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A Computerized Scheme for the Identification of Aeromonads and Members of the Family Enterobacteriaceae

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(Résumé en français)

INLAND WATERS DIRECTORATE,
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Contents

Tables

Illustrations

Abstract

A system is described which combines practical and theoretical concepts into a workable system for the routine and simultaneous identification of large numbers of *Aeromonas* isolates and members of the Family Enterobacteriaceae. The identification method is based on computerized determination of conditional probabilities of two-state characters, using estimations of likelihood rather than Bayesian probability. The multipoint inoculation procedures used, coupled with small quantities of media in non-disposable racks and vials, reduce the costs considerably below those of commercially available multitest single inoculation systems. The reliability of the system, determined for a range of hospital isolates, approaches 90%, and with isolates from water the accuracy approaches 100%.

Chapter 1 describes theoretical and general approaches. Chapter 2 deals with operation of the particular system.

Résumé

L'étude actuelle décrit un système, synthèse de la théorie et de la pratique, qui permet l'identification courante et simultanée d'un grand nombre d'isolats d'*Aeromonas* et des représentants de la famille des entérobactériacés. La méthode d'identification se fonde sur la détermination par ordinateur des probabilités conditionnelles de caractères dichotomiques, à l'aide de données estimatives de vraisemblance plutôt qu'à la probabilité bayésienne. L'utilisation des techniques d'ensemencement dispersé et de petites quantités de milieux de culture dans des tubes à essais et des portoirs réutilisables réduit les coûts bien en-dessous de ceux des dispositifs commerciaux d'ensemencement à usage unique applicables à plusieurs épreuves. Le système est sûr, dans le cas d'une série d'isolats provenant d'hôpitaux, à près de 90%, et l'exactitude atteint presque 100% lorsqu'il s'agit d'échantillons d'eau.

Le chapitre 1 traite de la théorie et des généralités, tandis que le chapitre 2 traite de l'application du système.

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CHAPTER 1

A Computerized Scheme for the Identification of Aeromonads and Members of the Family Enterobacteriaceae

INTRODUCTION

The simultaneous development of computer techniques and the application of a variety of mathematical disciplines has made fairly routine numerical taxonomic techniques possible (Sneath, 1957a, 1957b; Sokal and Sneath, 1963; Sneath and Sokal, 1973). More attention has been given, however, to problems of classification rather than to those of identification. Identification, following logically after classification, presupposes that individuals similar to those to be identified have been characterized and thoroughly described. This supposition is more true in some aspects of bacteriology (for example, in medical bacteriology with a family such as the Enterobacteriaceae) than in others (for example, in environmental bacteriology). In the latter, taxonomy is still at the stage of classification where organisms are being characterized and described. Where sufficient information is available concerning the characteristics of specific types of organisms, however, mathematical identifications of certain unknown organisms are a real possibility. While any calculations could be carried out manually, the greater processing speed and reliability of a computer are a definite asset.

In certain aspects of aquatic bacteriology, the routine identification of relatively large numbers of some members of the family Enterobacteriaceae has become increasingly necessary. The development of a reliable and time-saving system, which can accommodate a large number of individuals, is considered here. The system presented is neither unique nor does it take into account certain combinations of events which could render some identifications dubious. Rather, it was designed to combine practical and theoretical concepts into a workable system which fulfilled a particular need and which could serve as the starting point for a more refined system if required.

GENERAL CONSIDERATIONS

The objectives of any identification scheme are ease and certainty of identification (Davis and Heywood, 1963). In the present study, identification is regarded as an entity entirely separate from classification (that is, the delimitation of classes, clusters or taxa) and not as synonymous with it as often occurs in statistical usage (Sneath and Sokal, 1973).

For the numerical identification of bacteria, several possible lines of approach have been suggested (Beers and Lockhart, 1962). Numerical classifications (Sneath and Sokal, 1973) for each new isolate can be carried out, and unidentified strains compared with previously constructed taxonomic groups using numerical clustering methods (Quadling and Colwell, 1964; Gyllenberg, 1965). Such an approach is, however, time-consuming and expensive in terms of media, but will certainly be of use in determining the value of individual test criteria. Another approach is to construct mathematically monothetic or polythetic sequential keys and to use these to replace conventional keys. Rypka *et al.* (1967) and Rypka and Babb (1970) have described the mathematical models used initially to determine the most desirable tests for use in these keys. Strains are then identified by comparison of their test results, obtained by conventional methods, with those expected for each taxon. This results in a reduction in the number of tests required for identification but does not decrease the chances, inherent in intuitive identification, of misidentification of strains aberrant in one or several characters. An alternative method is to weight certain characters for identification (discriminant analyses; Sneath and Sokal, 1973). Sokal (1965) listed examples of the use of some such analyses in taxonomy, and new discriminant methods have been suggested by Hall (1968) and Saila and Flowers (1969). The value of such analyses lies primarily where identification is required for organisms belonging to relatively few overlapping taxa.

Consideration of the theoretical problems encountered in the identification of bacteria indicates that they are similar to the problems encountered in medical diagnoses (Ledley and Lusted, 1959). Here, records of previous cases are used to estimate the probability of a particular disease being present by means of a specific set of symptoms or test results. The disease with the highest probability is diagnosed and the value of the probability indicates the reliability of the diagnosis. A review of such methods has been given by Boyle *et al.* (1966). For bacterial identification, the unknown is compared in turn

with all the taxa being considered with the aim of obtaining an unambiguous identification with one of them in a single step. In such an approach there is a decreased likelihood of gross error if a single test is read incorrectly. In such simultaneous keys, any resemblance measure can be used to assess the best match. Cowan and Steel (1965) and Corlett *et al.* (1965) chose the number of agreements for two-state characters. Gyllenberg (1965) and Gyllenberg and Rauramaa (1966) considered each taxon to occupy a definite volume in A-space, an unknown being identified with the taxon closest to it. Considerable success has been achieved in bacterial identification with a method based on conditional probabilities of two-state characters (Beers and Lockhart, 1962; Dybowski *et al.*, 1963; Dybowski and Franklin, 1968; Lapage *et al.*, 1970; Lapage *et al.*, 1973). Willcox *et al.* (1973), Bascomb *et al.* (1973) and Friedman *et al.* (1973) have described identification methods based on Bayes' theorem (Uspensky, 1937), or derivations from it, as applied to both aerobic Gram-negative rods and to members of the family Enterobacteriaceae. Such an approach, however, requires the use of prior probabilities in diagnosis and identification; the use and estimation of these appears controversial (Lipkin, 1964; Boyle *et al.*, 1966). The method used in the present study is based on estimations of likelihood rather than Bayesian probability, and is similar to the method used by Dybowski and Franklin (1968) as applied to members of the family Enterobacteriaceae.

MATHEMATICAL THEORY (DYBOWSKI AND FRANKLIN, 1968)

From a total of N strains of a certain bacterial type, the conditional probability of obtaining a particular result, r_1, in a particular test, t_1, by N_1 of the bacteria tested is $\frac{N_1}{N}$.

Similarly, using the same N organisms for a second test, t_2, in which another specified result, r_2, is obtained, the likelihood of this result is $\frac{N_2}{N}$. The likelihood that a further strain of the organism will give the joint pattern r_1, r_2 as results for tests t_1, t_2 will be $\frac{N_1}{N} \times \frac{N_2}{N}$.

Such a relationship will hold true for further tests. If tests $t_1, t_2 \ldots t_m$ were performed on N established strains of a given bacterium, and N_1 gave result r_1 for test t_1, N_2 gave result r_2 for test t_2, etc., the likelihood of obtaining the pattern $r_1, r_2 \ldots r_m$, if the set of tests $t_1, t_2 \ldots t_m$ is performed on a further known strain of the organism, will be $\frac{N_1}{N} \times \frac{N_2}{N} \ldots \frac{N_m}{N}$.

Thus, given a known strain of the organism, the likelihood of obtaining a specific test pattern can be calculated. For identification purposes, individual likelihoods $\frac{N_1}{N}, \frac{N_2}{N} \ldots \frac{N_m}{N}$ will be stored in a matrix for each of the t characters and for all taxa being considered.

The problems in identification are, however, not to compute the likelihoods for known strains but to attempt to obtain some estimate of likelihoods for unknown strains and to compare them with the results previously obtained for known strains. Fisher's theory applied in the present case (Kendall and Stuart, 1963) indicates that it is necessary to compute the likelihoods for the observed pattern for each of the possibilities and then choose that bacterium which gives the greatest likelihood. The unknown is compared with each taxon in turn, the individual probabilities being multiplied together for as many characters as are available to give the joint likelihood. Such considerations will hold true as long as all tests are statistically independent (that is, the outcome of any test is unaffected by the outcome of any other).

If a small number of tests are used, problems can arise when assuming that joint probabilities or likelihoods are comparable even when they apply to different populations. Dybowski and Franklin (1968) attempted to overcome this problem by using a modal likelihood fraction for each suspect. This method endeavoured to obtain an estimate of the likelihood of obtaining a given set of results as compared to the maximum likelihood possible for any one taxon. However, the use of a relatively large number of tests, selected for their differentiating value for all the known organisms considered, seems to make such estimates unnecessary.

The use of a relatively large number of tests for the calculation of joint likelihoods does result in greatly decreasing values as the number of tests is increased. To avoid working with extremely small joint likelihoods, these are most conveniently scaled so that the maximum for any particular set of suspects has a value of 1, this being regarded as the relative likelihood.

METHODOLOGY

The organisms for which it was considered necessary to produce an identification scheme are listed in Table 1. In certain cases, test results obtained at two temperatures (22°C or 37.5°C) can be used for identification purposes; they are also indicated in the table. These organisms, with the exception of *Aeromonas*, belong to the family

Enterobacteriaceae. The *Aeromonas* species (including the C27 organisms; Ewing *et al.*, 1961) were included here because of their repeated occurrence in certain lake water samples and their appearance on some media used to select for coliforms.

Two methods are available for the selection of testing criteria for use in identifying these organisms. A minimum number of tests can be used which will allow discrimination between many types to be identified, but which will need additional confirmatory tests in certain cases. Lapage *et al.* (1970) and Friedman *et al.* (1973) describe procedures that give an identification score or scores and that also suggest additional discriminatory tests. Alternatively, a larger number of tests can be used, allowing good identification of all unknowns likely to be encountered without the need for additional tests. In the present study, where comparatively large numbers of isolates were to be identified at any one time, it was considered more practical to take the second approach.

In order to generate the matrix of probabilities used in the identification system, knowledge is required of the reactions of many representatives of the known organisms in all the tests being considered. Where adequate information is available in individual laboratories, it can serve to generate such a matrix. An alternative is to draw upon the large amount of information contained in the literature. The use of such data is valid if the testing conditions remain comparable. For the members of the family Enterobacteriaceae considered here, the data compiled by Edwards and Ewing (1972) were used, and for the aeromonads, the data of Ewing *et al.* (1961).

Examination of this data indicates that almost all Enterobacteriaceae and *Aeromonas* species considered here can be identified by the tests listed in Table 2. Friedman *et al.* (1973) found that a similar list accounted

Table 1. Taxa included in the identification scheme.

