ASPECTS OF THE TAXONOMY OF BORDETELLA AND RELATED

ORGANISMS

A thesis submitted for the degree of Doctor of Philosophy in the

University of Leicester

by

Raymond Johnson, B.Sc.

January 1972

UMI Number: U386459

X753069791

This thesis, submitted for the degree of Doctor of Philosophy, is based on work conducted by the author in the M.R.C. Microbial Systematics Unit at the University of Leicester. The work was carried out during the period between October 1968 and October 1971, and all the work recorded in this thesis is original, unless otherwise acknowledged in the text or by references. None of the work has been submitted for another degree in this or any other University.

Signed __Raymond Johnson__

Date __28th January 1972.__

# CONTENTS

# SUMMARY

Two hundred and one strains of Gram-negative bacteria representing the genera of the family Brucellaceae, together with species of Neisseria, Achromobacter, Acinetobacter, Alcaligenes and Agrobacterium have been subjected to a taxonomic examination. A numerical taxonomic analysis has separated the strains into fifteen clusters. Two of the clusters consisted of composite groups each containing two genera which could not be clearly separated on the tests carried out. The homogeneity of the clusters was substantiated by results obtained on selected strains when examined for GC base ratios ( by thermal denaturation ) and similarity of protein patterns ( examined by poly-acrylamide gel electrophoresis ).

The family Brucellaceae Breed, Murray and Smith 1957 contains a heterogeneous collection of genera which have little in common apart from often requiring special media for growth and in being potentially pathogenic.

The results of this study suggest that the Brucellaceae should be sub-divided into two families,

1) Pasteurellaceae Pribram 1933 or Fam. nov. This would contain the genera Haemophilus, Pasteurella and Actinobacillus. The close similarity between Pasteurella and Actinobacillus is recognised and it is suggested that further work on these two genera may reveal that they can be combined · in a single genus.

2) Brucellaceae Breed, Murray and Smith 1957 emend. This family would contain only three genera, Brucella, Bordetella and Alcaligenes.

Difficulties were found in separating oxidase-positive, motile Alcaligenes species from Bordetella bronchiseptica, although there is little evidence for separating B. bronchiseptica from the other species of Bordetella. Further work may suggest combining Alcaligenes and

Bordetella.

Oxidase-negative, non-motile Alcaligenes species should not be included in the genus Alcaligenes. Results from this study indicate that they should be transferred to the species Acinetobacter lwoffii.

Definitions for the families Pasteurellaceae and Brucellaceae are proposed.

INTRODUCTION

Among the families of bacteria listed in Bergey's manual, several
represent aggregations of genera, which at best, can only be described as
"loose associations". Instead of encompassing organisms which overall
are closely related, they have arisen or have been proposed because the
genera contained therein have one or two prominent characters in common.
Such a group is the Brucellaceae or its immediate ancestor the Parvobact-
eriaceae. This family represents the particular niche for those Gram-
negative, rod-shaped bacteria which require rich media for growth and
are primarily responsible for many human and animal infections.

For all their economic and medical importance, the Brucellaceae
has been sorely neglected by the taxonomist. No doubt their potential
pathogenicity and the difficulties of working with these organisms provide
excellent reasons for this. Many tend to die out or grow poorly on media
used for biochemical tests, while the addition of blood to the medium to
increase the amount of growth only serves to mask results which are in the
form of colour changes, zones of clearing or precipitation. Nevertheless
there has now been sufficient progress made in studies of the nutritional
requirements of the Brucellaceae, for a well-based study to be
undertaken.

This thesis deals with certain aspects of the taxonomy of
the genus Bordetella and related organisms. The related organisms
chosen for the study were the other members of the Brucellaceae and
also, those genera in which Bordetella species have been included at
one time or another.

In recent years there has been a great deal of interest
in applying objective methods to a field which has been traditionally
subjective in its interpretation of results. The development of
numerical taxonomy, with the advent of electronic computers, has
meant that the great mass of results that are obtained in a taxonomic

study can be analysed much more quickly and more systematically than ever before.

Sneath (1957a, 1957b), in a philosophical appraisal of taxonomy, reached four main conclusions. "First, that the ideal classification is the one which has the greatest content of information, Secondly, that overall similarity was the basic concept of such ideal classification and that it is measured in terms of the number of similar features possessed by two organisms. Thirdly, that every feature should have equal weight. Fourthly, that division into taxonomic groups is made upon correlated features". There has been a great deal of confusion and discussion over the use of terms such as ideal or natural when applied to classifications. Sneath (1962) and Rohlf (1964) distinguish between phylogenetic and cladistic classifications and classifications which are phenetic (a term introduced by Cain and Harrison (1960) to distinguish classifications based on overall similarity). Both types are natural classifications in their own way, yet are based on different approaches. For example, if the evolutionary relationships between organisms are known, then a cladistic classification will reflect these relationships in forming taxonomic groups. However, if the groups are based on overall similarity, without reference to phylogeny, they will be natural phenetic groups. The absence of fossil records and the simple morphology of bacteria eliminates any phylogenetic basis of bacterial taxonomy. It is therefore necessary to make use of phenetic evidence. This evidence is obtained by taking a large number of strains, referred to as operational taxonomic units or OTUs, and recording numerous characters of each. The overall similarity between each OTU is calculated, and those OTUs having high mutual similarity are grouped into clusters or phenons. Sokal and Sneath (1963) review the

mathematical coefficients which have been used to estimate taxonomic resemblance and also the methods of cluster analysis. Having obtained the groups, they can be represented diagramatically in the form of a hierarchical tree or dendrogram (Sneath, 1962). By using various mathematical procedures such as principle co-ordinates analysis, taxometric maps and multidimensional models, which represent the spatial arrangements of the groups, can be constructed (Carmichael and Sneath, 1969).

Numerical taxonomy has been used to good effect on a wide variety of bacteria and the results have provided real encouragement for its further use throughout bacterial taxonomy. Generally the results have been in agreement with those of existing classifications while, at the same time, throwing additional light on organisms which appear to be wrongly identified.

Another technique which is proving to be a very useful aid to numerical taxonomy is that of DNA base composition. This is reviewed by Hill (1966) and Rosypal and Rosypalova (1966). Various procedures have been devised for the measurement of GC ratios (i.e. the guanine-cytosine content in moles per cent). Isolation of the DNA is carried out according to the method of Marmur (1961), or a modification of it. Marmur and Doty (1962) have derived equations for calculating the GC ratio of purified DNA from its thermal denaturation temperature. The GC ratio can also be determined from buoyant density studies of DNA in caesium chloride gradients (Schildkraut, Marmur and Doty, 1962). More recently, modifications of these formulae, and the association between buoyant density and thermal denaturation temperatures have been derived (De Ley, 1970; Mandel, Igambi, Bergendahl, Dodson Jnr., and Scheltgen, 1970).

The GC ratio has proved a valuable tool in pointing out

species or strains which have been incorrectly classified. Closely
related organisms have very similar GC ratios, so that strains which
differ greatly in GC content cannot be closely related (Sueoka, 1961;
De Ley, 1969). The reverse, however, is not true because strains which
have identical GC ratios need not be closely related e.g. streptococci
and haemophili. GC ratios only indicate that two strains are
taxonomically different, they do not indicate which strains should
be grouped together. When taken in conjunction with the results
obtained from numerical taxonomy, however, GC ratios act as a check on
the homogeneity of the groups.

The success achieved by numerical taxonomy and DNA base
composition has stimulated research into other techniques which might
have applications in microbial taxonomy. Poly-acrylamide gel
electrophoresis is one of the most promising areas of research. The
basic assumption is that closely related organisms will have similar
proteins, and consequently they will give similar protein "patterns"
on electrophoresis. The results have been encouraging in the cases
of Bacillus thuringiensis, Microbacterium spp., Corynebacterium spp.
and group D streptococci, but less encouraging when applied to
streptomycetes. Norris (1968) reviews some of the literature. At
the present time, however, a deeper understanding of the problems
is needed. It is difficult to compare the patterns obtained from
different strains, (Rouatt, Skyring and Purkayastha, 1970; Johnson
and Thein, 1970) and even more difficult to understand what each
pattern represents. The results so far obtained are, nevertheless,
stimulating enough to justify further work.

For the taxonomist with an interest in innovations such as
numerical taxonomy, DNA base composition and gel electrophoresis, the
genus Bordetella and the Brucellaceae form a challenging area of study

because they are poorly classified. Bergey's manual (Breed, Murray and Smith, 1957) for example, places undue emphasis on pathogenicity and the type of growth media required, while offering little information on biochemical reactions.

The purpose of this thesis is to try and place the taxonomy of the Brucellaceae, and particularly Bordetella, on a firmer foundation. The more objective methods, already outlined, have been used to see whether they could be applied successfully in a taxonomic study and to see whether the results obtained would show a good correlation. As will be seen later, a considerable measure of success has been achieved.

CHAPTER   1


Literature Review of the Genus   Bordetella

## History and Nomenclature

The first recorded evidence of the connection between bacilli and whooping cough appears to be that of Burger (1883) who noted the presence of large numbers of small ellipsoidal rods in stained films of the expectoration from clinical cases. Other authors, (Affanassjew, 1887; Koplik, 1897; Jochmann and Krause, 1901, 1902; Wollstein, 1905; and others) claimed to have isolated bacteria from cases of whooping cough. The descriptions that are given, however, make it quite clear that they did not manage to isolate the whooping cough bacillus because their isolates grew readily on simple media or on blood agar.

The bacillus was eventually isolated by Bordet and Gengou (1906) using a potato-glycerine blood agar. They described the organism in some detail and gave evidence showing it to be the causative organism of the disease. Further work by Bordet and Gengou (1907, 1909), Fraenkel (1908), Arnheim (1909), Seiffert (1909) and Klimenko (1909) confirmed the earlier report of Bordet and Gengou. It is apparent from these papers that none of these authors gave the organism a legitimate latin binomial. The identity of the author(s) who first validly published the name of this organism is still a matter of considerable doubt. Breed, Murray and Smith (1957) attribute the species to Holland (1920) but this is clearly invalid because Holland admits that her list was only an index of reference and was not intended to deal with problems of specific identity. Lessel (1970) confirms that names published by Holland are not to be regarded as validly published. Buchanan, Holt and Lessel (1966) give two names which they state were validly published and are legitimate. The first is Bacillus tussis-convulsivae Affanassjew 1887. However,

Affanassjew did not isolate the whooping cough bacillus. Koplik (1897) isolated an organism, from a case of whooping cough, which he considered to be identical to the one isolated by Affanassjew but this bacillus was motile and grew anaerobically. These are not properties found in the pertussis bacillus. Migula (1900) described Bacillus pertussis, which he attributed to Affanassjew (1887), but this organism was able to grow on gelatin and grew anaerobically. Clearly, there is considerable doubt over the validity of Bacillus tussis-convulsivae Affanassjew 1887.

The other name cited by Buchanan, Holt and Lessel is that of Haemophilus pertussis Pribram 1933. This name is not entirely satisfactory. Pribram incorrectly attributes the genus Haemophilus to Castellani and Chalmers (1919) and, although this does not invalidate the name, he mentions H. pertussis only in the list of strains maintained in the Vienna Microbiological collection. The organism is attributed to Bordet (1906) and not, as it should have been, to Bordet and Gengou (1906). Buchanan, Holt and Lesell, however, consider that Pribram had validly published the name Haemophilus pertussis. Two points arise from this decision. Ferry and Noble (1918) used the term Bacterium pertussis for the whooping cough bacillus. They did not attribute the organism to Bordet and Gengou (1906) so presumably the species epithet, pertussis in this case, is regarded as a later homonym of Bacillus pertussis Migula 1900. Migula's organism was not the pertussis bacillus, so use of the epithet pertussis by Ferry and Noble would be illegitimate. More important is the fact that Bergey (1923) published a description of an organism, Haemophilus pertussis (Bordet and Gengou) Committee Society of American Bacteriologists, which is clearly the whooping cough bacillus. It appears that the Committee attributed the epithet pertussis to Bordet and Gengou, which is incorrect.

However, is this really any different from Pribram (1933, P68) who merely gives the label H.pertussis Bordet (1906)? Buchanan, Holt and Lessel do not comment on this anomaly.

The nomenclature of Bordetella parapertussis is much clearer. Eldering and Kendrick (1938) described and named an organism which they had discussed but not named or described, in an earlier paper (Eldering and Kendrick, 1937). This organism was named Bacillus para-pertussis. The authors concluded that this species together with Bacillus pertussis and Bacillus bronchisepticus should be in the same genus, but this genus should not be Haemophilus. For convenience, they chose the generic name Bacillus.

The organism now known as Bordetella bronchiseptica was isolated by Ferry (1910) and believed to be the causative organism of canine distemper. Ferry called this organism Bacillus bronchicanis. In a later paper, Ferry (1912), the name was changed to Bacillus bronchisepticus because the organism was able to produce disease in other animals as well as dogs. This argument is invalid under rule 23 of the International Code of Nomenclature of Bacteria (1966) which states that: " a legitimate name or epithet must not be rejected merely because it is inappropriate or disagreeable, or because another is preferable or better known, or because it has lost its original meaning". However, in the sixty years which have elapsed since Ferry first used the epithet bronchicanis, it has been used by few authors, a notable exception being Haupt (1935). It has become almost universally known as bronchiseptica, so that changing the name back to bronchicanis would undoubtedly cause much confusion. There are several cases where the Judicial Commission has accepted this line of argument; for example, the species epithet rhusiopathiae is

conserved over insidiosa (basionym) and, against all other specific
epithets, applied to Erysipelothrix rhusiopathiae (Opinion 32, 1970).
Conserving the name bronchisepticus Ferry 1912 over bronchicanis Ferry
1910 would not be unprecedented.   In this thesis the use of the
commoner name is continued for convenience.

Since the original isolation of the whooping cough bacillus,
the three species of the genus Bordetella have been placed, either
separately or en masse, in a variety of genera.   These include
Bacillus (Ferry 1911), Bacterium (Ferry and Noble 1918), Haemophilus
(Holland 1920), Alcaligenes (Bergey 1925), Brucella (Topley and Wilson
1929), Pasteurella (Pribram 1933), Bordetella (López 1952) and
Acinetobacter (Steel and Cowan 1964).   Several of these genera can be
easily discarded.   The type species of the genus Bacillus is a gram
positive, spore-forming rod.   The genus is based on the fact that all
its species produce heat-resistant endospores and, since no species of
Bordetella has ever been shown to be gram positive, or to form spores,
they cannot logically be placed there.

The revised version of Opinion 4 (1954) issued by the
International Committee on Bacteriological Nomenclature states:   "The
bacterial generic name Bacterium Ehrenberg 1828 is to be recognised as
a nomen genericum rejiciendium".   This has been accepted and
consequently the use of the generic name Bacterium is illegitimate.

There is little evidence to support the inclusion of species
of Bordetella in the genus Pasteurella.   Bordetella species are obligate
aerobes which do not attack carbohydrates and are responsible for respiratory
tract infections.   Pasteurella species are facultative anaerobes which
produce acid from carbohydrates and are responsible for septicaemic
infections.   Their GC ratios are sufficiently different (Bordetella 67-70%,
Pasteurella 36-39%) for them to have little common DNA and, finally,

there have not been any reports of genetic transfer between the two genera or of the existence of common antigens.

Many authors have placed Bordetella species in the genus Haemophilus. B. pertussis was originally included in this genus by Holland (1920). This was hardly surprising since the definition of Haemophilus proposed by the Committee: Society of American Bacteriologists (1917) was sufficiently vague as to allow not only the inclusion of Bordet-Gengou's bacillus but also, the Morax-Axenfeld bacillus (Moraxella lacunata). The vague nature of the definition led to considerable controversy over the taxonomic positions of these organisms. Wilson and Miles (1946, 1961) took the general view that if the genus was confined to species which required haematin or nicotinamide adenine dinucleotide or both, then organisms which did not require these growth factors would be excluded even though they were otherwise closely related. Wilson and Miles saw no reason why the genus should not be redefined to include B. pertussis, M. lacunata and B. bronchiseptica. As early as 1922 Kristensen had expressed strong opposition to the vague definition of the genus Haemophilus, on the grounds that species like the Bordet-Gengou bacillus and the Morax-Axenfeld bacillus should not be included. In a comparison between B. pertussis and Haemophilus influenzae, Kristensen points out the cultural and morphological differences. B. pertussis grows much more slowly on laboratory media. Except in the case of old laboratory strains, the growth is poor or non-existent on haemoglobin agar, Fildes' agar, Levinthal agar or heated blood agar. There are no satellitism effects shown in mixed cultures, a phenomenon common to H. influenzae. The cell morphology of B. pertussis is much more uniform, being normally a coccobacillus or short rod. B. pertussis is also more hardy to storage on agar media at 5°C. Fildes (1923) agreed with Kristensen, and recently work on the

growth of B. pertussis (Rowatt, 1957b) has thrown more light on the
nutritive dissimilarities between this organism and other Haemophilus
species.   H. influenzae requires blood in culture media because blood
contains haematin (X factor) and nicotinamide adenine dinucleotide
(V factor), important for cytochrome synthesis and consequently energy
production via the electron transport chain.   B. pertussis requires
blood only because of its ability to remove toxic substances (e.g.
organic peroxides) from growth media.   Blood can be replaced by starch,
charcoal or anionic exchange resins such as Dowex 1-X8 (British Drug
Houses Ltd.).   Bordetella species are obligate aerobes while Haemophilus
species are facultative anaerobes.

Further evidence which suggests that these two groups should
be separated comes from an examination of their DNA base composition.
Hill (1966) quotes examples of Haemophilus spp. having GC ratios of
38-42 moles per cent.   The few examples of Bordetella (Bacon,
Overend and Lloyd, 1967;  De Ley, 1968), show that they have a GC ratio
of 67-70 moles per cent.   Bacon, Overend and Lloyd also quote a value
of 57.5 moles per cent for a mixture of DNA from two strains of
B. pertussis.   This odd result can be dismissed because the organisms
concerned have now been re-identified as Brucella species, not
B. pertussis.

With regard to differences in biochemical tests Stillman and
Bourn (1920) showed that 119 strains of H. influenzae produced acid from
glucose, although sometimes irregularly and sixty three per cent were
indole positive.   Both B. pertussis and B. bronchiseptica produce
alkali from sugar media, by attacking the peptone and not the
carbohydrate.   They are both indole negative.

Very little comparative work has been carried out on the

Table 1          Characters of Bordetella species

| Character | B. pertussis | B. para-pertussis | B. bronchi septica |
|---|---|---|---|
| Morphology | minute coccobacilli | minute coccobacilli | minute coccobacilli |
| Motility | - | - | + at 12-24°C |
| Gram reaction | - | - | - |
| Growth on nutrient agar | - | + | + |
| Alkali produced in litmus milk | + (12-14d) | + (2-4d) | + (1-2d) |
| Indole reaction | - | - | - |
| Production of acid from sugars | - | - | - |
| Growth on citrate as sole carbon source | - | + | + |
| Nitrate reduction | - | - | + (often) |
| Urease | - | + (often) | + |
| Catalase | + | + | + |
| Oxygen requirements | aerobic | aerobic | aerobic |
| Optimum temperature | 35-37°C | 37°C | 37°C |

Key:          + positive reaction;          - negative reaction

(12-14d)  positive reaction in 12-14 days

serology of B. pertussis and H. influenzae, but little, if any, common
antigens have been found (Odaira, 1911; Kristensen, 1922). Finally,
Zinnemann (1967) believes that species without requirements for X or
V factors, or other coenzyme-like substances, should not be included in
the genus Haemophilus. This would exclude any Bordetella species from
this genus.

Having removed Bacillus, Bacterium, Pasteurella and Haemophilus
from the list of genera which may be suitable for the inclusion of
pertussis, parapertussis and bronchiseptica, we are left with a choice
of Bordetella, Alcaligenes, Acinetobacter or Brucella. Exclusion
from the latter three genera appears to be more difficult to justify
and is largely the motivation for the present work. The relationships
of Bordetella to these genera will be considered in more detail later.
However, before this can be done effectively, the relationships that
the three species have for each other should be clearly defined.


Morphological and Biochemical Relationships

Breed, Murray and Smith (1957) define the genus Bordetella
as follows:

" Minute coccobacilli. Motile and non-motile species occur. Gram
negative. On primary isolation some species are dependent on complex
media; all are haemolytic. Carbohydrates are not fermented. Litmus
milk becomes alkaline. A dermonecrotic toxin is produced. Parasitic.
Cause whooping cough or an infection clinically resembling it.

The type species is Bordetella pertussis (Holland) Moreno-López. "

The information the authors provide on each species is
summarised in Table 1.

Miller, Saito and Silverberg (1941) found that Bordetella

parapertussis was morphologically indistinguishable from B. pertussis.
Lautrop (1954) states that B. pertussis is more coccoid than
B. parapertussis although, after continual subculture, the cells of
B. pertussis are longer than at first isolation. Lautrop also found that
B. pertussis is frequently capsulated, whereas B. parapertussis never has
a capsule. Szturm and Bourdon (1948) could find no evidence of capsular
materials on B. bronchiseptica but Evans and Maitland (1939) found that
this species did have a similar capsule to B. pertussis. B. bronchiseptica
is the only motile species, although motility occurs only at 18-24°C and
not at 37°C. Szturm and Bourdon found that motility was achieved by
lophotrichous flagella.

On artificial media, B. parapertussis and B. bronchiseptica
initially grow much more rapidly than B. pertussis. Lautrop (1954) points
out that apart from growth rate, there are no other differences between
the colonial appearances of B. pertussis and B. parapertussis. All
three species produce an alkaline reaction when growing in media containing
peptone. On most media B. parapertussis, but not the others, produces
a soluble brown pigment thought by many workers to be due to the production
of inorganic iron salts from organic iron. Ensminger (1953), however,
showed that pigment production was dependent upon the presence of
tyrosine in the medium, which is converted into a melanin-like pigment.

Biochemical results have been published for species of
Bordetella, although little has been recorded for B. pertussis. The
more commonly recorded characters are shown in Table 1.

Portwood (1946) and Lautrop (1954) have both found that B. pertussis
tends to have little or no catalase activity although it is recorded as
positive by Breed, Murray and Smith (1957), in Bergey's manual.
Brumfitt (1959) points out that B. pertussis requires either catalase or
haematin for growth from small inocula and, in common with

Haemophilus influenzae, this species was unable to synthesis its porphyrin requirements from substances simpler than protoporphyrin IX. Even this substances is much less efficient than haematin.

Piéchaud and Szturm- Rubinstein (1965) found that B. parapertussis was oxidase negative, whereas B. bronchiseptica was oxidase positive. Other workers have found B. bronchiseptica to be unable to liquify gelatin, and to break down asparagine (Evans, 1918); aesculin negative, lysine decarboxylase positive (Farkas-Himsley, 1963); hydrogen sulphide production negative, methyl red negative, acetoin negative, reduction of methylene blue variable (Szturm and Bourdon, 1948).

## Isolation and Nutritional Requirements

These are excellently covered in a review by Rowatt (1957b). Bordetella species do not have complex nutritional requirements; for example, B. bronchiseptica grows abundantly on nutrient agar in twenty four hours, and Miller, Saito and Silverberg (1941) have found that B. parapertussis would grow on infusion agar on first subculture. On the other hand, B. pertussis has always required a very rich medium for its isolation. Those usually employed have been Bordet-Gengou medium or modifications thereof (Bailey, 1933; Madsen, 1937; Silverthorne and Cameron, 1942). Other media which have been used include those made selective for B. pertussis and B. parapertussis by the addition of penicillin and 4 : 4-diamidino-diphenylamine dihydrochloride (M and B 938), (Nicholson and Turner, 1954) and the DPF medium of Lacey (1960). More recently the charcoal blood agar produced by Oxoid Limited has found much favour and this can also be rendered selective by the addition of penicillin and M and B 938.

Table 2     Comparison of Synthetic and Semi-synthetic Media which

have been used for the cultivation of virulent

Bordetella pertussis  (concentrations in g/litre)

| Constituent | Hornibrook (1939) | Cohen and Wheeler (1946) | Verwey et al. (1949) | Sutherland and Wilkinson (1961) | Wilson (1963) | | Stainer and Scholte (1971) |
|---|---|---|---|---|---|---|---|
| | | | | | Synthetic | Semi-Synthetic | |
| Casein Hydrolysate | 7.0 | 10.0 | 14.0 | 10.0 | 1.6[1] | 10g | 0.91[2] |
| Starch | 1.0 | 1.5 | 1.0 | - | - | 1.0 | - |
| Yeast Extract | optional | 50ml dialysate | - | - | - | - | - |
| Glutathione | 0.01 | - | 0.01 | 0.01 | 0.025 | 0.025 | 0.1 |
| cysteine | - | 0.025 | - | - | - | - | - |
| L-cysteine | - | - | - | - | - | - | 0.04 |
| niacin | - | - | 0.02 | 0.02 | 0.001 | 0.001 | 0.004 |
| NaCl | 5.0 | 2.5 | - | - | 2.5 | 5.0 | 2.5 |
| KCl | 0.2 | - | 0.2 | - | 0.2 | - | 0.2 |
| $CaCl_2$ | 0.2 | 0.01 | - | 0.1 | 0.02 | - | 0.02 |
| $MgCl_2,6H_2O$ | 0.1 | 0.4 | 0.1 | 0.4 | 0.1 | - | 0.1 |
| $Na_2CO_3$ | 0.5 | - | - | - | - | - | - |
| Na or K phosphate | 0.25 | 0.5 | 0.5 | 0.5 | 0.5 | 1.0 | 0.5 |
| $FeSO_4,7H_2O$ | - | 0.01 | - | 0.01 | 0.01 | - | 0.01 |
| $CuSO_4,5H_2O$ | - | 0.0005 | - | - | 0.005 | - | - |
| tris buffer | - | - | - | 3.0 | 6.075 | - | 6.075 |
| Dowex | - | - | - | 1.0 | - | - | - |
| Charcoal | - | - | - | - | 0.2 | - | - |
| Growth factors | - | - | - | - | 0.227[3] | - | 0.020[4] |
| $H_2O$ | 1000 ml | 1000 ml | 1000 ml | 1000 ml | 1000ml | 1000 ml | 1000ml |

Key :     1  an amino acid mixture ;     2  L-proline and L-glutamic acid only;   3   mixture of nucleic acid and derivatives, ascorbic acid, ATP and liver coenzyme concentrates;   4   ascorbic acid.

Several synthetic or semi-synthetic media have been developed (see Table 2) but they have not been used for initial isolation of B. pertussis.   The media of Hornibrook (1939), Cohen and Wheeler (1946), Verwey, Thiele, Sage and Schuchardt (1949), Sutherland and Wilkinson (1961), Wilson (1963) and Stainer and Scholte (1971), have all achieved degrees of success in keeping freshly isolated strains of B. pertussis in the smooth form.

Pollock (1947), Proom (1955), Jebb and Tomlinson (1955, 1957), Rowett (1957a) and Turner (1961) have all pointed out that it is not the fastidious nutritional requirements of B. pertussis that make it difficult to grow, but its high sensitivity to toxic substances which are present in the growth media.   The inhibitory substances are of three kinds:

1.   Unsaturated fatty acids such as oleic, linoleic and linolenic acids. These occur in cotton wool plugs and in certain media constituents.

2.   Autoclaved cysteine, cystine and colloidal sulphides.

3.   Organic peroxides.

Unsaturated fatty acids can be eliminated by using capped containers and protein hydrolysates which are low in fatty acids, e.g. casamino acids.

Sulphur containing amino acids should be sterilised by filtration and not by autoclaving.   Woiwod (1954) showed that when cysteine is autoclaved in complex media, colloidal sulphur is formed and, in addition, colloidal copper sulphide if traces of copper ions are present.   Proom (1955) reported that B. pertussis was inhibited by these two substances but neither B. parapertussis nor B. bronchiseptica showed any sensitivity.

Peroxides in the medium may originate from two sources. Growing bacteria produce hydrogen peroxide which they eliminate by

the production of catalase.    More important are the peroxides formed
when media are autoclaved.    These include both hydrogen peroxide
and organic peroxides.    The organic peroxides are not readily
broken down by the presence of catalase or cysteine and consequently
inhibit growth.    It has been found that they can be removed by the
addition of red blood cells, haemin, filtered ferrous sulphate,
charcoal, albumen or starch.    Dawson, Farnworth, Mcleod and
Nicholson (1951) found that serum could be used to replace blood, but
it was less efficient.

Kuwajima, Matsui and Asano (1955) described a medium
containing an anionic exchange resin which they had used for the
production of toxin from phase I Haemophilus pertussis.    In a later
paper, Kuwajima, Matsui and Kishigami (1957) reported that most anionic
exchange resins allowed growth of phase I H. pertussis, and those resins
having the highest number of divinyl benzene cross links gave the
best results.    The growth promoting effect was not produced by
cationic exchange resins, nor by anionic resins in which the ion-
exchanging capacity has been destroyed.    Neither glass pieces, glass
beads nor sand increased growth.    It was important that the resin
was present in contact with the cells during growth since no growth took
place if the resin was removed from the medium before inoculation, nor
when the resin was separated from the cells by a cellophane membrane.
Sutherland and Wilkinson (1961) confirmed much of this work and also
found that the resin was more effective than starch for removing toxic
substances.    These authors also found that their medium could be
solidified with chloroform defatted New Zealand Agar but plates had to
be poured at $45^{\circ}C$ to maintain an even dispersal of the resin throughout
the solidified agar.

Bordetella pertussis has an absolute requirement for nicotinic

acid, a source of carbon, energy and starch (Rowatt, 1957b). The requirement for nicotinic acid is common to all species of Bordetella, while the requirement for starch seems to be peculiar to virulent strains of B. pertussis. James (1949a, 1949b) found that virulent strains grown in a liquid medium for twelve days, hydrolysed twenty per cent of the starch present. Avirulent strains did not break down starch, neither did two strains of B. parapertussis. Sutherland and Wilkinson (1961) found that an anionic exchange resin was more efficient than starch for increasing growth rate. When starch and resin are both included in the medium, the growth rate is faster than in a medium containing resin alone. However, after 10 days incubation, there is little difference in optical density of both media.

## Metabolism

All three species of Bordetella are obligate aerobes. Energy is obtained via the tricarboxylic acid cycle and the electron transport chain (Fukumi, Sayama, Tomisawa and Uchida, 1953). Fujita and Kodama (1934) found that a degraded strain of B. pertussis contained cytochromes a, b, c, $d_1$ and $d_2$. Fukumi et al. found that the cytochromes of the three species were identical with a main absorption peak at 552 m$\mu$. Sutherland (1963) identified two cytochromes in all three species, one he called cytochrome 553 and the other cytochrome 550. He suggested that they may play a role in the oxidation and reduction of azurin, a blue, copper-containing protein identified by Sutherland and Wilkinson (1963) and found in Bordetella, Alcaligenes and Pseudomonas. It is a water-soluble, auto-oxidisable protein having a molecular weight of 14,600 and a high

redox potential (+395 mV).    These authors considered that it may act as an alternative electron transport system existing between a cytochrome c and cytochrome oxidase.

None of the species of Bordetella oxidises or ferments any of the usual sugars, though the presence of nucleic acids and polysaccharides in cell constituents indicates that these organisms must be able to carry out metabolic reactions involving carbohydrates. By the use of labelled compounds, Inamdar, Bundeally and Ganapathi (1965a, 1965b) were able to show that B. pertussis did not metabolise sugars and no evidence could be found for the presence of kinase or permease  enzymes for sugars.    Krebs' cycle intermediates are oxidised and some carbon moieties become incorporated into cell proteins, lipids and nucleic acids.    Acetate is incorporated into lipid and stimulates growth.    Pyruvate and citrate were not incorporated but were oxidised. Succinate, fumarate and malate were all metabolised, malate appearing in the lipid fraction.    Of the nucleic acid derivatives, only guanine and uracil were incorporated.    The amino acids, glutamic acid, glycine, L- and D-alanine, and serine were all extensively taken up and appeared throughout the cell constituents, while leucine,iso-leucine, valine and phenylalanine were oxidised with subsequent loss of the carboxyl group.

The major metabolic activities of Bordetella appear to be concerned with the oxidation of amino acids (Rowatt, 1957b;  Goldner, Jakus and Wilson, 1962;  Goldner, Jakus, Rhodes and Wilson, 1966). Glutamic acid and proline are the preferred amino acids, followed by alanine, glycine and aspartic acid, which are taken up usually when the concentration of glutamic acid and proline in the medium has fallen.    Jebb and Tomlinson (1951) found that their virulent strain of B. pertussis did not utilise glycine, but most other workers have found that glycine is

metabolised fairly rapidly.   Rowatt (1955) found that whereas
Bordetella pertussis and B. parapertussis would deaminate only glutamate,
aspartate, serine, glycine, alanine and proline, B. bronchiseptica, in
addition to these, was able to deaminate threonine, valine, methionine,
leucine, iso-leucine and phenylalanine.   Neither histidine, lysine
nor arginine were metabolised by any species.

Vajdic, Goldner and Wilson (1964, 1966) correlated amino
acid uptake with the growth curve of Bordetella pertussis.   They
found that glutamic acid, glutamine, proline and glutathione disappeared during
the exponential phase.   Alanine, cystine, glycine, serine, histidine,
aspartic acid, threonine, tryptophan, tyrosine and cysteine disappeared
in the stationary phase.   They explained the pattern of utilisation in
terms of proline and glutamine being converted to glutamic acid during
the log phase.   This occurs until the immediate source of glutamic
acid is exhausted and then the other amino acids are taken up.
Glutamic acid is deaminated to $\alpha$-ketoglutaric acid and, from this, energy
is obtained via the tricarboxylic acid cycle.   The results of Vajdic,
Goldner and Wilson confirm the position of glutamic acid as a principal
source of carbon, nitrogen and energy in the genus Bordetella.


Serology

Bordet and Gengou (1906) and Bordet and Sleeswyk (1910) found
that Bordetella pertussis existed in one of two antigenic states.
Strains freshly isolated on a medium such as Bordet-Gengou agar belong
to a narrow homologous group.   Those strains within the group will
agglutinate to a high titre in an antiserum produced against any one
of them and are said to be in Bordet's state I.   Culture on an

unsuitable medium, however, or even continual growth on a medium
such as Bordet-Gengou agar, eventually leads to smooth to rough variation,
with a consequent change in antigenic make up, to Bordet's state II.

Krumweide, Mishulow and Oldenbusch (1923) used agglutination
and agglutinin-absorption tests and found that their strains of
B. pertussis fell into two groups; A and B. Unlike Bordet and Gengou,
and Bordet and Sleeswyk, these authors could not correlate the antigenic
type with the nature of the culture medium. They found that recent
isolates fell into group B, while old stock cultures were found to have
representatives of both groups. It is doubtful whether the groups
A and B of Krumweide, Mishulow and Oldenbusch correspond to Bordet's
states I and II since all the strains of B. pertussis used by Krumweide
and his colleagues were grown on chocolate agar, making it likely that
they were in the rough phase, or Bordet's state II.

Leslie and Gardner (1931) found that 32 strains of B. pertussis
fell into one or other of four well-marked agglutinative groups:
phases I, II, III or IV. Phases I and II were considered to represent
the smooth phase, and phases III and IV represent the rough phase.
Phase changes from I to IV occur on continuous subculture, but may be
to a large extent reversible. The authors found, however, that some
phase IV cultures could not be induced to revert back to phase I. Leslie
and Gardner compared their results with Bordet's and Krumweide's
records and found that they were in agreement, providing that Bordet's
state I corresponded to their phase I and that his second state,
together with Krumweide's type B, was their phase III. Krumweide's
type A corresponded completely with Leslie and Gardner's phase IV.

Great difficulty has been found in identifying the four

Table 3          Agglutination reactions of the three phases of <u>B. pertussis</u>
                 identified by Flosdorf et al. (1941)

| antiserum | antigen | | |
|-----------|:---:|:---:|:---:|
| phase | I | III | IV |
| I | ++ | + | − |
| III | − | ++ | + |
| IV | − | + | ++ |

phases of Leslie and Gardner. Toomey, Ranta, Robey and McClelland
(1935) could not distinguish between phases III and IV, and they concluded
that it was impractical to group B. pertussis into sharply defined
phases. Flosdorf, Dozois and Kimball (1941) agreed largely with
Leslie and Gardner, but found that there was more variation in minor
relationships between the phases than Leslie and Gardner had found.
They were unable to identify Leslie and Gardner's phase II. The
results of their cross-agglutination experiments are summarised in
Table 3. On the basis of these results and cross absorption, Flosdorf
and his colleagues postulated the simplest antigenic structure of
. B. pertussis as being:

| phase | antigens |
|-------|----------|
| I | a, (b, c, minor) |
| III | b (major), c or d |
| IV | c, d (major) |

Like most gram negative bacteria, the genus Bordetella has
somatic, capsular and flagella antigens corresponding to the O, K and
H antigens of the Enterobacteriaceae. Bordetella bronchiseptica is the
only motile species and therefore the only one with H antigens.

