorporated into the identification program, and at the same time identification would be enhanced if more precise definitions for taxa could be made, removing the weighting of characters so often associated with conventional identification schemes.

The matrix described here should prove of value in the examination of strains in the reference laboratory. With the increasing use of micro-computers, some readers may wish to adapt the matrix for routine laboratory use. This may well involve a reduction in both number of taxa and number of tests. To aid interested readers, we show in Table 1 those taxa we consider should be retained in any reduced matrix derived from our study. These taxa are those of known, or possible, clinical significance. By using our test selection programs, we have determined from the range of tests in the matrix the set of tests with the highest differential value for these taxa. These tests, listed in descending order of differential value, are given as a footnote to Table 1. This set of 33 tests separates most pairs of taxa in the matrix by at least two tests; 22 pairs of taxa are separated by only one test and three by none. The three pairs of taxa with no tests to separate them are *Actinobacillus lignieresii* and *Pasteurella pneumotropica*, *Pasteurella multocida* (atypical) and *Pasteurella pneumotropica*, and *V. cholerae* and *V. furnissii*; all these pairs require further taxonomic study. The total number of results obtainable from these 33 tests is 38 and the additional characters are given in parentheses in the footnote to Table 1. We have not constructed a reduced matrix ourselves, so we cannot vouch for its effectiveness. It is likely that fewer strains would exceed the threshold identification level of 0.999 on the reduced matrix than on the full matrix. This can be overcome by lowering the threshold level as has been done by the manufacturers of commercial identification systems who face similar problems (see, for example, API, 1983). Lowering the identification level, however, will increase the risk of misidentification (Lapage *et al.*, 1973).

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