1. *Escherichia coli*	18. *Enterobacter aerogenes*
2. *Alkalescens-dispar*	19. *Enterobacter hafniae* 37°C
3. *Shigella dysenteriae*	20. *Enterobacter hafniae* 22°C
4. *Shigella flexneri*	21. *Enterobacter liquefaciens* 37°C
5. *Shigella boydii*	22. *Enterobacter liquefaciens* 22°C
6. *Shigella sonnei*	23. *Pectobacterium* 37°C
7. *Edwardsiella tarda*	24. *Pectobacterium* 22°C
8. *Salmonella cholerae-suis*	25. *Serratia* spp.
9. *Salmonella typhi*	26. *Providencia alcalifaciens*
10. *Salmonella enteritidis*	27. *Providencia stuartii*
11. *Salmonella* spp.	28. *Proteus vulgaris*
12. *Arizona hinshawi*	29. *Proteus mirabilis*
13. *Citrobacter freundii*	30. *Proteus morganii*
14. *Klebsiella pneumoniae*	31. *Proteus rettgeri*
15. *Klebsiella ozaenae*	32. *Aeromonas* spp.
16. *Klebsiella rhinoscleramatis*	33. *Aeromonas salmonicida*
17. *Enterobacter cloacae*	34. C27

Table 2. Tests used in the identification scheme.

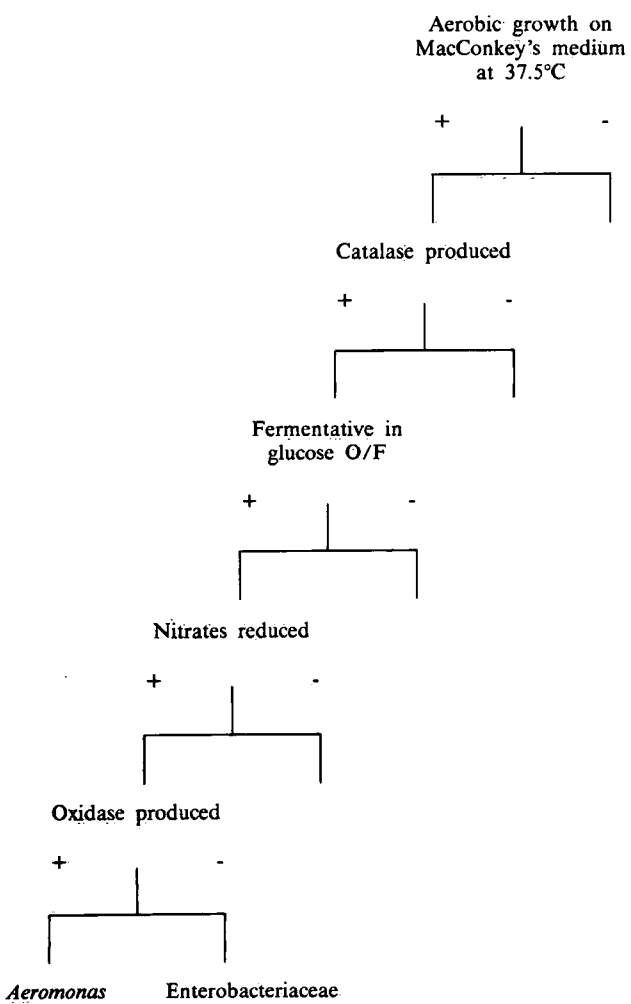
1. Indol production	17. Acid production from sucrose
2. Methyl red test	18. Acid production from mannitol
3. Voges-Proskauer test	19. Acid production from dulcitol
4. Citrate utilisation	20. Acid production from salicin
5. Hydrogen sulphide production	21. Acid production from adonitol
6. Urease production	22. Acid production from inositol
7. Potassium cyanide tolerance	23. Acid production from sorbitol
8. Motility	24. Acid production from arabinose
9. Gelatin hydrolysis	25. Acid production from raffinose
10. Lysine decarboxylase production	26. Acid production from rhamnose
11. Arginine decarboxylase production	27. Oxidase production
12. Ornithine decarboxylase production	28. Growth in MacConkey's medium at 37.5°C
13. Phenylalanine deamination	29. Fermentative in glucose O/F
14. Malonate utilisation	30. Catalase production
15. Gas production from glucose	31. Nitrate reduction
16. Acid production from lactose	

for 99.4% of all procedures used in their laboratory for identification of a similar list of organisms. The list given in Table 2 also includes certain tests (28-31) that are used, not to discriminate between members of the family Enterobacteriaceae or the aeromonads but to confirm that organisms to be identified do not fall outside of these taxa and so render the identification scheme ineffectual. These latter tests are not used, however, in the final calculations of joint probability values.

For the above system to function adequately, only organisms thought likely to be similar to those for which probabilities are already available can be considered. Some initial screening of organisms is, therefore, necessary to ensure that these conditions are met. The tests used for initial identification of Enterobacteriaceae and *Aeromonas* are given in Table 3.

In the present system, this screening can be conducted in two ways. (1) Organisms to be identified are put through the screening tests and only those fulfilling all the above requirements are then included in the identification scheme. This approach is chosen when there is no 'a priori' evidence to indicate that a large percentage of the individuals are either *Aeromonas* or Enterobacteriaceae. (2) Where there is evidence to indicate that the organisms concerned are highly likely to be members of the Enterobacteriaceae or *Aeromonas* [if, for example, they have been previously screened and need a confirmatory diagnosis, or if they have been isolated on certain selective media, such as m-FC agar (Difco) or m-Endo agar LES (Difco), and require further identification], organisms are simultaneously put through both the screening tests and the identification tests. Allowance is made for both approaches in the final analysis (see below). By using the two series of tests in this way, routine identification of a large number of organisms presumed to be Enterobacteriaceae or *Aeromonas* is possible without the need for prescreening. Such screening tests (27-31) are also included in Table 2.

Table 3. Screening procedures used for initial identification.



For the above species and the test reactions 1-27, the literature was searched to determine the relative number of times each test result was found to be positive. The 34 species and 27 tests were established as the boundaries of the data base. In this way it was possible to obtain probabilities for 904 of the 918 (27 tests x 34 taxa) entries in the data base. In the 14 cases where no data were available, or the available data were inadequate or incomplete, the entry was assigned a value of 9.999 and ignored in all subsequent calculations. The limits of individual probability values, theoretically set at 1 (positive in all cases) and 0 (negative in all cases), were changed to 0.999 and 0.001 in the present study. The reasons for this were that the inclusion of a test result with a zero probability in a test pattern would automatically eliminate the species in question, even if a perfect match was obtained in all other test results. It is probably undesirable to eliminate a possible identification solely on the basis of a single test result because (i) if data were gathered from a larger sample of bacteria of a particular species, one result might have been positive (or vice versa), and (ii) the test on the isolate being identified might have been misread.

All tests were assumed to have only two states (P_{nij}; j = positive finding or j = negative finding). The probability of a negative finding for test 'i' for species 'n' was calculated from:

$$P_{nij} = 1 - P_{nij}$$

j = positive j = negative

Table 4. Probability array.

PROBABILITY ARRAY

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	0.986	0.997	0.300	0.415	0.400	0.000	0.991	0.000	0.000	0.012	0.011	0.020	0.067	0.060	0.000	0.000	0.005	0.000	0.000	0.000
2	0.999	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.995	0.133	0.991	1.000	0.003	0.000	0.540	0.010
3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.911	0.000	0.000	0.995	1.000	0.650	0.990
4	0.006	0.022	0.000	0.000	0.000	0.000	0.000	0.900	0.000	0.908	0.871	1.000	0.947	0.977	0.629	0.000	0.995	0.937	0.580	0.820
5	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.700	0.943	0.937	0.924	0.987	0.840	0.000	0.000	0.000	0.000	0.000	0.000	0.000
6	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.763	0.945	0.198	0.000	0.647	0.027	0.030	0.054
7	0.024	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.006	0.087	0.971	0.979	0.886	1.000	0.980	0.987	0.970	1.000
8	0.691	0.000	0.000	0.000	0.000	0.000	0.982	1.000	1.000	0.940	0.946	1.000	0.957	0.000	0.000	0.000	0.945	0.973	0.930	0.986
9	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.011	0.011	0.960	0.009	0.033	0.000	0.000	0.970	0.773	0.999	0.000
10	0.895	0.682	0.000	0.000	0.000	0.000	1.000	0.900	1.000	0.949	0.946	1.000	0.000	0.972	0.480	0.000	0.005	0.987	1.000	1.000
11	0.508	0.590	0.173	0.173	0.173	0.173	0.000	0.900	0.813	0.928	0.925	0.973	0.880	0.009	0.060	0.000	0.965	0.000	0.090	0.050
12	0.695	0.478	0.000	0.000	0.067	0.990	1.000	1.000	0.000	0.967	0.927	1.000	0.174	0.000	0.040	0.000	0.960	0.987	1.000	1.000
13	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
14	0.000	0.999	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.005	0.933	0.225	0.925	0.040	0.955	0.806	0.747	0.740	0.760
15	0.911	0.000	0.021	0.021	0.021	0.021	0.994	0.900	0.000	0.961	0.919	0.993	0.909	0.965	0.660	0.000	1.000	1.000	1.000	1.000
16	0.959	0.381	0.117	0.117	0.117	0.117	0.000	0.000	0.000	0.609	0.008	0.780	0.992	0.996	0.948	0.728	1.000	0.974	0.230	0.085
17	0.545	0.229	0.320	0.320	0.320	0.320	0.003	0.000	0.000	0.006	0.005	0.047	0.247	0.989	0.336	1.000	0.970	1.000	0.120	0.210
18	0.968	0.975	0.000	0.689	0.938	0.990	0.000	1.000	1.000	1.000	0.997	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
19	0.645	0.503	0.100	0.266	0.235	0.010	0.000	0.200	0.313	0.960	0.892	0.600	0.601	0.315	0.000	0.000	0.129	0.040	0.810	0.061
20	0.540	0.184	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.009	0.008	0.080	0.271	1.000	1.000	1.000	0.940	1.000	0.210	0.146
21	0.063	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.877	1.000	1.000	0.284	0.987	0.000	0.000
22	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.391	0.353	0.000	0.052	0.987	0.862	1.000	0.343	1.000	0.000	0.000
23	0.945	0.874	0.510	0.510	0.510	0.510	0.003	1.000	1.000	0.979	0.981	0.990	0.990	0.997	0.880	1.000	0.950	1.000	0.000	0.000
24	0.997	0.984	0.790	0.790	0.790	0.790	0.096	1.000	0.063	0.994	1.000	0.990	1.000	0.999	1.000	1.000	0.995	1.000	0.960	1.000
25	0.519	0.249	0.000	0.411	0.000	0.840	0.000	0.000	0.000	0.036	0.033	0.060	0.151	0.997	0.900	1.000	0.970	0.960	0.000	0.000
26	0.848	0.820	0.196	0.094	0.053	0.980	0.000	1.000	0.000	0.952	0.914	0.980	0.994	0.997	0.680	1.000	0.920	0.987	0.930	0.910
27	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table 4 (cont'd)