Andersen (1953) found that freshly isolated strains of
B. pertussis could be divided serologically on the basis of K antigens.
She also showed that all Bordetella species have the same O antigen,
the same haemorrhagic toxin, and common, as well as species-specific,
K antigens. Andersen chose the term "K antigens" for the relatively
thermolabile antigens whose agglutinating, agglutinin-absorbing and
O agglutination-inhibiting powers were destroyed by heating at $120^{\circ}C$
for 60 minutes. She found that the K antigens could not be

Table 4    Antigenic structure of Four strains of Bordetella

(Andersen, 1953)

| strain | antigen | | | | | | |
|---|---|---|---|---|---|---|---|
| | O | | K | | | | |
| B. pertussis  3865 | I | a | | | e | f | (h) |
| B. parapertussis  21353 | I | | b | | e | | g (h) |
| B. bronchiseptica  7306 | I | | | c | e | f | g |
| B. bronchiseptica    458 | I | | | | d (e) | | (g) |

Key:  ( ) indicates partial antigens, which have one-sided antigenic
    relationships at low titres.   For example, B. pertussis absorbed
    with B. bronchiseptica agglutinates B. parapertussis, but
    B. parapertussis absorbed with B. bronchiseptica does not agglutinate
    B. pertussis.

differentiated on the basis of thermoresistance, but could be separated by absorbing antisera against living cells with culture which had been heated at 100°C for two hours (to remove O antigen); and then cross-absorbing with formalin-killed cultures. Using this technique, Andersen was able to identify five K antigens, designated 1 to 5, in six strains of B. pertussis. Three strains of B. parapertussis had identical O antigens and K antigens. Three strains of B. bronchiseptica had a common O antigen and combinations of two out of three K antigens. The antigenic formulae of the species of Bordetella are shown in Table 4.

Eldering, Hornbeck and Baker (1957) obtained similar results to those of Andersen. They identified 14 heat labile K antigens, which they numbered 1-14. All Bordetella species contained factor 7. Factors 1-6 were specific for B. pertussis. Factor 14 was specific for B. parapertussis, while factor 12 was specific for B. bronchiseptica. Factors 8, 9 and 10 were found in both B. parapertussis and B. bronchiseptica and help to explain some of the relationships between the two species. The heat stable antigens of the three species were identical and showed only quantitative differences.

Preston and Te Punga (1959) identified seven capsular antigens numbered 1, 2, 3, 4, X, Y, Z, in five strains of B. pertussis. Antigen 1 was common to all five strains and consisted of at least two factors, one of which was species-specific and the other shared with B. bronchiseptica. Antigens 2, 3 and 4 are identical to Andersen's 2, 3 and 4 K antigens. Antigens X, Y and Z are probably identical to some of the more numerous factors described by Eldering, Hornbeck and Baker (1957).

Although Andersen was not able to differentiate K antigens on the basis of thermoresistance, Kasuga (1957) designated two capsular

antigens, L and S, based on this property. L antigen was destroyed by heating at $100^{\circ}$C for 60 minutes. S antigen was more thermostable and resisted $100^{\circ}$C for two hours, but not $120^{\circ}$C for one hour. It appears that the K antigens of the previous authors are to be found in the L and S groups of Kasuga. Kasuga was also able to identify antigenic phases equivalent to those of Leslie and Gardner (1931). Kasuga identified three phases of which phase I was identical to phase I of Leslie and Gardner. Phases II and III of Leslie and Gardner were equivalent to the intermediate phase of Kasuga. Phase IV of Leslie and Gardner was renamed phase III by Kasuga.

Further work by Kasuga and Nakase has contributed greatly to current knowledge on the antigenic structure of Bordetella parapertussis and B. bronchiseptica. Nakase (1957a) and Kasuga, Ukishima and Nakase (1958) found that both species had definite capsules. In a series of papers, Nakase (1957a, b, c, d) was able to distinguish three smooth phases and one rough phase of B. bronchiseptica. These phases corresponded to the phases found by Kasuga (1957) in B. pertussis and also to the phases found by Kasuga, Ukishima and Nakase (1958) in B. parapertussis.

Phase I is the virulent phase. All species are capsulated and possess a haemagglutinin, high toxicity and protective antigenicity. The capsule inhibits O agglutination. In phase I, B. bronchiseptica has short peritrichous flagella.

Phase II strains of Bordetella are characterised by a thinner capsule. There are few antigenic changes and O agglutination is only weakly inhibited by the capsule.

There is no capsule present in phase III strains. Phase III is subdivided into phase III-1 and phase III-2 which are characterised by antigenic differences. In phase III, B. bronchiseptica has long peritrichous flagella.

Table 5        Antigenic Analysis of the genus <u>Bordetella</u>

(Kasuga and Nakase 1960)

| | B. pertussis | B. para-pertussis | B. bronchiseptica | |
| | | | guinea· pig strains | canine strains |
|---|---|---|---|---|
| Characteristic O antigen | 01 | 04 | 07 | 07 |
| Common L antigen | a, | a, | a, | a, |
| <u>Phases I and II</u> | | | | |
| L antigens | a,b,c | a,d | a,b | a,b |
| S antigens | 1 | 2 | 2,3 | 2,3 |
| O antigens | 01 | 02,04,(08) | 01,05,07 | 01,07,08 |
| <u>Phase III-1</u> | | | | |
| O antigens | 01 | 02,03,04, (08) | 01,(03), 07 | 01,(03), 07 |
| <u>Phase III-2</u> | | | | |
| O antigens | 01 | 03,04 | (03),05 07 | (03),07, 08 |

(  ) indicates a partly common antigen

Nakase points out that B. bronchiseptica changes from phase I to phase III very easily, with loss of capsular antigens and a part of the O antigen. Incubating cultures at 22°C or on poor media soon results in phase variation, but it is easily reversible by culturing on blood agar at 37°C.

The serological results obtained by Kasuga and Nakase show several differences to those obtained by Andersen and by Eldering, Hornbeck and Baker. These are accounted for by differences in the preparation techniques used to obtain factor sera. Kasuga and Nakase (1960) pointed out that Andersen prepared K antisera against B. bronchiseptica by absorbing the sera against living cultures with cultures which had been heated at 120°C for one hour. This leaves L, S and H antibodies in the absorbed sera. The L and S antibodies are not differentiated from each other by this method. Andersen also prepared K antisera against B. pertussis and B. parapertussis by absorbing sera against living cultures with cultures which had been heated at 100°C for 2 hours. The heated cultures still contained S and O antigens, so that the absorbed sera contained only L antibodies. Andersen prepared O antisera from heated cultures, whereas Kasuga and Nakase used unheated phase III cultures.

By using cross-agglutination and cross-absorption techniques, Kasuga and Nakase were able to subdivide the L, S and O antigens of the genus Bordetella. Their results are summarised in Table 5. In addition to the O antigen shown in Table 5, Kasuga and Nakase indicate that there is a common O antigen for the genus, but they do not give any details.

Lacey, in a series of papers (1951, 1953a, b, c, 1960 and 1961) has pointed out another phenomenon which occurs when Bordetella species

are grown on artificial media. For this, Lacey coined the term
antigenic "modulation". The antigenic structure of these organisms
is markedly influenced by the temperature of growth and the ionic
composition of the medium. Lacey named three antigenic "modes",
the X mode (xanthic), the I mode (intermediate) and the C mode (cyanic).
At $37^{o}C$ and with sodium chloride as the chief salt present in the
medium, colonies are in the X mode. At $25^{o}C$ on any medium or at
$37^{o}C$ with magnesium sulphate as the chief salt present, colonies are
in the C mode. The I mode occurs under conditions intermediate
between those leading to X and C modes.

The modes are not genetically determined because each is
highly reversible and reproducible. There is no selection of mutants
because modal changes occur throughout the population. Each mode
has a distinctive antigenic character, but with considerable overlap.
It is, however, possible to produce specific antisera for each mode.
Lacey points out that in the three species no single environment has
been found which will allow the simultaneous development of all the
potential antigens of any one strain.

In the X mode, cells have agglutinable X antigen on the
surface. This antigen is proteinaceous and capsular in origin, and
corresponds roughly with the K antigen of Andersen (1953). In the
C mode, only O antigens are present in B. pertussis and B. parapertussis
whereas B. bronchiseptica has O and H antigens. Strains of
B. bronchiseptica which are strongly in the X mode are not flagellated,
whereas those strains which are showing signs of moving towards the I
mode develop flagella. The X antigen is destroyed by heating at
$120^{o}C$; this also affects the specificity of the underlying O antigens
and at the same time appears to create a common O antigen for the genus.
This obviously explains why Andersen, and later Eldering, Hornbeck and
Baker, thought that there was only one O antigen and that it was common

to all species.    Lacey (1961) gives a table of agglutinable antigens
of Bordetella species (Table 2, page 352), in which he indicates the
existence of nine O antigens in the three species.    He does not,
however, provide experimental details.    Earlier, Lacey (1953a) had
found that all freshly isolated strains could be divided into three
principal O groups.    B. pertussis belonged to O group I;  B. parapertussis
and some strains of B. bronchiseptica belonged to O group II;  the rest
of the B. bronchiseptica strains belonged to O group III.    These
results show similarities to those of Kasuga and Nakase in that no
common O antigens were found for B. pertussis and B. parapertussis,
whereas there were common O antigens between B. parapertussis and
B. bronchiseptica.

Other Antigenically active fractions isolated from the genus Bordetella

In a serological review Munoz (1963) points out that
Bordetella pertussis has many antigens distributed throughout its
morphological elements,    the following of which have been recognised:

1.   agglutinogen

2.   heat-labile toxin

3.   heat-stable toxin or endotoxin

4.   haemagglutinin

5.   histamine-sensitising factor (HSF)

6.   protective antigen (PA)

Agglutinogen:    This appears to be a surface antigen loosely
associated with the cell wall.    It is easily extracted by washing cells
in saline (Maitland and Guerault, 1958).    At first it was thought to be
a nucleoprotein, but Onoue, Kitazawa and Yamamura (1961) found it to

be a simple protein of molecular weight 10,000. Many workers
(Evans and Maitland, 1937; Munoz, 1963; Schuchardt, Munoz, Verwey
and Sagin, 1963) have found it non-toxic and unable to confer protection
to mice against intra-cranial challenge by virulent strains of
B. pertussis. It has no histamine sensitising activity.

Haemagglutinin: This is either a surface substance or is liberated
from the cells as it is found in the supernatant of liquid cultures,
particularly during early growth. It is found in most virulent
strains of B. pertussis and also in B. parapertussis (Keogh, North and
Warburton, 1947; Ungar and Stevens, 1951). Avirulent strains of
B. pertussis do not produce haemagglutinin.

Heat Labile Toxin: This toxin is lethal and dermonecrotic. It is
found in the cell protoplasm and released on lysis (Munoz, Ribi and
Larson, 1959; Munoz, 1963); it is mainly proteinaceous in nature
(Banerjea and Munoz, 1962). Billaudelle et al. (1960) and Munoz (1963)
found that the heat-labile toxin had no protective activity or
histamine sensitising capabilities. However, Matosuoka, Yamamura
and Kitagawa (1965) found that their toxin had protective activity when
injected intra-cranially or intra-peritoneally. Evans and Maitland
(1939) were unable to demonstrate any antigenic effect, but Andersen
(1953) showed that with intensive immunisation procedures, an antitoxic
serum could be produced. Both these authors, and Bruckner and Evans
(1939) found that the toxins of all species of Bordetella were very
similar in their reactions to heat, formalin and filtration. They
produce identical lesions in infected animals, but the potency of the
toxin obtained from B. parapertussis is lower than the potencies of the
toxins from the other two species. The toxins of all three species
are neutralised by antitoxin produced against any one of them.

Heat Stable Toxin:    In common with that of many gram negative
bacteria, the endotoxin is a lipopolysaccharide (Maclennan, 1960;
Munoz, 1963).    It does not confer protection or sensitise to
histamine (Matosuoka, Yamamura and Kitagawa, 1965).    It is pyrogenic.
Nakase, Tateisi, Sekiya and Kasuga (1970) obtained an O antigen which
was pyrogenic and had an adjuvant effect on antibody production and
toxicity, but did not confer protection.    Maclennan (1960) isolated
specific lipopolysaccharides with warm aqueous phenol, from all three
species of Bordetella.    Each lipopolysaccharide contained an aldoheptose,
a hexose and hexosamine.    The endotoxin of B. bronchiseptica also
contained a small amount of galactosamine.    The lipopolysaccharides
were pyrogenic and differed in serological specificity.    B. pertussis
endotoxin was non-lethal and had a higher properdin activity than those
of the other three species.    The authors noted that serological
changes occur in the endotoxins when virulent strains become avirulent.
Ross, Munoz and Cameron (1969) obtained similar results.    They
extracted endotoxins from several strains of B. pertussis and found
them to be identical.    However, they were serologically distinct
from endotoxins extracted from B. parapertussis and B. bronchiseptica.

Protective Antigen and Histamine-Sensitising Factor:    Both these
substances are located in the cell wall (Munoz, 1963;  Billaudelle et
al., 1960;  Munoz, Ribi and Larson, 1959) and they can be extracted
from sonically disrupted cells.    Most workers believe that PA and HSF
are very closely associated, if not identical;    this view is not
supported by Griffiths and Mason (1964).    Sato and Nagase (1965)
sonicated cells of phase I B. pertussis and fractionated the sonicate
by differential centrifugation.    Theyisolated two components, 21S and
41S, and identified the 21S fraction as the protective antigen.    It

Table 6          Differential Characters of the Genus Bordetella

(After Moreno López 1952)

| | B. pertussis | B. para-pertussis | B. bronchi-septica |
|---|---|---|---|
| Cell Morphology | cocco-bacilli | cocco-bacilli | cocco-bacilli |
| Gram Reaction | - | - | - |
| Haemolysis on Bordet-Gengou Agar | + | + | + |
| Acid from Carbohydrates | - | - | - |
| Indole Reaction | - | - | - |
| Acetoin | - | - | - |
| Production of Alkaline reaction in growth media | + | + | + |
| Growth on agar without blood | - | + | + |
| Pigment from Tyrosine | - | + | - |
| Motility | - | - | + |
| Urease production | - | + | + |
| Growth on citrate as Sole carbon source | - | + | + |
| Nitrate reduction | - | - | + |

stimulated production of K agglutinins, but did not react with them. The 21S fraction was present in both pase I and phase II strains but there was no protective activity in the phase III strains. Ross, Munoz and Cameron (1969) found that whole cell vaccines and extracts of B. pertussis contained protective antigen and a histamine sensitising factor, whereas no HSF was found in B. parapertussis or B. bronchiseptica. Cells and saline extracts of B. pertussis or B. parapertussis protected against challenge by B. bronchiseptica, but cell and saline extracts of B. parapertussis or B. bronchiseptica did not protect against challenge by B. pertussis. These results are slightly different from those obtained by Eldering (1942) and Kendrick, Nadolski, Eldering and Baker (1953), who found that B. parapertussis gave very slight or no protection against challenge by B. pertussis, while B. bronchiseptica gave slight to good protection against challenge by B. pertussis.

A summary of the properties that suggested to Moreno López (1952) and Pittman (1955) that these three species should form the genus Bordetella, are shown in Table 6. These authors also pointed out that the three species have a common O antigen and a common dermonecrotic toxin. The toxin is neutralised by antitoxin produced against any of the species. There are also common, as well as species-specific, thermolabile capsular antigens. All three species produce infections of the respiratory tract, but B. pertussis and B. bronchiseptica are more virulent than B. parapertussis when tested by intra-cerebral inoculation.

Relationships between  Bordetella  and  Alcaligenes

Several authors have included species of <u>Bordetella</u> in the genus <u>Alcaligenes</u> (Bergey, 1925; Haupt, 1935; Hugh, 1953) and it is beyond question that <u>B. bronchiseptica</u> has many properties in common with <u>Alcaligenes faecalis</u>.

There has been a great deal of confusion over the legality of the genus <u>Alcaligenes</u> because the original description of Castellani and Chalmers (1919) was not very detailed.  They gave the following definition for the tribe Ebertheae, in which they had placed <u>Alcaligenes</u>: "Bacillaceae, growing well on ordinary media;  non-sporing;  aerobic and facultatively anaerobic;  non-fluorescent;  no pigment formation; gelatin negative;  no polar staining;  Gram negative and non capsulated".

<u>Alcaligenes</u> species do not clot milk or ferment glucose and lactose.  Alkaline reactions are produced in sugar media and in milk.  Castellani and Chalmers included three species in the genus: <u>A. metalcaligenes</u> which was non-motile;  <u>A. faecalis</u>, motile and did not attack sugars;  and <u>A. vivax</u> which was motile and produced acid from galactose and mannitol.  The type species is <u>A. faecalis</u>.

Conn, Wolfe and Ford (1940) thought that the limits of the genus <u>Alcaligenes</u> needed careful study as they had found numerous soil and milk organisms that differed only slightly from the intestinal inhabitant which is the type species.  The original description of <u>A. faecalis</u> by Petrushky (1896) makes it clear that it is a peritrichous, non-sporing rod of intestinal origin, producing no acid in sugar broths and causing alkalinity in milk.  Conn, Wolfe and Ford examined 22 strains· of <u>A. faecalis</u>, most of which agreed with Petrushky's description. Examination of strains obtained from other laboratories and culture collections, however, showed that there was disagreement over the identity of this species.  Some strains were non-motile, the rest

showed variable flagellation. About half reduced nitrate to nitrite.
Conn (1942) refuted his earlier suggestions that soil and intestinal
forms were similar, on the basis that the soil forms were able to grow
well on a synthetic medium containing glucose and could produce gas
from glucose. He placed the soil forms in a new genus, Agrobacterium
gen.nov. and, having decided that the definition of Alcaligenes faecalis
was vague, he suggested that the genus Alcaligenes should be retained
only if the type species could be recognised.

Hugh (1953), in an unpublished thesis, examined 282 strains
of gram negative rods. He divided them into three units based on
the ability to utilise dextrose. One hundred and twenty strains did
not utilise dextrose and these were divided into five groups based on
flagella arrangement. Group I contained the peritrichous non-oxidisers
such as Bordetella bronchiseptica, A. faecalis, A. denitrificans and
six strains which produced acid from xylose. Hugh agreed with
Conn (1942) that Petrushky's description of A. faecalis was too vague
to re-identify the organism. The description did fit B. bronchiseptica,
so Hugh placed B. bronchiseptica in the genus Alcaligenes in agreement
with Bergey (1934).

The lophotrichous strains were found in group III. They had
long been considered as A. faecalis, but Hugh placed them in a new genus,
Lophobacter. Presumably these are the same strains that were included
in Lophomonas gen.nov. (Galarneault and Leifson, 1956).

Group V contained the non-motile strains, most of which Hugh
labelled Alcaligenes viscosus, and also contained Bordetella parapertussis.

Moore and Pickett (1960) also placed heavy emphasis on
flagella arrangement when they examined 40 strains of gram negative rods

which did not utilise glucose. They also, questioned the validity of the genus _Alcaligenes_ because few of their strains resembled the original description and these strains could be included in the genus _Achromobacter_. Moore and Pickett agreed with Brisou (1953) in incorporating the species of _Alcaligenes_ into _Achromobacter_.

Holding (1960) placed little emphasis on flagellation. In a study of soil bacteria, he included all gram negative rods which did not oxidise glucose and were yellow or non-pigmented, in the genus _Alcaligenes_.

Hendrie, Hodgkiss and Shewan (1964) found that the genera _Achromobacter_ and _Alcaligenes_ fell into two morphological types, motile and non-motile - both types being found in each genus. The only apparent difference between the genera was the ability of _Alcaligenes_ to produce an alkaline reaction in litmus milk. However, De Ley, Bain and Shewan (1967), in a study of 300 strains of gram negative coccobacilli, found that the motile strains of _Achromobacter_ and _Alcaligenes_ were quite distinct from the non-motile strains. In addition, examination of the GC ratios of the motile strains showed a range of 56-70 moles per cent, indicating that they could not all belong to the same genus. Nevertheless, both Hendrie, Hodgkiss and Shewan (1964) and De Ley, Bain and Shewan (1967) favoured retaining temporarily, the genus _Achromobacter_ for the motile strains; while the non-motile strains could be placed in the genera _Acinetobacter_, _Moraxella_ or _Alcaligenes_.

It may well be possible that a great deal of the confusion that exists over the legality of the genus _Alcaligenes_ has arisen because too much emphasis has been placed on flagella arrangement and the source of isolation. It is quite possible that flagellation is a

variable character in <u>Alcaligenes</u>.    This would not be altogether
unusual as variation in flagella arrangement has been reported in
<u>Chromobacterium</u> (Sneath, 1956), <u>Aeromonas</u> (Stevenson, 1959), plant
pathogenic corynebacteria (Lelliot, 1966) and <u>Rhizobium</u>.

There is a tendency to combine the motile strains of
<u>Alcaligenes</u> and <u>Achromobacter</u> in a single genus, but one asks is this
necessarily a wise move?    In <u>A. faecalis</u> we now have a well-documented
species which could easily remain as the type species of a redefined
genus <u>Alcaligenes.</u>    This genus would contain aerobic, gram negative,
motile rods, which are oxidase positive;  have no action on
carbohydrates;  produce an alkaline reaction in litmus milk and have
a GC ratio in the range 65-70 moles per cent.

The genus <u>Achromobacter</u> is in the position of not even
having a recognisable type species on which a definition of the genus
can be based.    This group may correspond to the "Achromobacter 58%"
group of De Ley (1969), and contain aerobic, motile;  gram negative
rods which may or may not oxidise glucose and which have a GC content
of 56-61 moles per cent.

The non-motile strains of <u>Achromobacter</u> and <u>Alcaligenes</u>
appear to fit in the genus <u>Acinetobacter</u> Brisou and Prévot (1954).
Results reported in this thesis show that the non-motile species of
<u>Alcaligenes</u> have a GC ratio identical to <u>Acinetobacter</u> and they are
closely related to the non-saccharolytic <u>Acinetobacter lwoffi</u>.

However, regardless of the genus that <u>Alcaligenes faecalis</u>
is placed in, its existence as a species is well established;  and so
are the many characters that it has in common with <u>Bordetella bronchiseptica</u>.
Both organisms are gram negative coccobacilli or short rods.    They are
not usually pleomorphic.    They do not grow anaerobically, but will grow
aerobically on all the usual laboratory media.    Both species are

motile and non-pigmented. They are catalase positive; oxidase positive; produce ammonia from peptone; usually reduce nitrates to nitrite and produce an alkaline reaction in litmus milk. They do not produce acid from carbohydrates; and are methyl red negative and Voges-Proskauer negative. Gelatin is not liquefied. The only common difference between them is that A. faecalis is usually urease negative while B. bronchiseptica is urease positive. Ulrich and Needham (1953) report that A. faecalis grows on a simple glucose-inorganic salts medium, whereas B. bronchiseptica does not. Both species have a GC ratio in the region 67-70 moles per cent. As yet however, there have not been any published reports which show the presence of common antigens or genetic interchange.

Ambler (1968) has compared the amino acid sequences of azurins obtained from Pseudomonas fluorescens, Bordetella bronchiseptica, Alcaligenes denitrificans and A. faecalis. Each azurin differed from each of the others in about one-third of the amino acid positions, and the similarities and differences are distributed throughout the polypeptide chain. Although amino acid sequences may have some future role to play in taxonomy, Ambler's results did not indicate that Alcaligenes and Bordetella were any more closely related to each other than they were to Pseudomonas fluorescens.

Relationships between <u>Bordetella</u> and <u>Acinetobacter</u>

Acinetobacter Brisou and Prévot was proposed for the non-motile members of the genus Achromobacter, with type species Acinetobacter anitratum.

The genus contains non-pigmented, non-motile, gram negative short rods or coccobacilli, which are often capsulated. They grow well on all the usual laboratory media. Certain species are often found in clinical specimens from human urethritis, sinusitis and in urine. Litmus milk is turned feebly acid, or alkaline, or there is no change. Brisou and Prévot listed several species and differentiated them on the following characters: reduction of nitrate, gelatin liquifaction, ability to produce acid from carbohydrates, indole reaction and hydrogen sulphide production.

Further papers by Brisou (1957a, 1957b) left the definition of the genus virtually unchanged, but Baumann, Doudoroff and Stanier (1968b) consider it should contain only oxidase negative strains. Extensive reviews of the genus can be found in Rockwood (1962), Controni, Ballard and Griffith (1964), Henderson (1965), Gilardi (1967), Thornley (1967) and Baumann, Doudoroff and Stanier (1968b).

Steel and Cowan (1964) proposed that Bordetella parapertussis should be included in the genus Acinetobacter, their reason being that B. parapertussis is non-motile and oxidase negative. They also thought that the three species of Bordetella were not closely related, but the authors failed to provide any evidence to support breaking up the genus. There have not been any reports of antigenic cross reactions, similarities of clinical symptoms, or of genetic interchange between B. parapertussis and Acinetobacter, so that the evidence for including B. parapertussis in the genus Acinetobacter is very slim.

Relationships between <u>Bordetella</u> and <u>Brucella</u>

Topley and Wilson (1929) did not agree with Bergey (1925) in placing Brucella melitensis, Br. abortus and Br. bronchiseptica in the genus Alcaligenes, on the grounds that Bacillus alkaligenes (A. faecalis) was a larger organism, which had rather different cultural conditions and was often found in the intestinal tract. Topley and Wilson placed these three species in the genus Brucella Meyer and Shaw 1920; although they realised that objections may be raised against removing Br. bronchiseptica from Alcaligenes because of its motility. They also argued against it being placed with the whooping cough bacillus because the growth requirements of the two species were different.

Br. bronchiseptica was included in Brucella because it resembled the type species Br. melitensis in both individual and colonial morphology, and in its inability to ferment carbohydrates. However, Wilson and Miles (1961) recognised the conspicuous degree of antigenic similarity between Br. bronchiseptica and Haemophilus pertussis and included them both in the genus Haemophilus; at the same time, revising the definition of the genus. The position was further amended when Wilson and Miles (1964) recognised the genus Bordetella in which H. pertussis and H. bronchiseptica had been placed, together with H. parapertussis by Moreno López (1952).

Both the genera Bordetella and Brucella tend to give weak positive or negative reactions in many of the usual biochemical tests. There is a marked preference to metabolise amino acids rather than carbohydrates. It has already been mentioned that in Bordetella, glutamic acid and proline are the preferred amino acids; followed by alanine, glycine and aspartic acid, when the concentrations of glutamate and proline have fallen. Gerhadt and Wilson (1948) found that in Brucella DL-asparagine was the best nitrogen source, although L-glutamate and L-histidine may be utilised instead. Rowatt (1955) found that

Table 7         Comparison of Brucellae and Bordetella bronchiseptica

| Character | Brucellae | B. bronchi-septica |
|---|---|---|
| Catalase | + | + |
| Oxidase | (+) | + |
| Nitrate reduction | (+) | + |
| Urease | (+) | + |
| Growth on citrate as sole carbon source | - | + |
| Hydrogen Sulphide production | variable | - |
| Litmus Milk | - | alkaline |
| Acid from glucose | - | - |
| Liquefaction of Gelatin | - | - |
| Indole reaction | - | - |
| Methyl Red | - | - |
| Acetoin | - | - |

Key:  (+) most species give a positive reaction

L-histidine was not metabolised by B. bronchiseptica. According to Meyer and Cameron (1958) Br. suis is unable to metabolise L-asparagine and utilises L-glutamate and L-aspartic acid, slowly. All species were, however, able to oxidise D- and L-alanine rapidly. Other amino acids were used at varying rates according to the strain and the species.

Meyer and Cameron (1958) and Pickett (1955) showed that unlike Bordetella, the brucellae could oxidise carbohydrates. The rates of utilisation were slow and have to be determined manometrically as conventional carbohydrate media give negative results (Jones, 1968). Other characters of brucellae given by Jones (1968) are shown in Table 7 in a comparison with Bordetella bronchiseptica.

It is easy to see from Table 7 why Bordetella bronchiseptica had been included in the brucellae by Topley and Wilson. Apart from the alkaline reaction in litmus milk, there are few differences in the results of tests which have been carried out on both groups. More recently, the difficulties of identifying species within the genus Brucella have been recognised and a number of newer methods have been introduced. These include dye sensitivity (Pickett, Nelson, Hoyt and Eisenstein, 1952), resistance to phage (Jones, 1960), and utilisation of carbohydrates tested by manometric techniques (Pickett, 1955; Meyer and Cameron, 1958). Many of these tests have not been applied to B. bronchiseptica but, even if the results were negative, it would not be enough to eliminate it from the genus Brucella as several of the Brucella species also give negative results; for example, Br. melitensis and Br. ovis are not lysed by brucellaphage (Brinley Morgan and Gower, 1966).

There have not been any reports of antigenic relationships between brucellae and Bordetella. This suggests a more distant

relationship between them because all Brucella species agglutinate
in Brucella antiserum, showing the presence of common antigens.
Br. canis is anomolous in only agglutinating in antisera prepared
against rough strains of Brucella (Jones, 1968).

The International Sub-Committee on the Taxonomy of Brucella
issued the following definition of the genus (Stableforth and Jones,
1963): "Small, non-motile, non-sporing, gram negative coccobacilli.
Grow rather poorly on ordinary media or may require special media.
Aerobic, no growth under strict anaerobic conditions. Growth often
improved by $CO_2$. Little fermentative activity on carbohydrates in
usual media. Urea hydrolysed to a variable extent. Parasites
occurring in animals and producing characteristic infections in animals
and man".

The committee recommended a change in the definition
(Jones, 1966); the last sentence to read: "Facultative intracellular
parasites which produce acute and chronic infections in animals and
which may cause disease in man. The generalised infection is followed
by localisation which, in animals, is predominantly in the mammary
gland and reproductive tract".

It is now possible to see that B. bronchiseptica does not
appear to fit in with this definition. It is motile and never requires
special growth media or $CO_2$. Furthermore, B. bronchiseptica is not
an intracellular parasite and when causing infection, it does not
localise in the reproductive tract.

Hoyer and McCullough (1968) have also provided evidence of
distant relationships between B. bronchiseptica and the brucellae.
They found that Brucella canis has similar polynucleotide sequences in
DNA-DNA homology studies to those of the other Brucella species. There
was no detectable homology between Brucella and Bordetella bronchiseptica.

The few reports that have appeared on the DNA base composition of <u>Brucella</u> have given values of 55-58 moles per cent GC (Hill, 1966). These are substantially different from those of <u>Bordetella bronchiseptica</u> at 67-70 moles per cent.

The evidence available suggests that <u>Bordetella bronchiseptica</u> is not a member of the genus <u>Brucella</u>, yet there are definite morphological,colonial and biochemical similarities between them which cannot be overlooked.

CHAPTER 2

Materials and Methods

## Strains

Two hundred and one strains were selected for study and these are listed in Table 8. Type strains, as indicated by Sneath and Skerman (1966) or the American Type Culture Collection (1970), were included as far as possible. Recent isolates accounted for about 40 strains.

On receipt, all strains were subcultured onto agar and stained by Gram's method to check for purity. After a minimum of subculture, they were freeze-dried on a Speedivac centrifugal freeze drier (Model 5PS, Edwards High Vacuum Limited).

Stock cultures were maintained on one of three growth media and subcultured twice weekly; any cultures showing evidence of contamination were rejected and a new freeze-dried ampoule opened. A careful check was kept to ensure that strains did not undergo any serious degree of rough variation, or other change, during the subculturing. This check was reinforced by discarding all stock cultures after 20-25 subcultures and opening a new set of freeze-dried ampoules.

Table 8    List of strains used in the study

| Author's Strain Number | Strain Received as | Received from | Donor's Number | Type Status (if any) |
|---|---|---|---|---|
| J1 | Alcaligenes denitrificans | NCTC | 8582 | holotype[1] |
| J2 | A. odorans | NCTC | 10416 | holotype[1] |
| J3 | A. faecalis | NCTC | 8764 | |
| J4 | "      " | NCTC | 655 | |
| J5 | "      " | NCTC | 8769 | |
| J6 | "      " | NCIB | 8156 | |
| J7 | "      " | CCEB | 591 | |
| J8 | A. viscosus | NCTC | 3233 | |
| J9 | "      " | NCIB | 8154 | proposed working type[1] |
| J10 | "      " | NCIB | 8596 | |
| J11 | A. metalcaligenes | NCIB | 9018 | |
| J12 | "      " | NCIB | 9021 | |
| J13 | "      " | NCIB | 8734 | |
| J17 | Agrobacterium tumefaciens | NCPPB | 397 | |
| J18 | Agrobacterium sp. | MJT | ZH1 | |
| J19 | Actinobacillus equuli | NCTC | 8529 | proposed working type[1] |
| J20 | Act. lignieresii | NCTC | 4975 | |
| J21 | "      " | NCTC | 4976 | proposed working type[1] |
| J23 | Acinetobacter anitratus | NCTC | 8102 | |
| J24 | A. anitratus | ATCC | 14290 | |
| J25 | A. calco-aceticus | ATCC | 23055 | |

Table 8 (continued)

| Author's Strain Number | Strain received as | Received from | Donor's number | Type Status (if any) |
|---|---|---|---|---|
| J26 | A. haemolysans | ATCC | 17988 | |
| J27 | A. lwoffii | NCTC | 5866 | proposed neotype[1] |
| J28 | Acinetobacter sp. | MJT | F4/6/20 | |
| J29 | Acinetobacter sp. | Baumann | 19 | |
| J30 | Achromobacter sp. | MSU | – | |
| J31 | A. haemolyticus subsp. alcaligenes | NCTC | ·10306 | holotype[2] |
| J32 | A. haemolyticus | NCTC | 10305 | holotype[1] |
| J33 | A. citroalcaligenes | NCTC | 10308 | proposed neotype[2] |
| J34 | A. conjuctivae | NCTC | 10304 | holotype[1] |
| J35 | A. mucosus | NCTC | 10303 | proposed neotype[1] |
| J36 | A. liquefaciens | ATCC | 15716 | |
| J37 | A. lacticus | NCIB | 8208 | |
| J38 | A. anitratus | NCIB | 9212 | paratype[1] |
| J39 | "         " | NCIB | 9019 | |
| J40 | "         " | NCIB | 9301 | |
| J41 | Achromobacter sp. | NCIB | 8250 | |
| J42 | Bordetella pertussis | NCTC | 9797 | proposed working type[1] |
| J43 | "         " | NCTC | 8189 | |
| J44 | "         " | NCTC | 8631 | |
| J45 | Haemophilus pertussis | IID | 18-323 ph1 | |
| J46 | "         " | IID | Tohama ph.1 | |
| J47 | "         " | IID | Yamapaki ph.1 | |
| J48 | "         " | IID | Opaki ph.1 | |
| J49 | "         " | IID | Maeno ph.1 | |
| J50 | "         " | IID | Kawarada ph.1 | |
| J51 | "         " | IID | Tohama ph.III | |

Table 8 (continued)

| Author's Strain Number | Strain Received as | Received from | Donor's Number | Type status (if any) |
|---|---|---|---|---|
| J52 | Haemophilus pertussis | IID | Sakairi ph.111 | |
| J53 | " " | IID | Sugata ph.111 | |
| J54 | Bordetella parapertussis | NCTC | 5952 | cotype[1] |
| J55 | " " | ATCC | 15237 | |
| J56 | " " | ATCC | 15989 | |
| J57 | " " | ATCC | 9305 | |
| J58 | Bordetella bronchiseptica | MSU | – | |
| J59 | " " | NCTC | 452 | cotype[1] |
| J60 | " " | NCTC | 8344 | |
| J61 | " " | ATCC | 19 | |
| J62 | " " | ATCC | 780 | |
| J63 | " " | ATCC | 785 | |
| J64 | " " | ATCC | 786 | |
| J65 | " " | ATCC | 4617 | |
| J66 | " " | ATCC | 10580 | |
| J67 | " " | ATCC | 12714 | |
| J68 | " " | ATCC | 14064 | |
| J69 | " " | ATCC | 14065 | |
| J70 | " " | ATCC | 15101 | |
| J71 | " " | ATCC | 14455 | |
| J72 | Haemophilus bronchisepticus | IID | M12 | |
| J73 | Haemophilus aegyptius | NCTC | 8502 | proposed working type[1] |
| J74 | " " | ATCC | 11116 | |
| J75 | H. aphrophilus | NCTC | 5886 | holotype[1] |
| J78 | H.haemoglobinophilus | NCTC | 1659 | cotype[1] |
| J79 | " " | Zinneman | – | |

Table 8 (continued)

| Author's Strain Number | Strain Received as | Received from | Donor's Number | Type Status (if any) |
|---|---|---|---|---|
| J80 | H. haemolyticus | NCTC | 8479 | proposed working type[1] |
| J83 | H. influenzae | NCTC | 4560 | proposed working type[1] |
| J86 | "        " | ATCC | 9006 a | |
| J87 | "        " | NCTC | 8645 a | |
| J88 | "        " | NCTC | 7279 b | |
| J89 | "        " | NCTC | ·8469 c | |
| J90 | "        " | NCTC | 8470 d | |
| J91 | "        " | NCTC | 8472 e | |
| J92 | "        " | NCTC | 7918 f | |
| J93 | H. suis | NCTC | 4557 | cotype[1] |
| J94 | "    " | CAPM | 5111 | |
| J95 | "    " | CAPM | 5113 | |
| J99 | H. parainfluenzae | NCTC | 4101 | |
| J100 | "        " | ATCC | 7901 | |
| J101 | "        " | ATCC | 9796 | |
| J102 | H. gallinarum | CAPM | 5015 | |
| J103 | "        " | CAPM | 5066 | |
| J104 | "        " | CAPM | 5080 | |
| J108 | H. parasuis | CAPM | 5114 | |
| J120 | H. aphrophilus | NCTC | 5906 | holotype[3] |
| J121 | H. paraphrophilus | NCTC | 10557 | holotype[4] |
| J125 | Haemophilus sp. | CAPM | P392 | |
| J128 | Moraxella bovis | NCTC | 8561 | proposed working type[1] |
| J129 | "        " | NCTC | 9425 | |
| J130 | "        " | NCTC | 9426 | |