PROBABILITY ARRAY

	21	22	23	24	25	26	27	28	29	30	31	32	33	34
1	0.000	0.000	0.200	0.214	0.002	0.995	0.987	0.982	0.019	1.000	1.000	0.917	0.000	1.000
2	0.750	0.333	0.457	0.757	0.177	0.998	1.000	0.930	0.988	0.971	0.933	0.945	1.000	1.000
3	0.309	0.794	0.229	0.471	1.000	0.000	0.000	0.000	0.156	0.000	0.000	0.245	0.000	0.000
4	0.985	0.963	0.629	0.915	0.991	0.992	0.987	0.246	0.958	0.000	0.989	0.709	0.000	0.000
5	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.947	0.969	0.000	0.000	0.000	0.000	0.000
6	0.235	0.999	0.285	0.500	0.559	0.000	0.000	0.947	0.903	0.991	1.000	0.000	0.000	0.000
7	0.985	0.999	0.561	0.814	0.991	0.988	0.986	1.000	0.986	0.990	0.967	0.490	0.000	0.000
8	0.971	1.000	0.557	0.872	0.986	0.965	0.880	0.947	0.959	0.877	0.944	1.000	0.000	0.860
9	0.999	1.000	0.999	1.000	1.000	0.014	0.068	1.000	0.975	0.000	0.023	0.980	1.000	0.000
10	0.824	1.000	0.000	0.000	0.996	0.000	0.000	0.000	0.000	0.010	0.000	0.050	0.190	1.000
11	0.044	0.000	0.057	0.129	0.013	0.005	0.000	0.000	0.000	0.000	0.000	0.868	0.000	0.980
12	0.985	1.000	0.000	0.000	0.995	0.014	0.000	0.000	0.992	0.971	0.000	0.020	0.000	0.480
13	0.015	0.000	0.000	0.000	0.027	0.972	0.937	1.000	0.996	0.953	0.978	0.209	0.381	0.440
14	0.074	0.999	0.214	0.229	0.017	0.007	0.012	0.075	0.096	0.048	0.012	0.000	0.000	0.000
15	0.956	0.999	0.171	0.443	0.526	0.864	0.000	0.860	0.938	0.858	0.122	0.493	1.000	0.000
16	0.309	0.932	0.714	0.943	0.084	0.003	0.038	0.000	0.015	0.000	0.100	0.313	0.000	0.920
17	1.000	0.999	0.743	1.000	0.997	0.872	0.918	0.947	0.822	0.039	0.700	0.910	0.000	0.060
18	1.000	1.000	0.871	1.000	1.000	0.022	0.146	0.000	0.000	0.000	0.885	0.994	1.000	0.000
19	0.000	0.999	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000
20	1.000	0.999	0.714	1.000	0.986	0.009	0.019	0.691	0.306	0.000	0.366	0.627	0.952	0.340
21	0.117	0.999	0.000	0.000	0.719	0.947	0.038	0.000	0.000	0.000	0.865	0.000	0.000	0.000
22	0.985	0.979	0.157	0.057	0.867	0.006	1.000	0.000	0.000	0.000	0.973	0.000	0.000	0.000
23	0.970	0.999	0.028	0.014	1.000	0.012	0.443	0.000	0.000	0.000	0.108	0.104	0.000	0.000
24	0.926	0.946	0.714	1.000	0.000	0.014	0.083	0.000	0.000	0.000	0.000	0.448	1.000	0.000
25	0.897	0.981	0.685	0.971	0.029	0.020	0.078	0.000	0.010	0.000	0.095	0.020	0.286	0.000
26	0.000	0.999	0.700	0.857	0.003	0.013	0.000	0.094	0.015	0.000	0.679	0.015	0.000	0.000
27	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000

The matrix showing the probabilities of obtaining positive values (j = positive) for all test organisms in the complete series of tests is given in Table 4.

Because of the importance of the screening tests (Table 5) in the identification scheme (Edwards and Ewing, 1972; Ewing *et al.* 1961) they were treated differently from the remaining tests.

Table 5. Special case characters for inclusion in the identification scheme.

	Theoretical Maximum Positive Probability Values	
	Enterobacteriaceae	*Aeromonas*
27. Oxidase production	0	1
28. Growth in MacConkey's medium	1	1
29. Fermentative in glucose O/F	1	1
30. Catalase production	1	1
31. Nitrate reduction	1	1

Examination of the theoretical maximum probability values for tests 28-31 shows that, although of prime importance in separating the organisms under consideration from other organisms, they have no differentiating value among the organisms under study. Inclusion of these probabilities in the calculation of joint likelihoods will result only in an overall decrease in the magnitude of any joint likelihood as the number of individual probabilities is increased. For satisfactory operation of the identification scheme, however, individual test results for each of these characters should be positive. Even so, the chances of a misread test still apply and complete exclusion of an organism from the scheme purely because of the result of a single test, albeit a 'key' test, should probably be avoided.

Either positive or negative values occurring for these characters (28-31) result in computation of the joint likelihoods of characters 1-27, although the latter are not used in this calculation. However, if a negative value occurs for one of the characters 28-31, provision is made for additional computer output which indicates that disparity has occurred in a key feature (or features) and should be checked. Similarly, if no data are available, the output will indicate that the information available is inadequate for an acceptable identification.

Oxidase production, which is used as both a screening test and a differentiating test, is included in the calculation of joint likelihoods, but where a positive value is assigned, additional output indicates that this test is positive and that the organism is not a member of the Enterobacteriaceae.

For each unknown, the computer program is able to produce a list of joint probabilities and relative joint probabilities for each taxon considered, a list of characters in which the unknown differs from the taxon to which it appears most similar, plus the qualifying statements listed above. Complete program description is given in Appendix 1.

TESTING OF ISOLATES

If identification procedures are to be employed on a routine and time-saving basis using a large number of organisms, it is desirable that all inoculations be performed simultaneously. In addition test results should, if possible, be recorded at one time. Because of the necessity for varying incubation periods the latter is rarely feasible. For inoculation, however, any technique can be used, but where there are large numbers of organisms involved, a multiple inoculation procedure appears desirable.

In the routine procedures used in the author's laboratory, organisms are obtained in pure culture and either screened according to the above scheme or, where their probabilities of being Enterobacteriaceae or *Aeromonas* are high, tested for purity only. Organisms are then transferred into nutrient broth or tryptic soy broth in capped vials contained in racks of 105 (Lowe, 1974) and incubated for 24 hours at 37.5°C. These are then used as initial inocula for the inoculation of the test media, all media being inoculated at one time. A list of media and incubation periods used, based on the data of Edwards and Ewing (1972) and Ewing *et al.* (1961), is given in Table 6. All tests are read at the times indicated. Acid production from the range of carbohydrates should be checked daily from 48 hours to 4 days. The methods used are given in greater detail in Chapter 2.

DATA RECORDING

The test results are recorded as either + (1) or - (0) in tabular form such that transfer to computer cards may be carried out in the most simple fashion. Calculation of joint likelihoods is then carried out by the computer. Details of this are given in Chapter 2.

APPRAISAL OF METHODS

To assess the usefulness of the system described here it is necessary to obtain some estimation of the speed and

Table 6. Methods used for the identification of Enterobacteriaceae and aeromonads.

Test	Media	Incubation Time	Test Reagent/Notes
Indol	Tryptone water	48 hr	Kovacs
MR	Difco MRVP medium	4 days	Methyl red solution
VP	Difco MRVP medium	48 hr	5% α-napthol in absolute alcohol 40% KOH
Citrate	Difco Simmons citrate	48 hr - 4 days	After inoculation, replace lid with sterile cotton wool
H_2S	Difco TSI medium	48 hr	After inoculation, replace lid with sterile cotton wool
Urease	Difco broth base	48 hr - 4 days	After inoculation, replace lid with sterile cotton wool
KCN	Edwards *et al*, 1956	48 hr	
Motility	Difco motility S	24 hr - 4 days at room temp.	
Gelatin	NA broth charcoal gelatin capsules	4 days	
Lysine	Difco decarboxylase base	4 days	Oil after inoculation
Arginine	Difco decarboxylase base	4 days	Oil after inoculation
Ornithine	Difco decarboxylase base	4 days	Oil after inoculation
PA	Difco PA agar	24 hr	13% w/v ferric chloride
Malonate	Difco malonate broth	48 hr	After inoculation, replace lid with sterile cotton wool
Glucose (Gas)	1% Andrades	48 hr - 4 days	
Lactose	1% Andrades	48 hr - 4 days	
Sucrose	1% Andrades	48 hr - 4 days	
Mannitol	1% Andrades	48 hr - 4 days	
Dulcitol	1% Andrades	48 hr - 4 days	
Salicin	1% Andrades	48 hr - 4 days	
Adonitol	1% Andrades	48 hr - 4 days	
Inositol	1% Andrades	48 hr - 4 days	
Sorbitol	1% Andrades	48 hr - 4 days	
Arabinose	1% Andrades	48 hr - 4 days	
Raffinose	1% Andrades	48 hr - 4 days	
Rhamnose	1% Andrades	48 hr - 4 days	
Oxidase	NA plate	24 hr	1% para-aminodimethylaniline HCl 1% α-naphthol in 95% ethyl alcohol
Catalase	NA plate	24 hr	Hydrogen peroxide
Glucose O/F	Difco O/F basal medium	48 hr - 4 days	Set up x 2. Oil one set after inoculation
Nitrate	Potassium nitrate broth	48 hr	0.8% sulphanilic acid in 5 N acetic acid 0.5% α-naphthylamine in 5 N acetic acid
MacConkey's	MacConkey plates 0.15% sodium taurocholate	24 hr	

accuracy of both the inoculation procedures and the tests selected, and also of the identification program.

One hundred and sixty-three Enterobacteriaceae and *Aeromonas* isolates were obtained from two hospital laboratories (St. Joseph's in Hamilton, and Joseph Brant in Burlington). These strains were checked for purity in the laboratory by streaking onto nutrient agar and then inoculated, using the multipoint inoculation technique, into each of the identification media. After incubation, the results were read and recorded, then transferred onto computer cards. Identifications were then carried out in the laboratory by examination of the test data and according to the schemes of Edwards and Ewing (1972) and Ewing *et al.* (1961). These results, compared to the identities given by the hospitals, were used as an assessment of the adequacy of the multipoint inoculation technique in conjunction with the array of tests selected.

To test the validity of the computer program, the results were punched onto cards and the mathematical identities obtained. These were then compared to the identities given in both the hospital laboratories and the author's laboratory. The results are given in Table 7.

DISCUSSION

The system described here employs both multiple inoculation techniques and computerized mathematical calculations to produce a rapid and accurate identification scheme. The multiple inoculation system allows rapid transfer of large numbers of organisms within a short space of time and also reduces considerably the quantities of media normally used in conventional single incubation transfer systems. Because of the re-usability of the racks and vials, the cost per test is considerably less than commercially available multi-test single inoculation systems, such as the API (Analytab Products, Inc., N.Y.) and the Improved Enterotube (Roche Diagnostics, N.J.) systems.

The reliability of the system, as tested with a wide range of hospital isolates, approaches 90%. However,

Table 7. Comparison of identification of isolates by a hospital laboratory, the author's laboratory and by computer

Taxon	No. of Organisms Identified by Hospital	No. of Organisms Identified in Laboratory	Laboratory I.D. Compared to Hospital I.D.		Computer I.D. Compared to Hospital I.D.		Computer I.D. Compared to Laboratory I.D.	
			No.	%	No.	%	No.	%
Escherichia	69	73	62	90%	61	88%	68	93%
Shigella	4	3	3	75%	3	75%	3	100%
Arizona	1	1	1	100%	1	100%	1	100%
Providencia	4	3	3	75%	3	75%	3	100%
Proteus	22	22	21	96%	20	91%	21	96%
Enterobacter/ Pectobacterium	13	18	10	77%	10	77%	16	89%
Citrobacter	5	7	5	100%	5	100%	5	71%
Edwardsiella/ Salmonella	8	7	7	88%	8	100%	7	100%
Klebsiella	11	10	10	91%	9	82%	8	80%
Aeromonas	4	3	3	75%	3	75%	3	100%
Serratia	2	2	1	50%	2	100%	2	100%
Total Identified	143	149	126	88%	125	87%	137	92%
Total Unidentified	20	14	37	12%	38	13%	26	8%

with over 500 water isolates with a more restricted range of taxa, the accuracy of the system is close to 100%.

One great advantage of the system described here, compared to intuitive identification schemes, is that single aberrant results will not necessarily lead to a misidentification. In addition, the system eliminates personal bias and provides completely reproducible identifications.

CHAPTER 2

Practical Considerations for the Identification of Members of the Family Enterobacteriaceae and Aeromonads

ISOLATION AND PURIFICATION

Isolates to be identified can be grown and purified on any suitable medium. If isolates are to be screened prior to the calculation of joint likelihoods, they can be streaked onto MacConkey's agar and incubated for a minimum of 24 hours at 37.5°C; this can be used as both a screening test and to obtain pure cultures. Individual colonies should be picked from MacConkey's agar and inoculated into glucose O/F medium and nitrate broth, and streaked onto nutrient agar or other suitable growth medium for determination of catalase and oxidase production. All tests should be carried out at 37.5°C; for times of incubation see Table 6. Organisms which grow on MacConkey's agar, are fermentative in glucose O/F, reduce nitrate, produce catalase and do or do not produce oxidase can then be subjected to the identification tests.

Where organisms have been isolated from media selective for coliforms, for example, m-FC agar (Difco), or are considered highly likely to be Enterobacteriaceae or aeromonads, they should be streaked onto suitable growth medium (nutrient agar or tryptic soy agar) to test for purity and then inoculated simultaneously into both the screening and identification media.

PREPARATION OF INOCULA

Multipoint inoculation racks (Lowe, 1974), with foam-plugged sterile vials, each containing 2.5 ml nutrient broth, are used to prepare initial inocula. Organisms to be identified are picked from single colonies growing on purification plates and inoculated into the vials. It is not necessary to label each vial. Each rack of 105 vials can be oriented and identified by a label at one end of the rack; vials then follow sequentially (in rows of 7) from left to right. After the required number of vials have been inoculated, the plugged vials are incubated at 37.5°C for 24 hours.

INOCULATION

Racks containing the identification media and, if necessary, the screening media, are prepared (Table 6). In addition, a rack of nutrient broth is included at the end of each run to act as a control indicating that inoculation of viable organisms has occurred. The compositions of the media are given in Table 6.

The plugs are removed from the incubated vials, and organisms are transferred to each of the test media, using a multipoint inoculator. In this way, 105 organisms can be transferred at any one time. The multipoint inoculator is alcohol dipped and flamed between each transfer. After inoculation, racks containing test media lysine, ornithine, arginine and one set of glucose O/F are covered with sterile mineral oil. The covers are removed from TSI, urease, citrate and malonate and replaced with sterile non-absorbent cotton wool. All test media are incubated at 37.5°C for the periods given in Table 6. Glucose O/F and glucose media should be examined at 24-hour intervals for 4 days. All test reactions and media are given in detail in Edwards and Ewing (1972). Oxidase and catalase reactions can be performed after 24 hours, on the initial purity plates (if nutrient agar or some similar non-selective medium is used), on large plates of this medium inoculated with the multipoint inoculator or in vials of this medium.

RESULT READING

The test results are read (Edwards and Ewing, 1972) and recorded as two-state characters (+/- or 1/0) (see Figure 1). This order must be followed, as it is the order followed in the stored probability matrix. If data are not available, an NC character (9) is recorded.

COMPUTATION

Prior to computation, the results are transferred to 80-column computer cards (Figure 2).

Columns 1-3 inclusive are used as identifiers for each isolate. All numbers are right justified; organism numbers 001-999 can be accommodated here. Columns 4-6 inclusive are used as card identifiers; in this program these will always contain 001 (i.e. only one card is being used as input for each organism). The test results (1-31) are then

accommodated in this order by columns 7-37 inclusive. If no data are available, or an NC character has been recorded on the data record sheet, character 9 is used. All columns from 7 to 37 inclusive must contain either 0, 1 or 9; any other characters cannot be accommodated.

The program as designed contains several options. These are:

(1) All taxa, or only a specified set, can be considered.
(2) All characteristics, or only a specified set, can be considered.
(3) A probability matrix whose limits are set at 1.000 and 0.000, or whose limits are set at 0.999 and 0.001, can be used.
(4) Discrepancy values (i.e., characteristics in which an identified individual differs from a theoretical perfect match with any taxa) will, or will not, be printed.
(5) For certain taxa, characteristics determined at 37°C or 22°C can be considered.
(6) Where NC values appear for certain characteristics in the probability matrix, these can be ignored for all taxa in the calculation of joint likelihoods (reduced character set) or ignored only by those taxa in which they occur (full character set).

The options required should be examined before running the program. Selection of options and running the program should be carried out in conjunction with the author or Miss J. Dowell at CCIW. The program (LKLI) is included in the computer library at CCIW.

	ISOLATE NO.
Test	
Indol	1
MR	2
VP	3
Citrate	4
H_2S (TSI)	5
Urease	6
KCN	7
Motility	8
Gelatin	9
Lysine	10
Arginine	11
Ornithine	12
P.A.	13
Malonate	14
Glucose (gas)	15
Lactose	16
Sucrose	17
Mannitol	18
Dulcitol	19
Salicin	20
Adonitol	21
Inositol	22
Sorbitol	23
Arabinose	24
Raffinose	25
Rhamnose	26
Oxidase	27
MacConkey's	28
Catalase	29
Glucose O/F	30
Citrate	31

Figure 1. Data sheet format.

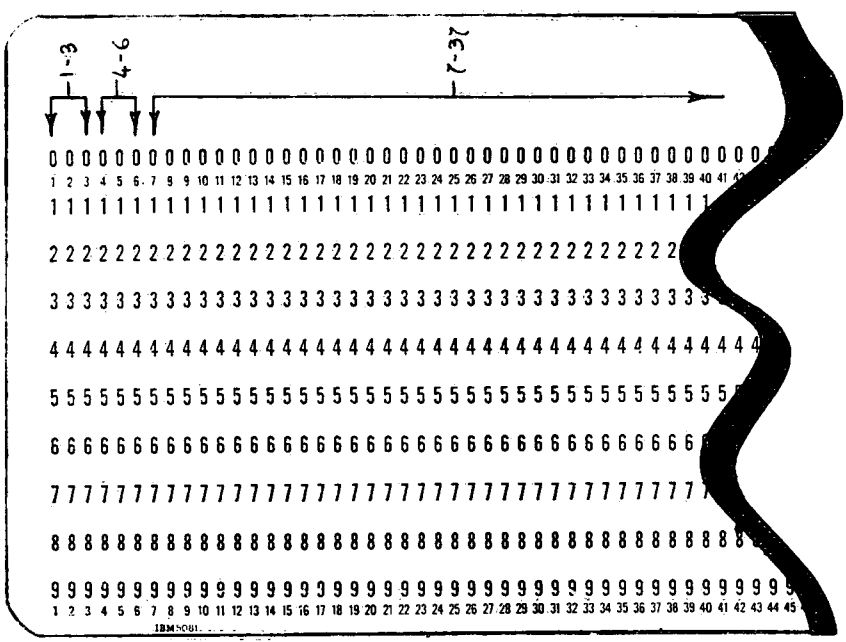


Figure 2. Computer card format.

COMPUTER OUTPUT

A sample output is given in Appendix 1. Under the conditions of this program, the output will be returned headed by the job identification (107LKL1) and, if the job is successfully completed, ended by STOP 0777. If the job is not successfully completed, JOB ABORTED will appear on page 1, together with an indication of the nature of the error. In such cases, the "Master Control Manual" should be consulted.

The special title given to the particular job being run appears on page 2 of the output.

Page 3 indicates the numbers of characters and families (taxa) being used, together with the probability matrix under consideration.

Page 4 lists the names and identifying numbers of taxa included in the probability matrix. The characters for which no data are recorded in the matrix are also given. The identifying numbers are also used in the likelihood listings given below.

Page 5 lists the characters used. Taxa for which no data for specific characters are recorded in the matrix are also indicated.

Pages 6 and 7 contain the probability array.

Pages 8 and 9 list the taxa being considered (either at 37°C or at 22°C) and the characters used in calculation of the joint likelihoods. Special case characters (i.e., those not used in calculation of the joint likelihoods) are also given if requested.

Page 9 onwards gives the identifications. In the examples given here (appendix page 9 — onwards), there is a relative likelihood of 1 of organism 001 occurring in Family 14, *K. pneumoniae* (see appendix page 4). Organism 002 has a relative likelihood of 1 of occurring in Family 32, *Aeromonas* sp.; here, a positive oxidase is indicated. Organism 003 is identified as *Pectobacterium,* etc.

For each organism, the isolate number is given, followed by the list of characters used as input for that organism. The sequence of characters is as on the input card. Next is a listing of a maximum of five characters; if any, or all, of these occur, it indicates that organisms differ in these characters from the expected reactions of organisms considered to be aeromonads or Enterobacteriaceae.

Following this is an indication of whether a full or reduced character set was used.

The identifications are then listed. Each family (taxa) has an identifying number (as given on page 4 of the program output) below which is listed a likelihood and a relative likelihood value. The taxon to which an unknown is most likely to belong will have a relative likelihood of 1.

If reduced character sets or discrepancy values are requested, they will appear before consideration of the next unknown to be identified.