Table 8 (continued)

| Author's Strain Number | Strain Received as | Received from | Donor's Number | Type Status (if any) |
|---|---|---|---|---|
| J131 | Morax. liquefaciens | NCTC | 7911 | working type |
| J132 | Morax. lwoffii | NCTC | 5867 | |
| J133 | "           " | NCTC | 7976 | |
| J134 | Morax. lacunata | NCTC | 7784 | proposed working type[1] |
| J136 | Mima polymorpha var. oxidans | ATCC | 10973 | |
| J137 | Morax. lacunata | H | 7911/SS | |
| J139 | Morax. bovis | H | 9425/SS | |
| J140 | Morax. osloensis | H | A1920 SS(S) | holotype[2] |
| J141 | Morax. phenylpyrouvica | H | 2863 SS | neotype |
| J142 | Morax. polymorpha | H | 17958 | holotype[2] |
| J143 | Morax. kingii (kingae) | NCTC | 10529 | holotype |
| J144 | Morax. ovis | H | 199/55 SS | |
| J145 | Moraxella sp. | MSU | - | |
| J146 | Neisseria caviae | NCTC | 10293 | holotype |
| J147 | N. flavescens | NCTC | 8263 | holotype[1] |
| J148 | N. catarrhalis | NCTC | 3622 | |
| J149 | "           " | NCTC | 4103 | |
| J150 | N. pharyngis "flavus" | NCTC | 4590 | |
| J152 | N. pharyngis "siccus" | NCTC | 4591 | |
| J153 | Noguchia granulosis | ATCC | 11479 | |
| J154 | Brucella abortus | Wey | 19 biotype 1. | |
| J158 | "           " | Wey | 64/51 biotype 9 | |
| J159 | Br. melitensis | Wey | Rev.1 biotype 1 | |

Table 8 (continued)

| Author's Strain Number | Strain Received as | Received from | Donor's Number | Type Status (if any) |
|---|---|---|---|---|
| J161 | Br. suis | Wey | Thomson biotype 1 | |
| J162 | "      " | Wey | 63/258 biotype 1 | |
| J165 | Br. canis | Wey | RM6/66 | |
| J167 | Pasteurella multocida | NCTC | 3195 | proposed working type [1]. |
| J168 | P. pfaffi | NCTC | 1102 | |
| J169 | P. pneumotropica | NCTC | 8141 | |
| J170 | P. ureae | NCTC | 10222 | |
| J171 | P. haemolytica A | NCTC | 10365 | |
| J172 | P. haemolytica T | NCTC | 10371 | |
| J173 | Yersinia pestis | NCTC | 5923 | proposed working type [1]. |
| J174 | "          " | NCTC | 5924 | |
| J175 | Y. pseudotuberculosis | NCTC | 8315 | |
| J176 | "          " | Mair | 1647 | |
| J177 | Y. enterocolitica | Mair | L301 | |
| J178 | "          " | Mair | PA120 | |
| J179 | Francisella tularensis | Owen | Jap | |
| J180 | Fr. novicida | Owen | U112 | |
| J181 | Bordetella pertussis | PHL.Leics. | - | |
| J182 | Haemophilus sp. | PHL.Leics. | - | |
| J183 | Haemophilus sp. | PHL.Leics. | - | |

Table 8 (continued)

| Author's Strain Number | Strain Received as | | Received from | Donor's Number | Type Status (if any) |
|---|---|---|---|---|---|
| J186 | Bordetella pertussis | | Lacey | – | |
| J187 | " | " | Lacey | H36 | |
| J188 | " | " | Lacey | H52 | |
| J189 | " | " | Lacey | H103 | |
| J190 | " | " | Lacey | H104 | |
| J191 | " | " | Lacey | H105 | |
| J192 | Bordetella bronchiseptica | | Lacey | BR2 | |
| J193 | " | " | Lacey | BR4 | |
| J194 | " | " | Lacey | BR58 | |
| J195 | " | " | Lacey | BR62 | |
| J196 | " | " | Lacey | BR88 | |
| J197 | " | " | Lacey | BR106 | |
| J198 | " | " | Wey | P21 | |
| J199 | " | " | Wey | S23 | |
| J200 | " | " | Wey | DB80 | |
| J201 | " | " | Wey | DB82 | |
| J202 | " | " | Wey | DB84 A | |
| J203 | " | " | Wey | DB85 | |
| J204 | " | " | Wey | DB86 | |
| J205 | " | " | Wey | DB87D | |
| J206 | " | " | Wey | DB91 | |
| J207 | " | " | Wey | DB92 | |
| J208 | " | " | Wey | DB105 B2 | |
| J209 | " | " | Wey | DB8 V16 | |
| J210 | Yellow pigmented rod | | IID | – | |

Table 8 (continued)

| Author's Strain Number | Strain Received as | | Received from | Donor's Number | Type Status (if any) |
|---|---|---|---|---|---|
| J211 | Bordetella parapertussis | | NCTC | 10523 | |
| J212 | " | " | NCTC | 10524 | |
| J214 | " | " | Lautrop | 4380 | |
| J215 | " | " | Lautrop | 4840 | |
| J216 | " | " | Lautrop | 4847 | |
| J217 | " | " | Lautrop | 4888 | |
| J218 | " | " | Lautrop | 5725 | |
| J219 | " | " | Lautrop | 5743 | |
| J220 | " | " | Lautrop | 5954 | |
| J221 | " | " | Lautrop | 6002 | |
| J222 | " | " | Lautrop | 6006 | |
| J223 | " | " | Lautrop | 6084 | |
| J224 | " | " | Lautrop | 6137 | |
| J225 | " | " | Lautrop | 6225 | |
| J226 | " | " | Lautrop | 6231 | |
| J227 | " | " | Lautrop | 7806 | |
| J228 | " | " | Lautrop | 7915 | |
| J229 | Bordetella pertussis | | Lautrop | 3234 | |
| J230 | " | " | Lautrop | 3252 | |
| J231 | " | " | Lautrop | 3286 | |
| J232 | " | " | Lautrop | 3210 | |
| J233 | " | " | Lautrop | 3239 | |
| J234 | " | " | Lautrop | 3254 | |
| J235 | " | " | Lautrop | 3255 | |
| J236 | " | " | Lautrop | 3272 | |
| J237 | " | " | Lautrop | 3277 | |

Table 8 (continued)

| Author's Strain Number | Strain Received as | Received from | Donor's Number | Type Status (if any) |
|---|---|---|---|---|
| J238 | Bordetella pertussis | Lautrop | 3281 | |
| J239 | " " | Lautrop | P.5759 | |
| J240 | " " | Lautrop | P.5767 | |
| J241 | Bordetella bronchiseptica | Lautrop | AB65 | |
| J242 | " " | Lautrop | AB108 | |
| J243 | " " | Lautrop | AB1043 | |
| J244 | " " | Lautrop | Ole | |
| J245 | Pasteurella piscicida | ATCC | 17911 | |
| J246 | P. ureae | Mair | - | |

Key

1.          Sneath and Skerman (1966).

2.          American Type Culture Collection Catalogue (1970).

3.          Boyce, Frazer and Zinnemann (1969).

4.          Zinnemann, Rogers, Frazer and Boyce (1968).

NCTC:       National Collection of Type Cultures, Central Public Health
            Laboratory, Colindale Avenue, London N.W.9.

CCEB:       Czechoslovak Collection of Entomogenous Bacteria, Na cvičišti 2,
            Prague 6.

NCIB:       National Collection of Industrial Bacteria, Torry Research
            Station, Aberdeen.

MJT:        Dr. M.J. Thornley, Low Temperature Research Station, Cambridge.

ATCC:       American Type Culture Collection, 12301, Parklawn Drive,
            Rockville, Maryland.

IID:        Institute of Infectious Diseases, University of Tokyo, Japan.

Zinnemann:  Dr. K. Zinnemann, Department of Bacteriology, University of Leeds.

CAPM:       Czechoslovak Collection of Animal Pathogenic Micro-organisms,
            Brno-Medlánky.

H:          Professor S.D. Henriksen, Kaptein W. Wilhelmsen og Frues
            Bakteriologiske Institutt, University of Oslo, Norway.

Wey:        Central Veterinary Laboratory, Weybridge.

Mair:       Dr. N.S. Mair, Public Health Laboratory, Leicester.

Owen:       Dr. C.R. Owen, National Institute of Allergy and Infectious
            Diseases, Rocky Mountain Laboratory, Hamilton, Montana.

PHL.Leics.: Public Health Laboratory, Leicester.

Lacey:      Professor B.W. Lacey, Department of Bacteriology, Westminster
            Medical School, London.

Lautrop:    Dr. H. Lautrop, Statens Seruminstitut, Copenhagen.

Key

NCPPB:  National Collection of Plant Pathogenic Bacteria, Ministry of
        Agriculture, Fisheries and Food, Plant Pathology Laboratory,
        Milton Road, Harpenden, Herts.

Baumann: Dr. P. Baumann, Retina Foundation, Boston, Massachusetts.

MSU:    M.R.C., Microbial Systematics Unit, University of Leicester,
        England.

Numerical Taxonomy

Maintenance Media

i)  Heated blood Agar ("chocolate")

|  |  |
|---|---|
| Nutrient broth No. 2 (Oxoid) | 25g. |
| Agar No. 1 (Oxoid) | 11g. |
| Nicotinic acid | 0.02g. |
| distilled water | 1000 ml. |
| pH 7.2 | |

The medium was sterilised by autoclaving at 15 lbs. pressure for 15 minutes, cooled to 70°C and 10 ml. of a sterile 2.5% (w/v) aq. solution of cysteine hydrochloride was added, followed by 100 ml. of sterile horse blood (Burroughs Wellcome Ltd.). The complete medium was further held at 70°C until it turned chocolate-brown in colour, and then dispensed in sterile plastic Petri dishes.

ii)  Charcoal Blood Agar

One hundred millilitres of sterile horse blood was added per litre of charcoal agar (Oxoid Ltd.), and the medium dispensed in Petri dishes.

iii)  Serum Agar

|  |  |
|---|---|
| Nutrient broth No. 2 (Oxoid) | 25g. |
| Agar No. 1 (Oxoid) | 11g. |
| Nicotinic acid | 0.02g. |
| distilled water | 930 ml. |
| pH 7.2 | |

The medium was autoclaved at 15 lbs. pressure for 15 minutes, cooled to 50°C and the following sterile solutions added before dispensing:

haematin, 0.05% (w/v) soln.                                    5 ml.

nicotinamide adenine dinucleotide (NAD), 0.05% (w/v) soln.  5 ml.

cysteine hydrochloride, 2.5% (w/v) soln.                    10 ml.

inactivated horse serum, No. 5  (Burroughs Wellcome Ltd.) 50 ml.

The haematin solution (Koch-Light Laboratories Ltd.) was prepared by the method of Butler (1962), and sterilised by autoclaving at 15 lbs. for 15 minutes.   The solutions of NAD and cysteine hydrochloride were prepared by dissolving in distilled water and sterilised by membrane filtration.

The particular maintenance medium upon which each strain was grown is listed below:

chocolate agar:      J73-J125, J182 and J183,

charcoal blood agar:     J42-J57, J181, J186-J191, and J211-J240,

serum agar:          the remaining strains.

### Basal Medium for Tests

In order to standardise the test conditions, a modification of serum agar had to be developed so that satisfactory growth of all the strains could be obtained.   The problems encountered after adding substances like blood or charcoal to test media have already been mentioned. Many tests such as methyl red, Voges Proskauer, indole reaction, urease and hydrolysis reactions, require media which must be transparent so that colour changes or zones of clearing can be observed.

Serum agar, containing haematin and NAD, allowed the growth

of all strains except pertussis;  for these strains it was necessary to add charcoal and starch to the medium.   Many transparent synthetic or seru-synthetic media have been used to grow smooth strains of <u>Bordetella</u> <u>pertussis</u> for vaccine production, but they have all been liquid media which gave unsatisfactory growth when solidified with agar.   The reports of Kuwagima, Matsui and Kishigami (1957) and Sutherland and Wilkinson (1961) on the use of anionic exchange resins incorporated into media have received little attention by other authors, but seemed to have possibilities for overcoming some of the problems mentioned.   The resin does not dissolve, but is used in a low concentration and therefore has a negligible effect on the opacity of the medium.   Sutherland and Wilkinson have also found that their medium could be solidified with agar and still give satisfactory growth of smooth <u>Bordetella pertussis</u>.

Preliminary experiments confirmed the suitability of Sutherland and Wilkinson's medium for growing <u>B. pertussis</u> but, even after adding haematin and NAD, some of the other strains would not grow.   Eventually a compromise between serum agar and Sutherland and Wilkinson's medium was found to give satisfactory growth of all the strains used in this study.

The modified medium, designated BM agar, had the following formula:

BM Agar:

| | |
|---|---|
| Nutrient broth No. 2 (Oxoid) | 20g. |
| Casein hydrolysate (Oxoid) | 5g. |
| Agar No. 1 (Oxoid) | 11g. |
| Nicotinic acid | 0.02g. |
| $KH_2PO_4$ | 0.5g. |
| $MgCl_2,6H_2O$ | 0.4g. |
| $FeSO_4,7H_2O$ | 0.01g. |
| $CaCl_2$ | 0.1g. |
| Dowex 1-X8 (British Drug Houses) | 5g. |
| distilled water | 930 ml. |

pH 7.2

The medium was autoclaved at 15 lbs. pressure for 15 minutes, cooled to 50°C and the following sterile constituents added:

| | |
|---|---|
| haematin, 0.05% (w/v) soln. | 5 ml. |
| NAD, 0.05% (w/v) soln. | 5 ml. |
| cysteine hydrochloride, 2.5% (w/v) soln. | 10 ml. |
| inactivated horse serum | 50 ml. |

The complete agar was further cooled to 44°C and thoroughly mixed before pouring. The agar should set quickly so that the resin remained suspended. BM broth was prepared from the same formula, omitting the agar.

Before the resin was included in the medium it had to be activated. The following procedure was used during this study: Dowex 1-X8 (20-50 U.S. mesh) was washed in tap water and then activated by washing in N/2 sodium hydroxide for two hours. The activated resin was washed repeatedly with distilled water until the pH of the effluent was below 7.5. The most practical way of carrying out the final washing process, was to pack a glass column with the resin and slowly pass distilled water through, from a 50 litre tank for about 24 hours.

Temperature of Incubation

Stock cultures and tests, for all except eleven strains, were incubated at 35°C. The following 11 strains grew poorly at 35°C and were consequently incubated at 25°C: J10, J12, J13, J17, J24, J25, J26, J28, J29, J34 and J245.

Test results, unless otherwise stated, were recorded after 2, 5 and 7 days.

## Staining and Morphology

Strains were grown on BM agar and examined after 1, 3 and 5 days.
In the case of the slower growing parapertussis and pertussis cultures,
examination was after 2, 3 and 5 days. Cell shape and arrangement were
studied by the hanging drop technique.

Heat fixed films were stained by Kopeloff and Beerman's
modification of Gram's method (Cruickshank, 1965, p. 646). Dilute
carbol fuchsin , which was used as the counterstain, was made up as follows:
basic fuchsin 1g., was dissolved in 10 ml. absolute alcohol and the
solution added to 100 ml. of 5% (w/v) phenol in water. This solution
was then made up to one litre with distilled water.

## Cultural Methods

Colonial morphology was described from 48 hour cultures on
BM agar. The colour and diffusibility of any pigment produced was also
recorded.

The ability to grow anaerobically was determined by inoculating
a loopful of 48 hr. agar culture onto BM agar, and incubating in an
anaerobic jar (Baird and Tatlock Ltd.). Anaerobic conditions were
attained by three passages of hydrogen through the jar; anaerobiosis
being indicated by loss of colour of the indicator in the side-arm.
Growth anaerobically in the presence of nitrate was tested similarly,
on BM agar containing 0.1% (w/v) potassium nitrate.

Additional $CO_2$ requirement was determined by inoculating a
duplicate set of cultures on BM agar and incubating one set under an
increased pressure of $CO_2$ according to Cruickshank (1965, p. 799).

X and V factor requirements were determined on BM agar from

which the haematin and NAD had been omitted. Cultures were streaked
onto the surface of the medium and X, V and X + V factor discs (Oxoid
Ltd.) placed onto the streaked areas before incubation.

Growth in BM broth was recorded for turbidity, type of
sediment and the presence of surface growth. The ability to grow on
nutrient agar was determined by streaking 48 hr. agar cultures onto
Blood Agar Base (Oxoid Ltd.). Growth on blood agar was tested on
layered blood agar base containing 10% (v/v) horse blood in the upper
layer. Five millilitres of haematin solution and 5 ml. NAD solution
were also included in each litre of the blood agar. Haemolysis of
horse and sheep blood was tested on layered BM agar containing 10% (v/v)
horse or sheep blood, instead of the serum.

Motility was examined by three methods:

i)   BM broths were inoculated with a loopful of 48 hr. agar culture and
incubated at 25°C and 35°C. Hanging drops were prepared after 1 and
2 days, and examined microscopically.

ii)  The condensation water of BM agar slopes was inoculated and
examined by hanging drop after 1 and 2 days incubation.

iii) Craigie's method (Craigie, 1931). Semi-solid BM agar, containing
0.25% (w/v) Davis agar, was dispensed aseptically in 15 ml. volumes into
1 oz. universal bottles containing a glass tube about 2 inches long.
Cultures were inoculated inside the protruding end of the glass tube and
incubated at 35°C and at room temperature. Any cultures showing growth
in the medium outside the glass tube, were subcultured into BM broth and
incubated at 25°C for up to 24 hours. Hanging drops were prepared from
the BM broth after 3 hrs. and 24 hrs. and examined microscopically for
motile cells.

## Resistance Tests

Many of the pertussis cultures produced better growth on charcoal blood agar than on BM agar, particularly when growth was required over the complete agar surface. Similarly, several of the haemophili which required X and V factors, were capable of better growth on heated blood agar than on BM agar. It was therefore decided to inoculate duplicate sets of plates for many of the resistance tests. If growth in the presence of 5% (w/v) bile salts is taken as an example, the following media were prepared: BM agar containing 5% (w/v) bile salts, charcoal blood agar containing 5% (w/v) bile salts, and heated blood agar containing 5% (w/v) bile salts. The pertussis and parapertussis cultures were inoculated onto both the BM and charcoal blood bile salts agar, while the haemophili requiring X or V factors or both, were inoculated onto both the BM and heated blood bile salts media. The remaining strains were inoculated only onto BM bile agar. This procedure was adopted for the following tests:

        growth in the presence of bile salts,

        tolerance of sodium chloride,

        sensitivity to the pteridine compound, 0/129,

        sensitivity to dyes,

        sensitivity to antibiotics,

        growth in the presence of potassium tellurite,

        growth in the presence of sodium azide,

        growth in the presence of thallous acetate,

        growth in the presence of optochin,

        growth in the presence of 0.5% (w/v) sodium nitrite.

## Growth in the presence of bile salts

BM agar, charcoal blood agar and heated blood agar, each

containing 5, 10 and 40% (w/v) bile salts (Oxoid Ltd.), were inoculated from 48 hr. agar cultures.

## Tolerance of sodium chloride

BM agar, charcoal blood agar and heated blood agar containing 0, 0.5, 3.0, 4.5, 6.0, 7.5 and 9.0% (w/v) sodium chloride were inoculated from 48 hr. agar cultures.

## Sensitivity to the pteridine compound, O/129

The pteridine compound, O/129 (2,4 diamino, 6-7 di-iso-propyl pteridine), was dissolved in ethanol to give a 0.4% (w/v) solution. Paper discs, five millimetres in diameter, were punched from Whatman No. 1 filter paper with an office paper punch. Batches of 100 discs were then placed in 1 oz. universal containers and sterilised at $140^{o}C$ for 2 hours. To each universal, 1 ml. of O/129 solution was added and allowed to soak into the discs, which were then dried at $37^{o}C$. BM agar, charcoal blood agar and heated blood agar were streaked with 48 hr. agar cultures and an O/129 disc placed onto the streaked area before incubation.

## Sensitivity to dyes

Each dye was dissolved in distilled water to give a 0.1% (w/v) solution, which was sterilised by steaming for 1 hour. Further dilutions were prepared in sterile distilled water to give the following concentrations:

| | |
|---|---|
| basic fuchsin (BDH) | 1 in 200 |
| methyl violet (Edward Gurr Ltd.) | 1 in 400 |
| pyronin G (BDH) | 1 in 800 |
| safranin O (BDH) | 1 in 800 |
| thionine (BDH) | 1 in 800. |

Filter paper strips, measuring 6 x 0.5 cm, were cut from Whatman No. 1 filter paper and sterilised at $140^{o}C$ for 2 hours. The paper strips

were then soaked in the dye solutions and dried at $37^{o}$C.   BM agar,
charcoal blood agar and heated blood agar plates were poured and, when
set, a paper strip containing an appropriate dye was placed on the
agar surface and overlayed with 10 ml. of the same agar.   Each plate
was inoculated from 48 hr. agar cultures, at right angles to the
direction of the dye strip.

## Sensitivity to antibiotics

Antibiotic sensitivity was measured using high potency
"Sentest" discs and a "Sentest" dispenser (Evans Medical Ltd.).
Four antibiotic discs were placed on the surface of each agar plate,
after it had been streaked from a 48 hr. agar culture.   The following
antibiotics were used:  penicillin, streptomycin, chloramphenicol,
aureomycin, terramycin, erythromycin, tetracyline, sulphonamide,
neomycin, novobiocin, oleondomycin, nitrofurantoin, and bacitracin.

Sensitivity discs, containing 25µg. Bactrim Roche (trimethoprim-
sulphamethoxazole, Oxoid Ltd.), were tested in a similar manner to the
sentest antibiotics.

## Sensitivity to M and B 938    (4:4'-Diamidinodiphenylamine dihydrochloride, May and Baker Ltd.)

Forty-eight hour agar cultures were streaked onto BM agar
containing 12.5 ml. of a 0.1% (w/v) aq. solution of M and B 938, per
litre of agar.

## Growth in the presence of potassium tellurite

BM agar, charcoal blood agar and heated blood agar,
containing 16 ml. of a 2% (w/v) aq. solution of potassium tellurite per
litre were dispensed in Petri dishes and inoculated from a 48 hr. agar
culture.

## Growth in the presence of sodium azide

BM agar, charcoal blood agar and heated blood agar, containing

7.5 ml, of a 1% (w/v) aq. solution of sodium azide per litre, were each inoculated from 48 hr agar cultures.

## Growth in the presence of thallous acetate

BM agar, charcoal blood agar and heated blood agar, containing 0.5 g. per litre of thallous acetate were inoculated from 48 hr agar cultures.

## Sensitivity to optochin

Optochin discs (Oxoid Ltd.) were placed onto the streaked surface of BM agar, heated blood agar and charcoal blood agar before incubation.

## Growth in the presence of sodium nitrite

BM agar, charcoal blood agar and heated blood agar, containing 0.5% (w/v) sodium nitrite, were inoculated from 48 hr. agar cultures.

## Growth in the presence of phenol

A 10% (w/v) solution of phenol was prepared in sterile distilled water and added to BM agar to give final concentrations of 0.1, 0.2, 0.3, 0.4 and 0.5% (w/v) of phenol. The medium was dispensed in Petri dishes and inoculated from 48 hr. agar cultures.

## Growth temperature

BM agar plates, inoculated from 48 hr. agar cultures, were incubated for up to 10 days at the following temperatures: 5, 10, 15, 25, 30, 35, 37 and 44°C.

## Heat Resistance

Nutrient broth No. 2 (Oxoid Ltd.) was dispensed in 4.5 ml. volumes in 6 x 5/8 inch test tubes, and sterilised by autoclaving at 15 lbs. pressure for 15 minutes. During the test, broths were heated to 56°C in a water bath and 0.5 ml. of bacterial suspension added to each. A loopful was removed to BM agar, heated blood agar or charcoal blood

agar after 5, 10, 15 and 30 minutes.   The plates were then incubated for up to 5 days and checked for growth.

## Biochemical Tests

### Catalase

Hydrogen peroxide solution (20 vol.%) was added dropwise to 48 hr. BM agar cultures and observed for effervescence.   An uninoculated BM agar plate was also tested to confirm that there was no catalase present in the medium.

### Peroxidase

Peroxidase reagent was made up as follows:  0.5g. of crystalline benzidine hydrochloride was dissolved in 3 ml.of glacial acetic acid and an equal volume of hydrogen peroxide added.

One drop of the reagent was added to 48 hr. BM agar cultures and observed for the development of a blue colour.

### Kovacs' Oxidase (Kovacs, 1956)

Two or three drops of a 1% (w/v) aq. solution of N:N:N':N'-tetramethyl-p-phenylenediamine dihydrochloride (British Drug Houses Ltd.) were placed on a filter paper.   A colony from a 48 hr. BM agar culture was smeared on to the paper with a platinum loop.   A positive reaction was indicated by the appearance of a dark blue colour within 10 seconds.

### Hydrogen Sulphide production

BM agar, heated blood agar and charcoal blood agar, containing 0.025% (w/v) cysteine hydrochloride, were dispensed as slopes in 6 x 5/8 inch test tubes, and inoculated from 48 hr. agar cultures. Lead acetate paper was placed between the test tube plug and the side of the tube and observed for blackening over a period of 7 days incubation.

## Phosphatase

BM agar, containing 10 ml. per litre of a sterile 1% (w/v) aq. solution of phenolphthalein diphosphate (British Drug.Houses Ltd.), was dispensed in Petri dishes and inoculated from 48 hr. agar cultures. Phosphatase activity was determined by inverting the dishes and placing 3 drops of 0.880 ammonia solution in the lid. The development of a pink colour around the colonies indicated a positive reaction.

## Gelatin liquefaction

Gelatinase activity was deterined by three methods:

i) Plate method. BM agar, containing 0.4% (w/v) gelatin (Oxoid Ltd.), was inoculated from 48 hr. agar cultures. After incubation, the plates were flooded with acid mercuric chloride solution and examined for clear zones around the colonies.

ii) Slope method.(Edmunds, 1962). Charcoal blood agar, heated blood agar and BM agar were dispensed as slopes, overlayed with 5 ml. sterile nutrient gelatin (Oxoid Ltd.), and incubated at $37^{\circ}C$ for 48 hours. The slopes were then inoculated from 48 hr. agar cultures and incubated for up to 30 days. The medium was examined for liquefaction by cooling the tubes to $5^{\circ}C$ for 1 hour.

iii) Gelatin stabs. BM broth, containing 12% (w/v) nutrient gelatin, was dispensed in 15 ml. volumes in 1 oz. universal containers. Each universal was stab-inoculated from 48 hr. agar culture and incubated for up to 30 days. Liquefaction was determined by holding the tubes at $5^{\circ}C$ for 1 hour.

## Litmus Milk

Sterile BM broth, containing 100g. litmus milk (Oxoid Ltd.), was dispensed in 15 ml. volumes in 1 oz. universal containers. They were inoculated from 48 hr. agar cultures and incubated at optimum temperature for up to 30 days.

### Starch hydrolysis

BM agar, containing 200 ml. of a 2.5% (w/v) aq. solution of
starch per litre, was dispensed in Petri dishes and inoculated from 48 hr.
agar cultures.    After incubation, some of the growth on each plate
was scraped aside and the plates then flooded with Gram's iodine.

### Egg-yolk reaction

BM agar, containing 10% (v/v) concentrated egg-yolk emulsion
(Oxoid Ltd.) and an additional 1% (w/v) sodium chloride, was dispensed
in Petri dishes and inoculated from 48 hr. agar cultures.    After
incubation, plates were examined for zones of opacity.

### Casein hydrolysis

BM agar, containing 20% (v/v) sterile skim-milk, was
inoculated from 48 hr. agar cultures and, after incubation, examined
for zones of clearing around the colonies.

### Aesculin hydrolysis

BM agar, containing 0.05% (w/v) Ferric ammonium citrate
and 0.5% (w/v) aesculin (British Drug Houses Ltd.), was inoculated
from 48 hr. agar cultures and, after incubation, examined for areas of
dark-brown precipitation.

### Dextran or levan production

BM agar, containing 5% (w/v) sucrose, was inoculated from
48 hr. agar cultures and incubated at optimum temperature.    The growth
of large mucoid colonies was taken as an indication of dextran or levan
production.

### Tetrazolium reduction

BM agar adjusted to pH 6.0, and containing 1% (w/v) glucose
and 7.5 ml. of a 1% (w/v) sterile aq. solution of 2:3:5 - triphenyl-
tetrazolium chloride (British Drug Houses Ltd.) per litre of medium, was
dispensed in Petri dishes and inoculated from 48 hr. agar cultures.

After incubation, plates were examined for the production of red
insoluble formazan.

## Production of ammonia from peptone

BM agar, containing 10g. peptone (L37, Oxoid Ltd.), in place of
nutrient broth No. 2 and casein hydrolysate, was inoculated from 48 hr.
agar cultures.    After incubation, 1 ml. of Nessler's reagent was added
to each plate;  a brown coloration or precipitation indicated the
presence of ammonia.

## Urease production

Tested according to the micro-method of Lautrop (1960).

## Arginine hydrolysis

Arginine hydrolysis was determined by a micro-method using a
modification of Thornley's (1960) method.    The following medium was used:

| | |
|---|---|
| peptone (Oxoid, L37) | 0.1g. |
| NaCl | 0.5g. |
| $K_2HPO_4$ | 0.03g. |
| Arginine hydrochloride (British Drug Houses) | 1.0g. |
| Phenol red, 0.2% (w/v) soln. | 0.5 ml. |
| distilled water | 100 ml. |

pH 7.2

The medium was dispensed in 0.6 ml. volumes in 4 x $\frac{1}{2}$ inch test tubes
and sterilised by autoclaving at 10 lbs. pressure for 10 minutes.    The
tubes were inoculated heavily from 48 hr. agar cultures and the
development of any pink coloration recorded after 2 and 24 hours.

## Nitrate reduction

BM agar, containing 0.1% (w/v) $KNO_3$, was inoculated from 48 hr.
agar cultures, and, after incubation, nitrate reduction tested for by the
addition of Griess-Ilosvay's solutions, A and B.    A deep red coloration
indicated the presence of nitrite.    It was impractical to test for

complete reduction of nitrate by adding zinc dust, because even the
most vigorous nitrate reducers were incapable of reducing all the nitrate
in the agar medium.

Deoxyribonuclease activity

The following medium was used:

| | |
|---|---|
| DNase agar (Oxoid Ltd.) | 39g. |
| Dowex 1-X8 | 5g. |
| distilled water | 940 ml. |

After sterilising by autoclaving at 15 lbs. pressure for 15 minutes, the
medium was cooled to $44^{o}C$ and the following constituents added:

| | |
|---|---|
| haematin, 0.05% (w/v) soln. | 5 ml. |
| NAD, 0.05% (w/v) soln. | 5 ml. |
| horse serum | 50 ml. |

It was then dispensed in sterile plastic Petri dishes and inoculated
from 48 hr. agar cultures. After incubation, the plates were flooded
with normal hydrochloric acid, which reacted with nucleic acid in the
medium to form a cloudy precipitate. DNase producing colonies were
surrounded by clear zones.

Production of hydrogen cyanide

Hydrogen cyanide production was determined on BM agar
containing 10% (v/v) horse blood, using the method of Sneath (1956).
Picric acid-sodium carbonate indicator papers (Sneath, 1966) were used
instead of the original benzidine acetate-cupric acetate papers.

Methyl Red and Voges-Proskauer

These tests were performed only on the strains which were able
to produce acid from glucose in one of the conventional fermentation
media. The following medium was used:

| | |
|---|---|
| Brain Heart Infusion (Oxoid) | 37g. |
| Glucose | 5g. |
| $K_2HPO_4$ | 5g. |
| distilled water | 940 ml. |

The medium was sterilised by autoclaving and, after cooling to 45°C, the following constituents added:

| | |
|---|---|
| haematin soln. | 5 ml. |
| NAD soln. | 5 ml. |
| horse serum | 50 ml. |

Ten millilitre volumes were dispensed into sterile 1 oz. universal containers and inoculated from 48 hr. agar cultures. Methyl red indicator and O'Meara's reagent were used as the test reagents (Cruickshank, 1965, p.818).

## Tyrosine hydrolysis and production of pigment from tyrosine

Tyrosine hydrolysis was determined by the method of Gordon and Smith (1955) using BM agar containing 0.5% (w/v) tyrosine. Plates were inoculated from 48 hr. agar cultures and, after incubation, examined for zones of clearing and pigment production.

## Xanthine hydrolysis

Hydrolysis of xanthine was determined by the method of Gordon and Smith (1955) using BM agar containing 0.4% (w/v) xanthine. Plates were inoculated from 48 hr. agar cultures and, after incubation, examined for zones of clearing.

## Phenylalanine deamination

The following medium was used:

| | |
|---|---|
| Agar No. 1 (Oxoid) | 11g. |
| nicotinic acid | 0.02g. |
| Dowex 1-X8 | 6g. |
| DL-β-phenylalanine | 2g. |
| distilled water | 930 ml. |

pH 7.2

After autoclaving at 15 lbs. pressure for 15 minutes, the medium was cooled to 44°C and the following sterile constituents added:

| | |
|---|---|
| haematin solution, 0.05% (w/v) | 5 ml. |
| NAD solution, 0.05% (w/v) | 5 ml. |
| cysteine hydrochloride solution, 2.5% (w/v) | 10 ml. |
| horse serum | 50 ml. |

Plates were inoculated from 48 hr. agar cultures and, after incubation, 1 ml. 10% (w/v) $FeCl_3$ aq. solution was run over the surface of the agar. The appearance of a green colour indicates a positive reaction.

Tween hydrolysis

Hydrolysis of tweens 20, 40, 60 and 80 were determined on BM agar containing 1% (v/v) tween. Each medium was inoculated from 48 hr. agar cultures and, after incubation, examined for precipitation around colonies. Many strains produced zones of clearing on tween 80 medium; this character was recorded in addition to the precipitation reaction.

Production of acid from carbohydrates

Two media were used to determine the production of acid from carbohydrates,

i) BM agar containing 1% (w/v) carbohydrate.

ii) BM agar containing 0.2% (w/v) tryptone in place of nutrient broth No. 2, and only 0.1% (w/v) casein hydrolysate. This medium also contained 1% (w/v) carbohydrate.

All carbohydrates were dissolved in distilled water to give 10% (w/v) solutions. They were sterilised by membrane filtration and 100 ml. added to the medium after it had been autoclaved. The media were dispensed in Petri dishes and inoculated from 48 hr. agar cultures. After incubation, acid production was detected by adding 2 drops of sterile 0.04% (w/v) aq. bromothymol blue at pH 7.2, and noting the colour change. The following carbohydrates were tested: glucose, lactose,

xylose, salicin, raffinose, α-methyl d glucoside, ribose, rhamnose, mannose, inositol, cellobiase, fructose, inulin, dextrin, dulcitol, arabinose, adonitol, sorbitol, galactose, maltose, sucrose, mannitol, glycerol, erythritol and glucosamine hydrochloride. Acid production was also determined in the same media, containing 10% (w/v) glucose and 10% (w/v) lactose. This time, the carbohydrates were incorporated into the media before autoclaving.

The mode of glucose metabolism, i.e. oxidation or fermentation, was determined by incubating, in duplicate, the carbohydrate media containing 1% (w/v) glucose and incubating one set of plates under anaerobic conditions in an anaerobic jar.

## Computer Methods

The computing was performed on an Elliott 4130 electronic computer, using a numerical taxonomy program written at the Microbial Systematics Unit. The majority of characters were two-state and were coded 1 for positive, 0 for negative. Multistate characters were scored 0 for negative and 1, 2 or 3, if there was more than one form of positive response, for example, sizes of zones of inhibition.

The final t by n matrix measured 201 OTU's by 145 characters, of which 11 characters were excluded from the computing because they gave identical results for all strains, or were judged to be unreliable. The limitations of the computer prevented all 201 OTU's being sorted in a single run; this was overcome by selecting OTU's with the species epithets pertussis, parapertussis or bronchiseptica, and sorting these first.

The t by n matrix for the first run measured 89 OTU's by 134 characters; this was sorted into three well-defined clusters from which 38 representative strains were chosen and included with the remaining OTU's, in the second run.

The similarity coefficient of Jaccard, which excludes negative matches, and Gower's similarity coefficient (to be published), which utilises both positive and negative similarities, were tried on initial computing runs. Apart from the levels of similarity, however, the results obtained after clustering were practically identical, so it was decided to use only Gower's coefficient.

The program allows three different clustering methods: single linkage (Sneath, 1957b), unweighted average linkage, or weighted average linkage (Sokal and Michener, 1958). Both single linkage and unweighted average linkage methods were tried and, again, the results obtained were very similar.

In order to cut down on the sheer volume of computer output, the similarities were calculated only by Gower's coefficient, and clustered by the unweighted average linkage method. The output data consisted of a sorted similarity matrix, an abbreviated similarity matrix, a rough dendrogram, and a punched paper tape which makes use of plotting facilities to draw an accurate dendrogram.

The program also has options for analysing the Vigour and Pattern statistics (Sneath, 1968) of the data matrix. Sneath pointed out that the Simple Matching Coefficient, $S_{sm}$, was the complement of Total Difference, $D_T$, so that $D_T = 1 - S_{sm}$. However,

$$(\text{Total Difference})^2 = \text{Vigour Difference})^2 + (\text{Pattern Difference})^2.$$

Algebraic definitions were provided for each function in this equation and are used by the program to calculate triangular matrices corresponding to Vigour Difference and Pattern Difference. The matrices are clustered by an identical procedure to that used for a normal similarity matrix.