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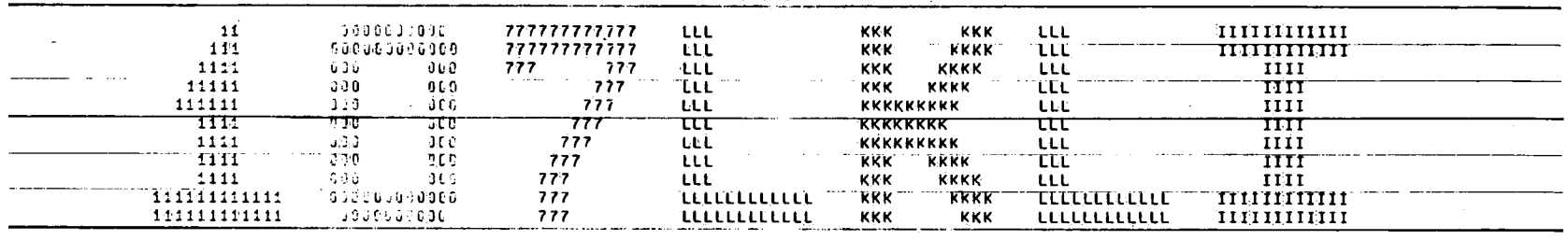
APPENDIX 1a

Sample Program Without Discrepancy Values Printed



Page 1, sample program.

```
JOB,231,107LKLI,2,3100,,,ST.LAWRENCE.ENTEROBACT.BILL LOWE.
SCHED,CORE=96,SCR=3,TIME=2
*DEF(O,,AEDL,865/123,NUM-TAX-ANAL-PGMS,,,I)
LKLI,A90L
```

Page 2, sample program.

```
PROGRAM  LIKELI

  31 CHARACTERISTICS
  34 FAMILIES
READ PROBABILITIES FROM DSI 60

CHANGE  100%  PROBABILITY VALUES TO  99.9%
CHANGE    0%  PROBABILITY VALUES TO   .1%
```

Page 3, sample program.

	NAMES OF FAMILIES	OMIT CHARACTERISTICS
1	ESCHERICHIA COLI	
2	ALKALESCENS-DISP	14
3	S.DYSENTERIAE	
4	S.FLEXNERI	
5	S.BOYDII	
6	S.SONNEI	
7	EDWARDSIELLA	
8	S.CHOLERAE-SUIS	
9	S.TYPHI	
10	S.ENTERITIDIS	
11	SALMONELLA SPP	
12	ARIZONA HINSHAWI	
13	CITROBACTER FREU	
14	K.PNEUMONIAE	
15	K.OZAENAE	
16	K.RHINOSCLEROMAT	
17	E.CLOACAE	
18	E.AEROGENES	
19	E.HAFNIAE 37	9
20	E.HAFNIAE 22	
21	E.LIQUEFAC 37	9
22	E.LIQUEFAC 22	6 7 14 15 17 19 20 21 23 26
23	PECTOBACT 37	9
24	PECTOBACT 22	
25	SERRATIA SPP	
26	P.ALCALIFACIENS	
27	P.STUARTII	
28	PROTEUS VULGARIS	
29	PROTEUS MIRABIL	
30	PROTEUS MORGANII	
31	PROTEUS RETTGERI	
32	AEROMONAS SP	
33	A.SALMONICIDA	
34	C27	

Page 4, sample program.

NAMES OF CHARACTERISTICS	OMITTED BY FAMILIES
1 INDOL	
2 MR	
3 VP	
4 CITRATE(S)	
5 H2 S (TS1)	
6 UREASE	22
7 RCN	22
8 MOTILITY	
9 GELATIN	19 21 23
10 LYSINE	
11 ARGININE	
12 ORNITHINE	
13 PHENYLALANIN	
14 MALONATE	2 22
15 GLUCOSE/GAS	22
16 LACTOSE	
17 SUCROSE	22
18 MANNITOL	
19 DULCITOL	22
20 SALICIN	22
21 ACONITOL	22
22 INOSITOL	
23 SORBITOL	22
24 ARABINOSE	
25 RAFFINOSE	
26 RHAMNOSE	22
27 OXIDASE	
28 MACCONKEY	
29 GLUCOSE O/F	
30 CATALASE	
31 NITRATE	

Page 5, sample program.

PROBABILITY ARRAY

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	0.986	0.997	0.300	0.415	0.400	0.001	0.991	0.001	0.001	0.012	0.011	0.026	0.067	0.060	0.001	0.001	0.005	0.001	0.001	0.001
2	0.999	0.999	0.999	0.999	0.999	0.999	0.999	0.999	0.999	0.999	0.999	0.999	0.995	0.133	0.391	0.999	0.003	0.001	0.540	0.010
3	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.911	0.001	0.001	0.995	0.999	0.650	0.990
4	0.006	0.022	0.001	0.001	0.001	0.001	0.001	0.900	0.001	0.908	0.871	0.999	0.947	0.977	0.629	0.001	0.995	0.937	0.580	0.820
5	0.001	0.001	0.001	0.001	0.001	0.001	0.999	0.700	0.943	0.937	0.924	0.987	0.840	0.001	0.001	0.001	0.001	0.001	0.001	0.001
6	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.763	0.945	0.198	0.001	0.647	0.027	0.030	0.024
7	0.024	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.006	0.006	0.087	0.971	0.979	0.880	0.999	0.980	0.987	0.970	0.999
8	0.661	0.041	0.001	0.001	0.001	0.001	0.982	0.999	0.999	0.940	0.946	0.999	0.957	0.001	0.001	0.001	0.945	0.973	0.930	0.986
9	0.001	0.001	0.001	0.001	0.001	0.001	0.003	0.001	0.001	0.011	0.011	0.960	0.009	0.033	0.001	0.001	0.970	0.773	0.999	0.001
10	0.895	0.682	0.001	0.001	0.001	0.001	0.999	0.900	0.999	0.949	0.946	0.999	0.001	0.972	0.480	0.001	0.005	0.987	0.999	0.999
11	0.508	0.599	0.173	0.173	0.173	0.173	0.001	0.900	0.813	0.928	0.925	0.973	0.886	0.009	0.060	0.001	0.965	0.001	0.090	0.050
12	0.695	0.478	0.001	0.001	0.667	0.998	0.999	0.999	0.001	0.967	0.927	0.999	0.174	0.001	0.040	0.001	0.960	0.987	0.999	0.999
13	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
14	0.001	0.999	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.999	0.015	0.933	0.225	0.925	0.340	0.955	0.806	0.747	0.740	0.760
15	0.911	0.001	0.021	0.021	0.021	0.121	0.994	0.900	0.001	0.961	0.919	0.993	0.909	0.965	0.660	0.001	0.999	0.999	0.999	0.999
16	0.959	0.381	0.117	0.117	0.117	0.117	0.001	0.001	0.001	0.069	0.018	0.780	0.902	0.996	0.948	0.728	0.999	0.974	0.230	0.085
17	0.545	0.229	0.320	0.320	0.320	0.320	0.003	0.001	0.001	0.006	0.005	0.047	0.247	0.989	0.336	0.999	0.970	0.999	0.120	0.210
18	0.960	0.975	0.001	0.689	0.930	0.998	0.001	0.999	0.999	0.999	0.997	0.999	0.999	0.999	0.999	0.999	0.999	0.999	0.999	0.999
19	0.645	0.503	0.100	0.266	0.235	0.010	0.001	0.200	0.313	0.960	0.892	0.001	0.601	0.315	0.001	0.001	0.129	0.040	0.010	0.001
20	0.540	0.184	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.009	0.008	0.080	0.271	0.999	0.999	0.999	0.940	0.999	0.210	0.140
21	0.083	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.877	0.999	0.999	0.284	0.987	0.001	0.001
22	0.013	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.391	0.353	0.001	0.052	0.987	0.862	0.999	0.343	0.999	0.001	0.001
23	0.945	0.874	0.510	0.510	0.510	0.510	0.003	0.999	0.999	0.979	0.981	0.990	0.990	0.997	0.886	0.999	0.950	0.999	0.001	0.001
24	0.997	0.994	0.798	0.796	0.796	0.798	0.996	0.001	0.063	0.994	0.908	0.998	0.999	0.999	0.999	0.999	0.995	0.999	0.960	0.999
25	0.519	0.249	0.001	0.411	0.001	0.840	0.001	0.001	0.001	0.036	0.033	0.060	0.151	0.997	0.900	0.999	0.970	0.960	0.001	0.001
26	0.848	0.826	0.196	0.094	0.053	0.980	0.001	0.999	0.001	0.952	0.914	0.980	0.994	0.997	0.680	0.999	0.926	0.987	0.930	0.910
27	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
28	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
29	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
30	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
31	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

Page 6, sample program.

PROBABILITY ARRAY

	21	22	23	24	25	26	27	28	29	30	31	32	33	34
1	0.001	0.001	0.200	0.214	0.002	0.995	0.987	0.982	0.019	0.999	0.999	0.917	0.001	0.999
2	0.750	0.333	0.457	0.757	0.177	0.998	0.999	0.930	0.988	0.971	0.933	0.945	0.999	0.999
3	0.309	0.794	0.229	0.471	0.999	0.001	0.001	0.001	0.156	0.001	0.001	0.245	0.001	0.001
4	0.985	0.963	0.629	0.915	0.991	0.992	0.987	0.246	0.958	0.001	0.989	0.709	0.001	0.001
5	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.947	0.969	0.001	0.001	0.001	0.001	0.001
6	0.235	0.999	0.285	0.500	0.559	0.001	0.001	0.947	0.903	0.991	0.999	0.001	0.001	0.001
7	0.985	0.999	0.561	0.814	0.991	0.988	0.986	0.999	0.996	0.990	0.967	0.490	0.001	0.001
8	0.971	0.999	0.557	0.872	0.986	0.965	0.880	0.947	0.959	0.877	0.944	0.999	0.001	0.860
9	0.999	0.999	0.999	0.999	0.999	0.014	0.068	0.999	0.975	0.001	0.023	0.980	0.999	0.001
10	0.824	0.999	0.001	0.001	0.996	0.001	0.001	0.001	0.001	0.010	0.001	0.050	0.190	0.999
11	0.344	0.001	0.057	0.129	0.013	0.005	0.001	0.001	0.001	0.001	0.001	0.868	0.001	0.980
12	0.985	0.999	0.001	0.001	0.995	0.114	0.001	0.001	0.992	0.971	0.001	0.020	0.001	0.480
13	0.015	0.001	0.001	0.001	0.027	0.972	0.937	0.999	0.996	0.953	0.978	0.209	0.381	0.440
14	0.174	0.999	0.214	0.229	0.017	0.007	0.012	0.075	0.096	0.048	0.012	0.001	0.001	0.001
15	0.956	0.999	0.171	0.443	0.526	0.864	0.001	0.860	0.938	0.858	0.122	0.493	0.999	0.001
16	0.305	0.932	0.714	0.943	0.084	0.003	0.038	0.001	0.015	0.001	0.100	0.313	0.001	0.920
17	0.999	0.999	0.743	0.999	0.997	0.872	0.918	0.947	0.822	0.039	0.750	0.910	0.001	0.960
18	0.999	0.999	0.871	0.999	0.999	0.022	0.146	0.001	0.001	0.001	0.885	0.994	0.999	0.001
19	0.001	0.999	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.999	0.001
20	0.999	0.999	0.714	0.999	0.986	0.009	0.019	0.691	0.306	0.001	0.366	0.627	0.952	0.340
21	0.117	0.999	0.001	0.001	0.719	0.947	0.038	0.001	0.001	0.001	0.865	0.001	0.001	0.001
22	0.985	0.979	0.157	0.057	0.867	0.006	0.999	0.001	0.001	0.001	0.973	0.001	0.001	0.001
23	0.978	0.999	0.128	0.014	0.999	0.012	0.443	0.001	0.001	0.001	0.168	0.104	0.001	0.001
24	0.926	0.946	0.714	0.999	0.001	0.014	0.083	0.001	0.001	0.001	0.001	0.448	0.999	0.001
25	0.897	0.981	0.685	0.971	0.029	0.020	0.078	0.001	0.010	0.001	0.095	0.020	0.286	0.001
26	0.001	0.999	0.700	0.857	0.003	0.013	0.001	0.094	0.015	0.001	0.679	0.015	0.001	0.001
27	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.999	0.999	0.999
28	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
29	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
30	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
31	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

Page 7, sample program.