Poly-acrylamide Gel Electrophoresis

Strains were not subjected to the procedures of gel
electrophor̂sis and the determination of DNA base composition until
the results of the numerical taxonomy had been carefully analysed.
On the basis of these results, representative strains of the groups,
showing close similarity to the "bordetella" clusters, were chosen
for further study.

## Growth medium for cultures

Strains which were able to grow on simple nutrient media
were inoculated into 600 ml. of Brain Heart Infusion broth (Oxoid Ltd.)
in a 2-litre Flask.    A 24 hr. broth culture was used as the inoculum
and the flasks were incubated at $35^{o}$C in a gyrotory shaker (New
Brunswick Scientific Company), for 3 days.

Brucella cultures were grown in a similar medium containing
5% (v/v) sterile inactivated horse serum and 1% (w/v) glucose.
Cultures of Bordetella pertussis were grown in 600 ml. of Hornibrook's
medium (Hornibrook, 1939) and incubated for 5 days.    A 48 hr. agar
culture, suspended in 3 ml. of broth, was used as the inoculum.

## Cell disintegrates

Cells were harvested by centrifugation in a High Speed 18
centrifuge (Measuring and Scientific Equipment Company), at 10,000 rpm
(15,000xg) for 20 minutes.    The harvested cells were washed twice in
0.05 M tris buffer, pH 7.2, and resuspended in 0.05 M 20 ml. of the same tris
buffer.    Each suspension was stained by Gram's method to check for
purity.    The cells were then disintegrated by ultrasonic vibration using
a type 1130 A Soniprobe (Dawe Instruments Ltd.).    Each suspension was
subjected to ultrasonic vibration for 5 minutes, using 30 second pulses
alternating with 30 second cooling periods.    The cell disintegrates were
clarified by centrifugation at 18,000 rpm (38,000xg), for 30 minutes, and
stored at $5^{o}$C without the addition of a preservative.    The amount of

protein present in each extract was determined according to Lowry, Rosebrough, Farr and Randall (1951).

Electrophoresis

       Protein extracts were analysed within 2 days of preparation, on a Shandon disc electrophoresis apparatus (Shandon Scientific Company Ltd.), using polyacrylamide gels.   The procedure for preparation and polymerisation of gels was similar to that described in the Shandon instructions.

       The following stock solutions were used:

| | | |
|---|---|---|
| small pore acrylamide | - | acrylamide (B.D.H.) 30g., NN'-methylene bis acrylamide (Bis,B.D.H.) 0.8g., $K_3Fe(CN)_6$ 0.015g., distilled water to 100 ml. |
| Large pore acrylamide | - | acrylamide 10g., bis 2.5g., distilled water to 100 ml. |
| Small pore tris buffer pH 9.0 | - | tris (hydroxymethyl) methylamine (B.D.H.) 36.3g., N hydrochloric acid 48 ml., N,N,N',N'-tetramethylethylenediamine (temed Kodak Ltd.) 0.46 ml, distilled water to 100 ml. |
| Large pore tris buffer pH 9.0 | - | tris 5.7g., N $H_3PO_4$ 25.6 ml, distilled water to 100 ml. |
| Small pore initiator | - | ammonium persulphate (B.D.H.) 0.14g., distilled water to 100 ml. |
| Large pore initiator | - | ammonium persulphate 0.35g., temed 0.32 ml, distilled water to 100ml. |
| Reservoir buffer pH 9.0 | - | glycine (B.D.H.) 28.8g., tris 6.0g., distilled water to 1,000 ml. |
| Protein stain | - | amido black (B.D.H.) 1.0g., 7% (v/v) acetic acid 100 ml. |
| Destaining fluid | - | 7% (v/v) acetic acid. |

The small pore gel was made up from the stock solutions in
the following proportions: tris buffer 1 part: acrylamide, 2 parts:
initiator, 4 parts: $H_2O$, 1 part. A suitable volume of about 30 ml.
was de-aerated for 10 minutes and dispensed into precision bore glass
tubes, 7.5 cm. in length with an internal diameter of 0.5 cm. Each
tube was filled to about 3mm. below the rim and the gel surface gently
overlayed with distilled water in order to obtain a flat meniscus.
Polymerisation was allowed to take place at ambient temperature.

Large pore gel was made up from the stock solutions in the
following proportions: tris buffer, 1 part: acrylamide, 2 parts:
initiator, 1 part: $H_2O$, 4 parts. A small volume of about 8 ml. was
de-aerated for 10 minutes. The water on the surface of the small
pore gels, which by now should have solidified, was removed with a
soft paper tissue and replaced with an equal volume of large pore gel.
Polymerisation was again allowed to take place at room temperature.
The reservoir buffer was diluted four times before use and, when the
apparatus was set up, 300 ml. of buffer were poured into the lower
reservoir and 250 ml. into the upper reservoir.

Protein extracts were diluted in 0.05 M tris buffer pH 7.2,
to give final concentrations of 4 mg/1 ml., and then made more dense
by the addition of 10% sucrose. Before application to the gel surface,
the protein extracts were stained by adding a tiny amount of bromophenol
blue (B.D.H.). This enabled the progress of the electrophoresis to
be followed. Protein samples containing 200 µg. protein were pipetted
gently onto the surface of each gel and the Vokam power supply switched
on to give an initial current of 1.25 ma. per tube. After 10 minutes
the current was increased to 2.5 ma. per tube and electrophoresis allowed
to proceed at constant current for a further 80 minutes, by which time
the bromophenol blue marker dye had reached the bottom of the tubes.

The gels were removed by passing water from a 10 ml. hypodermic syringe between the gel and the tube wall. They were then stained with 1% (w/v) amido black in 7% (v/v) acetic acid, for 60 minutes, and destained by successive changes in 7% (v/v) acetic acid. After staining the final position of the marker dye was still visible.

Photography

The gels were photographed using transmitted light on Pan F film ( Ilford Ltd.).

DNA  Base  Composition

The GC ratio of purified DNA was determined by the thermal denaturation method. Marmur and Doty (1959, 1962) have shown that when DNA was heated in solution, a sharp increase in the optical density occurred at the temperature where the double-stranded structure unwound to form the denatured state. The temperature corresponding to the midpoint of the absorbance rise, the Tm, was linearly related to the average DNA base composition. For a solvent containing 0.2 M - Na$^+$, Tm is given by

$$Tm = 69.3 + 0.41 \, (GC)$$

where Tm is in degrees Centigrade.

De Ley (1970) revised the formula, in accordance with more recent studies to

$$Tm = 69.4 + 0.41 \, (GC)$$

## Growth medium for cultures

Strains which were able to grow on simple media were inoculated into two 2-litre flasks, each containing 600 ml. Brain Heart Infusion Broth (Oxoid Ltd.). A 24 hr. broth culture was used at the inoculum and the flasks were incubated on a gyrotory shaker at 35°C for 24 hours.

Bordetella pertussis and B. parapertussis were grown in Hornibrook's medium (Hornibrook, 1939), and incubated for 3 days.

## Isolation of Deoxyribonucleic acid

DNA was isolated by the method Marmur (1961) with certain minor modifications. Firstly, after harvesting the cells by centrifugation, they were resuspended in saline-EDTA (0.15M NaCl plus 0.1 M ethylenediaminetetra-acetic acid, di-sodium salt at pH 8.0), to give a final concentration of 1g. wet weight in 25 ml. saline-EDTA. Secondly, shakings during deproteinisations were performed vigorously

by hand for 5 minutes. Thirdly, the DNA was precipitated with ethyl alcohol after the first deproteinisation and, thereafter, only when there was very little or no protein at the interface, i.e. immediately before treatment with ribonuclease and before the treatment with iso-propyl alcohol.

The purified DNA was dissolved in dilute saline citrate (0.015 M NaCl plus 0.0015 M tri-sodium citrate, pH 7.0), and the thermal denaturation temperature determined at least twice for each sample of DNA.

Determination of the thermal denaturation temperature

The system chosen was identical to the one in use at the National Collection of Type Cultures, Colindale. It consisted of a series one SP500 spectrophotometer (Unicam Instruments Ltd.), fitted with a thermostatically controlled heated cell holder (Type U; Adkins and Sons Ltd., Leicester). The accurate temperature inside the heated cuvette was monitored with a probe attached to a thermistor thermometer (Model C; Grant Instruments (Developments) Ltd., Cambridge).

The DNA was diluted in dilute saline citrate to obtain an initial optical density of about 0.1 on the scale. A similar cell containing only dilute saline citrate served as the blank. The initial optical density at 260 nm.was read and recorded with the temperature. The heating was then switched on and optical density readings taken at preset temperatures.

In the denaturation region, there was a sharp rise in optical density, and the temperature was then increased only a degree at a time; each time allowing the system to equilibrate, before the optical density and temperature were recorded. At the end of the denaturation zone, the optical density did not increase with subsequent rise in temperature, and

at this point the heating was switched off.

The melting temperature was determined by plotting a graph of relative optical density against temperature. Relative optical density is given by

$$\text{Relative OD} = \frac{\text{OD at temp } t^{\circ}C}{\text{minimum OD}}$$

The rise in optical density which occurs when denaturation is taking place is called the hyperchromicity, and the Tm is defined as that temperature corresponding to 50% of the hyperchromicity.

The melting temperature is not only dependent on the base composition of the DNA, but also on the ionic strength of the solvent in which the DNA is dissolved. The denaturation temperature is linearly related to the logarithm of the solvent ionic strength; thus when the DNA is dissolved in standard saline citrate, Tm is given by

$$Tm = 69.4 + 0.41 \ (GC)$$

However, when DNA is dissolved in dilute saline citrate, the Tm is given by

$$Tm = 16.6 \log M\text{-}Na^{+} + 0.41 \ (GC) + 81.5$$

(Schildkraut and Lifson, 1965; Hill, 1968).

Throughout this study, thermal denaturation temperatures were determined on DNA dissolved in dilute saline citrate.

CHAPTER 3


Results

NUMERICAL  TAXONOMY

Figure 1        Dendrogram showing the arrangement of 89 strains

of Bordetella included in computation 1, clustered

by Average Linkage.  The strain numbers are shown

on the right and correspond to those in table 8.

The horizontal scale shows the Similarity calculated

by Gower's coefficient.
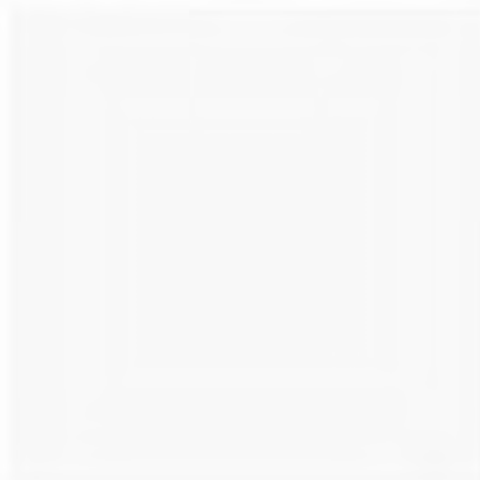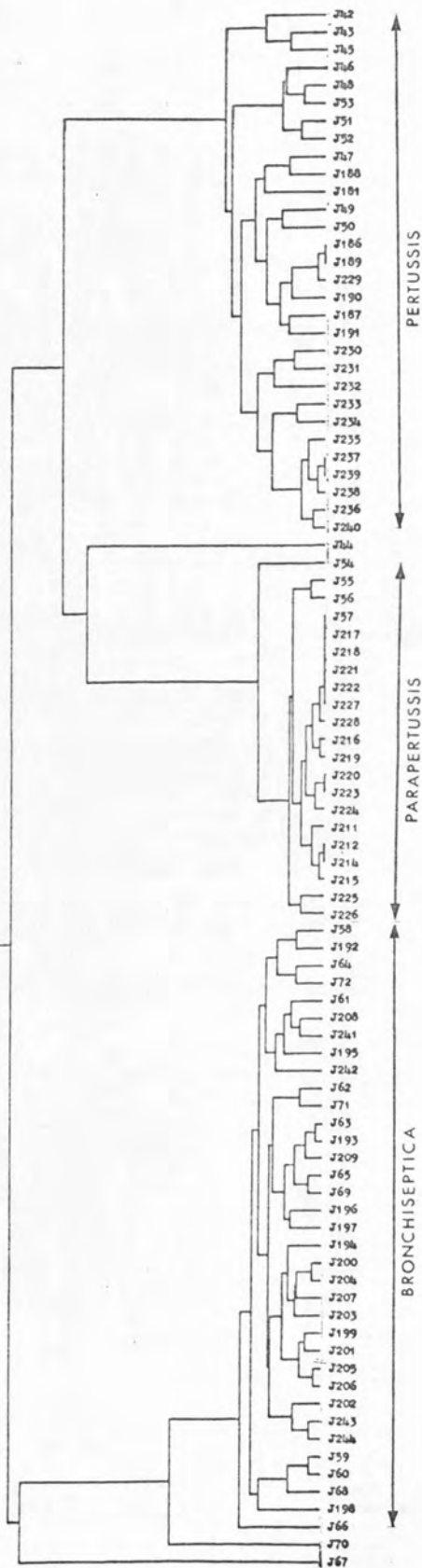
### The arrangement of strains into groups

The results of two computations are presented as dendrograms (Figs. 1 and 2), and as abbreviated similarity matrices (Appendix 1 and Appendix 2).

Figure 1 illustrates that all but three of the 89 OTU's shown in the dendrogram, fell into one of three very tight clusters. Cluster 1 contained strains received as Haemophilus or Bordetella pertussis. Cluster 2 contained strains received as Haemophilus or Bordetella parapertussis, while cluster 3 contained strains received as Haemophilus or Bordetella bronchiseptica. In order to avoid confusion between strains of Bordetella received as Haemophilus pertussis, H. parapertussis and H. bronchisepticus, and the true Haemophilus species which require X and V factors, only the latter group will be referred to as haemophili or Haemophilus species.

Strain J70 (Bordetella bronchiseptica, ATCC 15101), fell slightly outside cluster 3, but still retained an average similarity of over 91% with the rest of the cluster.

Two strains, J44 (B. pertussis, NCTC 8631) and J67 (B. bronchiseptica, ATCC 12714), fell significantly outside all three clusters.

Figure 2 represents the arrangement of the 112 OTU's not included in the first computation, together with 38 representative strains taken from Fig. 1. It was immediately apparent that the clusters in Fig. 1 having such high intra-group similarities, could be included on an enlarged dendrogram showing the arrangement of all 201 OTU's. This did not necessitate a further computer run, nor did it alter the arrangement of the other groups. The enlarged dendrogram is shown in a simplified form in Fig. 3.

Both Figs. 2 and 3 show that the 201 OTU's were divided into

Figure 2    Dendrogram showing the arrangement of 150

            Gram-negative rods included in computation 2,

            clustered by unweighted Average linkage and

            based on Similarities calculated from

            Gower's coefficient. The clusters are numbered

            as follows;

1   BRONCHISEPTICA-ALCALIGENES          9   ANITRATUS

2   LWOFFII                             10  ACTINOBACILLUS-PASTEURELLA

3   PERTUSSIS                           11  HAEM I

4   PARAPERTUSSIS                       12  HAEM II

5   M-N I                               13  HAEM III

6   M-N II                              14  M-N IV

7   M-N III                             15  YERSINIA.

8   BRUCELLA

Fig. 2

UNWEIGHTED AVERAGE LINKAGE

S (GOWER)
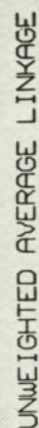
two large groups at the 66% similarity level.    Group A contained
51 OTU's which divided into three sub-groups A1, A2 and A3 at 68%.
Group B contained 150 OTU's which also divided into three sub-groups B1,
B2 and B3, but at the higher level of 75%.

It is worth noting that a line drawn across the dendrogram
at 75% divided the 201 strains into seven sub-groups.    Sub-groups A1,
A2, B1, B2 and B3 remained intact, but sub-group A3 was split into two
branches;  one containing the ACTINOBACILLUS-PASTEURELLA cluster, the
other containing clusters HAEM I, HAEM II and HAEM III.


The sub-groups of group A

The sub-groups A1, A2 and A3 were divided into six clusters,
accounting for 49 OTU's.    Two remaining strains, J153 Noguchia
granulosis and J172 Pasteurella haemolytica T, were associated with
sub-group A1, but did not have high similarities with the cluster found
in this sub-group.

Sub-group A1 had only one cluster, designated YERSINIA, the
strains of which joined together at 84.2% similarity.    This cluster
contained seven strains, 6 of which were received as Yersinia species and
the seventh as Pasteurella pfaffi.

Sub-group A2 contained four strains;  two were received as
Moraxella bovis, and two received as Neisseria pharyngis.    The one
cluster in this sub-group had a relatively low similarity of 74.4%, and
was labelled as M-N IV.

Sub-group A3 contained 38 strains which formed 4 clusters at
about 76%.    The cluster ACTINOBACILLUS-PASTEURELLA consisted of 3
strains of Actinobacillus which formed a tight group at 94.5%, and then
joined onto 6 strains of Pasteurella at 81.2%.    The three remaining
clusters all contained strains of Haemophilus.    Those included in HAEM I
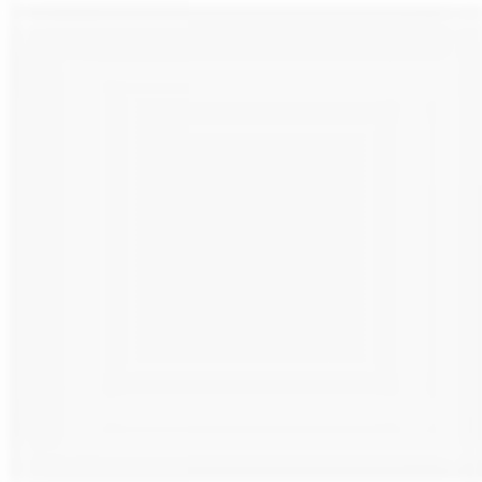
Figure 3    Simplified dendrogram showing the arrangement of

all 201 OTU's.   Dotted lines indicate strains which

fell outside the clusters.   The total number of

such strains is also included.  The similarities

were calculated from Gower's coefficient.
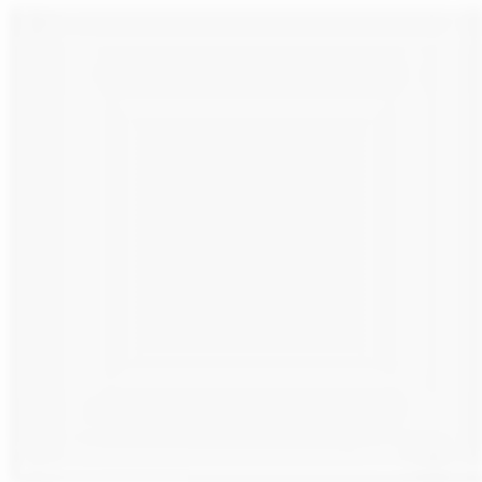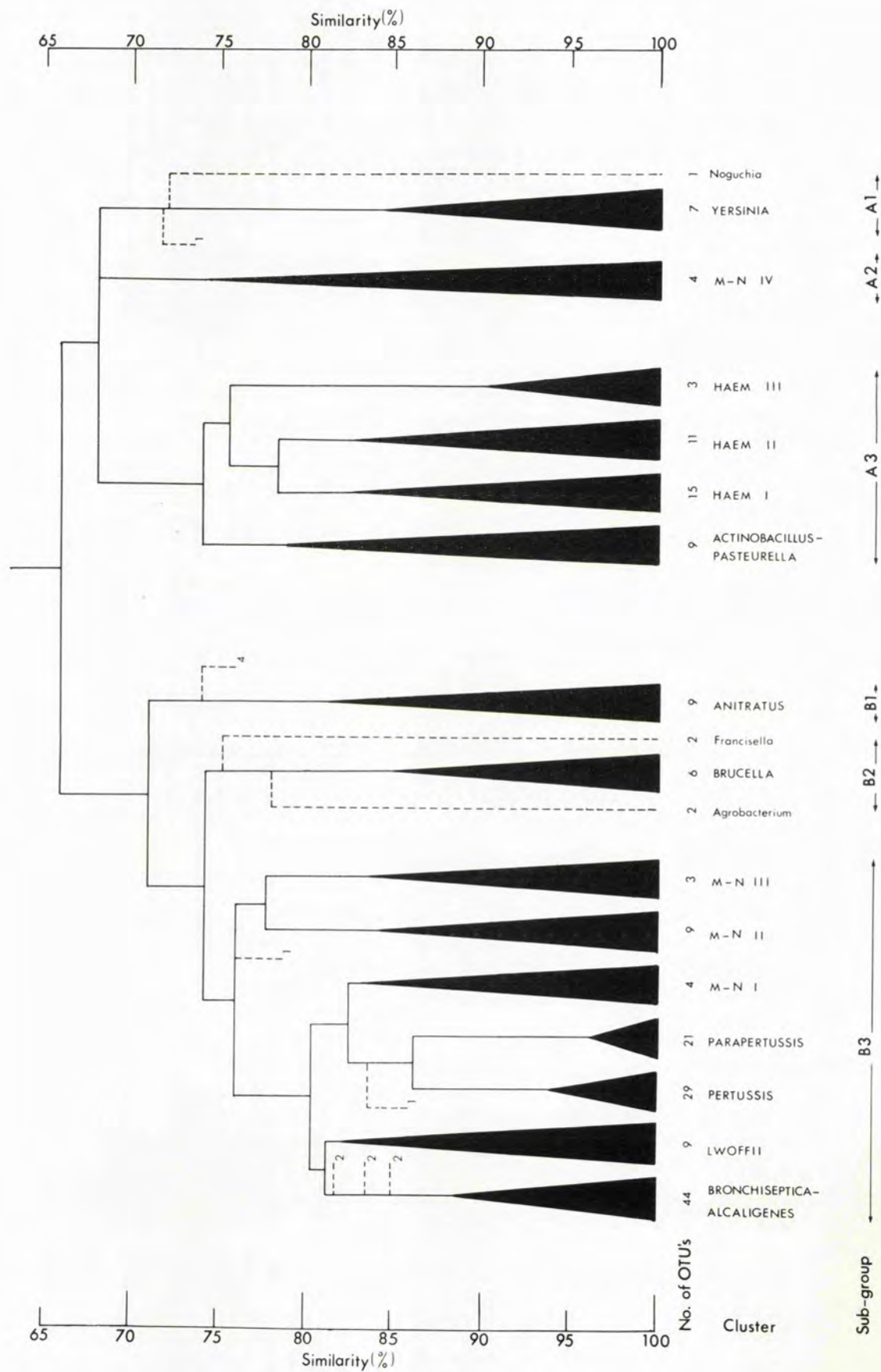
had an absolute requirement for X factor.    V factor was required

by some strains of this cluster but not by all.    HAEM II strains had

an absolute requirement for V factor, but did not require X factor.

HAEM III contained three strains which required additional $CO_2$ for growth,

regardless of their X and V factor requirements.


## The sub-groups of group B

The three sub-groups of group B were divided into nine clusters

containing 134 OTU's.    Two strains of Agrobacterium, J17 and J18, and

two  strains of Francisella J179 and J180, were attached to the BRUCELLA

cluster in sub-group B2.    A further 12 strains of assorted genera,

showed similarities with other clusters, but were not part of them.

Sub-group B1 contained a group of 9 strains received as

Achromobacter and Acinetobacter species, which clustered at 81.2%, and

formed the ANITRATUS cluster.    An additional 4 strains were attached

to this cluster at the 74% similarity level.

Sub-group B2 contained 10 strains, 6 of which were received

as Brucella spp and formed the BRUCELLA cluster at 85%.    Two strains of

Agrobacterium and two strains of Francisella joined onto BRUCELLA at

78% and 75.2% respectively.

Sub-group B3 represented the largest of the sub-groups and

contained 127 OTU's of which 119 were sorted into seven clusters.    This

sub-group could be split into two major branches at the 77% similarity

level, each branch containing several well-defined clusters.

Except for the 4 strains of Moraxella and Neisseria which

appeared in sub-group A2, and for 2 strains of Moraxella lwoffii which

occurred in the LWOFFII cluster, the remaining strains of Moraxella

and·Neisseria fell into three clusters designated M-N I, M-N II and

M-N III.    Figure 3 shows that clusters M-N II and M-N III fell together

Figure 4    Intra- and Inter-group similarities determined on

17 clusters.    The variance and standard deviation

are also recorded. The first line of each box shows

the mean inter-group similarity, the second line the

variance, while the third line shows the standard

deviation.

| | YERSINIA | HAEM III | HAEM II | HAEM I | PASTEURELLA | ACTINOBACILLUS | M-N IV | M-N III | M-N II | M-N I | PERTUSSIS | PARAPERTUSSIS | BRONCHISEPTICA | ALCALIGENES | LWOFFII | ANITRATUS | BRUCELLA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **BRUCELLA** | 87.14/18.49/4.30 | 91.20/5.25/2.29 | 85.81/15.04/3.87 | 85.43/15.62/3.95 | 78.90/12.42/3.52 | 94.87/0.49/0.70 | 76.66/17.71/4.21 | 84.63/2.54/1.59 | 86.70/19.75/4.44 | 84.87/7.96/2.82 | 95.27/2.89/1.70 | 98.43/1.41/1.19 | 95.93/4.04/2.02 | 89.08/15.64/3.96 | 85.08/14.52/3.81 | 85.53/12.75/3.57 | 87.70/12.12/3.48 |
| **ANITRATUS** | 64.67/8.02/2.83 | 76.50/8.54/2.92 | 78.59/12.21/3.49 | 73.11/14.08/3.75 | 80.09/4.64/2.15 | 69.85/7.46/2.73 | 64.50/42.38/6.51 | 77.85/8.79/2.96 | 82.15/8.17/2.86 | 81.20/5.29/2.30 | 86.06/4.87/2.21 | 83.37/1.89/1.37 | 89.84/6.56/2.56 | 79.96/13.13/3.62 | 72.61/13.13/3.62 | 72.38/32.28/5.68 | |
| **LWOFFII** | 68.11/13.52/3.68 | 75.09/10.27/3.20 | 74.96/7.44/2.73 | 75.56/7.56/2.75 | 71.55/15.54/3.94 | 63.87/6.26/2.50 | 73.32/36.97/6.08 | 76.65/16.53/4.06 | 77.97/6.03/2.45 | 84.52/2.18/1.48 | 82.05/5.41/2.33 | 79.63/4.56/2.14 | 82.23/8.11/2.84 | 75.48/9.35/3.06 | 72.10/19.73/4.44 | | |
| **ALCALIGENES** | 65.14/11.82/3.44 | 71.91/12.19/3.49 | 74.86/2.91/1.71 | 67.63/27.65/5.89 | 61.92/16.99/4.12 | 67.45/10.38/3.22 | 72.87/47.87/6.92 | 78.25/3.47/1.86 | 80.64/5.60/2.36 | 79.40/6.19/2.49 | 78.38/3.93/1.98 | 80.20/5.28/2.30 | 75.91/14.56/3.82 | 79.68/22.67/4.76 | | | |
| **BRONCHISEPTICA** | 70.49/18.52/4.30 | 71.59/2.46/1.57 | 67.27/27.65/5.28 | 64.27/8.57/2.93 | 65.56/16.85/4.10 | 69.89/6.81/2.61 | 72.67/47.08/6.41 | 76.74/7.36/2.71 | 73.09/12.38/3.52 | 77.46/4.27/2.07 | 80.85/6.28/2.51 | 70.13/9.11/3.02 | 80.31/24.67/4.97 | | | | |
| **PARAPERTUSSIS** | 73.73/6.51/2.55 | 64.87/9.14/3.02 | 60.13/15.50/3.94 | 66.48/14.52/3.82 | 69.49/15.80/3.98 | 69.22/2.34/1.55 | 68.31/23.09/4.80 | 79.13/1.78/1.33 | 71.19/13.29/3.65 | 80.84/8.90/2.98 | 68.07/5.28/2.30 | 78.62/20.02/4.47 | | | | | |
| **PERTUSSIS** | 66.58/11.20/3.35 | 54.90/35.61/5.97 | 62.01/23.78/4.88 | 70.98/10.33/3.21 | 69.67/2.85/1.69 | 67.35/1.51/1.23 | 64.30/23.30/4.83 | 75.76/3.10/1.76 | 76.70/9.96/3.16 | 65.79/7.06/2.65 | 77.08/10.09/3.18 | | | | | | |
| **M-N I** | 59.69/10.58/3.25 | 58.72/21.79/4.67 | 67.50/18.90/4.35 | 73.05/14.04/3.75 | 65.60/14.42/3.79 | 67.11/5.37/2.31 | 62.80/25.20/5.02 | 78.52/8.98/2.99 | 62.42/8.87/2.98 | 75.63/9.37/3.06 | | | | | | | |
| **M-N II** | 58.79/17.87/4.23 | 61.38/3.22/1.79 | 69.12/10.55/3.25 | 69.11/10.95/3.31 | 66.03/17.07/4.13 | 63.87/6.08/2.46 | 66.83/32.56/5.71 | 66.79/5.68/2.38 | 71.76/40.13/6.33 | | | | | | | | |
| **M-N III** | 62.51/7.16/2.68 | 64.86/2.42/1.56 | 64.78/10.86/3.29 | 66.03/8.34/3.23 | 63.74/21.12/4.50 | 68.07/5.54/2.35 | 63.29/7.07/2.66 | 70.47/13.31/4.16 | | | | | | | | | |
| **M-N IV** | 62.02/5.04/2.25 | 60.47/1.41/1.19 | 64.94/2.15/1.79 | 64.26/10.46/3.23 | 65.55/11.86/3.44 | 66.22/7.14/2.67 | 69.71/30.80/5.55 | | | | | | | | | | |
| **ACTINOBACILLUS** | 65.60/4.09/2.02 | 58.59/5.01/2.24 | 64.02/4.74/2.18 | 68.86/8.60/2.93 | 64.71/24.30/4.93 | 70.70/15.84/3.98 | | | | | | | | | | | |
| **PASTEURELLA** | 70.16/7.23/2.69 | 58.88/3.01/1.74 | 64.27/10.97/3.31 | 62.89/14.68/3.83 | 68.50/32.07/5.66 | | | | | | | | | | | | |
| **HAEM I** | 69.68/6.73/2.96 | 64.36/5.56/2.43 | 63.24/16.11/4.01 | 66.22/19.93/4.46 | | | | | | | | | | | | | |
| **HAEM II** | 65.06/5.95/2.44 | 61.98/10.59/3.25 | 64.98/8.16/2.86 | | | | | | | | | | | | | | |
| **HAEM III** | 72.03/9.34/3.05 | | | | | | | | | | | | | | | | |
| **YERSINIA** | 76.27/19.15/4.38 | | | | | | | | | | | | | | | | |

in the same branch, whereas M-N I appears to be more closely related
to the clusters in the other branch of sub-group B3.

Both the PERTUSSIS and PARAPERTUSSIS clusters remained
extremely tight and well-defined, as was shown in Fig. 1.

The LWOFFII cluster contained 9 strains, four of which were
received as Moraxella or Acinetobacter lwoffii.  The remaining 5 strains
were non-motile Alcaligenes species, received as A. viscosus or
A. metalcaligenes.

The boundary of the remaining cluster BRONCHISEPTICA-
ALCALIGENES, was less well-defined in Fig. 2.    It was possible to draw
the line either between OTU's J67 and J9, or between OTU's J8 and J7.
Examination of the biochemical characters of the OTU's of this cluster
indicated, however, that it would be more appropriate to draw the line
between J8 and J7 and regard the six strains J7, J44, J30, J31, J26 and
J67 as satellite organisms of this cluster.  This will be further
discussed later.

<u>Intra-group and inter-group average similarities</u>

It quickly became obvious to disciples of numerical taxonomy
that the only way to accurately represent the taxonomic relationships
of n OTU's, was to arrange the OTU's in a multi-dimensional space, where
the number of dimensions required was equal to n-1.  A dendrogram
reduces the number of dimensions to two, if it is accepted that the
similarity scale forms one dimension, and the order of the OTU's forms
the second dimension.   The second dimension incidentally, is not a
fixed entity since it is possible to twist groups about their vertical
stems.    Nevertheless, the gross reduction in the number of dimensions,
naturally results in loss of information.    Some of this information
can be recovered by calculating intra-group and inter-group average

| CLUSTER | NEAREST NEIGHBOURS | | | | | | WITH PER CENT SIMILARITY |
|---|---|---|---|---|---|---|---|
| **Sub-group A1** | | | | | | | |
| YERSINIA | BRUCELLA | 76.27 | ACTINOBACILLUS | 73.73 | ANITRATUS | 72.03 | PASTEURELLA 70.49 |
| **Sub-group A3** | | | | | | | |
| HAEM III | HAEM II | 76.50 | HAEM I | 75.09 | PASTEURELLA | 71.91 | ACTINOBACILLUS 71.59 |
| HAEM II | HAEM I | 78.39 | HAEM II | 76.50 | PASTEURELLA | 74.96 | ACTINOBACILLUS 74.86 |
| HAEM I | HAEM II | 78.39 | ACTINOBACILLUS | 75.56 | HAEM III | 75.09 | PASTEURELLA 73.11 |
| PASTEURELLA | ACTINOBACILLUS | 80.09 | HAEM II | 74.96 | HAEM I | 73.11 | HAEM III 71.91 |
| ACTINOBACILLUS | PASTEURELLA | 80.09 | HAEM I | 75.56 | HAEM II | 74.86 | YERSINIA 73.73 |
| **Sub-group A2** | | | | | | | |
| M-N IV | M-N II | 73.32 | M-N I | 72.87 | PERTUSSIS | 72.67 | PASTEURELLA 71.55 |
| **Sub-group B3** | | | | | | | |
| M-N III | BRONCHISEPTICA | 79.13 | LWOFFII | 78.52 | PERTUSSIS | 78.25 | M-N II 77.85 |
| M-N II | M-N I | 82.15 | PARAPERTUSSIS | 80.64 | PERTUSSIS | 77.97 | M-N III 77.85 |
| M-N I | PARAPERTUSSIS | 84.52 | M-N II | 82.15 | PERTUSSIS | 81.20 | LWOFFII 80.84 |
| PERTUSSIS | PARAPERTUSSIS | 86.06 | BRONCHISEPTICA | 82.05 | M-N I | 81.20 | LWOFFII 80.85 |
| PARAPERTUSSIS | PERTUSSIS | 86.06 | M-N I | 84.52 | BRONCHISEPTICA | 83.37 | M-N II 80.64 |
| BRONCHISEPTICA | ALCALIGENES | 89.84 | PARAPERTUSSIS | 83.37 | LWOFFII | 82.23 | PERTUSSIS 82.05 |
| ALCALIGENES | BRONCHISEPTICA | 89.84 | LWOFFII | 79.96 | BRUCELLA | 79.68 | PARAPERTUSSIS 79.63 |
| LWOFFII | BRONCHISEPTICA | 82.23 | PERTUSSIS | 80.85 | M-N I | 80.84 | PARAPERTUSSIS 80.20 |
| **Sub-group B1** | | | | | | | |
| ANITRATUS | BRONCHISEPTICA | 75.91 | ALCALIGENES | 73.48 | LWOFFII | 72.61 | BRUCELLA 72.38 |
| **Sub-group B2** | | | | | | | |
| BRUCELLA | BRONCHISEPTICA | 80.31 | ALCALIGENES | 79.68 | PARAPERTUSSIS | 78.62 | PERTUSSIS 77.08 |

Table 9   Inter-group nearest neighbours (adapted from results shown in Figure 4).

similarities, and representing the results in the form of a simple
model.

The 15 clusters shown in Fig. 3 were taken as the basis for
this exercise.    However,  for the purpose of calculating intra-group
and inter-group similarities, the ACTINOBACILLUS-PASTEURELLA and the
BRONCHISEPTICA-ALCALIGENES clusters were sub-divided, and the resulting
four groups each given cluster status.   The new clusters contained the
following strains, shown in order in Fig. 2:

|  |  |
|---|---|
| BRONCHISEPTICA | J58 - J68, |
| ALCALIGENES | J1, J70 - J8, |
| ACTINOBACILLUS | J19, J20, J21, |
| PASTEURELLA | J245 - J171. |

Average similarities, together with variance and standard
deviation, were calculated between each of the 17 clusters and the
results are shown in Fig. 4.    When calculating intra-group similarities,
the values of 100% obtained when strains were compared with themselves,
were ignored because they introduced a bias towards a high similarity.

The four nearest neighbours for each cluster were extracted
from Fig. 4 and are shown in Table 9.   It can be seen that with one
exception (ACTINOBACILLUS-YERSINIA), all the clusters within sub-group A3,
had their nearest neighbours within sub-group A3.   The same applied to
sub-group B3, indicating that the two major sub-groups containing 165
OTU's, were distinct and homogeneous.   The clusters of three other
sub-groups, A1, A2 and B1 did not show high similarities with either
sub-group A3 or B3, indicating that they may be intermediate in their
taxonomic relationships, or more likely, that they were related to
organisms not represented  in this study.   Examination of sub-group A2,
indicated that it was not a natural cluster, but rather a collection of

Figure 5.  Taxonomic model based on inter-group similarities.
Each node represents a cluster, while the inter-nodal
distances D, are based on the formula, $D = 1 - S_{Gower}$.

This is a view of a three dimensional model, where the
distances were as close to those determined from the
mean inter-group similarities as was possible to allow
in constructing the model manually.
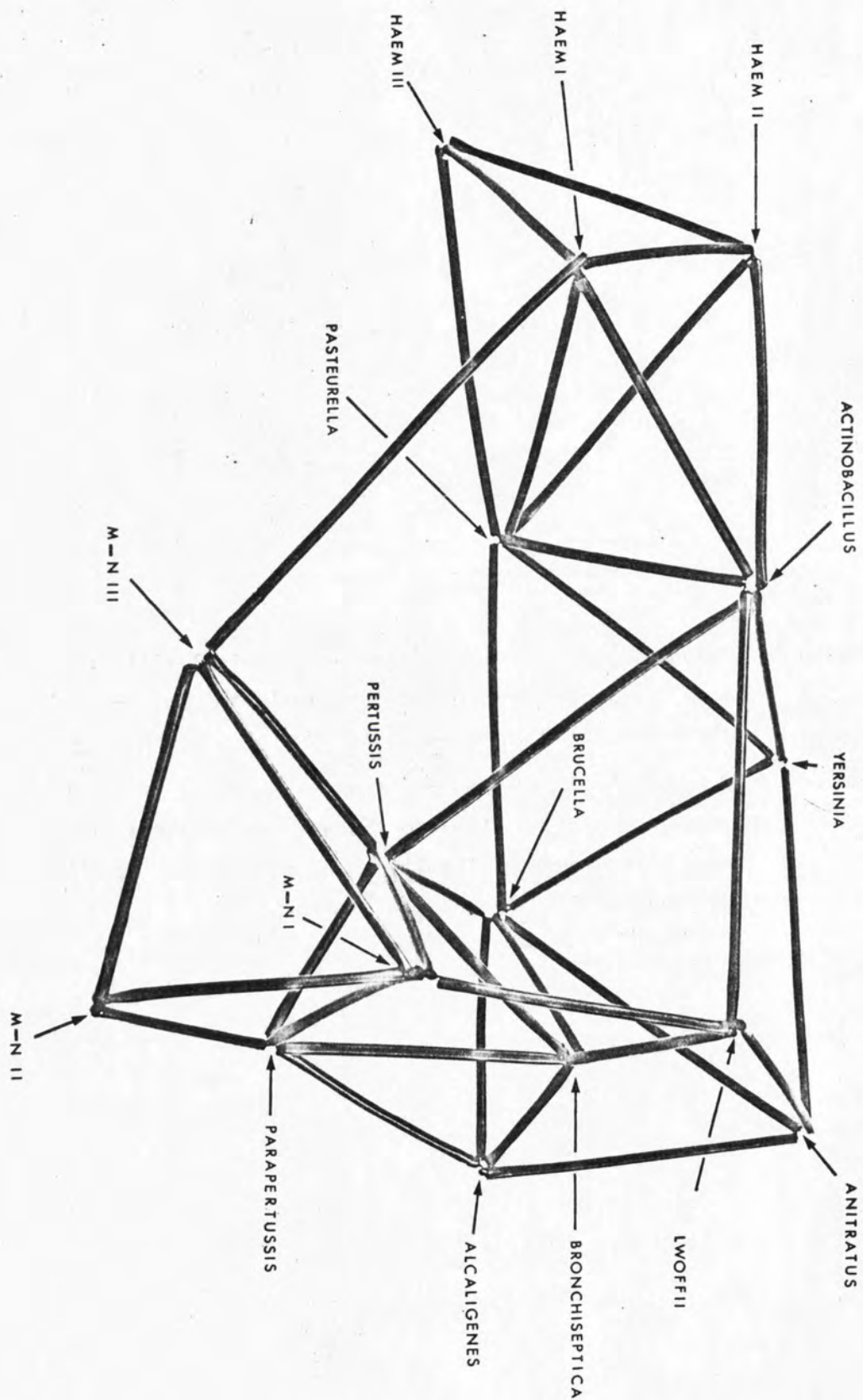
two pairs of OTU's.    This was reflected in the low intra-group similarity of M-N IV.

Sub-group B2, represented by the BRUCELLA cluster, had similarities which were sufficiently high to enable it to be considered as a satellite cluster of sub-group B3.

The inter-group similarity values were used to construct the model shown in Fig. 5.    The junctions on the model corresponded to one of the named clusters, while the distance  D, between each cluster was based on the formula $D = 1 - S_{SM}$, where $S_{SM}$ is the Simple Matching Coefficient (Sokal and Sneath, 1963).    In this study $S_{SM}$ was replaced by Gower's Similarity Coefficient.    In actual fact, Sokal and Sneath have pointed out that taxonomic distance D, is given by the formula

$$D^2 = 1 - S_{SM}.$$

In the construction of the model, however, use of this formula for calculating distance, exaggerated the distance between clusters having a high similarity to each other, so that the modification seemed empirically justified. Cluster M-N IV has been omitted from the model because of its suspect nature as a homogeneous cluster.

### The position of sub-group A2

Because the four strains of M-N IV were received as Moraxella and Neisseria spp., it was somewhat surprising that they did not fall into sub-group B3, near to clusters M-N I, M-N II or M-N III.    This point was investigated by a third computation in which the four OTU's of M-N IV were combined with the 150 OTU's of group B.

The limits of the computer demanded that four OTU's be omitted;  this was overcome by removing four strains of Bordetella parapertussis which had 100% similarities with other strains of

Figure 6    Dendrogram showing the relationship of cluster
           M-N IV to group B, based on a third computation
           in which sub-groups A1 and A3 had been omitted.

Fig.6

UNWEIGHTED AVERAGE LINKAGE

opp. 85
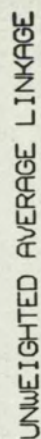
B. parapertussis.

     The resulting dendrogram is shown in Fig. 6, and it was noticable that the groups obtained bear remarkable resemblance to the corresponding groups shown in Fig. 2. Except for the enlargement of the clusters containing B. bronchiseptica, B. pertussis and B. parapertussis the position of cluster M-N III, and the positions of the following satellite strains, J44, J26, J67, J9, J146, J33, J179 and J180, the corresponding clusters of both dendrograms were identical.

     The four strains of sub-group A2 still remained outside the groups formed by the other OTU's, indicating a definite lack of relationship to group B clusters.

### The properties of the groups and clusters

     The complete data matrix (in coded form) is shown in Appendix 3, while a shortened table of characters is included in Appendix 4. Strains belonging to the named clusters were Gram-negative or Gram-variable. They all grew aerobically on BM agar, while some were also capable of anaerobic growth.

### The properties of the Bordetella clusters shown in Fig.1

     The strains which fell into three clusters in Fig. 1 were all Gram-negative coccobacilli or short rods, which showed no tendency to retain methyl violet. Pleomorphism, in the form of chains or filaments, was found in only two strains, both received as Bordetella pertussis. None of the strains would grow anaerobically, even in the presence of nitrate as a terminal electron acceptor. Additional $CO_2$ or growth factors, such as haematin or nicotinamide adenine dinucleotide, were not required by any strain.

Table 10   Characters which differentiate the three clusters of Figure 1

| Character | BRONCHI-SEPTICA | PERTUSSIS | PARA-PERTUSSIS |
|---|---|---|---|
| Pigment from tyrosine | − | − | + |
| Motility | + | − | − |
| Growth on 5% Bile salts | + | − | + |
| "      40% Bile salts | + | − | − |
| Growth on 3% NaCl | + | − | + |
| "      6% NaCl | + | − | − |
| Sensitivity to O/129 | − | + | − |
| Growth in the presence of safranine | + | − | − |
| Growth on 0.1% phenol agar | + | − | − |
| Sensitivity to penicillin | − | + | − |
| Kovac's oxidase | + | + | − |
| Urease | + | − | + |
| Nitrate reduction | + | − | − |
| Hydrolysis of tyrosine | + | − | − |
| Clearing of tween 80 | + | − | − |
| Growth at 15°C | + | − | + |

Key:   + more than 80% of strains positive

− more than 80% of strains negative

Table 11      Differentiation of groups A and B shown in Figs. 2 and 3

| Group | A | | | B | | |
|---|---|---|---|---|---|---|
| Sub-group | A1 | A2 | A3 | B1 | B2 | B3 |
| **Character** | | | | | | |
| Growth anaerobically | + | v | + | – | – | – |
| Growth anaerobically on media containing nitrate | + | v | + | – | + | – |
| Acid from 1% glucose, aerobically | + | + | + | + | – | – |
| Acid from 1% glucose, anaerobically | + | v | + | – | – | – |
| Acid from 10% glucose | + | + | v | + | + | – |

Neither thionine, pyronine, sodium azide nor M and B 938 inhibited growth, whereas no growth took place in the presence of potassium tellurite, thallous acetate or most antibiotics. Bacitracin, however, did not inhibit growth. Positive reactions were obtained for the following biochemical tests: catalase, peroxidase, production of ammonia from peptone, and production of ammonia from arginine.

The following tests gave negative results: gelatin liquefaction, phosphatase, hydrolysis of starch, lecithinase, hydrolysis of aesculin, hydrolysis of casein, deoxyribonuclease, methyl red, Voges Proskauer, hydrolysis of xanthine, hydrolysis of tweens, hydrogen cyanide production, and the production of acid from carbohydrates.

Properties which were useful for the differentiation of the three clusters are shown in Table 10.

## The properties of the clusters shown in Figs. 2 and 3
Differentiation of group A from group B

Examination of the abbreviated data matrix (Appendix 4), indicated that the ability to grow anaerobically, and the ability to produce acid from 1% (w/v) glucose, played a major role in differentiating group A and group B. They were also useful characters in the differentiation of the six sub-groups as shown in Table 11.

The heterogeneity of M-N IV as a cluster was again apparent from the variable results shown by sub-group A2 in Table 11. If the four strains of this cluster were excluded, the results indicated that group A strains were fermentative, being able to produce acid from glucose anaerobically. Group B strains were oxidative, being able to produce acid from glucose only aerobically, or not at all.

Table 12    Differentiation of the sub-groups and clusters of group A

| Sub-group | A3 | | | | | A2 | A1 |
|---|---|---|---|---|---|---|---|
| Cluster | ACTINO-BACILLUS | PASTEUR-ELLA | HAEM I | HAEM II | HAEM III | M-N IV | YERSINIA |
| **Character** | | | | | | | |
| Growth on nutrient agar | + | + | - | - | - | + | + |
| Requirement for $CO_2$ | - | - | - | - | + | - | - |
| X factor requirement | - | - | + | - | v | - | - |
| V factor requirement | - | - | v | + | v | - | - |
| Growth at 15°C | + | v | - | - | - | v | + |
| Growth on 0% NaCl | + | v | + | + | + | - | + |
| Growth on 4.5% NaCl | - | - | - | - | - | v | + |
| Growth in the presence of basic fuchsin | v | v | - | + | + | - | + |
| Sensitivity to 0/129 | + | + | v | - | v | v | - |
| Catalase | + | + | + | + | - | + | + |
| Oxidase | + | + | - | v | - | + | - |
| Phosphatase | + | + | + | + | + | - | - |
| Hydrolysis of starch | + | + | v | v | - | + | v |
| Tween 80 clearing | - | - | - | - | - | - | + |
| Acid from 10% glucose | + | + | - | - | + | + | + |
| Acid from 1% lactose | - | - | - | - | + | - | - |
| fructose | + | v | - | + | + | + | + |
| arabinose | + | - | - | v | - | - | + |
| sucrose | - | + | - | + | + | + | v |
| mannitol | + | + | - | - | - | v | + |

Key:    +    more than 80% of strains positive

     -    more than 80% of strains negative

     v    variable

Characters associated with group A

Strains belonging to group A were Gram-negative, only three strains of sub-group A2, showing any tendency to retain methyl violet. Pleomorphism was quite common, particularly in clusters HAEM I and HAEM II. Colonies were not pigmented and pigment was not produced from tyrosine. Growth did not take place on 40% bile salts agar, 6% sodium chloride agar or on 0.2% phenol agar. They were sensitive to most antibiotics, but not bacitracin. Sodium azide did not inhibit growth. Strains of sub-groups A1 and A3 produced hydrogen sulphide, and produced acid from 1% glucose, aerobically and anaerobically.

The following tests gave negative results: gelatin liquefaction, lecithinase, hydrolysis of casein, deoxyribonuclease, hydrogen cyanide production, Voges Proskauer, hydrolysis of tyrosine, xanthine and tweens.

The characters which have proved useful in the identification and differentiation of the clusters and sub-groups of group A are shown in Table 12.

Characters associated with group B

Strains belonging to group B were Gram-negative, although strains in clusters M-N II and M-N III showed some retention of methyl violet. Between strains, cells varied in shape from coccobacilli to medium length rods, but showed little evidence of pleomorphism. Colonies were usually non-pigmented but some strains, particularly those of the PARAPERTUSSIS cluster, were able to produce pigment from tyrosine. No growth took place anaerobically, although strains of the BRUCELLA cluster would grow anaerobically on media containing nitrate. Additional $CO_2$ or X or V factors were not required for growth on artificial media. Most strains grew on nutrient agar. No growth occurred on 0.3% phenol

Table 13    Differentiation of the sub-groups and clusters of group B

| Sub-group | B3 | | | | | | | | B2 | B1 |
|---|---|---|---|---|---|---|---|---|---|---|
| Cluster | ALCALIGENES | BRONCHISEPTICA | PERTUSSIS | PARAPERTUSSIS | LWOFFII | M-NI | M-N II | M-N III | BRUCELLA | ANITRATUS |
| **Character** | | | | | | | | | | |
| Pigment from tyrosine | - | - | - | + | - | - | - | + | - | - |
| Growth anaerobically in the presence of nitrate | - | - | - | - | - | - | - | - | + | - |
| Motility | + | + | - | - | - | - | - | - | - | v |
| Growth on 5% bile salts | + | + | - | + | + | - | - | + | v | + |
| Growth on 6% NaCl | v | + | - | - | - | - | - | + | - | - |
| Growth on 0.1% phenol agar | + | + | - | - | + | - | - | v | v | + |
| Sensitivity to penicillin | - | - | + | - | v | + | + | + | + | - |
| Sensitivity to bacitracin | - | - | - | - | - | - | + | - | - | - |
| Growth in the presence of potassium tellurite | + | - | - | - | - | - | - | - | * | - |
| Growth in the presence of M and B 938 | + | + | + | + | - | - | - | v | * | + |
| Oxidase | + | + | + | - | - | + | + | + | + | - |
| Hydrolysis of starch | - | - | - | - | - | + | + | + | - | - |
| Urease | v | + | - | + | - | - | - | v | + | + |
| Nitrate reduction | + | + | - | - | - | - | v | v | + | - |
| Hydrolysis of tyrosine | + | + | - | - | v | - | - | - | - | + |
| Hydrolysis of tween 60 | - | - | - | - | v | - | + | + | * | v |
| Acid from 1% Glucose | - | - | - | - | - | - | - | - | - | + |
| Acid from 10% Glucose | - | - | - | - | - | - | - | - | + | + |

Key:    +    more than 80% of strains positive

         -    more than 80% of strains negative

         v    variable

         *    no comparison, test not done

agar or in the presence of most antibiotics.   Reactions to penicillin, nitrofurantoin and bacitracin, however, were variable both within and among clusters.   Sodium azide did not inhibit growth.   The following biochemical characters gave positive results: catalase, peroxidase, production of ammonia from peptone and arginine.

Negative reactions were given in the following: aesculin hydrolysis, deoxyribonuclease, hydrogen cyanide production, methyl red, Voges Proskauer, and the production of acid from 1% glucose, anaerobically.

Table 13 indicates the characters which were found most useful for the differentiation of the clusters of group B.

## The production of acid from carbohydrates

The results have clearly indicated that the production of acid from glucose was a most useful property in differentiating the sub-groups.   Baumann, Doudoroff and Stainer (1968b) have legitimately pointed out that in the oxidase-negative moraxellas, glucose is metabolised by a non-specific aldose dehydrogenase enzyme, which can also oxidise D-galactose, D-mannose, D-ribose, D-xylose, L-arabinose, L-rhamnose, maltose, lactose and cellobiose.   Thus, production of acid from these sugars is highly correlated with the ability to produce acid from glucose.   This raises serious doubts as to whether the results of "sugar tests" are truly independent characters, and whether they bias the similarities between organisms capable of producing acid from glucose, and those which are not.

In this study, tests on the production of acid from carbohydrates contributed 28 unit characters in the data matrix.   This number would seem to be sufficiently large to exert an influential effect on the formation of clusters.

Table 14    Inter-strain similarities between the LWOFFI and ANITRATUS clusters,

based on the complete data matrix, and after the deletion of 22

sugar characters.

| | | LWOFFII | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | J9 | J10 | J12 | J13 | J11 | J27 | J132 | J133 | J29 |
| ANITRATUS | J23 | 69.7 | 74.1 | 76.7 | 71.3 | 70.1 | 70.4 | 71.7 | 69.2 | 73.6 |
| | | 70.8 | 75.9 | 79.2 | 72.4 | 72.4 | 71.4 | 73.3 | 70.5 | 75.2 |
| | J37 | 71.7 | 78.4 | 81.9 | 74.5 | 75.7 | 73.1 | 74.9 | 71.4 | 76.3 |
| | | 73.8 | 81.9 | 85.2 | 76.6 | 78.1 | 74.8 | 77.6 | 72.9 | 78.5 |
| | J40 | 66.3 | 74.3 | 77.0 | 74.7 | 71.8 | 74.5 | 73.5 | 70.5 | 76.0 |
| | | 66.7 | 76.9 | 79.4 | 76.6 | 73.3 | 76.6 | 75.7 | 71.9 | 78.3 |
| | J38 | 71.2 | 76.3 | 77.8 | 73.7 | 70.3 | 74.3 | 76.6 | 74.2 | 76.5 |
| | | 72.8 | 80.0 | 80.5 | 75.7 | 71.4 | 76.6 | 79.4 | 76.6 | 79.4 |
| | J35 | 62.9 | 71.7 | 71.8 | 70.3 | 64.4 | 68.6 | 69.1 | 67.7 | 70.7 |
| | | 65.0 | 75.2 | 75.0 | 73.8 | 66.7 | 71.0 | 71.9 | 71.4 | 73.8 |
| | J24 | 66.4 | 74.9 | 80.5 | 75.8 | 69.0 | 72.3 | 72.5 | 69.5 | 75.7 |
| | | 67.9 | 78.1 | 85.0 | 79.2 | 71.4 | 74.5 | 75.5 | 71.7 | 79.2 |
| | J34 | 68.3 | 75.2 | 81.3 | 76.0 | 72.5 | 69.4 | 71.7 | 68.2 | 72.3 |
| | | 70.8 | 79.0 | 87.0 | 80.4 | 76.2 | 72.9 | 74.8 | 71.0 | 75.7 |
| | J39 | 68.5 | 72.5 | 77.9 | 74.0 | 69.8 | 75.1 | 72.3 | 71.4 | 74.4 |
| | | 69.9 | 74.3 | 80.5 | 75.7 | 71.4 | 71.0 | 77.6 | 72.9 | 76.6 |
| | J32 | 67.2 | 73.9 | 76.7 | 72.9 | 72.2 | 72.3 | 70.2 | 68.7 | 68.1 |
| | | 67.6 | 75.9 | 79.2 | 74.5 | 74.3 | 74.5 | 71.7 | 69.8 | 68.6 |

Key:        the upper figure in each box refers to the inter-strain

similarities based on the complete data matrix.

The lower figure refers to the similarities, after the deletion

of the sugar characters.

Figure 7    Dendrogram of the strains shown in Fig. 2, based on

a similarity matrix from which 22 sugar characters

had been deleted . This is computation four.
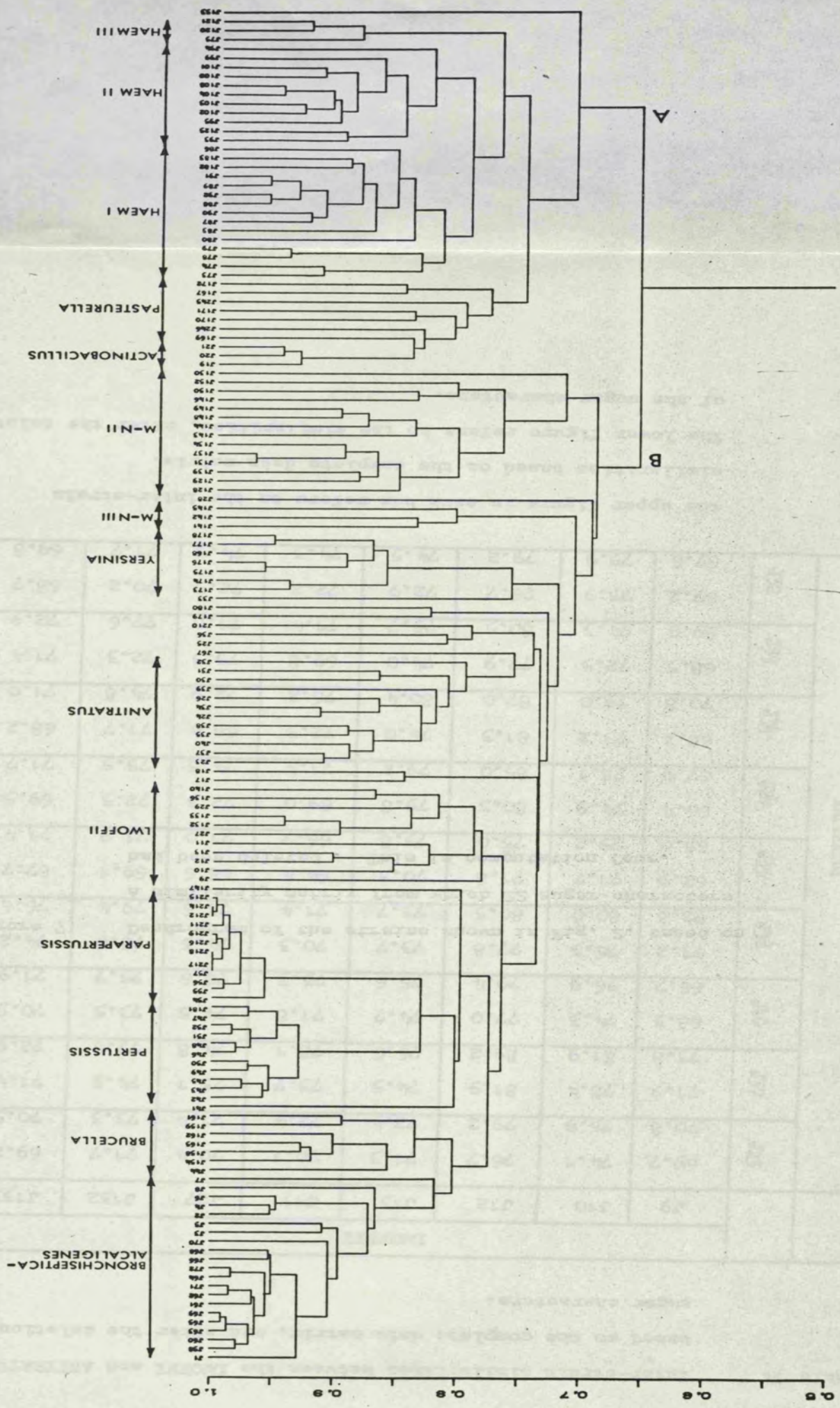
UNWEIGHTED AVERAGE LINKAGE

Fig. 7

A preliminary investigation was made by selecting the strains of two clusters LWOFFII and ANITRATUS, and comparing their inter-strain similarities calculated from the complete data matrix, and from a data matrix from which the last 22 sugar characters (Table A1, xylose to glucosamine hydrochloride) had been deleted. Table 14 indicates how little the inter-strain similarities altered. The inter-group average similarity rose from 72.61 to 74.92 per cent, indicating that although the sugar characters did tend to bias the similarities, the effect was small and would not grossly affect the clusters formed.

The strains shown in Figs. 2 and 6 were re-sorted by the computer, after deleting the 22 sugar characters from the data matrix. The dendrograms obtained are shown in Figs. 7 and 8.

Comparison of Figs. 7 and 2 indicated that sub-group A3 remained virtually identical, except for the inclusion of J172 into the PASTEURELLA cluster. The YERSINIA and M-N IV clusters have moved from group A to group B, endorsing their lack of close relationships with sub-group A3.

Group B has undergone considerable re-organisation in the arrangement of the sub-groups and clusters, although most of the original clusters were still easily recognisable. Sub-group B3 now contained BRUCELLA and ANITRATUS, and had lost M-N II and M-N III. Several of the clusters have had strains added to them, for example, J44 has joined BRUCELLA; J136 and J140 have joined LWOFFII; J26, J30, J31 and J67 have joined ANITRATUS. The strains of the original M-N IV cluster have attached themselves to M-N II, together with J146. The only cluster to be seriously affected by the omission of sugar characters was M-N I, which was actually non-existent in Fig. 7.

A glance at the strains which have altered their positions,

Figure 8    Dendrogram of the strains shown in Fig. 6, based on

a similarity matrix from which 22 sugar characters

had been deleted.  This is computation five.

Fig.8

UNWEIGHTED AVERAGE LINKAGE

opp. 90
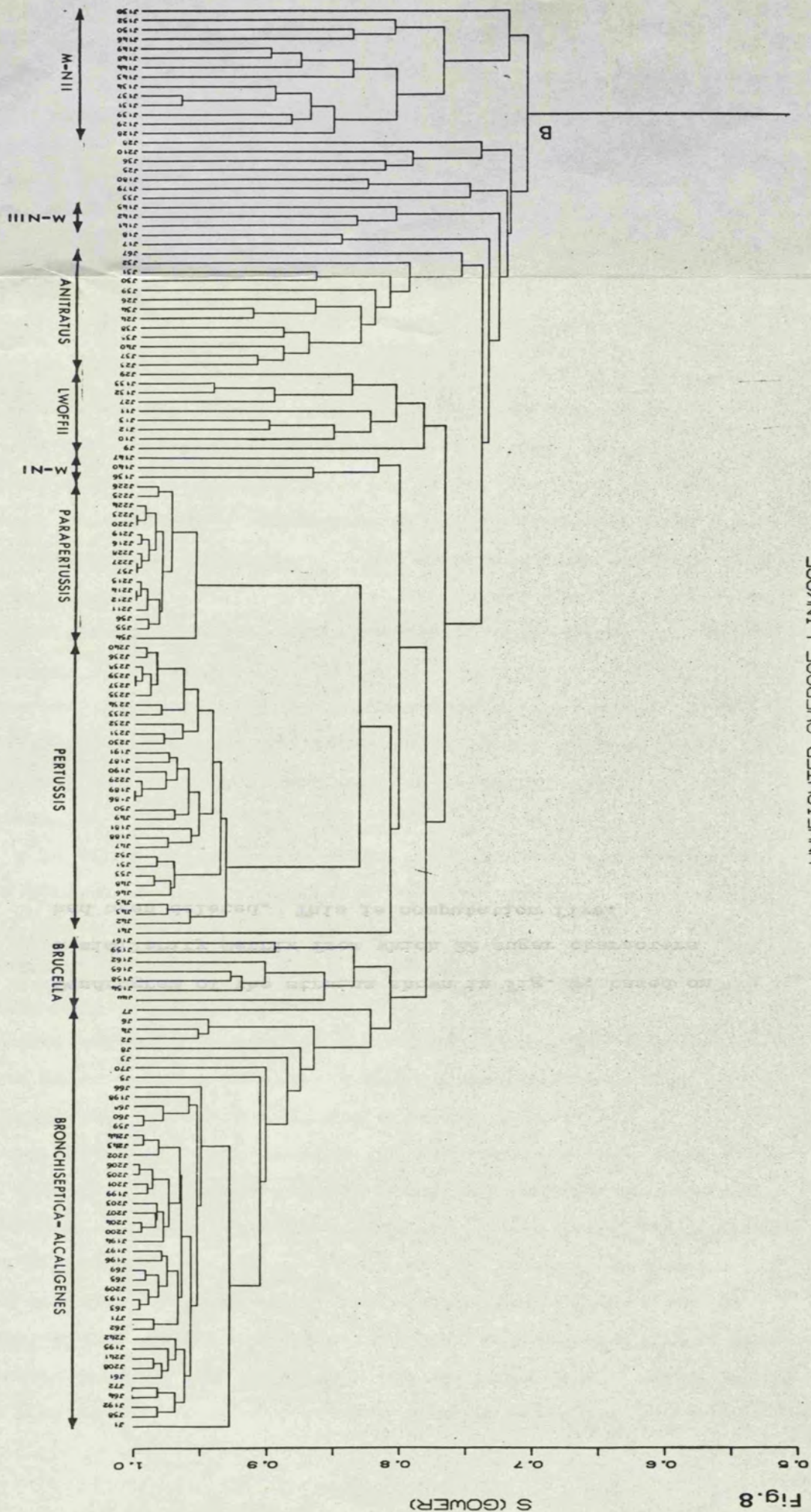
reveals that they were largely the satellite strains shown in Fig. 2, i.e. J44, J26, J30, J31, J67, J33, J28 and J172. This situation would not be entirely unexpected, because if these strains shared high similarities with more than one cluster, they would tend to alternate between them if the data matrix is altered. Similar conclusions are reached if Fig. 8 is compared with Fig. 6, indicating that any changes were of only minor taxonomic importance.

### The position of strains which fell outside the clusters

Figure 2 indicates that 18 strains fell outside the named clusters. Of these strains, J17 and J18, and J179 and J180, formed two pairs which shared their closest similarities with BRUCELLA and were included in the same sub-group, B2. Table 15 shows that whether the similarities were based on the complete data matrix, or on the data matrix without the twenty-two sugar characters, the nearest neighbours to these four strains were, on the whole, members of the BRUCELLA cluster. Characters useful for differentiating BRUCELLA from the two associated pairs are shown in Table 16.

Although similarities of over 80% existed between the strains of sub-group B2, the total number of strains involved was too small to make valid taxonomic conclusions. The two strains J17 and J18 were received as <u>Agrobacterium</u> spp; this genus is included with <u>Rhizobium</u> and <u>Chromobacterium</u> in the family Rhizobiaceae by Breed, Murray and Smith (1957), so it would be presumptive to state that <u>Agrobacterium</u> should be placed with <u>Brucella</u> when the other genera were not represented in the study.

The organism <u>Noguchia granulosis</u>, J153, shared its highest similarities with members of sub-group A3. The similarities are, however, quite low and tend to suggest only a loose relationship. More probably,

Table 15  Nearest neighbours of the strains which remained outside the fifteen clusters shown in Fig. 3.

| Strain | Nearest neighbours  (with % similarity) | | | | | | | |
|--------|------|------|------|------|------|------|------|------|
| J153 | J246 | 75.7 | J176 | 75.4 | J171 | 75.1 | J100 | 74.7 |
|       | J246 | 78.5 | J171 | 76.6 | J93  | 76.3 | J100 | 76.1 |
| J172 | J132 | 79.6 | J171 | 78.8 | J167 | 78.7 | J133 | 78.1 |
|       | J167 | 84.8 | J171 | 83.0 | J169 | 81.6 | J20  | 81.3 |
| J179 | J162 | 85.9 | J180 | 82.5 | J27  | 81.0 | J211 | 80.7 |
|       | J162 | 85.9 | J180 | 82.5 | J27  | 81.0 | J211 | 80.7 |
| J180 | J179 | 82.5 | J154 | 80.2 | J165 | 79.7 | J158 | 77.4 |
|       | J179 | 82.5 | J154 | 80.2 | J165 | 79.7 | J158 | 77.4 |
| J17 | J18 | 85.6 | J165 | 81.2 | J162 | 81.2 | J158 | 79.7 |
|      | J18 | 84.5 | J165 | 81.2 | J162 | 81.2 | J158 | 79.7 |
| J18 | J17 | 85.6 | J158 | 81.8 | J165 | 81.2 | J61 | 79.7 |
|      | J17 | 84.5 | J158 | 81.8 | J165 | 81.2 | J61 | 80.3 |
| J25 | J24 | 81.4 | J38 | 80.9 | J37 | 79.9 | J32 | 79.6 |
|      | J36 | 81.2 | J210 | 80.6 | J24 | 80.4 | J10 | 78.5 |
| J28 | J24 | 78.7 | J25 | 78.4 | J29 | 75.5 | J32 | 75.1 |
|      | J25 | 77.7 | J24 | 77.1 | J179 | 73.7 | J35 | 73.6 |
| J36 | J25 | 78.8 | J30 | 76.5 | J54 | 75.9 | J154 | 75.6 |
|      | J25 | 81.2 | J210 | 77.5 | J37 | 76.7 | J17 | 76.6 |
| J210 | J54 | 83.2 | J55 | 83.2 | J56 | 82.2 | J136 | 81.5 |
|       | J54 | 81.4 | J55 | 81.4 | J24 | 81.1 | J37 | 80.8 |
| J33 | J27 | 83.3 | J30 | 83.2 | J132 | 81.5 | J11 | 81.3 |
|      | J27 | 79.9 | J30 | 79.8 | J175 | 78.3 | J174 | 78.1 |
| J41 | J51 | 87.4 | J52 | 87.2 | J46 | 86.1 | J48 | 86.1 |
|      | J51 | 84.7 | J52 | 84.3 | J48 | 83.0 | J46 | 83.0 |
| J7 | J44 | 86.8 | J63 | 86.7 | J60 | 86.6 | J71 | 86.2 |
|     | J44 | 84.0 | J63 | 84.0 | J154 | 84.0 | J60 | 83.8 |
| J44 | J46 | 88.3 | J48 | 88.3 | J154 | 88.2 | J51 | 88.0 |
|      | J154 | 88.2 | J158 | 86.0 | J46 | 85.7 | J48 | 85.7 |
| J30 | J31 | 88.9 | J12 | 88.7 | J4 | 87.7 | J66 | 87.1 |
|      | J31 | 86.6 | J12 | 86.4 | J37 | 86.3 | J4 | 85.2 |
| J31 | J30 | 88.9 | J66 | 86.4 | J12 | 85.3 | J58 | 85.1 |
|      | J30 | 86.6 | J66 | 83.6 | J32 | 82.7 | J12 | 82.2 |

Table 15 continued.

| Strain | Nearest neighbours (with % similarity) | | | | | | | |
|--------|------|------|------|------|------|------|------|------|
| J26 | J67 | 87.2 | J12 | 85.5 | J31 | 84.7 | J64 | 84.0 |
|     | J24 | 87.9 | J34 | 85.3 | J67 | 84.5 | J12 | 82.4 |
| J67 | J26 | 87.2 | J65 | 85.0 | J7 | 84.9 | J69 | 84.8 |
|     | J26 | 84.5 | J34 | 82.4 | J65 | 81.9 | J69 | 81.6 |

Key:

The upper row denotes nearest neighbours based on the complete data matrix.
The lower row denotes nearest neighbours based on the data matrix after the deletion of 22 sugar characters.

it shares a closer  association to strains not included in the study.

In many ways it is an interesting organism.    It is a
Gram-negative rod, sometimes showing pleomorphism.    It grows on simple
media but is inhibited by bile salts.    The metabolism is fermentative
but acid is not produced from lactose.    It is non-motile, sensitive to
0/129, catalase-positive and reduces nitrate to nitrite.    The oxidase
reaction is moderately positive.    It differs from the published
descriptions (Tilden and Tyler, 1930;  Olitsky, Syverton and Tyler, 1934)
in not fermenting lactose, its non-motility and in having a dull matt
colony.    This particular strain was found to be non-motile when tested
by Craigie's method or on blood agar plates incubated at $15^{o}C$ and $30^{o}C$.
The rough  nature of the colonies, however, suggested that the organism
may have been in a rough phase with a subsequent loss of motility.

The strain of Pasteurella haemolyticaT, J172, differed from
the other pasteurellas in being able to grow on 4.5% sodium chloride
and 0.2% phenol agar, and in being phosphatase-negative and $H_2S$-negative.
It differed in the description of P. haemolytica T given by Smith (1961)
in being unable to produce acid from xylose, rhamnose, fructose or dextrin.
Table 15 indicates that its sugar reactions were probably responsible
for it being displaced from the other Pasteurella strains.    However,
both Smith and Thal (1965) and Stevens (1969) found that the similarities
between P. haemolytica T and other species of Pasteurella were not very
high.

Five strains, J25, J28, J36, J210 and J33, did not share high
average similarities with any of the clusters, although Table 15 indicates
that some strains have moderately high similarities with some strains
belonging to clusters, e.g. J210 and strains of the PARAPERTUSSIS cluster.

Baumann, Doudoroff and Stanier (1968b) included J25 in their
study, and found it to be one of three unassigned strains.    It shows

Table 16      Differentiation of BRUCELLA from Agrobacterium and

Francisella

| Character | BRUCELLA | J17, J18 Agrobacterium | J179, J180 Francisella |
|---|---|---|---|
| growth anaerobically in the presence of nitrate | + | - | + |
| sensitivity to penicillin | + | - | - |
| sulphonamide | - | + | - |
| oxidase | + | + | - |
| urease | + | + | - |
| nitrate reduction | + | + | - |
| acid from 1% glucose aerobically | - | - | + |
| 10% glucose | + | + | + |

only moderately high similarities to other strains, although the ANITRATUS cluster would appear to be its nearest neighbour.

A poultry isolate, J28, obtained from Dr. M.J. Thornley, was attached to the ANITRATUS cluster at under 80% similarity. It was the representative strain of Thornley's (1967) phenon 3.

Achromobacter liquefaciens ATCC 15716, strain J36, did not share high similarities with any one cluster. Among its nearest neighbours were ANITRATUS, PARAPERTUSSIS and BRUCELLA. Thornley (1967) examined this strain and found most of the cells to be Gram-positive. This was confirmed in this study although several of the results obtained, particularly hydrogen sulphide production, acid from various sugars and ammonia from arginine, differed to those obtained by Thornley.