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COMPARE PUGS TO  31 FAMILIES

      1 ESCHERICHIA COLI
      2 ALKALESCENS-DISP
      3 S.DYSENTERIAE
      4 S.FLEXNERI
      5 S.BOYDII
      6 S.SONNEI
      7 EDWARDSIELLA
      8 S.CHOLERAE-SUIS
      9 S.TYPHI
     10 S.ENTERITIDIS
     11 SALMONELLA SPP
     12 ARIZONA HINSHAWI
     13 CITROBACTER FREU
     14 K.PNEUMONIAE
     15 K.OZAENAE
     16 K.RHINOSCLEROMAT
     17 E.CLOACAE
     18 E.AEROGENES
     19 E.HAFNIAE 37
     21 E.LIQUEFAC 37
     23 PECTOBACT  37
     25 SERRATIA  SPP
     26 P.ALCALIFACIENS
     27 P.STUARTII
     28 PROTEUS VULGARIS
     29 PROTEUS MIRABIL
     30 PROTEUS MORGANII
     31 PROTEUS RETTGERI
     32 AEROMONAS SP
     33 A.SALMONICIDA
     34 G27

TEST   27 CHARACTERISTICS

      1 INDOL
      2 MR
      3 VP
      4 CITRATE(S)
      5 H2-S (TS1)
      6 UREASE
      7 KCN
      8 MOTILITY
      9 GELATIN
     10 LYSINE
     11 ARGININE
     12 ORNITHINE
     13 PHENYLALANIN
     14 MALONATE
     15 GLUCOSE/GAS
     16 LACTOSE
     17 SUCROSE
     18 MANNITOL
     19 DULCITOL
     20 SALICIN
     21 ADONITOL
     22 INOSITOL
     23 SORBITOL
     24 ARABINOSE
     25 RAFFINOSE
     26 RHAMNOSE
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Page 8, sample program.

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   27 OXIDASE

MAKE    4 SPECIAL 0/9 CHARACTERISTIC TESTS

   28 MACCONKEY
   29 GLUCOSE O/F
   30 CATALASE
   31 NITRATE

MAKE    1 SPECIAL 1/9 CHARACTERISTIC TESTS

   27 OXIDASE

BUG 001 HAS CHARACTERISTICS 0091009991000111199991111101111

        FULL CHARACTERISTIC SET

            FAMILY      1           2           3           4           5           6           7           8           9
        LIKELIHOOD  0.198E-14  0.258E-17  0.112E-21  0.447E-22  0.242E-22  0.797E-23  0.451E-34  0.217E-25  0.667E-30
        REL.LIKELIHOOD 0.619E-13  0.803E-16  0.348E-20  0.140E-20  0.753E-21  0.249E-21  0.144E-32  0.678E-24  0.208E-28

            FAMILY     10          11          12          13          14          15          16          17          18
        LIKELIHOOD  0.239E-13  0.191E-15  0.238E-18  0.672E-14  0.321E-01  0.235E-04  0.686E-12  0.911E-08  0.222E-03
        REL.LIKELIHOOD 0.744E-12  0.596E-14  0.741E-17  0.210E-12  1.0        0.731E-03  0.214E-10  0.284E-06  0.691E-02

            FAMILY     19          21          23          25          26          27          28          29          30
        LIKELIHOOD  0.249E-14  0.143E-39  0.467E-11  0.337E-13  0.214E-23  0.614E-22  0.279E-30  0.900E-30  0.180E-34
        REL.LIKELIHOOD 0.776E-13  0.447E-08  0.145E-39  0.105E-11  0.667E-22  0.192E-20  0.870E-29  0.280E-28  0.560E-33

            FAMILY     31          32          33          34
        LIKELIHOOD  0.735E-21  0.178E-23  0.117E-27  0.748E-36
        REL.LIKELIHOOD 0.229E-19  0.556E-22  0.364E-26  0.233E-34

BUG 002 HAS CHARACTERISTICS 1010006090010000019999001190111111
                        POSITIVE OXIDASE      - CONFIRM

        FULL CHARACTERISTIC SET

            FAMILY      1           2           3           4           5           6           7           8           9
        LIKELIHOOD  0.555E-13  0.559E-11  0.191E-11  0.297E-11  0.280E-11  0.158E-17  0.306E-31  0.268E-30  0.289E-23
        REL.LIKELIHOOD 0.333E-05  0.336E-03  0.115E-03  0.179E-03  0.168E-03  0.951E-10  0.184E-23  0.161E-22  0.174E-15

            FAMILY     10          11          12          13          14          15          16          17          18
        LIKELIHOOD  0.649E-25  0.671E-21  0.181E-29  0.751E-17  0.188E-18  0.138E-17  0.324E-25  0.127E-15  0.116E-25
        REL.LIKELIHOOD 0.390E-17  0.403E-13  0.109E-21  0.451E-09  0.113E-10  0.828E-10  0.195E-17  0.765E-08  0.696E-18

            FAMILY     19          21          23          25          26          27          28          29          30
        LIKELIHOOD  0.337E-23  0.440E-18  0.390E-09  0.336E-22  0.271E-24  0.128E-21  0.902E-27  0.487E-29  0.582E-26
        REL.LIKELIHOOD 0.185E-15  0.264E-10  0.234E-01  0.202E-14  0.163E-16  0.770E-14  0.542E-19  0.292E-21  0.350E-18

            FAMILY     31          32          33          34
        LIKELIHOOD  0.991E-26  0.166E-07  0.496E-21  0.364E-16
        REL.LIKELIHOOD 0.596E-18  1.0        0.298E-13  0.219E-08

BUG 003 HAS CHARACTERISTICS 1011006090010000199990011911111
                        POSITIVE OXIDASE      - CONFIRM
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Page 9, sample program.

FULL CHARACTERISTIC SET

FAMILY	1	2	3	4	5	6	7	8	9
LIKELIHOOD	0.197E-14	0.573E-12	0.465E-15	0.309E-15	0.157E-15	0.776E-19	0.307E-37	0.241E-26	0.290E-29
REL.LIKELIHOOD	0.121E-05	0.372E-03	0.302E-06	0.200E-06	0.102E-06	0.503E-10	0.199E-28	0.156E-17	0.188E-20

FAMILY	10	11	12	13	14	15	16	17	18
LIKELIHOOD	0.127E-22	0.481E-19	0.884E-25	0.222E-13	0.265E-14	0.497E-17	0.324E-25	0.291E-12	0.131E-22
REL.LIKELIHOOD	0.823E-14	0.312E-10	0.573E-16	0.144E-04	0.172E-05	0.322E-08	0.210E-16	0.189E-03	0.848E-14

FAMILY	19	21	23	25	26	27	28	29	30
LIKELIHOOD	0.564E-22	0.289E-19	0.154E-08	0.111E-22	0.443E-24	0.973E-23	0.305E-28	0.169E-29	0.583E-32
REL.LIKELIHOOD	0.365E-13	0.187E-10	1.0	0.723E-14	0.287E-15	0.631E-14	0.198E-19	0.110E-20	0.378E-23

FAMILY	31	32	33	34
LIKELIHOOD	0.189E-23	0.617E-09	0.497E-27	0.365E-22
REL.LIKELIHOOD	0.122E-14	0.400E+00	0.322E-18	0.236E-13

BUG 004 HAS CHARACTERISTICS 01000090901100019999900119101111

FULL CHARACTERISTIC SET

FAMILY	1	2	3	4	5	6	7	8	9
LIKELIHOOD	0.998E-05	0.700E-04	0.108E-05	0.434E-06	0.168E-04	0.765E-02	0.277E-24	0.267E-12	0.288E-17
REL.LIKELIHOOD	0.131E-02	0.915E-02	0.142E-03	0.567E-04	0.220E-02	1.0	0.362E-22	0.349E-10	0.377E-15

FAMILY	10	11	12	13	14	15	16	17	18
LIKELIHOOD	0.310E-11	0.811E-08	0.432E-14	0.725E-06	0.147E-16	0.134E-07	0.323E-13	0.106E-12	0.667E-22
REL.LIKELIHOOD	0.495E-09	0.106E-05	0.565E-12	0.948E-04	0.192E-14	0.175E-05	0.423E-11	0.138E-10	0.873E-20

FAMILY	19	21	23	25	26	27	28	29	30
LIKELIHOOD	0.257E-13	0.194E-12	0.103E-07	0.216E-20	0.127E-21	0.168E-20	0.228E-25	0.211E-21	0.653E-23
REL.LIKELIHOOD	0.336E-11	0.253E-10	0.135E-05	0.283E-18	0.166E-19	0.220E-18	0.298E-23	0.276E-19	0.854E-21

FAMILY	31	32	33	34
LIKELIHOOD	0.292E-24	0.248E-13	0.496E-21	0.336E-19
REL.LIKELIHOOD	0.382E-22	0.324E-11	0.649E-19	0.440E-17

BUG 005 HAS CHARACTERISTICS 11100090913000119999900119101111

FULL CHARACTERISTIC SET

FAMILY	1	2	3	4	5	6	7	8	9
LIKELIHOOD	0.261E-04	0.379E-07	0.476E-10	0.316E-10	0.160E-10	0.794E-14	0.506E-20	0.241E-20	0.664E-21
REL.LIKELIHOOD	1.0	0.145E-02	0.182E-05	0.121E-05	0.614E-06	0.304E-09	0.194E-15	0.923E-16	0.254E-16

FAMILY	10	11	12	13	14	15	16	17	18
LIKELIHOOD	0.457E-16	0.115E-12	0.347E-18	0.337E-12	0.101E-08	0.904E-11	0.324E-19	0.801E-15	0.666E-16
REL.LIKELIHOOD	0.175E-11	0.439E-08	0.133E-13	0.129E-07	0.387E-04	0.347E-06	0.124E-14	0.307E-10	0.255E-11

FAMILY	19	21	23	25	26	27	28	29	30
LIKELIHOOD	0.483E-12	0.292E-14	0.261E-08	0.456E-18	0.226E-20	0.128E-21	0.763E-23	0.922E-25	0.119E-22
REL.LIKELIHOOD	0.185E-07	0.112E-09	0.100E-03	0.175E-13	0.864E-16	0.491E-17	0.292E-18	0.353E-20	0.455E-18

FAMILY	31	32	33	34
LIKELIHOOD	0.405E-22	0.339E-13	0.116E-18	0.743E-21
REL.LIKELIHOOD	0.155E-17	0.130E-08	0.445E-14	0.285E-16

Page 10, sample program.