Strain J210 was isolated as a contaminant from a freeze-dried culture. It had a bright-yellow, non-diffusible pigment, and as such differed from every other strain. It produced acid from 10% glucose but not 1% glucose, and shared its highest similarities with the PARAPERTUSSIS cluster, although it could not be regarded as a member of the cluster.

A. citroalcaligenes, J33, was included in Baumann's sub-group B1. In this study, its nearest neighbours were mainly members of the LWOFFI cluster, although the similarities were not sufficiently high for its association to be beyond doubt.

Four strains, J30, J31, J26 and J67, showed high similarities to strains of several clusters including ANITRATUS, ALCALIGENES, LWOFFII and BRONCHISEPTICA. They would appear to represent intermediate strains of clusters in group B. Examination of Figs. 7 and 8 show that if the sugar reactions are excluded they take up positions within the ANITRATUS cluster. None of these four strains was able to produce

acid from carbohydrates, this giving rise to a difference of ten
characters in the initial computation.    J67, Bordetella bronchiseptica
ATCC 12714, differed in seventeen characters from the strains of the
BRONCHISEPTICA cluster and could not logically be retained in that
species.

The results obtained for J41 agreed largely with those
obtained by Fewson (1967a, 1967b).    It shared high similarities
with the PERTUSSIS cluster and differed from members of this cluster in
being oxidase-negative, hydrogen sulphide-positive, growing on nutrient
agar, lecithinase-positive, tyrosinase-positive and in clearing tween 80.
It was insensitive to penicillin but sensitive to sulphonamide.

Of the two remaining strains, J7 Alcaligenes faecalis retained
a high similarity to the BRONCHISEPTICA cluster although also sharing a
similarity of 84% with J154, Brucella abortus.

Bordetella pertussis NCTC 8631, my strain J44, is the strain
most similar to J7, yet its own nearest neighbours are members of the
PERTUSSIS and BRUCELLA clusters.    Figures 2, 6, 7 and 8 show J44 taking
up positions with J7, between PERTUSSIS and PARAPERTUSSIS or with the
BRUCELLA cluster.    The results given in Table 15 suggest that the
strain is intermediate between PERTUSSIS and BRUCELLA clusters.    When
tested by slide-agglutination against Bordetella pertussis antiserum and
Brucella antiserum, J44 gave a strong agglutination in the Brucella
antiserum and did not agglutinate in the pertussis antiserum.    This
suggested that this organism is incorrectly labelled as Bordetella pertussis
and is in fact a member of the genus Brucella, albeit an atypical member.
This view is further substantiated by its deoxyribonucleic acid base
composition discussed later.    Characters which proved useful for
differentiating J44 from the PERTUSSIS and BRUCELLA clusters, included
the ability to grow anaerobically in the presence of nitrate;  growth on

nutrient agar, 5% bile salts and 3% sodium chloride; sensitivity to 0/129, penicillin and Bactrim Roche; urease production; nitrate reduction and the ability to produce acid from 10% glucose. These results together with the results for the other unclustered strains are shown in Table A3 (Appendix 3).

REPRODUCIBILITY OF RESULTS

A great deal of importance is currently being attached to the validity and particularly, the reproducibility of results obtained in numerical taxonomic studies. Gottlieb (1961), Liston, Weibe and Colwell (1963), Lapage, Bascomb, Willcox and Curtis (1970) and Taylor, Guthrie and Shirling (1970) have indicated that errors affecting the reproducibility of results, both within a single laboratory and between laboratories, were larger than commonly realised. Error values of 4 - 19% have been recorded. Clearly, if 20% of the results obtained in a taxonomic study are erroneous, calculated similarities would have little meaning.

Sneath (1971) has derived the following equation relating Similarity and test errors,

$$S' = S(2p-1)^2 + 2p(1-p),$$

where S is the true Similarity, S' the observed Similarity[*] and p the statistical probability of errors calculated from the proportion of errors obtained. The errors reduce the Similarity of a strain when compared to itself, so that, although an $S_{SM}$ value of 1 would normally be expected, if p has a value of 0.19 (19% error), the observed $S_{SM}$ would be only 0.69.

With this in mind, part of the numerical taxonomic study was repeated to determine the accuracy of the results obtained. Time did not allow a full-scale replication of all tests on all strains, so a more managable but less accurate procedure was adopted.

Twenty-one strains were selected and tests repeated once to enable 83 characters to be re-examined. The following strains were used:

[*] In reality this is a mean value for the observed similarity.

| J1,   | J3,   | J9,   | J11,  | J23,  |
|-------|-------|-------|-------|-------|
| J27,  | J31,  | J35,  | J42,  | J43,  |
| J55,  | J56,  | .J58, | J59,  | J60,  |
| J128, | J45,  | J167, | J178, | J181, |
| J211. |       |       |       |       |

Results were coded as in Table A1. In addition, acid production from 1% and 10% glucose was repeated on 193 strains (six strains of Brucella and two strains of Francisella were omitted), and production of gelatinase was examined by three methods over the same 193 strains.

In all, a total of 2,708 results were recorded, containing 93 discrepancies. This is equivalent to an error rate of 3.42% which compares favourably with the value of 6.9% obtained by Lapage et al. (1970).

The results were examined statistically using a modification of Fisher's Analysis of Variance, the following example illustrating the procedure.

The assumption was made that the total variation observed was due to variation in strains, variation in replicates and residual variation due to interaction of strains and replicates. Thus, when the strains sum of squares was subtracted from the total sum of squares, the remainder represented replicate and residual variation, from which the variance and the probability p of erroneous results could be calculated. It is important to realise that the following procedure only holds for data coded as 1 or 0, it does not hold for quantitative data at least not without a modification of the method.

Table 17     The variation of the probability p, of erroneous results based on the replication of eighty-six characters.

| Value of p | Character number (reference Table A1) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 0 | 1 | 2 | 6 | 7 | 8 | 9 | 10 | 11 |
| | 12 | 13 | 14 | 15 | 16 | 17 | 19 | 20 |
| | 21 | 22 | 23 | 24 | 25 | 29 | 33 | 34 |
| | 39 | 40 | 45 | 46 | 47 | 48 | 53 | 56 |
| | 62 | 63 | 71 | 72 | 73 | 74 | 80 | 86 |
| | 87 | 88 | 107 | 108 | 109 | 110 | 111 | 112 |
| | sensitivity to optochin, production of hydrogen cyanide and growth at 30°C | | | | | | | |
| 0.0132 | 109 (determined on 193 strains) | | | | | | | |
| 0.0244 | 5 | 18 | 31 | 35 | 36 | 41 | 42 | 43 |
| | 44 | 49 | 51 | 52 | 54 | 61 | 70 | 82 |
| | 84 | 93 | 94 | 99 | | | | |
| 0.0357 | 85 (determined by three methods on 193 strains) | | | | | | | |
| 0.0502 | 30 | 92 | 95 | | | | | |
| 0.0774 | 27 | 50 | 55 | 58 | 83 | | | |
| 0.1066 | 3 | 4 | 57 | | | | | |
| 0.1381 | 81 | | | | | | | |

Average value of p measured over 86 characters = 0.01825

| strains | replicate | results | row totals |
|---------|-----------|---------|------------|
| 1 | 1 | 1 | 2 |
| 2 | 1 | 1 | 2 |
| 3 | 0 | 1 | 1 |
| 4 | 1 | 0 | 1 |
| 5 | 0 | 0 | 0 |
| 6 | 1 | 1 | 2 |
| 7 | 1 | 1 | 2 |
| 8 | 0 | 0 | 0 |
| 9 | 0 | 0 | 0 |
| 10 | 0 | 0 | 0 |
| Column totals | 5 | 5 | 10    grand total, G |

The correction factor C.F. $= \dfrac{G^2}{N}$, where N is the total number of observations.

The total sum of squares $= \sum x^2 - \dfrac{G^2}{N}$,

the strains sum of squares $= \dfrac{\sum (\text{row totals})^2}{\text{number of replicates}} - \dfrac{G^2}{N}$.

The replicate and residual sum of squares is equal to the total sum of squares - the strains sum of squares, and the variance is given by,

$$\text{Variance} = \dfrac{\text{total sum of squares} - \text{strains sum of squares}}{\text{total degress of freedom} - \text{strains degrees of freedom}}.$$

The probability p of erroneous results is given by,

$$\text{Variance} = p(1-p),$$

which solves to the quadratic equation, $-p^2 + p - \text{variance} = 0$.

Table 17 shows the values of p calculated over the 86 characters.

the average value being 0.01825 or 1.8% error. Those characters showing the greatest degree of error included: peroxidase; morphological characters such as the presence of chains or filaments and cell arrangement; haemolysis of horse blood; antibiotic sensitivity using bacitracin, erythromycin, oleandomycin and Bactrim Roche and, finally, production of hydrogen sulphide.

The value of p obtained, when substituted in the original formula, indicated that if a strain should be compared with itself in this particular study, an observed Similarity of around 0.964 could be expected. As was previously mentioned the estimated value of S' obtained is a mean value, which is dependent on the mean value of p over the series of tests. The distribution of p is binomial and this approximates to the distribution of S'. Sneath gives the variance of S' as being

$$\text{Variance of } S' = \frac{2p(1-p)}{n} \times \left[(2S-1) + S(1-S)\right]$$

where n is the number of tests.

Substituting the values of p, S and n from this study we obtain a variance of 0.0004168 with a standard error of 0.02042 or $\pm$ 2.042%. Calculation of the 95% bounds of the expected mean S' gives a range of $\pm$ 4.002% on either side of S', i.e. 0.9242 –1.0042. The upper limit exceeds 100% because the standard error is only an approximation, which is not quite accurate when S approaches 1.0. The 90% bounds would perhaps be more suitable, in this case a range of 0.9306 – 0.9978 being obtained.

By an oversight, one strain was included twice under the numbers J60 and J65. One culture was obtained from the National Collection of Type Cultures and the other from the American Type Culture Collection. The observed Similarity between J60 and J65 was in fact 0.976, which was well inside the expected limits. The result of this oversight taken into consideration with the low value of p, indicates that in this study the reproducibility of the tests was entirely satisfactory.

APPLICATION OF VIGOUR AND PATTERN STATISTICS

Sneath (1968) pointed out that size and shape differences in higher organisms were analogous to his concepts of Vigour and Pattern differences in bacteria. The Vigour of a bacterial strain was equivalent to the proportion of positive results obtained over a given battery of tests. The Pattern was dependent on what tests gave positive results. Unlike Vigour, Pattern cannot be defined mathematically, because it is not an absolute entity in itself, it can only be defined in terms of the Pattern Difference between two strains.

Both Vigour and Pattern are affected by the set of tests chosen, but one assumes that if the set is a purely random choice and is sufficiently large, then both Vigour and Pattern differences will have small standard deviations.

After comparing two strains over a series of tests, a mathematical Similarity between the two organisms can be calculated. It is then a short step from Similarity to its complement, Total Difference $D_T$. The Total Difference between two strains is made up of two additive components, Vigour Difference $D_V$, and Pattern Difference $D_P$, such that,

$$D_T{}^2 = D_V{}^2 + D_P{}^2. \tag{1}$$

If we now consider the possible results that occur over a batch of $\underline{n}$ tests, we have

|  | Strain 1 | Strain 2 | number of tests |
|---|---|---|---|
|  | + | + | a |
| result | + | − | b |
|  | − | + | c |
|  | − | − | d |
|  |  |  | n |

in which the total number of positive results for Strain 1 is (a + b), and for strain 2 is (a + c). Formula (1) now becomes

$$\frac{(b + c)^2}{n^2} = \frac{(c - b)^2}{n^2} + \frac{4\,bc}{n^2} \quad ,$$

from which $D_V$ is given by $\frac{c - b}{n}$, $D_P$ by $\frac{2\sqrt{bc}}{n}$ and $D_T$ by $\frac{b + c}{n}$.

Having deduced these formulae it is easy to see that given a t x n data matrix, Vigour Differences and Pattern Differences between all strains, can be calculated, and the results represented as a triangular matrix which can be clustered by one of several clustering methods. One can now validly enquire, why should phenetic difference be split up into Vigour and Pattern differences, and what results would be expected when Vigour Differences and Pattern Differences are clustered? The answer to the first question is most obvious when comparing strains which give similar results over a series of tests, but some strains grow slowly, while the others grow rapidly. Over short incubation periods the fast-growing strains will give more positive results and consequently the $D_V$ will be large. Over long incubation periods, all strains will reach maximum growth and give the maximum number of positives, since in bacteriology it is rare for a positive result to revert to negative. At this point $D_V$ will be small and the similarity of the strains to each other will depend largely on Pattern Difference. Over short incubation periods elimination of $D_V$ allows slow-growing strains to show their true similarity by basing the clustering only on Pattern Differences.

The answer to the second question will depend largely on the computer program. Clustering procedures are usually based on Similarity or Taxonomic Distance D. Vigour Difference takes the range -1 to +1. A value of -1 or +1 indicates that over one hundred tests, one strain

Table 18     Variation in Vigour of the strains in computation 2.

| CLUSTER | NUMBER OF STRAINS | RANGE OF VIGOUR | AVERAGE VIGOUR |
|---|---|---|---|
| ALCALIGENES | 7 | 0.354 - 0.424 | 0.386 |
| BRONCHISEPTICA | 14 | 0.353 - 0.400 | 0.390 |
| PERTUSSIS | 11 | 0.317 - 0.344 | 0.330 |
| PARAPERTUSSIS | 12 | 0.386 - 0.405 | 0.398 |
| LWOFFII | 9 | 0.325 - 0.406 | 0.368 |
| ANITRATUS | 9 | 0.445 - 0.504 | 0.473 |
| BRUCELLA | 7 | 0.393 - 0.519 | 0.471 |
| M-N I | 4 | 0.328 - 0.403 | 0.369 |
| M-N II | 9 | 0.393 - 0.468 | 0.424 |
| M-N III | 3 | 0.426 - 0.496 | 0.465 |
| M-N IV | 4 | 0.425 - 0.581 | 0.490 |
| ACTINOBACILLUS | 3 | 0.492 - 0.500 | 0.497 |
| PASTEURELLA | 7 | 0.445 - 0.508 | 0.479 |
| YERSINIA | 7 | 0.504 - 0.575 | 0.546 |
| HAEM I | 15 | 0.345 - 0.450 | 0.394 |
| HAEM II | 11 | 0.385 - 0.464 | 0.430 |
| HAEM III | 3 | 0.383 - 0.451 | 0.414 |
| Francisella | 2 | 0.442 - 0.468 | 0.455 |
| Agrobacterium | 2 | 0.440 - 0.472 | 0.456 |
| J25 | 1 | | 0.488 |
| J26 | 1 | | 0.419 |
| J28 | 1 | | 0.472 |
| J30 | 1 | | 0.408 |
| J31 | 1 | | 0.408 |
| J33 | 1 | | 0.447 |
| J36 | 1 | | 0.527 |
| J41 | 1 | | 0.371 |
| J67 | 1 | | 0.411 |
| J153 | 1 | | 0.603 |
| J210 | 1 | | 0.433 |

gives 100 positives and the other gives 100 negatives. A Vigour Difference of 0 indicates that both strains give an equal number of positive results. The computer program transforms the Vigour range $-1$ to $+1$, into a Similarity range 0 to 1, so that a Vigour Difference of 0 is taken as complete Similarity i.e. 1, while a Vigour Difference of 1 is taken as complete dissimilarity i.e. 0.

If bacterial genera always had specific values of Vigour, then clustering on $D_V$ would place species belonging to one genus together, and species belonging to another genus also together. Unfortunately, this is not the case, because neither genera nor species have constant Vigour. In many cases the variation of Vigour within a species is as much as within the genus and between genera. Table 18 shows the variation of Vigour among the clusters formed in Fig. 2. There does not appear to be any specificity about Vigour, and many genera have overlapping values. Clustering based on Vigour Difference would therefore be meaningless.

On the other hand, clustering on Pattern Difference yields as much information as clustering on Similarity. It is not difficult to calculate that $D_P$ falls into the range 0 to $+1$. This transforms naturally into similarity, and clustering becomes more logical. Strains having low values of $D_P$ invariably have high similarities. The exceptions arise when one strain does not have any positive results which the strain with which it is being compared, does not also have, i.e. in the original example, b or c becomes zero.

In a study of a large number of strains Vigour Difference falls into a defined range within the limits $-1$ to $+1$. Removal of the $D_V$ therefore tends to affect the similarity of all strains fairly equally and clustering based on $D_P$ will approximate to clustering based on Similarity. This is clearly seen in Figure 9, which compared with

Figure 9     Dendrogram showing the arrangement of 150 Gram-negative

rods based on Pattern Difference .  This is computation

six.
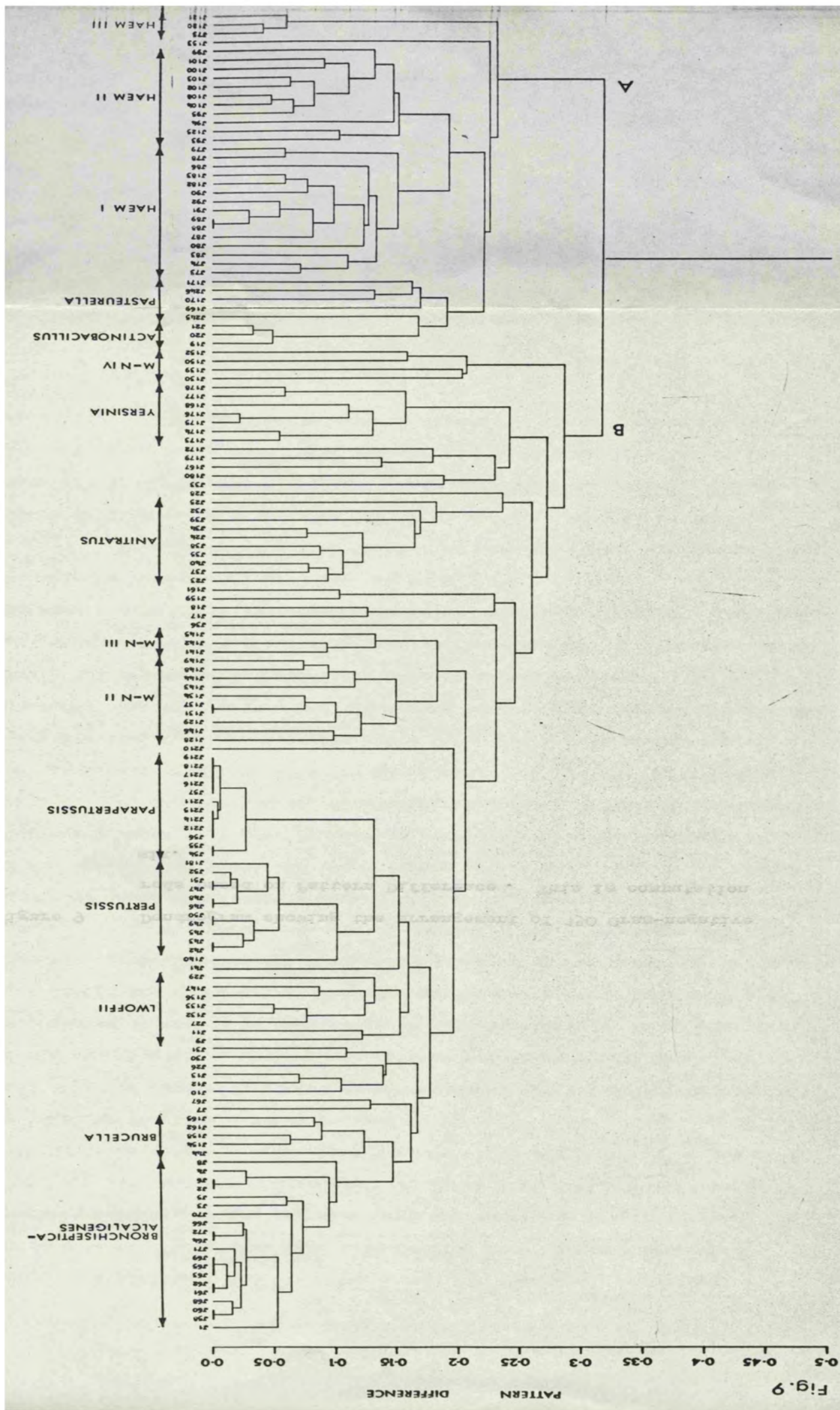
Fig. 9

UNWEIGHTED AVERAGE LINKAGE

Fig. 2 shows close similarity, although some movement of strains has occurred.    YERSINIA and M-N IV have moved into group B as in Fig. 7. PASTEURELLA has become split up, strains J167 and J172 being relocated in group B.    Two strains of BRUCELLA, J159 and J161 have been removed from the cluster, and the LWOFFII cluster has been reorganised.

POLY ACRYLAMIDE GEL ELECTROPHORESIS

The protein extracts of 53 strains were examined by disc electrophoresis in poly-acrylamide gels, and the protein patterns of 47 of these strains are shown in Plates 1-9. Plates 1-7 show respectively, representative strains of the following clusters; ALCALIGENES, BRONCHISEPTICA, PARAPERTUSSIS, PERTUSSIS, BRUCELLA, LWOFFII and ANITRATUS. Plate 8 shows the protein patterns obtained from four unclustered strains, while Plate 9 compares the patterns of single strains selected from each of the above named clusters.

Time did not allow detailed examination of each of the gels, particularly along the lines used by Johnson and Thein (1970). Indeed that would have become a full-scale project in itself. The examination of the protein patterns was undertaken mainly to investigate the possibility that the technique might be useful in the taxonomy of this group of bacteria, and to see whether any useful information could be gleaned from such a preliminary investigation. Basically the object was to observe any visible similarities in the protein patterns, both within clusters and between clusters, but also of extreme importance was the recognition of the problems involved and the means to overcome them.

For the photographs the strains were arranged such that gels with the most similar protein patterns were adjacent. The reproducibility of the band patterns was good between duplicates of the same protein sample, even when run on different days. One problem which did arise however, concerned variation in the rate of migration on different days. Both the marker dye and the protein sample would travel down the gels at slightly varying rates on different days, so that although the electrophoresis was stopped when the marker dye had reached the bottom of the running tubes, the protein sample had not always moved the same distance. This is particularly noticable in Plate 1, where the gels are obviously different in length.

Plate 1   Polyacrylamide gels showing patterns obtained from crude protein
extracts of the ALCALIGENES cluster.
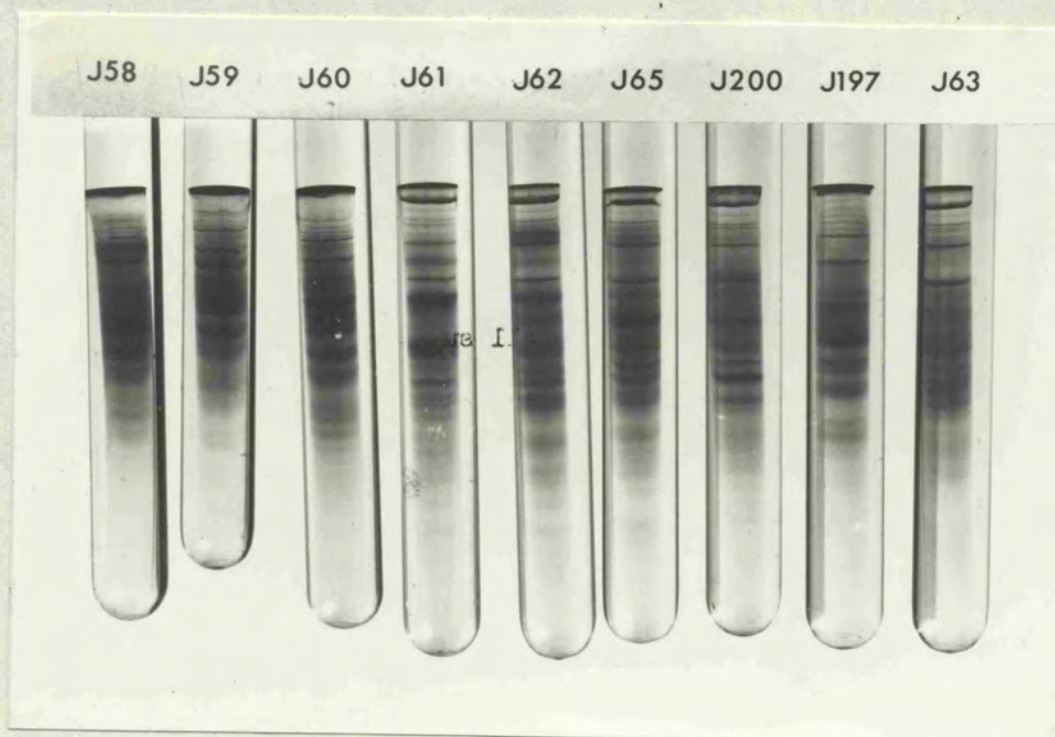


Plate 2   Polyacrylamide gels showing patterns obtained from crude protein
extracts of the BRONCHISEPTICA cluster.

ALCALIGENES.        The seven strains shown in Plate 1 exhibit

considerable variation in patterns.  Four nomenspecies are represented;

       J1     Alcaligenes denitrificans

       J2     A. odorans

       J3     A. faecalis

       J4      "

       J5      "

       J6      "

       J8     A. viscosus.

Only two strains J4 and J6 showed any evidence of pattern similarity.
This was particularly noticable in the arrangement of the darker-staining
bands.

BRONCHISEPTICA.      Plate 2 shows that obvious pattern similarities

existed in the strains of Bordetella bronchiseptica examined.  The

nine strains were obtained from culture collections or were fresh

isolates, and the high phenetic simlarity of the strains of this species

shown in the numerical taxonomy, is also reflected in the protein

patterns.

PARAPERTUSSIS.       The gels shown all suffered from not receiving

a sufficient concentration of protein in the sample.  However, as in

case of the strains of B. bronchiseptica, obvious similarities exist

which can be seen in the photograph.  This again coincides with the

high phenetic similarity of the strains found in the PARAPERTUSSIS

cluster.

PERTUSSIS.       The gels for the the three strains of Bordetella

pertussis that were examined were not too successful, mainly because

it proved difficult to obtain sufficient growth in the culture medium.
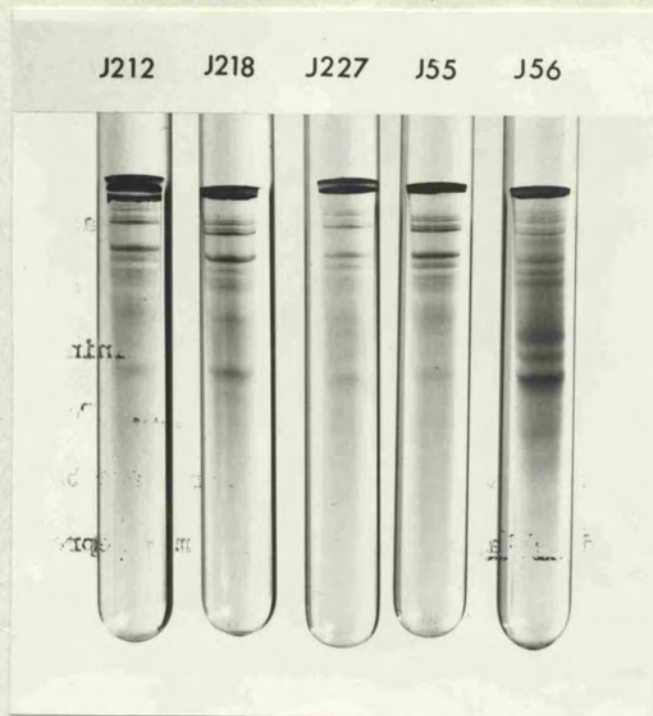
Plate 3   Polyacrylamide gels showing patterns obtained from crude protein
extracts of the PARAPERTUSSIS cluster.
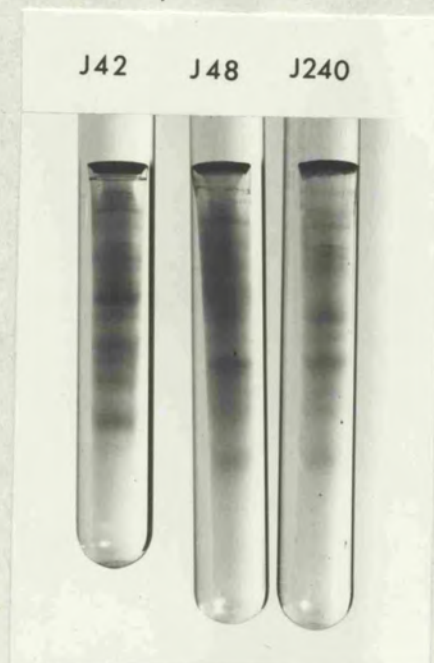


Plate 4   Polyacrylamide gels showing patterns obtained from crude protein
extracts of the PERTUSSIS cluster.

Consequently, the amount of protein isolated was also low. Strain J42 suffered from the migration problem already mentioned, nevertheless similarities were observed between J48 and J240.

When comparing the above clusters, it was noticable that each of the patterns for BRONCHISEPTICA, PARAPERTUSSIS and PERTUSSIS was characterised by the presence of two darkly-staining bands about 3-4mm. apart and about one centimetre from the origin. Further studies would have to be carried out to determine whether these bands are characteristic for the genus Bordetella, and whether they may represent particular proteins such as the azurins which have been described in this genus as well as in Alcaligenes and Pseudomonas ( Sutherland and Wilkinson, 1963; Ambler, 1968).

BRUCELLA.     The five gels shown in the photograph represent four species of Brucella;

| | |
|---|---|
| J154 | B. abortus |
| J159 | B. melitensis |
| J162 | B. suis |
| J165 | B. canis |

J44     was a supposed phase IV strain of Bordetella pertussis , but has now been reidentified as a Brucella species.

Most of the strains did not show similar protein patterns, but of interest and confirming the results of the numerical taxonomy, are the similar patterns obtained from J44 and J159. These two strains had a high phenetic similarity and also appear to have similar proteins. Incidentally, the National Collection of Type Cultures are currently listing J44 as a strain of Brucella melitensis.

LWOFFII.     Again, the strains of this cluster showed evidence of protein similarities, particularly J12, J132, J133 and J27. This tends

**Plate 5**  Polyacrylamide gels showing patterns obtained from crude protein extracts of the BRUCELLA cluster.



**Plate 6**  Polyacrylamide gels showing patterns obtained from crude protein extracts of the LWOFFII cluster.

to confirm the numerical taxonomy results indicating that <u>Acinetobacter</u> <u>lwoffii</u> is in fact a "good" species.

<u>ANITRATUS</u>.    Plate 7 shows that this cluster contains strains such as J37 and J40 which have very similar patterns, but on the other hand there appears to be considerable variation in the cluster as a whole.

<u>Strains not clustered</u>.    Four strains were examined, and as expected they showed very little evidence of pattern similarity.

Plate 9 shows that when a single strain of each cluster is compared, they show very little similarity. This is obviously a reflection of differences between the groups as a whole and also of the particular strains chosen for comparison. The photographic evidence of the gels has indicated that the preliminary investigation has proved useful. Pattern similarities do exist particularly in certain clusters such as BRONCHISEPTICA and PARAPERTUSSIS. Most clusters however, show a healthy division between similar and dissimilar patterns and clearly further studies are necessary to determine if the procedure has practical uses in identification work on these organisms.

## Practical problems in gel electrophoresis

The problems were found to exist in two basic forms. Firstly, experimental details involved in the preparation of the gels and the actual electrophoresis "run". Secondly, comparison of the protein patterns.
<u>Experimental problems</u>.

Many of these came to light during the early stages of the work carried out on the 53 strains. Still more were revealed during discussions with Professor J. De Ley and Dr. Martin Kersters after the work was completed. Many of the problems have already been discussed by other workers who have used the technique,
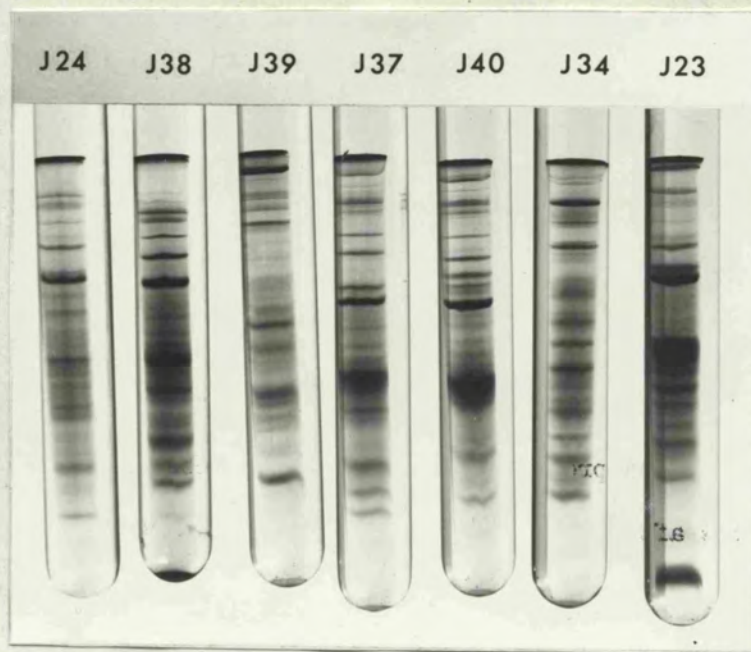
Plate 7  Polyacrylamide gels showing patterns obtained from crude protein extracts of the ANITRATUS cluster.



Plate 8  Polyacrylamide gels showing patterns obtained from crude protein extracts  of strains which did not fall into any cluster.

and in standard text books. However, two problems became obvious during the work.

The first problem concerns the effect of "bombing". This is the disturbing of the still-liquid running gel surface when covering it with the small amount of water required to obtain a perfectly flat meniscus. Whenever this occurred the gel was discarded, for although it would still solidify and have a flat surface, the internal matrix was altered and horizontal bands could never be obtained.

The second problem concerned the process of centrifugation of the broken cells after sonication, to obtain the crude protein extract. If the centrifugation is not carried out at the correct speed and for a sufficiently long time, some cell debris will still remain in the protein sample to be applied to the gel surface. The debris then precipitates on to the gel surface, making it impossible for the soluble proteins to enter evenly. This again results in uneven bands. This problem was appreciated to some extent and consequently the final centrifugation at 18,000rpm was increased to 40 minutes. Dr. Kersters however, employs a much more rigorous centrifugation procedure consisting of three stages. An initial centrifugation of the broken cells is carried out at 15,000rpm for 20 minutes. The supernatent is removed and centrifuged at 40,000rpm for 1hr. before being removed again for a final centrifugation at 40,000rpm for 2 hours.

### Comparison of protein patterns.

In the past, the comparison of protein patterns has been undertaken by using one of three methods;

1. Visual comparison as used in this study, and by Lund (1965) on group D streptococci, Razin (1968) on mycoplasmas and by Sacks, Haas and Razin (1969) on the Enterobacteriaceae.
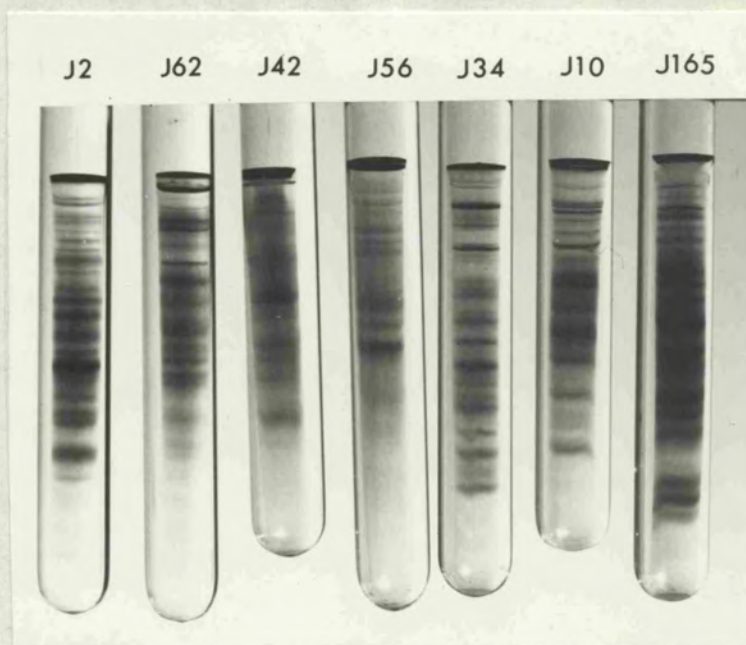
Plate 9   Comparison of the protein patterns of selected strains from each
of the seven clusters examined.

The disadvantages of this method lie in the subjective interpretations of the observer.

2. Calculation of $E_f$ or $R_f$ values. This usually involves measuring distances moved by marker dyes or marker proteins and the distance moved by individual protein bands, ( Gottlieb and Hepden, 1966; Baptist, Shaw and Mandel, 1969 ). More recently Rouatt, Skyring and Purkayastha (1970) tried to analyse the $E_f$ values obtained from 47 soil bacteria, by calculating distance coefficients and using a centroid clustering method. They found however, that their results required considerable subjective editing and were not very successful.

3. The most promising method to date has been used by Johnson and Thein (1970) . Using rigorously standardised conditions, the protein samples are prepared and run; the gels are then scanned on a densitometer. The traces obtained are divided horizontally ( i.e. the migration scale ) into about one hundred equal divisions and the optical density or wave height measured at each division. The trace of each gel is thus converted into a long column of figures which can be compared statistically using correlation coefficients. These can be transformed into Fisher's Z scores and clustered.

The results so far obtained have been extremely encouraging, although certain practical difficulties in obtaining exactly reproducible gels have yet to be overcome.

A more fundamental point that perhaps should receive greater consideration concerns the fact that when protein patterns are being compared we still do not know what it is that we are looking at. If we put two gels side by side, how do we know that bands at corresponding loci are identical? In actual fact, bands at corresponding loci could be;

a) two different proteins with the same rate of migration, or

b) mixtures of proteins, some of which may be absent in one of the bands.

Furthermore, differences in band intensity could be due to differences in concentration of protein, or the total number of proteins in one band or merely that some proteins take up the protein stain more readily than others.

Recently, Kaltschmidt and Wittmann (1970) outlined a method of two-dimensional electrophoresis, which overcomes the problem of a single band containing more than one protein. With their technique which was entirely reproducible, they were able to separate more than fifty ribosomal proteins. It would appear that in separating proteins, two-dimensional electrophoresis has definite advantages over disc electrophoresis. On the other hand, having successfully separated the proteins we may still have the problem of comparing protein patterns.

DNA BASE COMPOSITION

Table 19          DNA base composition of selected strains

| CLUSTER | STRAIN | % GC content | | METHOD |
|---------|--------|--------------|------------|--------|
| | | From this study | From the literature | |
| BRONCHISEPTICA | J1 | 74.0 | | Tm |
| | J58 | 74.1 | | Tm |
| | J59 | 75.0 | | Tm |
| | J60 | 74.7 | 65.2 | Tm |
| | J61 | 74.2 | | Tm |
| | J62 | 74.3 | | Tm |
| | J63 | 74.6 | | Tm |
| | J197 | 75.8 | | Tm |
| ALCALIGENES | J2 | 61.4 | | Tm |
| | J3 | | 66.0 | bd |
| | J4 | 63.0 | 63.3 | Tm |
| | J5 | 63.9 | 63.0 | Tm, bd |
| | J6 | | 58.9 | Tm |
| | J8 | 70.2 | | Tm |
| PERTUSSIS | J43 | 67.5 | | Tm |
| | J48 | 68.4 | | Tm |
| | J181 | 67.0 | | Tm |
| | J189 | 69.5 | | Tm |
| | J231 | 69.2 | | Tm |
| | J240 | 67.5 | | Tm |
| PARAPERTUSSIS | J54 | 69.3 | | Tm |
| | J55 | 70.0 | | Tm |
| | J56 | 68.7 | | Tm |
| | J211 | 67.7 | | Tm |

Table 19 (continued)

| CLUSTER | STRAIN | % GC content | | METHOD |
| --- | --- | --- | --- | --- |
| | | From this study | From the literature | |
| PARAPERTUSSIS | J218 | 69.5 | | Tm |
| (continued) | J227 | 69.4 | | Tm |
| LWOFFII | J10 | 44.6 | | Tm |
| | J11 | 45.3 | | Tm |
| | J12 | 40.0 | | Tm |
| | J13 | 45.1 | | Tm |
| | J27 | | 45.9 | Tm |
| | J29 | 49.5 | | Tm |
| | J132 | | 43.5 | Tm |
| | J133 | 46.6 | | Tm |
| ANITRATUS | J23 | | 41.0 | Tm |
| | J24 | 42.2 | | Tm |
| | J32 | | 43.0 | Tm |
| | J34 | | 44.0 | Tm |
| | J35 | | 42.0 | Tm |
| | J39 | | 45.0 | Tm |
| UNCLUSTERED | J25 | 42.2 | | Tm |
| STRAINS | J26 | | 44.0 | Tm |
| | J31 | | 43.0 | Tm |
| | J33 | | 44.0 | Tm |
| | J41 | | 39.0 | Tm |
| BRUCELLA | J44 | | 57.5 | Tm |
| | J154 | | 55.0 | Tm |
| | J159 | 57.0 | | Tm |

| CLUSTER | STRAIN | % GC content | | METHOD |
| --- | --- | --- | --- | --- |

Table 19 (continued)

| CLUSTER | STRAIN | % GC content | | METHOD |
| | | From this study | From the literature | |
|---|---|---|---|---|
| BRUCELLA (continued) | J162 | 57.5 | | Tm |
| M-N I | J136 | | 44.6 | hyd |
| | J140 | | 43.5 | bd |
| | J146 | | 44.5 | bd |
| | J147 | | 46.5 | bd |
| M-N II | J128 | | 42.5 | bd |
| | J129 | | 42.5 | bd |
| | J131 | | 41.5 | bd |
| | J134 | | 41.0 | bd |
| | J137 | | 41.5 | bd |
| | J143 | | 44.5 | bd |
| | J144 | | 45.0 | bd |
| | J149 | | 42.5 | bd |
| M-N III | J141 | | 43.0 | bd |
| | J142 | | 43.5 | bd |
| | J145 | 43.0 | | Tm |
| M-N IV | J130 | | 43.0 | bd |
| | J139 | | 42.5 | bd |
| Agrobacterium | | | 58-65.5 | Tm,hyd |
| Francisella | | | 33-36 | Tm,hyd |
| Actinobacillus | | | 34.8-42.6 | hyd |
| Pasteurella | | | 36-41.5 | hyd |

Table 19 (continued)

| CLUSTER | STRAIN | % GC content | | METHOD |
| --- | --- | --- | --- | --- |
| | | From this study | From the literature | |
| Haemophilus | | | 38-42 | Tm, hyd |
| Yersinia | | | 43-47 | Tm |
| | J168 | | 43.5 | Tm |

key:    Tm        thermal denaturation

        bd        buoyant density

        hyd       hydrolysis methods

The following references were used for results obtained from the literature:

Bacon, Overend and Lloyd (1967), Bøvre (1967), Bøvre, Fiandt and Szybalski (1969), Catlin and Cunningham (1964), De Ley (1968), De Ley (1970), De Ley, Kersters et al. (1970), Hill (1966), Johnson, Anderson and Ordal (1970), Marmur and Doty (1962), National Collection of Type Cultures, unpublished information, Rogul, Brendle, Haapala and Alexander (1970) and Rosypal and Rosypalova (1966).

As it was impossible to determine the base composition of all 201 strains included in the study, extensive use was made of the literature to find results which were applicable to the strains used. In all, the base composition was determined on 35 strains, while the results for a further 32 strains were located in the literature.

Table 19 lists the results obtained together with results for other genera represented in the study. De Ley (1969) has pointed out that the average standard deviation of the GC content of bacterial genera was 4.06%. This was based on a study of 5000 thermal denaturation curves on 36 genera. From this it would be expected that most species belonging to the same genus would have a GC content within 8% of any other species contained in the same genus. In the majority of cases this is true, although Pseudomonas is a notable exception, having a range of 58 - 70 moles per cent.

The clusters for which results are available readily confirm previously published findings that numerical taxonomy and DNA base composition show agreement with each other ( Colwell and Mandel, 1964; Rosypal, Rosypalova and Hořejš, 1966 and De Ley, 1968 ).

Three clusters BRONCHISEPTICA, ALCALIGENES and LWOFFII, exhibited ranges of GC content in excess of 9.5%. In each case however, this was invariably due to one or perhaps two strains having results which differed significantly from the results of the other strains in the cluster. In two cases the particular results concerned J60 and J6, were obtained from the literature or by personal communication. Furthermore, the result for J60 was not confirmed when measured in this study.

Apart from these three clusters, the remaining ones examined showed a narrow range of GC content within each cluster although showing differences between clusters.

The unclustered strains examined had GC percentages between 39

and 44 moles.   For J25, J26 and J31, this supported their association with the ANITRATUS cluster revealed in Figs. 7 and 8.

The results obtained for the BRONCHISEPTICA cluster are very high.   There is only one previously published report on the base composition of Bordetella bronchiseptica, that of De Ley (1968), who obtained a value of 69.5% for a strain from the National Collection of Type Cultures, NCTC 8761.   An unpublished result from L.R. Hill gave a value of 65.2% for J60, but this was not confirmed in this study.   The base composition was determined on eight BRONCHISEPTICA strains and all fell within 74-75.8%.   The range 65.2-75.8% is considerable in view of the tightness of the cluster when examined by numerical taxonomy.   In many ways the situation would appear to be somewhat unique and it raises the question that some results may be inaccurate.   The fact that I have examined several strains and found their GC content to agree within 2% suggests that the cluster is homogeneous without throwing any light on the reasons for the discrepancy.   To date I have been unable to locate any report which discusses why values of base composition should be in error by being too high.   For the present, I will take the results I have obtained as correct for the particular strains examined.

The somewhat anomolous position of J44 is clarified by examination of its base ratio.   Bacon, Overend and Lloyd (1967) obtained a value of 57.5% for this organism, which suggested that it was somewhat removed from the genus Bordetella.   The position of this strain in the BRUCELLA cluster shown in Figs. 7 and 8 is confirmed after comparing the base composition of J44 with strains of PERTUSSIS and BRUCELLA.   Both clusters have narrow ranges of base ratios which do not overlap, thus the similarity of the GC ratios of J44 and the BRUCELLA cluster provides more evidence for the re-identification of J44 as a species of the genus Brucella.

The ALCALIGENES cluster showed a comparatively wide range of GC content, 58.9-70.2%. This is reflected in the shape of the cluster in Fig. 2, where rather than forming an obvious cluster, the strains tended to join on separately, or in pairs, to the BRONCHISEPTICA cluster. The position of the non-motile, oxidase-negative Alcaligenes species in the LWOFFII cluster is confirmed on base composition. The four strains examined had values ranging from 40-45.3% which is compatible with values obtained for strains labelled as Acinetobacter lwoffii or Moraxella lwoffii, whether measured in this study or extracted from the literature.

The GC content of specific strains in group A were not measured, and the value for only one strain, J168 (43.5%), could be obtained from the literature. However, strains not included in this study have had their base ratios determined. It can be seen that the sub-group A3 would appear to have a range of 34-43% which extends over three genera, Actinobacillus, Pasteurella and Haemophilus. This suggests that this sub-group A3 is homogeneous both phenetically and genetically.

CHAPTER 4


Discussion and Conclusions

<u>The usefulness of numerical taxonomy, gel electrophoresis and DNA base</u>
<u>composition and their compatibility.</u>

Having applied the three techniques in varying degrees to some or all of the 201 strains, it is now possible to see how useful and how mutually reinforcing the results have been.

The numerical taxonomy has divided 183 strains into fifteen clusters each containing from 3-44 strains. Eighteen strains were not placed in any of the clusters, although several were closely associated. Two of the fifteen clusters were composite groups and could possibly be subdivided into separate clusters, making a total of seventeen in all.

Closer examination of the clusters reveals that some are equivalent to established bacterial species, for example

| | | |
|---|---|---|
| PARAPERTUSSIS | - | <u>Bordetella parapertussis</u> |
| PERTUSSIS | - | <u>Bordetella pertussis</u> |
| LWOFFII | - | <u>Acinetobacter lwoffii</u> |
| ANITRATUS | - | <u>Acinetobacter anitratus</u>. |

Others are equivalent to genera,

| | | |
|---|---|---|
| YERSINIA | - | <u>Yersinia</u> |
| BRUCELLA | - | <u>Brucella</u>. |

Clusters HAEM I, HAEM II and HAEM III when combined represent the genus <u>Haemophilus</u>, while clusters M-N I, M-N II, M-N III and M-N IV represent the genera <u>Neisseria</u> and <u>Moraxella</u>, although in this case they are not well differentiated from each other. Furthermore, it is not clear whether the classical pathogenic neisseria, the gonococcus and the meningococcus would fall here, as they were not studied.

Of the two composite clusters, PASTEURELLA — ACTINOBACILLUS is currently recognised by most bacteriologists as two separate genera,

_Pasteurella_ and _Actinobacillus_. Further work on this group however, may suggest that only one genus is necessary.

The last composite cluster, BRONCHISEPTICA-ALCALIGENES consists of the genus _Alcaligenes_ and the species _Bordetella bronchiseptica_. This is probably due to the fact that tests which would serve to separate the two were not included (Pickett and Pedersen, 1970). Nevertheless, it has long been recognised that there is very little difference between _B. bronchiseptica_ and _Alcaligenes faecalis_ ( Torrey and Rahe, 1913; Szturm and Bourdon, 1948; Beer, 1960 ), and the results obtained in this study have tended to confirm this view.

Without too much difficulty, the clusters can be combined into groups and sub-groups, some of which may or may not correspond to a higher taxonomic category e.g. family. Furthermore, each of the groups or sub-groups have specific and definitive properties which have proved useful for differentiating them from each other.

The preliminary studies on poly-acrylamide gel electrophoresis have indicated that strains within several of the clusters also showed similarities in their protein patterns. This was particularly apparent in _Bordetella bronchiseptica_ and _B. parapertussis_. Other clusters contained some strains having similar protein patterns while the remaining strains had differing patterns.

The results of the DNA base compositions were very encouraging, apart from the high results obtained with strains of _B. bronchiseptica_. Invariably each cluster has a fairly narrowly-defined range of GC content, this being consistent with the conclusion that they are phenetically and genetically homogeneous.

Both gel electrophoresis and DNA base composition provide important information and real encouragement when the results obtained confirm the groupings of numerical taxonomic studies. However, the absence of protein

pattern similarities does not necessarily mean that strains are not related. The situation is analogous to that occurring in DNA base compositions, where phenetically dissimilar bacteria can have identical base ratios e.g. micrococci and pseudomonads both have base ratios in the range 65-70 moles per cent.

In conclusion it can be stated that the three techniques have proved extremely useful in defining the taxonomic relationships among the strains studied here, and the close agreement between the techniques has also proved rewarding.

## Taxonomic Conclusions

Before discussing the taxonomy in detail, it seems worthwhile to consider individually certain points of interest which have arisen in previously published reports and which have arisen again in this work.

In numerical taxonomy many authors have defined their taxonomic groups by drawing lines ( phenon lines ) across dendrograms, at what appeared to them to be the most suitable point. This has advantages in that it is partially objective in that all groups are decided by the same line, but it remains subjective as to what level the line is drawn.

One might well ask, whether we can in fact draw a line across a dendrogram and say at this point the OTU's are divided into n groups which we shall call species or genera. Bacteriologists are faced with the problem of genera in one family being much more similar to each other than genera in another family. For instance, it is doubtful taxonomically that the genera of the Enterobacteriaceae should receive generic status, since they are probably more aligned to a "species" level, ( Cowan, 1956; Krieg and Lockhart, 1966; Stanier, Doudoroff and Adelberg, 1970 ).

In a taxonomic study, the taxonomist usually includes more strains of the species which areof particular interest to him than he does of those species which he includes as " marker" organisms. This is the case in this study where 89 of the 201 strains were received as

or were identified as <u>Bordetella</u> species.

When the number of OTU's in a cluster increases, the average
intra-group similarity tends to alter, either upwards or downwards.
Although the arrangement of the groups to each other may not change,
the phenon level at which a group forms may change considerably as the size
of the group increases, particularly if the group initially consisted of
a small number of OTU's and the phenon level is to take in all OTU's of
the group. As an example the average intra-group similarity of the
BRUCELLA cluster was 87.7%. However, this was based on only six strains
representing four nomenspecies. It is quite probable that if forty strains
had been studied, the average intra-group similarity would have been
significantly different and may even have transcended a line drawn across
the dendrogram at say 80% similarity. For this reason the boundaries of
the clusters have been based on experience and not solely on drawing a
line across the dendrogram. As was already mentioned in the results
however, a line drawn at a similarity level of 75% split the strains into
seven sub-groups, whereas six where eventually recognised. The only
difference occurred in sub-group A3, and this depended on whether it
was thought worthwhile to split it into two branches, one containing
<u>Actinobacillus</u> and <u>Pasteurella</u> and the other containing the <u>Haemophilus</u>
clusters.

### The position of families in bacterial taxonomy.

Both Stanier, Doudoroff and Adelberg (1970) and Cowan (1971)
have expressed their dissatisfactions about designating bacterial taxa
at higher levels than genera. Their arguments are forceful, and one can
think of many families, not least of which is the Brucellaceae, which
contain a heterogeneous collection of genera. However, it defeats the
purpose of hierarchical taxonomy if we stop at the genus level. We may

as well adopt a coding system ( Cowan, 1965) and forget about a taxonomy.

By their very nature some genera are more similar to each other than they are to other genera. One only needs "reliable and sensible" criteria to group them together. If we can move away from the idea of defining families on abstract characters such as pathogenicity and requirements for rich growth media, and move toward definitions based on more readily measurable characters which can be systematically applied to all genera, then there is no reason why bacterial families cannot be more clearly and accurately defined.

### Taxonomic status of the clusters and sub-groups.

The strains used in this study, when classified according to the seventh edition of Bergey's Manual, fell into four families;

Brucellaceae

Neisseriaceae

Achromobacteraceae

Rhizobiaceae.

It is apparent from Figs. 2 and 3 that the limits of these families are not clearly visible. On the basis of Fig. 3 and the table of nearest neighbours ( Table 9 ) there are probably only two higher taxa, roughly corresponding to sub-groups A3 and B3. But this data blurs what appears to be a truer picture of the inter-group similarities, by taking into consideration a large batch of sugar characters. It is doubtful whether production of acid from each of a wide variety of sugars should be considered as a truly independent character if most of the species are incapable of producing acid from glucose. The truer relationship of the clusters is approximated to more closely in Fig. 7, and here three higher taxa could be discerned. Sub-group A3 is still intact. The clusters M-N I, M-N II, M-N III and M-N IV have adopted closer positions to each other and consequently show agreement with the family Neisseriaceae ( Lessel, 1971 ).

A third taxon incorporating <u>Bordetella</u>, <u>Brucella</u> and <u>Alcaligenes</u> has also
appeared, while the position of <u>Acinetobacter anitratus</u> and <u>A. lwoffii</u>
is still indeterminate although seeming to take up an intermediate position
between the <u>Bordetella</u> group and <u>Moraxella</u> group.

<u>The significance of Group A.</u>

There are few reasons why sub-group A3 should not be considered as
representing a single family. Several points have arisen from the arrange-
ment of the clusters in this sub-group. It can be argued that the four
clusters should each be given generic status, although this would result
in dividing the genus <u>Haemophilus</u> into three separate genera. On the
basis of studying only twenty-nine strains, this would be presumptuous.
It appears more logical to recognise the existence of three sub-generic
divisions within the genus. One would contain those strains which have
an obligate requirement for haematin. A second would contain those
strains which require nicotinamide adenine dinucleotide but do not require
haematin. The third division would contain those strains which require
additional $CO_2$ on primary isolation.

The three sub-divisions would then contain the following species;

<u>Haemophilus</u> I   ( require haematin )

  <u>H. aegyptius</u>   ( probably a biotype of <u>H. influenzae</u> ).

  <u>H. influenzae</u>

  <u>H. canis</u>

  <u>H. haemolyticus</u>

<u>Haemophilus</u> II   ( require $NAD^+$ but not haematin )

  <u>H. suis</u>

  <u>H. gallinarum</u>

  <u>H. parasuis</u>

  <u>H. parainfluenzae</u>

  <u>H. parahaemolyticus</u>

<u>Haemophilus</u> III   (require $CO_2$ on primary isolation )

        <u>H. aphrophilus</u>

        <u>H. paraphrophilus</u>

The species names used above are taken from the names of strains as they were received by the author.

Recently, the Subcommittee on the Taxonomy of <u>Haemophilus</u> ( Biberstein and Zinnemann, 1971 ) have adopted the view that species which require only $NAD^+$ should have a specific epithet beginning with <u>para</u>. On this basis those species <u>H. suis</u> and <u>H. gallinarum</u> in <u>Haemophilus</u> II should be renamed <u>H. parasuis</u> and <u>H. paragallinarum</u> respectively.

It appears that there are no genuine strains of haematin-requiring <u>H. suis</u> and <u>H. gallinarum</u> available for study. Until such strains have been reisolated the specific epithets <u>suis</u> and <u>gallinarum</u> should not be used. Biberstein and White (1969) have already commented on this anomaly and have proposed two new species <u>H. parasuis</u> and <u>H. paragallinarum</u> for those strains which require only $NAD^+$.

Apart from this minor change in nomenclature and the recognition of sub-generic divisions within the genus <u>Haemophilus</u>, there was little evidence to suggest any radical changes in the taxonomy of this genus. I have therefore taken the view that the three clusters HAEM I, HAEM II and HAEM III should be considered as belonging to the single genus <u>Haemophilus</u>.

The separation of the ACTINOBACILLUS-PASTEURELLA cluster into two genera is more difficult to justify. It is difficult to base conclusions on the results of a small number of strains, but it is beyond doubt that the particular strains of <u>Actinobacillus</u> that were studied, had a higher inter-group average similarity to the strains of <u>Pasteurella</u> than the <u>Pasteurella</u> strains had for each other.

Neither Talbot and Sneath (1960), Smith and Thal (1965) nor

Stevens (1969) included strains of <u>Actinobacillus</u> in their taxonomic studies on <u>Pasteurella</u>, although Sneath and Cowan (1958) had already found that there was a close similarity between <u>Actinobacillus lignieresii</u> and <u>Pasteurella septica</u>. Furthermore, Jones (1962) had found that <u>P. haemolytica var. ureae</u> had a similar morphological appearance to <u>A. lignieresii</u> and many strains shared the ability to produce starch from maltose.

More recently, a series of reports by Mraz (1964; 1968; 1969a; 1969b and 1969c) have suggested that there are in fact close relationships between species of the two genera. He found that forty-six strains of <u>P. haemolytica</u> shared a higher average similarity (95%) with ninety-nine strains of <u>A. lignieresii</u> than they did with <u>P. multocida</u> (80%). On the basis of this evidence Mraz suggested that <u>P. haemolytica</u> be transferred to the genus <u>Actinobacillus</u> and renamed <u>A. haemolyticus</u>.

In addition to this direct evidence for combining the two genera, indirect evidence was obtained after a detailed literature search was undertaken. This revealed that there has not been a single report of characters which can effectively separate the two genera from each other. It is possible to identify individual species on the basis of production of indole, $\beta$-galactosidase and the ability to produce acid from mannitol, salicin and sorbitol, then only after having identified the species can it be assigned to a genus.

Some evidence for separating the two genera can be obtained from pathogenicity and symptoms of disease, if they are known. For instance, infections caused by pasteurellas are usually acute and characterised by septicaemia with widespread invasion of the viscera. The illness produced by actinobacilli is usually chronic and localised, often in the lungs, neck, tongue and regional lymph nodes. It is characterised by the presence of fibrous granulomatous lesions, and there is little or no invasion of the body viscera.

The balance of evidence would appear to favour combining the two genera, into a single genus which by priority would have to be called Pasteurella. However, before definitely proposing this amalgamation it would probably be worthwhile to carry out a more detailed study.

The literature survey of DNA base ratios offered further evidence that sub-group A3 is homogeneous and could act as the nucleus for the establishment of a new family. The GC content of strains in this family fell into a range 34.8-42 moles per cent, indicating that not only were there phenetic similarities but probably also genetic similarities.

A name suitable for this group has already been used by Pribram (1933). He used the family name Pasteurellaceae, in which he included four genera; Pasteurella, Brucella, Haemophilus and Neisseria. It is doubtful whether Pribram's Pasteurellaceae was ever validly published, nevertheless the name is appropriate for sub-group A3 and as such I have retained it as the family name for the three genera Haemophilus, Pasteurella and Actinobacillus.

The family Pasteurellaceae would then have the following definition;

## Pasteurellaceae Pribram 1933 or Fam. Nov.

Small coccoid to rod-shaped cells usually irregularly arranged. Occasionally, pleomorphic thread-like forms may occur. Bipolar staining may be shown by special methods. Non-pigmented. They do not grow abundantly on agar media and frequently require special media containing nicotinamide adenine dinucleotide and/or haematin. All species are non-motile. Sometimes encapsulated, particularly on primary isolation.

Facultative anaerobes with a fermentative metabolism. Many biochemical test media have to be enriched to ensure adequate growth. Carbohydrates are attacked without the production of gas. Gelatin is not liquefied. Oxidase reaction frequently positive. Nitrates often reduced.

Methyl red negative, Voges-Proskauer usually negative. They do not attack Tweens. Growth range 25 - 37°C. Optimum temperature 37°C. They are strict parasites on man and animals and are frequently pathogenic causing septicaemias, respiratory tract infections or diseases of joints and muscle. GC content 34 - 43 moles per cent.

Key to the genera of the family Pasteurellaceae,

I    Require NAD$^+$ and/or haematin.

<p style="text-align:center">Genus I    <u>Haemophilus</u>.</p>

II   Do not require NAD$^+$ or haematin.

<p style="text-align:center">Composite group</p>

<p style="text-align:center">Genus II    <u>Pasteurella</u>.</p>

<p style="text-align:center">Genus III  <u>Actinobacillus</u>.</p>

<u>The relationship of Yersinia to the Pasteurellaceae.</u>

The position of sub-group A1 containing the cluster YERSINIA appears from both Fig. 3 and Fig. 7 to be outside the family Pasteurellaceae. The seven strains included in the cluster formed a tight group and there appears to be no reason why it should not be considered as corresponding to the genus <u>Yersinia</u>.

Both Martinevskij (1968) and the report of the Sub-committee on <u>Pasteurella</u>, <u>Yersinia</u> and <u>Francisella</u> (Mollaret and Knapp, 1971) have indicated that there is a close similarity between <u>Yersinia</u> and the enterobacteria. This agrees with earlier reports of Thal (1954), and Sneath and Cowan (1958). Stevens (1969) also found that <u>Yersinia</u> had a greater similarity to the Enterobacteriaceae than to <u>Pasteurella</u>, but was of the opinion that <u>Yersinia</u> fell only at the edge of the Enterobacteriaceae.

There have been reports of common antigens shared by <u>Yersinia</u> and the enterobacteria ( Wetzler, Eitzen, Currie and Marshall, 1968 ).

On the other hand there have also been reports of antigens shared by Yersinia enterocolitica and Brucella abortus ( Ahvonen and Sievers, 1969), and by Yersinia and Pasteurella multocida ( Wilson and Miles, 1964 ).

Genetic studies on Yersinia have also tended to support its inclusion in the Enterobacteriaceae. Both Lazarus and Gunnison (1947) and Timofeeva (1968) have found phage cross-reactions between Y. pestis, Y. pseudotuberculosis and Salmonella and Shigella, but not with Pasteurella multocida.

Lawton, Morris and Burrows (1968) have reported genetic transfer between Y. pseudotuberculosis and Escherichia coli, and also refer to an earlier report ( Martin and Jacob, 1962 ) of transfer between Y. pestis and E. coli.

Ritter and Gerloff (1966) examined nucleic acid hybridisation between Yersinia , Pasteurella, Francisella and E. coli and obtained a higher rate of hybridisation between Y. pestis and E. coli, than between Y. pestis and P. multocida.

Further evidence of close genetic relationships between Yersinia and the Enterobacteriaceae has also been obtained from studies on pesticins and colicins. Smith and Burrows (1962) reported that some strains of Y. pestis, Y. pseudotuberculosis and E. coli were sensitive to the same pesticins and colicins. Additional reports can be obtained from the reviews of Reeves (1965) and Jones and Sneath (1970).

The majority of reports in the literature seem to favour placing Yersinia in the Enterobacteriaceae, but it should not be overlooked that many did not study the relationships between Yersinia and the true pasteurellae. Because of this the evidence must still be regarded as inconclusive.

The Taxonomic Sub-committee have not yet included Pasteurella "X" ( Yersinia enterocolitica ) in the genus Yersinia, although the results from this study and those of Smith and Thal (1965) and Stevens (1969)

strongly suggest that it should belong there.

Table 9 shows that the nearest neighbours to Yersinia are Brucella, Actinobacillus, Acinetobacter anitratus and Pasteurella respectively. The heterogeneity of these groups tends to suggest that Yersinia is not closely related to the Pasteurellaceae. As a genus it differs from the others in this family in being able to grow on 4.5% sodium chloride agar. Two species are motile and all species usually give a positive methyl red test. Yersinia species are oxidase-negative, and phosphatase-negative. They show similarities to the Pasteurellaceae in being fermentative, reducing nitrates and often showing bipolar staining. The GC base ratio falls into the range 43 - 47 moles per cent, which is slightly higher than the genera in the Pasteurellaceae.

The evidence from this study indicates that Yersinia has certain characters in common with the Pasteurellaceae, but overall does not share a high phenetic similarity. In addition, the lack of any enterobacteria in this study effectively prevents any direct comparison between Yersinia and that group.

### The taxonomic significance of Group B.

The removal of three genera of the Brucellaceae to a new family Pasteurellaceae raises the question of the status of the remaining genera in the Brucellaceae. Apart from requiring rich media for growth and their potential pathogenicity, the original genera of this family shared little in common.

Of those genera that are left, I was unable to obtain a strain of Calymmatobacterium, and the only strain of Noguchia granulosis available, ATCC 11479, differs from the original description, thereby raising doubts of its authenticity. The position of these genera must therefore remain in doubt.

The last three genera, Bordetella, Brucella and Moraxella were

all closely associated with, and showed greatest similarity to strains in group B, received as species of Alcaligenes, Acinetobacter, Neisseria, Agrobacterium and Francisella.

The strains of this group as a whole, shared the properties of not attacking carbohydrates, or only doing so oxidatively. They did not grow anaerobically; however, some strains would grow anaerobically in the presence of nitrate. The only exceptions were the two strains of Francisella, which were fermentative.

If we accept the principle of grouping bacterial genera into families, then a number of possibilities exist in group B. Figure 3 indicates that within this group are strains which fall into one of four families listed in Bergey's Manual- the Brucellaceae, Rhizobiaceae, Achromobacteraceae and the Neisseriaceae. However, the arrangement of the strains in Fig. 3 makes it virtually impossible to recognise these four families. Removal of the twenty-two sugar characters indicates that the arrangement of the clusters of group B is not too strictly determined. Fig. 7 shows that YERSINIA has moved into group B, but this is not a serious problem if we accept that as a genus it holds an intermediate position between several families.

Clusters M-N II and M-N III have moved to the edge of the group, as is indicated in the model, (Fig. 5). This suggests that the family Neisseriaceae as envisaged by the Sub-committee on the Taxonomy of Moraxella and Allied Bacteria ( Lessel, 1971 ), is in fact a reasonable grouping. This family would consist of Moraxella and Neisseria, with the genus Acinetobacter at least temporarily associated.

Figure 7 also shows Acinetobacter anitratus taking up a position much closer to A. lwoffii. Brucella has fallen in amongst Bordetella and Alcaligenes, which again is more appropriate in view of the confusion which hasexisted between species of these three genera.

We can now look at group B from two viewpoints. Firstly, as

indicated in Fig. 3 it could be envisaged as one complete family containing Gram-negative organisms which do not attack carbohydrates, or do so only oxidatively. Secondly, as indicated in Fig. 7 it could be split into two or three families.

I.   Neisseriaceae, containing Moraxella and Neisseria.

II.  A family containing Bordetella, Brucella and Alcaligenes.

A third family if required, would cater for Acinetobacter and those Achromobacter species which appear to be closely related, but as yet cannot be identified because there is no suitable type species on which to base the definition of the genus Achromobacter.

The first possibility is based on insufficient evidence to be justifiable at the present time. Furthermore, the DNA base composition would range from 40 - 75 moles per cent, a range which indicates that many of the strains have little genetic similarity.

The second possibility appears to be much more reasonable, and is more in keeping with the views of the International Sub-committee on Moraxella and Related Bacteria, however more work is needed before the position is clarified.

### Family I: the Neisseriaceae.

This family contains Moraxella and Neisseria although the position of the classical neisserias such as N. meningitidis, N. gonorrhoeae and the neisseria of the upper respiratory tract is still not clear from this study. The classical neisserias must however, remain within the genus Neisseria since its type species is N. gonorrhoeae. The uncertainty surrounds species such as N. catarrhalis, N. ovis and N. caviae which may possibly be transferred to the genus Moraxella.

The bacteria in this family fall within the definitions of Henriksen and Bövre (1968b) and include cocci and short rods which often

Table 20    Differentiation of the species of Moraxella and Neisseria.

| | M.osloensis | M.bovis | M. lacunata var. liq. | M.nonlique-faciens | M. kingae | M.phenyl-pyruvica | M.catarrhalis | M.caviae | M.ovis | N.flavescens | N.pharyngis |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | J136 J140 | J128 J129 J139 | J131 J137 | J134 | J143 | J141 J142 J145 | J148 J148 | J146 | J144 | J147 | J150 J152 |
| pigment from tyrosine | - | - | - | - | - | + | - | - | - | ± | - |
| haemolysis | - | + | - | - | - | - | - | - | - | - | - |
| growth on 5% bile | - | - | - | - | - | + | - | - | - | - | - |
| growth on 0% NaCl | + | - | - | - | + | + | + | - | + | - | - |
| catalase | + | + | + | + | + | + | + | + | - | + | + |
| lecithinase | - | + | + | - | - | (+) | - | + | - | - | - |
| casein hydrolysis | - | + | + | - | - | - | - | - | - | - | - |
| phenylalanine deamination | - | - | - | - | + | + | + | - | + | - | + |
| nitrate reduction | - | - | + | + | + | (+) | - | + | + | - | v |
| phosphatase | + | - | - | - | + | - | - | + | - | - | - |
| starch hydrolysis | + | + | + | + | + | + | + | + | + | - | + |
| acid from glucose | - | - | - | - | - | - | - | - | - | - | + |

Key:  +  positive,   -  negative,   (+)  usually positive,

±  moderate positive,   v  variable.

require rich media for culture and maintenance. They rarely grow well on culture media and die out fairly rapidly. They are inactive biochemically and are sensitive to a wide range of antibiotics and bacteria-inhibitory substances. The GC base ratios fall within the range 40 - 50 moles per cent, 40 - 45 per cent for Moraxella, 47 - 50 per cent for Neisseria.

The species recognised by Henriksen and Bovre are discernible in the four clusters M-N I - M-N IV. With the exclusion of the sugar reactions only three clusters are formed, M-N IV and J146 Neisseria caviae having merged with M-N II. Thus, seven of the nine species of Moraxella listed by Henriksen and Bovre appear in cluster M-N II. The two remaining species M. osloensis and M. phenylpyrouvica, fall in separate clusters but lie close to M-N II.

Table 20 shows how the nine Moraxella species plus two species of Neisseria may be differentiated. The list of strains below each species' name indicates strains which were received as typical of the species, or were identified as belonging to that species on the results obtained.

The results for each species, with the exception of M. kingae, correspond closely with those given by Henriksen and Bovre (1968a). Moraxella kingae, when tested in this study differed from the original description in catalase, phenylalanine deamination, haemolysis and production of acid from glucose. Since these characters are definitive for M. kingae one cannot exclude the possibility that the culture was contaminated.

Two strains J130 and J139, both received as Moraxella bovis, produced unexpected positive results when tested for the production of acid from glucose. After the computer runs were completed these strains were retested; J139 was found to be negative, while J130 was still positive. Confirmation of the results was obtained by further replicates, and thus, J139 takes up its correct position with J128 and J129, while the result and position of J130 is still not clear.

The position of the oxidase-negative Acinetobacter species.

Whether Acinetobacter should be included in the Neisseriaceae is still a matter for discussion. As a group, Acinetobacter species grow well on ordinary media and remain viable without sub-culture for quite long periods.

The GC base ratios agree closely with Moraxella and Neisseria, but Johnson, Anderson and Ordal (1970) found little evidence of nucleic acid hybridisation between oxidase-positive moraxellas and oxidase-negative acinetobacters. On this evidence they reached similar conclusions to Stenzel and Mannheim (1963) and Baumann, Doudoroff and Stanier (1968a) that the two should be kept separate.

The reliability of the oxidase reaction for separating Moraxella and Acinetobacter is disputed by Thornley (1967) who found that many of her phenon 4 strains, equivalent to Acinetobacter lwoffii were oxidase-positive. In this study two out of nine strains of A. lwoffii were oxidase-positive, so it would seem that the proposal of Baumann et al. to split the moraxellas and acinetobacters on the basis of the oxidase reaction may not be entirely satisfactory.

The relationship between Acinetobacter anitratus and A. lwoffii.

For the present, I propose to recognise the two clusters ANITRATUS and LWOFFII as separate species of the genus Acinetobacter. In both Fig. 3 and Fig. 7 they are well-defined and sufficiently distinct.

The results obtained indicate that except for a few strains which do not produce acid from glucose, strains belonging to the species A. anitratus are saccharolytic, whereas non-saccharolytic strains should be placed in the species A. lwoffii. This contradicts the report of the Sub-committee on Moraxella ( Lessel, 1971 ), who propose to recognise only one species, Acinetobacter calcoaceticus. Once again in this study as in

previous studies ( Pinter and Bende, 1967, 1968; Thornley, 1967 and Baumann, Doudoroff and Stanier, 1968b ), there is evidence of two separate groups. Furthermore, A. lwoffii appears to be more similar to Bordetella parapertussis than to A. anitratus ( see Fig. 4 ).

Baumann et al. criticised the excessive emphasis that was being placed on the production of acid from carbohydrates. They pointed out that in A. anitratus the metabolism of at least ten sugars is mediated by a non-specific aldose dehydrogenase. Thus, if acid is produced from glucose it is extremely likely that it will be produced from several sugars. In order to test their hypothesis, twenty-two of the twenty-eight sugars were deleted from the data matrix and the data rerun. It can be seen from Figs. 7 and 8 that the two clusters ANITRATUS and LWOFFII have come closer together, but have not overlapped. Three previously unclustered strains have joined the ANITRATUS cluster, making the boundaries of the species less clearly defined because none of the three strains concerned produced acid from carbohydrates.