BUG 006 HAS CHARACTERISTICS 0110100900110011999900119101111

FULL CHARACTERISTIC SET

FAMILY	1	2	3	4	5	6	7	8	9
LIKELIHOOD	0.617E-06	0.158E-08	0.232E-10	0.931E-11	0.361E-09	0.164E-06	0.460E-25	0.216E-10	0.289E-23
REL.LIKELIHOOD	0.477E-02	0.122E-04	0.180E-06	0.720E-07	0.279E-05	0.127E-02	0.355E-21	0.167E-06	0.223E-19

FAMILY	10	11	12	13	14	15	16	17	18
LIKELIHOOD	0.753E-09	0.621E-06	0.612E-09	0.129E-03	0.172E-13	0.441E-07	0.324E-19	0.210E-07	0.992E-18
REL.LIKELIHOOD	0.582E-05	0.481E-02	0.473E-05	1.0	0.133E-09	0.341E-03	0.250E-15	0.162E-03	0.767E-14

FAMILY	19	21	23	25	26	27	28	29	30
LIKELIHOOD	0.395E-10	0.276E-09	0.361E-08	0.264E-18	0.100E-18	0.128E-21	0.456E-25	0.729E-19	0.395E-25
REL.LIKELIHOOD	0.274E-06	0.213E-05	0.279E-04	0.204E-14	0.774E-15	0.990E-18	0.353E-21	0.563E-15	0.305E-21

FAMILY	31	32	33	34
LIKELIHOOD	0.365E-23	0.588E-13	0.496E-21	0.337E-25
REL.LIKELIHOOD	0.282E-19	0.454E-09	0.383E-17	0.261E-21

BUG 007 HAS CHARACTERISTICS 0011000900110011999900119101111

FULL CHARACTERISTIC SET

FAMILY	1	2	3	4	5	6	7	8	9
LIKELIHOOD	0.618E-12	0.158E-14	0.233E-16	0.933E-17	0.362E-15	0.164E-12	0.461E-31	0.217E-16	0.290E-29
REL.LIKELIHOOD	0.445E-09	0.114E-11	0.168E-13	0.672E-14	0.261E-12	0.119E-09	0.332E-28	0.156E-13	0.209E-26

FAMILY	10	11	12	13	14	15	16	17	18
LIKELIHOOD	0.754E-15	0.623E-12	0.614E-15	0.651E-09	0.115E-11	0.401E-12	0.324E-25	0.139E-02	0.951E-12
REL.LIKELIHOOD	0.543E-12	0.449E-09	0.442E-12	0.469E-06	0.826E-09	0.289E-09	0.234E-22	1.0	0.713E-09

FAMILY	19	21	23	25	26	27	28	29	30
LIKELIHOOD	0.561E-10	0.412E-10	0.127E-08	0.123E-14	0.201E-24	0.128E-27	0.344E-29	0.164E-21	0.118E-29
REL.LIKELIHOOD	0.404E-07	0.297E-07	0.917E-06	0.884E-12	0.145E-21	0.924E-25	0.248E-26	0.118E-18	0.850E-27

FAMILY	31	32	33	34
LIKELIHOOD	0.262E-27	0.111E-14	0.497E-27	0.338E-31
REL.LIKELIHOOD	0.189E-24	0.800E-12	0.358E-24	0.243E-28

BUG 008 HAS CHARACTERISTICS 0100000900100000199999001190111111
POSITIVE OXIDASE - CONFIRM

FULL CHARACTERISTIC SET

FAMILY	1	2	3	4	5	6	7	8	9
LIKELIHOOD	0.796E-09	0.168E-07	0.444E-05	0.418E-05	0.419E-05	0.158E-08	0.277E-27	0.268E-21	0.288E-14
REL.LIKELIHOOD	0.177E-03	0.378E-02	1.0	0.942E+00	0.943E+00	0.355E-03	0.625E-22	0.603E-16	0.649E-09

FAMILY	10	11	12	13	14	15	16	17	18
LIKELIHOOD	0.533E-17	0.602E-13	0.884E-22	0.208E-10	0.441E-19	0.151E-09	0.323E-16	0.383E-18	0.116E-28
REL.LIKELIHOOD	0.120E-11	0.136E-07	0.199E-16	0.468E-05	0.994E-14	0.341E-04	0.728E-11	0.862E-13	0.261E-23

FAMILY	19	21	23	25	26	27	28	29	30
LIKELIHOOD	0.134E-20	0.295E-14	0.442E-08	0.361E-23	0.680E-21	0.168E-17	0.219E-24	0.112E-24	0.195E-24
REL.LIKELIHOOD	0.437E-15	0.664E-09	0.995E-03	0.814E-18	0.153E-15	0.379E-12	0.494E-19	0.252E-19	0.439E-19

Page 11, sample program.

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          FAMILY      31          32          33          34
     LIKELIHOOD    0.138E-24   0.797E-07   0.494E-12   0.364E-13
     REL.LIKELIHOOD  0.321E-19   0.185E-01   0.111E-06   0.819E-08

BUG 009 HAS CHARACTERISTICS 011100090901011199990001910111111

     FULL CHARACTERISTIC SET

          FAMILY      1           2           3           4           5           6           7           8           9
     LIKELIHOOD    0.348E-10   0.158E-09   0.107E-12   0.428E-13   0.166E-11   0.755E-09   0.153E-22   0.241E-17   0.666E-30
     REL.LIKELIHOOD  0.341E-04   0.155E-03   0.105E-06   0.420E-07   0.163E-05   0.740E-03   0.150E-16   0.236E-11   0.653E-24

          FAMILY      10          11          12          13          14          15          16          17          18
     LIKELIHOOD    0.125E-08   0.490E-11   0.239E-11   0.517E-07   0.702E-13   0.392E-08   0.687E-18   0.166E-09   0.293E-17
     REL.LIKELIHOOD  0.123E-02   0.481E-05   0.234E-05   0.507E-01   0.688E-07   0.385E-02   0.673E-12   0.163E-03   0.287E-11

          FAMILY      19          21          23          25          26          27          28          29          30
     LIKELIHOOD    0.102E-05   0.148E-10   0.564E-06   0.347E-21   0.116E-16   0.195E-20   0.369E-20   0.772E-14   0.199E-20
     REL.LIKELIHOOD  1.0         0.145E-04   0.553E+00   0.340E-15   0.113E-10   0.192E-14   0.362E-14   0.757E-08   0.195E-14

          FAMILY      31          32          33          34
     LIKELIHOOD    0.366E-21   0.771E-16   0.495E-18   0.688E-27
     REL.LIKELIHOOD  0.358E-15   0.756E-10   0.486E-12   0.674E-21

BUG 010 HAS CHARACTERISTICS 111000909100001199991001191999999
                    NO DATA FOR MACCONKEY     - CONFIRM
                    NO DATA FOR GLUCOSE O/F   - CONFIRM
                    NO DATA FOR CATALASE      - CONFIRM
                    NO DATA FOR NITRATE       - CONFIRM
                    NO DATA FOR OXIDASE       - CONFIRM

     FULL CHARACTERISTIC SET

          FAMILY      1           2           3           4           5           6           7           8           9
     LIKELIHOOD    9.251E-04   0.390E-07   0.477E-10   0.316E-16   0.160E-10   0.795E-14   0.536E-20   0.241E-20   0.665E-21
     REL.LIKELIHOOD  1.0         0.145E-02   0.182E-05   0.121E-05   0.614E-06   0.304E-09   0.194E-15   0.923E-16   0.254E-16

          FAMILY      10          11          12          13          14          15          16          17          18
     LIKELIHOOD    0.457E-16   0.115E-12   0.348E-18   0.337E-12   0.101E-08   0.905E-11   0.324E-19   0.802E-15   0.667E-16
     REL.LIKELIHOOD  0.175E-11   0.439E-08   0.133E-13   0.129E-07   0.387E-04   0.347E-06   0.124E-14   0.307E-10   0.255E-11

          FAMILY      19          21          23          25          26          27          28          29          30
     LIKELIHOOD    0.483E-12   0.292E-14   0.262E-08   0.457E-18   0.226E-20   0.128E-21   0.764E-23   0.923E-25   0.115E-22
     REL.LIKELIHOOD  0.185E-07   0.112E-09   0.100E-03   0.175E-13   0.864E-16   0.491E-17   0.292E-18   0.353E-20   0.455E-18

          FAMILY      31          32          33          34
     LIKELIHOOD    0.496E-22   0.339E-10   0.116E-15   0.743E-18
     REL.LIKELIHOOD  0.155E-17   0.130E-05   0.445E-11   0.285E-13

STOP     77777
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Page 12, sample program.

APPENDIX 1b

Portion of Sample Program With Discrepancy Values Printed

BUG 001 HAS CHARACTERISTICS 0091010901000111999911119101111

DISCREPANCY AT CHARACTER	1	FOR FAMILY	30	BUG VALUE IS	0.0	PROBABILITY VALUE IS	0.999
DISCREPANCY AT CHARACTER	1	FOR FAMILY	31	BUG VALUE IS	0.0	PROBABILITY VALUE IS	0.999
DISCREPANCY AT CHARACTER	1	FOR FAMILY	34	BUG VALUE IS	0.0	PROBABILITY VALUE IS	0.999
DISCREPANCY AT CHARACTER	2	FOR FAMILY	1	BUG VALUE IS	0.0	PROBABILITY VALUE IS	0.999
DISCREPANCY AT CHARACTER	2	FOR FAMILY	2	BUG VALUE IS	0.0	PROBABILITY VALUE IS	0.999
DISCREPANCY AT CHARACTER	2	FOR FAMILY	3	BUG VALUE IS	0.0	PROBABILITY VALUE IS	0.999
DISCREPANCY AT CHARACTER	2	FOR FAMILY	4	BUG VALUE IS	0.0	PROBABILITY VALUE IS	0.999
DISCREPANCY AT CHARACTER	2	FOR FAMILY	5	BUG VALUE IS	0.0	PROBABILITY VALUE IS	0.999
DISCREPANCY AT CHARACTER	2	FOR FAMILY	6	BUG VALUE IS	0.0	PROBABILITY VALUE IS	0.999
DISCREPANCY AT CHARACTER	2	FOR FAMILY	7	BUG VALUE IS	0.0	PROBABILITY VALUE IS	0.999
DISCREPANCY AT CHARACTER	2	FOR FAMILY	8	BUG VALUE IS	0.0	PROBABILITY VALUE IS	0.999
DISCREPANCY AT CHARACTER	2	FOR FAMILY	9	BUG VALUE IS	0.0	PROBABILITY VALUE IS	0.999
DISCREPANCY AT CHARACTER	2	FOR FAMILY	10	BUG VALUE IS	0.0	PROBABILITY VALUE IS	0.999
DISCREPANCY AT CHARACTER	2	FOR FAMILY	11	BUG VALUE IS	0.0	PROBABILITY VALUE IS	0.999
DISCREPANCY AT CHARACTER	2	FOR FAMILY	12	BUG VALUE IS	0.0	PROBABILITY VALUE IS	0.999
DISCREPANCY AT CHARACTER	2	FOR FAMILY	16	BUG VALUE IS	0.0	PROBABILITY VALUE IS	0.999
DISCREPANCY AT CHARACTER	2	FOR FAMILY	27	BUG VALUE IS	0.0	PROBABILITY VALUE IS	0.999
DISCREPANCY AT CHARACTER	2	FOR FAMILY	33	BUG VALUE IS	0.0	PROBABILITY VALUE IS	0.999
DISCREPANCY AT CHARACTER	2	FOR FAMILY	34	BUG VALUE IS	0.0	PROBABILITY VALUE IS	0.999
DISCREPANCY AT CHARACTER	4	FOR FAMILY	3	BUG VALUE IS	1.0	PROBABILITY VALUE IS	0.001
DISCREPANCY AT CHARACTER	4	FOR FAMILY	4	BUG VALUE IS	1.0	PROBABILITY VALUE IS	0.001
DISCREPANCY AT CHARACTER	4	FOR FAMILY	5	BUG VALUE IS	1.0	PROBABILITY VALUE IS	0.001
DISCREPANCY AT CHARACTER	4	FOR FAMILY	6	BUG VALUE IS	1.0	PROBABILITY VALUE IS	0.001
DISCREPANCY AT CHARACTER	4	FOR FAMILY	7	BUG VALUE IS	1.0	PROBABILITY VALUE IS	0.001
DISCREPANCY AT CHARACTER	4	FOR FAMILY	9	BUG VALUE IS	1.0	PROBABILITY VALUE IS	0.001
DISCREPANCY AT CHARACTER	4	FOR FAMILY	16	BUG VALUE IS	1.0	PROBABILITY VALUE IS	0.001
DISCREPANCY AT CHARACTER	4	FOR FAMILY	30	BUG VALUE IS	1.0	PROBABILITY VALUE IS	0.001
DISCREPANCY AT CHARACTER	4	FOR FAMILY	33	BUG VALUE IS	1.0	PROBABILITY VALUE IS	0.001
DISCREPANCY AT CHARACTER	4	FOR FAMILY	34	BUG VALUE IS	1.0	PROBABILITY VALUE IS	0.001
DISCREPANCY AT CHARACTER	6	FOR FAMILY	7	BUG VALUE IS	0.0	PROBABILITY VALUE IS	0.999
DISCREPANCY AT CHARACTER	6	FOR FAMILY	31	BUG VALUE IS	0.0	PROBABILITY VALUE IS	0.999
DISCREPANCY AT CHARACTER	8	FOR FAMILY	8	BUG VALUE IS	0.0	PROBABILITY VALUE IS	0.999
DISCREPANCY AT CHARACTER	8	FOR FAMILY	9	BUG VALUE IS	0.0	PROBABILITY VALUE IS	0.999
DISCREPANCY AT CHARACTER	8	FOR FAMILY	12	BUG VALUE IS	0.0	PROBABILITY VALUE IS	0.999
DISCREPANCY AT CHARACTER	8	FOR FAMILY	32	BUG VALUE IS	0.0	PROBABILITY VALUE IS	0.999
DISCREPANCY AT CHARACTER	10	FOR FAMILY	3	BUG VALUE IS	1.0	PROBABILITY VALUE IS	0.001
DISCREPANCY AT CHARACTER	10	FOR FAMILY	4	BUG VALUE IS	1.0	PROBABILITY VALUE IS	0.001
DISCREPANCY AT CHARACTER	10	FOR FAMILY	5	BUG VALUE IS	1.0	PROBABILITY VALUE IS	0.001
DISCREPANCY AT CHARACTER	10	FOR FAMILY	6	BUG VALUE IS	1.0	PROBABILITY VALUE IS	0.001
DISCREPANCY AT CHARACTER	10	FOR FAMILY	13	BUG VALUE IS	1.0	PROBABILITY VALUE IS	0.001
DISCREPANCY AT CHARACTER	10	FOR FAMILY	16	BUG VALUE IS	1.0	PROBABILITY VALUE IS	0.001
DISCREPANCY AT CHARACTER	10	FOR FAMILY	23	BUG VALUE IS	1.0	PROBABILITY VALUE IS	0.001
DISCREPANCY AT CHARACTER	10	FOR FAMILY	26	BUG VALUE IS	1.0	PROBABILITY VALUE IS	0.001
DISCREPANCY AT CHARACTER	10	FOR FAMILY	27	BUG VALUE IS	1.0	PROBABILITY VALUE IS	0.001
DISCREPANCY AT CHARACTER	10	FOR FAMILY	28	BUG VALUE IS	1.0	PROBABILITY VALUE IS	0.001
DISCREPANCY AT CHARACTER	10	FOR FAMILY	29	BUG VALUE IS	1.0	PROBABILITY VALUE IS	0.001
DISCREPANCY AT CHARACTER	10	FOR FAMILY	31	BUG VALUE IS	1.0	PROBABILITY VALUE IS	0.001

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DISCREPANCY AT CHARACTER  12 FOR FAMILY   7   BUG VALUE IS  0.0 PROBABILITY VALUE IS  0.999
DISCREPANCY AT CHARACTER  12 FOR FAMILY   8   BUG VALUE IS  0.0 PROBABILITY VALUE IS  0.999
DISCREPANCY AT CHARACTER  12 FOR FAMILY  12   BUG VALUE IS  0.0 PROBABILITY VALUE IS  0.999
DISCREPANCY AT CHARACTER  12 FOR FAMILY  19   BUG VALUE IS  0.0 PROBABILITY VALUE IS  0.999
DISCREPANCY AT CHARACTER  13 FOR FAMILY  28   BUG VALUE IS  0.0 PROBABILITY VALUE IS  0.999
DISCREPANCY AT CHARACTER  14 FOR FAMILY   1   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  14 FOR FAMILY   3   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  14 FOR FAMILY   4   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  14 FOR FAMILY   5   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  14 FOR FAMILY   6   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  14 FOR FAMILY   7   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  14 FOR FAMILY   8   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  14 FOR FAMILY   9   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  14 FOR FAMILY  32   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  14 FOR FAMILY  33   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  14 FOR FAMILY  34   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  15 FOR FAMILY   2   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  15 FOR FAMILY   9   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  15 FOR FAMILY  16   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  15 FOR FAMILY  27   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  15 FOR FAMILY  34   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  16 FOR FAMILY   7   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  16 FOR FAMILY   8   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  16 FOR FAMILY   9   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  16 FOR FAMILY  28   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  16 FOR FAMILY  30   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  16 FOR FAMILY  33   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  21 FOR FAMILY   2   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  21 FOR FAMILY   3   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  21 FOR FAMILY   4   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  21 FOR FAMILY   5   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  21 FOR FAMILY   6   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  21 FOR FAMILY   7   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  21 FOR FAMILY   8   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  21 FOR FAMILY   9   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  21 FOR FAMILY  10   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  21 FOR FAMILY  11   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  21 FOR FAMILY  12   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  21 FOR FAMILY  13   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  21 FOR FAMILY  19   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  21 FOR FAMILY  23   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  21 FOR FAMILY  28   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  21 FOR FAMILY  29   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  21 FOR FAMILY  30   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  21 FOR FAMILY  32   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  21 FOR FAMILY  33   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  21 FOR FAMILY  34   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  22 FOR FAMILY   2   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  22 FOR FAMILY   3   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  22 FOR FAMILY   4   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  22 FOR FAMILY   5   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  22 FOR FAMILY   6   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  22 FOR FAMILY   7   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  22 FOR FAMILY   8   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  22 FOR FAMILY   9   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  22 FOR FAMILY  12   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  22 FOR FAMILY  19   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  22 FOR FAMILY  28   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  22 FOR FAMILY  29   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  22 FOR FAMILY  30   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  22 FOR FAMILY  32   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  22 FOR FAMILY  33   BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
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DISCREPANCY AT CHARACTER  22 FOR FAMILY  34    BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  23 FOR FAMILY  19    BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  23 FOR FAMILY  28    BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  23 FOR FAMILY  29    BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  23 FOR FAMILY  30    BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  23 FOR FAMILY  33    BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  23 FOR FAMILY  34    BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  24 FOR FAMILY   8    BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  24 FOR FAMILY  25    BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  24 FOR FAMILY  28    BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  24 FOR FAMILY  29    BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  24 FOR FAMILY  30    BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  24 FOR FAMILY  31    BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  24 FOR FAMILY  34    BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  26 FOR FAMILY   7    BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  26 FOR FAMILY   9    BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  26 FOR FAMILY  21    BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  26 FOR FAMILY  27    BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  26 FOR FAMILY  30    BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  26 FOR FAMILY  33    BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  26 FOR FAMILY  34    BUG VALUE IS  1.0 PROBABILITY VALUE IS  0.001
DISCREPANCY AT CHARACTER  27 FOR FAMILY  32    BUG VALUE IS  0.0 PROBABILITY VALUE IS  0.999
DISCREPANCY AT CHARACTER  27 FOR FAMILY  33    BUG VALUE IS  0.0 PROBABILITY VALUE IS  0.999
DISCREPANCY AT CHARACTER  27 FOR FAMILY  34    BUG VALUE IS  0.0 PROBABILITY VALUE IS  0.999
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FULL CHARACTERISTIC SET

FAMILY	1	2	3	4	5	6	7	8	9
LIKELIHOOD	0.198E-14	0.258E-17	0.112E-21	0.447E-22	0.242E-22	0.797E-23	0.461E-34	0.217E-25	0.667E-30
REL.LIKELIHOOD	0.619E-13	0.803E-16	0.348E-20	0.140E-20	0.753E-21	0.249E-21	0.144E-32	0.678E-24	0.208E-28

FAMILY	10	11	12	13	14	15	16	17	18
LIKELIHOOD	0.239E-13	0.191E-15	0.238E-18	0.672E-14	0.321E-01	0.235E-04	0.686E-12	0.911E-08	0.222E-03
REL.LIKELIHOOD	0.744E-12	0.596E-14	0.741E-17	0.210E-12	1.0	0.731E-03	0.214E-10	0.284E-06	0.691E-02

FAMILY	19	21	23	25	26	27	28	29	30
LIKELIHOOD	0.249E-14	0.143E-09	0.467E-11	0.337E-13	0.214E-23	0.614E-22	0.279E-30	0.900E-30	0.180E-34
REL.LIKELIHOOD	0.776E-13	0.447E-08	0.145E-09	0.105E-11	0.667E-22	0.192E-20	0.870E-29	0.280E-28	0.560E-33

FAMILY	31	32	33	34
LIKELIHOOD	0.735E-21	0.178E-23	0.117E-27	0.748E-36
REL.LIKELIHOOD	0.229E-19	0.556E-22	0.364E-26	0.233E-34