The grouping is further confused by results obtained when acid production is determined in the ammonium salt sugar medium of Smith, Gordon and Clark (1952). Henderson (1967) examined five strains of A. lwoffii and found them to be non-saccharolytic, whereas 28 out of 29 strains of A. anitratus produced acid from glucose. These results agree with the pattern obtained from the carbohydrate medium used in this study. In contrast, Snell and Lapage (1971) have found that many strains of A. lwoffii do in fact produce acid from glucose in ammonium salt sugar media.

Bell and Marus (1966) and Marus and Bell (1966) postulated an abortive catabolism of glucose in A. lwoffii. Partial hexose monophosphate and Entner - Doudoroff pathways were identified, but there was a complete lack of kinase enzymes to enable phosphorylation of carbohydrates to proceed. Glucose could be directly oxidised to gluconic acid however, and the authors suggested that the energy might come from a cytochrome-linked

reaction of glucose dehydrogenase. They showed that the yield of cells on an acetate medium was increased in the presence of glucose and that some of the glucose was metabolised.

In summary it appears that two groups do exist - a predominantly saccharolytic group, A. anitratus and a predominantly non-saccharolytic group, A. lwoffii. Occasional intermediate strains exist giving the impression that strains of Acinetobacter form a continuous spectrum.

Further study of these groups may well reveal the existence of other characters which are useful for the differentiation of the two species.

## Family II: containing Brucella, Bordetella and Alcaligenes.

The genera included in this family all have a marked preference for metabolising amino acids. Brucella species can oxidise carbohydrates feebly, but only in the absence of amino acids. There is a requirement for part of the vitamin B complex; Bordetella and Alcaligenes require nicotinamide, while Brucella requires thiamine.

Morphologically, they are all Gram-negative coccobacilli which show little evidence of pleomorphism. They are usually oxidase-positive, although Bordetella parapertussis is an exception. Finally, all species have high GC base ratios, usually above 55moles per cent.

The species of Alcaligenes included in this family are those which are motile and oxidase-positive. This distinguishes them from the non-motile, oxidase-negative species which in my experience are invariably mis-identified strains of Acinetobacter lwoffii.

Two problems remain in defining the limits of this family and its constituent genera. Firstly, there is the apparent phenetic similarity

between Bordetella parapertussis and Acinetobacter lwoffii, which prompted Steel and Cowan (1964) to place B.parapertussis in the genus Acinetobacter.

There are difficulties in distinguishing between these two species, particularly if the strains of A.lwoffii produce alkali in Hugh and Leifson's oxidation-fermentation medium. Both species are Gram-negative, oxidase-negative rods or coccobacilli which do not attack carbohydrates in most test media. They also give negative results in many of the conventional biochemical tests. The high phenetic similarity between them is shown by the average inter-group similarity of 80.2% obtained in this study.

Only two clearly differential characters were found, and these depended on the ability of Bordetella parapertussis to produce pigment from tyrosine, and to grow in the presence of M and B 938. Urease production, haemolysis of sheep red blood cells, production of hydrogen sulphide in a medium containing cysteine, growth on 10% bile salts and growth on 4.5% sodium chloride media were however, useful tests for separating the two species ( see Table A3 ). Growth rate is also invaluable in their differentiation. Bordetella parapertussis takes two to three days to achieve confluent growth whereas Acinetobacter lwoffii takes only twenty-four hours. Finally, the difference of around 25 per cent in their GC base ratios is clear enough evidence that they should not be included within the same genus.

The second and more complicated problem concerns the relation-ship between Alcaligenes and Bordetella bronchiseptica. Both are found in the same cluster in all the dendrograms with the seven strains of Alcaligenes having a higher average similarity to B. bronchiseptica than they had for each other.

It has been apparent for some time that there is very little difference between these species over most bacteriological tests. Most workers have found that urease production is the most useful but in

this study three out of seven strains of Alcaligenes were urease-positive, so even this character has severe limitations. The most useful characters I have found are growth in the presence of potassium tellurite and hydrogen sulphide production.

Pickett and Pedersen (1970) tested strains for their ability to produce alkali from amides and organic salts. They found several substrates were useful in differentiating Alcaligenes and Bordetella bronchiseptica. These included, acetamide, allantoin, formamide, malonamide, isobutyrate and hexanoate. It may well be that more work is needed in developing tests that will distinguish these two groups from each other.

There is ample evidence for retaining Bordetella bronchiseptica in the same genus as Bordetella pertussis and Bordetella parapertussis on physiological, biochemical, serological and genetic grounds. The problem is whether Alcaligenes should be included in the same genus. This would pose nomenclatural problems which would require on grounds of priority, that the combined generic name should be Alcaligenes. In view of the present confusion over the validity of the genus Alcaligenes , the prospect of further arguments is far too daunting to comtemplate here. Let us first adopt a neotype strain of Alcaligenes faecalis in order to define the genus satisfactorily and unambiguously. Once this has been done, Alcaligenes can validly be compared with Bordetella. For the present I intend to recognise three genera in this family.

Having decided on the three genera Brucella, Bordetella and Alcaligenes, one of them has to be nominated as the type genus and the family named accordingly. The oldest generic name is Alcaligenes Castellani and Chalmers 1919, but choosing this name would again cause confusion in view of the arguments over the validity of the genus. The genus Brucella was listed as the type genus of the family Brucellaceae by Breed, Murray and Smith (1957), for seven genera, including Bordetella but not Alcaligenes, although Biberstein and Cameron(1961) have

Table 21          Differentiation of the Brucellaceae.

| Character | Alcaligenes | Bordetella | | | Brucella |
|---|---|---|---|---|---|
| | | bronchi septica | pertussis | para- pertussis | |
| growth anaerobically in the presence of nitrate | - | - | - | - | + |
| tyrosine hydrolysis | + | + | - | - | - |
| pigment from tyrosine | - | - | - | + | - |
| motility | + | + | - | - | - |
| oxidase | + | + | + | - | + |
| urease | v | + | - | + | + |
| nitrate reduction | + | + | - | - | + |
| sensitivity to | | | | | |
| penicillin | - | - | + | - | + |
| growth in the presence of potassium tellurite | + | - | - | - | - |

Key:   + positive,    - negative, v variable.

subsequently expressed the view that it is not a suitable genus on which
to base a family. In this study its nearest neighbours were two strains
of Agrobacterium and two strains of Francisella. Of the clusters, Table 9
indicates that BRONCHISEPTICA, ALCALIGENES, PARAPERTUSSIS and PERTUSSIS
were the most similar. Until more strains of Agrobacterium can be
studied it is better to leave the position of that genus in abeyance.
Apart from this there does not appear to be any serious obstacle to
retaining the genus Brucella as the type genus of the family Brucellaceae
with an emended generic composition. The following definition can be
given;

## Brucellaceae Breed, Murray and Smith 1957. emend.

Coccoid or rod-shaped cells irregularly arranged. There is little evi-
dence of pleomorphism. Non-pigmented. Motile and non-motile forms occur.
Certain species do not grow on nutrient agar on primary isolation.
Isolation can be favoured by incubating in 10 per cent carbon dioxide.
One species, Bordetella pertussis is extremely sensitive to toxic substances
found in culture media; the addition of charcoal and blood favours its
isolation. Nutritional requirements are relatively simple. Growth occurs
via the metabolism of amino acids so there is a marked tendency for media
to become alkaline. Carbohydrates are not attacked in conventional test
media. Usually biochemically inactive. They are obligate aerobes, although
some species can grow anaerobically in the presence of nitrate.
Catalase-positive and usually oxidase-positive. Litmus milk becomes
alkaline. Optimum growth temperature 30-37°C. Many are strict parasites
affecting man and animals, although saprophytic soil and water forms occur.
GC base ratios fall into the range 55-75 moles per cent.

The type genus is Brucella, Meyer and Shaw 1920.
Two other genera are included; Bordetella, Moreno-Lopez,

Alcaligenes, Castellani and Chalmers.

present study

| Order | Family | Genera |
|---|---|---|

Eubacteriales

- Pasteurellaceae —— <u>Pasteurella</u>, <u>Actinobacillus</u>, <u>Haemophilus</u>.

- Enterobacteriaceae ? ---- <u>Yersinia</u>.

  ? ---- <u>Francisella</u>.

- Brucellaceae —— <u>Bordetella</u>, <u>Alcaligenes</u>, <u>Brucella</u>.

  r---- <u>Acinetobacter</u>.

- Neisseriaceae —— <u>Neisseria</u>, <u>Moraxella</u>.

Bergey's Manual

| Order | Family | Genera |
|---|---|---|

Eubacteriales

- Achromobacteraceae —— <u>Achromobacter</u>, <u>Alcaligenes</u>.

- Brucellaceae —— <u>Pasteurella</u>, <u>Bordetella</u>, <u>Brucella</u>, <u>Haemophilus</u>, <u>Actinobacillus</u>, <u>Moraxella</u>, <u>Noguchia</u>, <u>Calymatobacterium</u>.

- Neisseriaceae —— <u>Neisseria</u>

Figure 10  A comparison of the classification obtained from the present
study with that given in Bergey's Manual ( 7th. edition).
Dotted lines indicate temporary associations still awaiting
further studies.

Table 21 indicates characters which are useful in differentiating the three genera.

## Comparison of the classification obtained from the present study with that given in Bergey's Manual ( 7th. edition ).

Comparison of the two classifications is made more difficult because Breed, Murray and Smith (1957) did not recognise the genera Yersinia, Francisella and Acinetobacter. However, all are now accepted and will undoubtedly be appearing in the eighth edition of the Manual.

The main differences appear in the rearrangement of the Brucellaceae and the establishment of a new family, Pasteurellaceae to accomodate Pasteurella, Actinobacillus and Haemophilus.

In accordance with the recommendation of the Subcommittee on the taxonomy of Moraxella and related bacteria, Moraxella is removed from the Brucellaceae and included in the Neisseriaceae ( see Fig. 10 ). Acinetobacter for the present, is loosely attached to this family, but its correct position probably lies in a separate family containing a revised genus Achromobacter, or a suitable substitute.

The Brucellaceae should contain only three genera, Brucella, Bordetella and Alcaligenes. Further studies on Francisella may indicate that it could also be included here, however, its fermentative metabolism and low GC base ratio (33-36 per cent) suggest that another family may be more suitable.

Within the Brucellaceae, the genera have a marked preference for metabolising amino acids to obtain their carbon, nitrogen and energy. They tend to be inactive in many biochemical characterisation tests, and all have a high GC base ratio. Alcaligenes is retained as a separate genus for convenience until more strains have been studied. Brucella is retained as the type genus and its definition is well documented by the

Subcommittee on the Taxonomy of the genus Brucella (Stableforth and Jones, 1963; Jones, 1967).

In the light of the results obtained here, the definition of the third genus Bordetella, can be extended to read;

## Bordetella, Moreno-Lopez 1952

Gram-negative small coccobacilli which show little evidence of pleomorphism. Motile and non-motile forms occur. Flagella, when present, are peritrichous. Fresh isolates may be encapsulated. Obligate aerobes. Metabolism is based on the primary utilisation of amino acids, usually with consequent production of alkali in culture media. Litmus milk becomes alkaline. Carbohydrates are not attacked. Catalase-positive. Growth requirements are simple although all species have an obligate requirement for nicotinamide. Some species are extremely sensitive to toxic by-products present in media, consequently substances rich in absorbing power (e.g. blood, starch, charcoal or anionic exchange resins) are often incorporated to improve growth. Optimum temperature 33-37$^\circ$C. All species possess common as well as species-specific antigens and produce an identical dermonecrotic toxin. They are parasites on warm-blooded animals causing respiratory tract infections such as Whooping Cough.

Type species is Bordetella pertussis.
Two other species are included in the genus; B. parapertussis,

B. bronchiseptica.

They all possess the following characters in common: they do not require added carbon dioxide or X and V factors for growth on artificial media. Catalase-positive, peroxidase-positive. They produce ammonia from peptone. Negative reactions are given in the following tests; phosphatase, hydrolysis of gelatin, starch, egg yolk, aesculin and casein; DNase, methyl red, Voges-Proskauer, production of acid from carbohydrates and

hydrolysis of Tweens.

Growth occurs on media containing any one of the following; 1 in 800 thionine, 1 in 800 pyronin G, 0.0075%(w/v) sodium azide or 0.00125%(w/v) 4:4'-Diamidinodiphenylamine dihydrochloride ( M and B 938 ). Growth does not occur on media containing 0.05%(w/v) thallous acetate or 0.032%(w/v) potassium tellurite.

They are sensitive to a wide range of antibiotics including streptomycin, chloramphenicol, aureomycin, terramycin, erythromycin, tetracycline, neomycin, novobiocin and oleandomycin, but are resistant to bacitracin.

## Future changes in the taxonomy of Bordetella and related organisms.

Inevitably in a taxonomic study one cannot hope to tie up all the loose ends. If the project is to be worthwhile, it must serve to clarify some of the confusion which has existed in the past, and it must also identify the areas where further research is required. In these respects the work presented here has achieved considerable success.

The results have shown that the Brucellaceae as defined by Breed, Murray and Smith (1957), contained a heterogeneous collection of genera which was in need of considerable revision. The taxonomy of the group has, in my opinion, been improved firstly, by emending the definition of the Brucellaceae and decreasing the number of genera contained therein to three. Secondly, the family Pasteurellaceae has been proposed for the three fermentative genera, Haemophilus, Pasteurella and Actinobacillus which were originally included in the Brucellaceae by Breed, Murray and Smith.

The problems that remain are much more specific in nature and involve changes within the two families or within individual genera. Of immediate interest in the Brucellaceae is the relationship between

Bordetella and Alcaligenes. There is also a need for a numerical taxonomic study of the genus Brucella in order to establish the relatioships, firstly, between its species and biotypes, and secondly, between that genus and those of Bordetella and Alcaligenes.

The main problem in the Pasteurellaceae concerns the relationship between Pasteurella and Actinobacillus; should there be two genera or only one? This again could be solved by using numerical taxonomic techniques.

Two areas of research highlighted and discussed in this study, are currently being investigated by taxonomic subcommittees. These areas are concerned with the speciation within Acinetobacter, and the relationship of Yersinia to the Enterobacteriaceae and to Pasteurella. One hopes that official guidance on the taxonomy of these groups will shortly be forthcoming.

In the past the main inconsistencies in the taxonomy of Bordetella and related organisms have arisen because many of the conventional tests have just not been applied. This has invariably been due to the difficulties encountered when working with fastidious or pathogenic species. Nevertheless, provided that the bacteriologist is prepared to spend some time on devising a suitable medium on which to culture these bacteria, on standardising test conditions and in applying tests to all strains under study, there is no reason why useful results cannot be obtained. This study was undertaken on these lines and has yielded enough information to place the taxonomy on a firmer basis. There remains a great deal of work to be carried out in investigating and applying new biochemical tests. Bacteria such as Bordetella, Alcaligenes, Brucella and Acinetobacter lwoffii give a large number of negative results in currently used identification tests. The development of more useful characterisation tests and perhaps the inclusion of nutritional studies on carbon and nitrogen utilisation will go a long way towards completing the taxonomic picture of the groups represented in this study.

The newer techniques of DNA base composition and gel electrophoresis have provided real encouragement for further work. In addition, large areas of productive research in nucleic acid pairing, genetic transfer, bacteriophage studies and serology have as yet been largely untapped. Future work on the taxonomy of Bordetella and related organisms will undoubtedly make use of these more sophisticated techniques to clear up any remaining problems that arise in this most challenging group of bacteria.

APPENDIX 1


Similarity matrix of <u>Bordetella</u> strains

Fig. A1. Similarity matrix of the first computation.
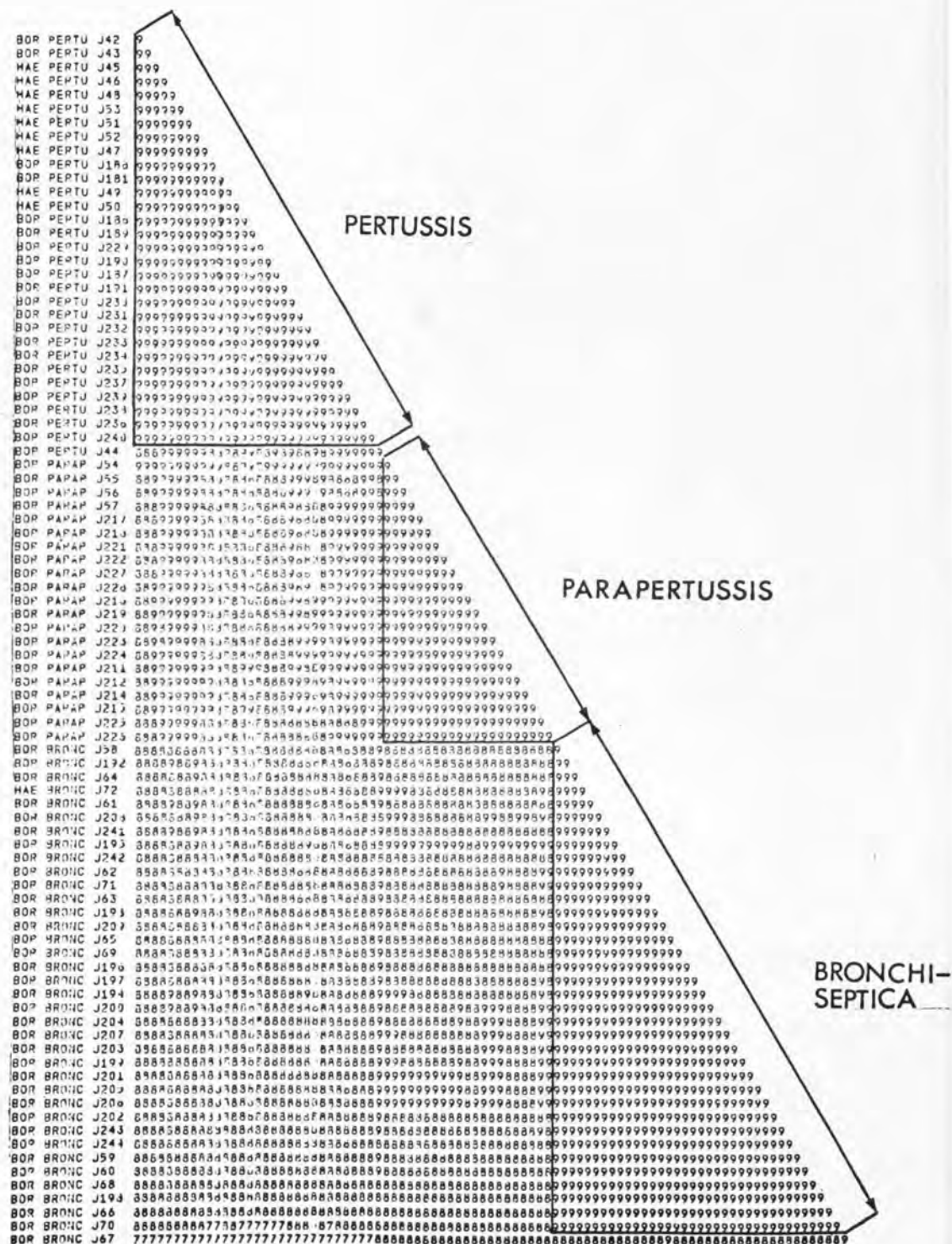
APPENDIX 2


Similarity matrix of the remaining strains

Fig. A2  Similarity matrix of the 150 Gram-negative rods included in
computation two.

1 BRONCHISEPTICA–ALCALIGENES

2 LWOFFII

3 PERTUSSIS

4 PARAPERTUSSIS

5 M–N I

6 M–N II

7 M–N III

8 BRUCELLA

9 ANITRATUS

10 ACTINOBACILLUS

11 PASTEURELLA

12 HAEM I

13 HAEM II

14 HAEM III

15 M–N IV

16 YERSINIA

APPENDIX 3


Coded data matrices

Eight strains were received as <u>Brucella</u> or <u>Francisella</u> species and were of dubious pathogenicity. It was therefore decided to carry out the tests on these strains after all tests on the remaining 193 strains had been completed. For this same reason, the eight strains were surveyed over a smaller range of tests and were coded for only 84 characters, as opposed to the 145 characters coded for the other strains.

The complete set of characters, and the choice of coding used for the computer analysis is shown in Table A1. The coded data matrix is shown in Table A2. In this table many characters were coded as "no comparison", even though the test had been carried out and the result obtained. The logic of this procedure rested in the example of growth of sodium chloride. If growth did not occur on 4.5% sodium chloride, growth on 6 and 9% sodium chloride could no longer be regarded as independent characters and were consequently scored as no comparison rather than negative.

Of the 145 characters, eleven were omitted from the computer analysis because the results appeared unreliable, or were identical for all strains. Growth on 0.5% sodium chloride, growth at 30°C, and growth in the presence of optochin were positive for all strains. No strain was capable of producing hydrogen cyanide or fermenting dulcitol. The results obtained from litmus milk (five characters), were dubious because it was difficult to know whether many of the strains had grown in the medium or, alternatively, were capable of producing sufficient growth to bring about a reaction. Lastly, an attempt was made to find out whether cells which occurred in pairs were joined end on, or side by side. In many of the strains, the cells were either short rods or coccobacilli and consequently it was impossible to determine the plane of division by light microscopy. Furthermore, in many cases it was impossible to distinguish between a coccus and a coccobacillus, so throughout this thesis both shapes of cell were referred to as coccobacilli.

Table A1          Characters and choice of coding.

| Character number | Character | Coding |
|---|---|---|
| 1 | Gram reaction | 1, 0 |
| 2 | retention of methyl violet | 1, 0 |
| 3 | cells arranged irregularly (I) or in sheets (S) | I=1, S=0 |
| 4 | presence of chains or filaments | 1, 0 |
| 5 | presence of pleomorphism | 1, 0 |
| 6 | cell shape | 0, 1, 2 |
| 7 | colony opaque | 1, 0 |
| 8 | colony glossy | 1, 0 |
| 9 | colony edge regular | 1, 0 |
| 10 | colony viscid | 1, 0 |
| 11 | colony raised | 1, 0 |
| 12 | production of a diffusible pigment | 1, 0 |
| 13 | pigment colour | 0, 1, 2 |
| 14 | pigment from tyrosine | 1, 0 |
| 15 | broth culture turbid | 1, 0 |
| 16 | flaky sediment in broth culture | 1, 0 |
| 17 | viscous sediment in broth culture | 1, 0 |
| 18 | presence of surface growth in broth culture | 1, 0 |
| 19 | growth anaerobically | 1, 0 |
| 20 | growth anaerobically in the presence of nitrate | 1, 0 |
| 21 | requirement for $CO_2$ | 1, 0 |
| 22 | requirement for V factor | 1, 0 |
| 23 | requirement for X factor | 1, 0 |
| 24 | requirement for X+V factors | 1, 0 |
| 25 | growth on nutrient agar | 1, 0 |
| 26 | growth on blood agar containing X+V factors | 1, 0 |
| 27 | haemolysis of horse red cells | 1, 0 |
| 28 | haemolysis of sheep red cells | 1, 0 |
| 29 | motility | 1, 0 |
| 30 | growth on 5% bile salts agar | 1, 0 |
| 31 | growth on 10% bile salts agar | 1, 0 |
| 32 | growth on 40% bile salts agar | 1, 0 |
| 33 | growth on 0% sodium chloride | 1, 0 |
| 34 | growth on 3% sodium chloride | 1, 0 |
| 35 | growth on 4.5% sodium chloride | 1, 0 |

Table A1 (continued)

| Character number | Character | Coding |
|---|---|---|
| 36 | growth on 6% sodium chloride | 1, 0 |
| 37 | growth on 7.5% sodium chloride | 1, 0 |
| 38 | growth on 9% sodium chloride | 1, 0 |
| 39 | sensitivity to 0/129 | 1, 0 |
| 40 | sensitivity to thionine | 1, 0 |
| 41 | sensitivity to basic fuchsin | 1, 0 |
| 42 | sensitivity to methyl violet | 1, 0 |
| 43 | sensitivity to safranine O | 1, 0 |
| 44 | sensitivity to pyronin G | 1, 0 |
| 45 | sensitivity to penicillin | 0, 1, 2, 3 |
| 46 | streptomycin | 0, 1, 2, 3 |
| 47 | chloramphenicol | 0, 1, 2, 3 |
| 48 | aureomycin | 0, 1, 2, 3 |
| 49 | terramycin | 0, 1, 2, 3 |
| 50 | erythromycin | 0, 1, 2, 3 |
| 51 | tetracycline | 0, 1, 2, 3 |
| 52 | sulphonamide | 0, 1, 2, 3 |
| 53 | neomycin | 0, 1, 2, 3 |
| 54 | novobiocin | 0, 1, 2, 3 |
| 55 | oleandomycin | 0, 1, 2, 3 |
| 56 | nitrofurantoin | 0, 1, 2, 3 |
| 57 | bacitracin | 0, 1, 2, 3 |
| 58 | Bactrim Roche | 0, 1, 2, 3 |
| 59 | growth at $5^{\circ}$C. | 1, 0 |
| 60 | growth at $10^{\circ}$C. | 1, 0 |
| 61 | growth at $15^{\circ}$C. | 1, 0 |
| 62 | growth at $25^{\circ}$C. | 1, 0 |
| 63 | growth at $35^{\circ}$C. | 1, 0 |
| 64 | growth at $37^{\circ}$C. | 1, 0 |
| 65 | growth at $44^{\circ}$C. | 1, 0 |
| 66 | resistance to $56^{\circ}$C. for 5 minutes | 1, 0 |
| 67 | resistance to $56^{\circ}$C. for 10 minutes | 1, 0 |
| 68 | resistance to $56^{\circ}$C. for 15 minutes | 1, 0 |
| 69 | resistance to $56^{\circ}$C. for 20 minutes | 1, 0 |
| 70 | growth on 0.1% phenol | 1, 0 |

Table A1 (continued)

| Character number | Character | Coding |
|---|---|---|
| 71 | growth on 0.2% phenol | 1, 0 |
| 72 | growth on 0.3% phenol | 1, 0 |
| 73 | growth on 0.4% phenol | 1, 0 |
| 74 | growth on 0.5% phenol | 1, 0 |
| 75 | growth in the presence of potassium tellurite | 1, 0 |
| 76 | growth in the presence of sodium azide | 1, 0 |
| 77 | growth in the presence of thallous acetate | 1, 0 |
| 78 | growth in the presence of 0.5% sodium nitrite | 1, 0 |
| 79 | growth in the presence of M and B 938 | 1, 0 |
| 80 | catalase | 1, 0 |
| 81 | peroxidase | 1, 0 |
| 82 | Kovac's oxidase | 1, 0 |
| 83 | hydrogen sulphide production | 1, 0 |
| 84 | phosphatase | 1, 0 |
| 85 | liquefaction of gelatin | 1, 0 |
| 86 | hydrolysis of starch | 1, 0 |
| 87 | egg yolk reaction | 1, 0 |
| 88 | hydrolysis of casein | 1, 0 |
| 89 | hydrolysis of aesculin | 1, 0 |
| 90 | dextran or levan production | 1, 0 |
| 91 | breakdown of T.P.T.C. | 1, 0 |
| 92 | ammonia from peptone | 1, 0 |
| 93 | urease | 1, 0 |
| 94 | ammonia from arginine | 1, 0 |
| 95 | reduction of nitrate | 1, 0 |
| 96 | deoxyribonuclease | 1, 0 |
| 97 | methyl red | 1, 0 |
| 98 | Voges-Proskauer | 1, 0 |
| 99 | hydrolysis of tyrosine | 1, 0 |
| 100 | hydrolysis of xanthine | 1, 0 |
| 101 | phenylalanine deaminase | 1, 0 |
| 102 | hydrolysis of tween 20 | 1, 0 |
| 103 | hydrolysis of tween 40 | 1, 0 |
| 104 | hydrolysis of tween 60 | 1, 0 |
| 105 | hydrolysis of tween 80 | 1, 0 |

Table A1 (continued)

| Character number | Character | Coding |
|---|---|---|
| 106 | clearing of tween 80 | 1, 0 |
| 107 | acid from 1% glucose, aerobically | 1, 0 |
| 108 | acid from 1% glucose, anaerobically | 1, 0 |
| 109 | acid from 10% glucose | 1, 0 |
| 110 | acid from 1% lactose | 1, 0 |
| 111 | acid from 10% lactose | 1, 0 |
| 112 | alkali from 1% lactose | 1, 0 |
| 113 | acid from xylose | 1, 0 |
| 114 | salicin | 1, 0 |
| 115 | raffinose | 1, 0 |
| 116 | $\alpha$-methyl d glucoside | 1, 0 |
| 117 | ribose | 1, 0 |
| 118 | rhamnose | 1, 0 |
| 119 | mannose | 1, 0 |
| 120 | inositol | 1, 0 |
| 121 | cellobiose | 1, 0 |
| 122 | fructose | 1, 0 |
| 123 | inulin | 1, 0 |
| 124 | dextrin | 1, 0 |
| 125 | arabinose | 1, 0 |
| 126 | adonitol | 1, 0 |
| 127 | sorbitol | 1, 0 |
| 128 | galactose | 1, 0 |
| 129 | maltose | 1, 0 |
| 130 | sucrose | 1, 0 |
| 131 | mannitol | 1, 0 |
| 132 | glycerol | 1, 0 |
| 133 | erythritol | 1, 0 |
| 134 | glucosamine hydrochloride | 1, 0 |

Key to coding:     1 = positive     0 = negative
cell shape     0 = coccobacillus     1 = short rod     2 = rod
pigment colour     0 = none     1 = brown     2 = yellow
antibiotic sensitivity     0 = insensitive     1 = zone up to 5mm.
    2 = zone up to 9mm.     3 = zone greater than 10mm.

Table A2.                    Coded data matrix.

| Strain No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| J1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| J2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 |
| J3 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | |
| J4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 |
| J5 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 |
| J6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| J7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | * | * | 1 | 1 |
| J8 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 |
| J9 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 |
| J10 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | * | 1 | 1 |
| J11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | * | * | 1 | 1 |
| J12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 |
| J13 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | * | 1 | 1 |
| J17 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | | |
| J18 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 |
| J19 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | * | 1 | 0 |
| J20 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | * | 1 | 1 |
| J21 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | * | 1 | 1 | |
| J23 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 |
| J24 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 |
| J25 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | * | * | 1 | 1 |
| J26 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 |
| J27 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | * | 1 | 1 | |
| J28 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 |
| J29 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | * | * | 1 | 1 |
| J30 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 |
| J31 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | |
| J32 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | |
| J33 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | |
| J34 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | | |
| J35 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | * | 1 | 1 |
| J36 | 1 | * | 0 | 0 | 0 | 2 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 |
| J37 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 |
| J38 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | * | 1 | 1 |
| J39 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | |
| J40 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | |
| J41 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | * | * | 1 | 0 | |

Strain No.                          Character number

Table A2 (continued).

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| J42 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | * | * | 1 | 0 |
| J43 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | * | * | 1 | 0 |
| J44 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 |
| J45 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | * | * | 1 | 0 |
| J46 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | * | * | 1 | 0 |
| J47 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | * | * | 1 | 0 |
| J48 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | * | * | 1 | 1 |
| J49 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | * | * | 1 | 0 |
| J50 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | * | * | 1 | 0 |
| J51 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | * | * | 1 | 0 |
| J52 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | * | * | 1 | 0 |
| J53 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | * | * | 1 | 1 |
| J54 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 |
| J55 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 |
| J56 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 |
| J57 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 |
| J58 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| J59 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| J60 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| J61 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| J62 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| J63 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| J64 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 |
| J65 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| J66 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| J67 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 |
| J68 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| J69 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| J70 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 |
| J71 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| J72 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 |
| J73 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | * | * | 1 | 0 |
| J74 | 0 | 0 | 0 | 1 | 1 | 2 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | * | * | 1 | 0 |
| J75 | 0 | 0 | 0 | 1 | 1 | 2 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | * | 1 | 0 |
| J78 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | * | * | 1 | 0 |
| J79 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | * | * | 1 | 0 |
| J80 | 0 | 0 | 0 | 1 | 1 | 2 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | * | * | 1 | 0 |

Table A2 (continued).

```
           1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34
J83   0 0 0 1 1 1 0 1 1 0 1 0 0 0 0 1 0 0 1 1 0 0 0 1 0 1 1 0 0 1 0 * 1 0
J86   0 0 0 1 1 1 0 1 1 0 1 0 0 0 1 0 1 0 1 1 0 0 0 1 0 1 0 0 0 1 1 0 1 0
J87   0 0 0 1 1 1 0 1 1 0 1 0 0 0 1 1 0 0 1 1 0 0 0 1 0 1 0 0 0 1 0 * 1 0
J88   0 0 0 1 1 1 0 1 1 0 1 0 0 0 1 0 1 0 1 1 0 0 0 1 0 1 0 0 0 1 0 * 1 0
J89   0 0 0 1 1 1 0 1 1 0 1 0 0 0 1 0 1 0 1 1 0 0 0 1 0 1 0 0 0 1 0 * 1 0
J90   0 0 0 1 1 1 0 1 1 0 1 0 0 0 0 1 0 0 1 1 0 0 0 1 0 1 0 0 0 1 0 * 1 0
J91   0 0 0 1 1 1 0 1 1 0 1 0 0 0 1 0 1 0 1 1 0 0 0 1 0 1 1 0 0 1 0 * 1 0
J92   0 0 0 1 1 1 0 1 1 0 1 0 0 0 1 0 1 0 1 1 0 0 0 1 0 1 0 0 0 1 0 * 1 0
J93   0 0 0 1 1 2 0 1 1 0 1 0 0 0 1 1 0 0 1 1 0 1 0 0 0 1 0 0 0 0 * * 1 0
J94   0 0 0 1 1 2 0 1 1 0 1 0 0 0 1 1 0 1 1 1 0 1 0 0 0 1 1 0 0 1 0 * 1 1
J95   0 0 0 1 1 2 0 1 1 0 1 0 0 0 1 1 0 0 1 1 0 1 1 0 1 0 0 0 1 0 0 0 1 1
J99   0 0 0 1 1 2 0 1 1 0 1 0 0 0 1 0 1 0 1 1 0 1 0 0 0 1 0 0 0 0 * * 1 1
J100  0 0 0 1 1 2 0 1 1 0 1 0 0 0 0 1 0 0 1 1 0 1 0 0 0 1 0 0 0 0 * * 1 0
J101  0 0 0 1 1 2 0 1 1 0 1 0 0 0 0 1 0 0 1 1 0 1 0 0 0 1 0 0 0 1 0 * 1 0
J102  0 0 0 1 1 1 0 1 1 0 1 0 0 0 1 1 0 0 1 1 0 1 0 0 0 1 1 0 0 1 0 * 1 1
J103  0 0 0 1 1 2 0 1 1 0 1 0 0 0 1 1 0 0 1 1 0 1 0 0 0 1 1 0 0 1 1 0 1 1
J104  0 0 0 1 1 1 0 1 1 0 1 0 0 0 1 1 0 0 1 1 0 1 0 0 0 1 0 0 0 0 * * 1 1
J108  0 0 0 1 1 1 0 1 1 0 1 0 0 0 1 1 0 0 1 1 0 1 0 0 0 1 0 0 0 1 1 0 1 0
J120  0 0 0 0 0 1 0 1 1 0 1 0 0 0 1 1 0 0 1 1 1 0 1 0 0 1 0 0 0 1 0 * 1 0
J121  0 0 0 0 0 0 1 1 0 1 0 0 0 0 0 1 0 1 1 1 1 0 0 0 1 0 0 0 1 0 * 1 0
J125  0 0 0 1 1 1 0 1 1 0 1 0 0 0 1 0 1 0 1 1 0 1 0 0 0 1 0 0 0 0 * * 1 0
J128  0 1 0 1 1 0 1 1 1 0 1 0 1 1 0 1 0 0 0 0 0 0 1 1 1 1 0 0 * * 0 1
J129  0 1 0 1 0 1 1 1 1 0 1 0 0 0 1 0 1 0 0 0 0 0 0 1 1 1 1 0 0 * * 0 1
J130  0 1 0 0 0 0 1 1 1 0 1 0 0 0 1 0 1 0 1 1 0 0 0 0 1 1 1 1 0 0 * * 0 1
J131  0 1 0 0 1 0 1 1 1 0 1 0 0 0 1 0 1 0 0 0 0 0 0 1 1 0 0 0 0 * * 0 1
J132  0 0 0 0 0 0 1 1 1 1 0 0 0 1 0 1 0 0 0 0 0 0 1 1 0 0 0 1 0 * 1 1
J133  0 0 0 0 0 0 1 1 1 1 0 0 0 0 0 1 0 0 0 0 0 0 1 1 0 0 0 1 0 * 1 1
J134  0 1 0 0 0 1 1 1 1 0 1 0 0 0 1 0 1 0 0 1 0 0 0 0 1 1 0 0 0 0 * * 0 1
J136  0 0 0 0 0 0 1 1 1 0 1 0 0 0 1 0 1 1 0 0 0 0 0 0 1 1 0 0 0 0 * * 1 0
J137  0 1 0 0 1 0 1 1 1 0 1 0 0 0 1 0 1 0 0 0 0 0 0 1 1 0 0 0 0 * * 0 1
J139  0 1 0 0 1 0 0 1 1 0 1 0 0 0 1 0 1 0 0 0 0 0 0 1 1 1 1 0 0 * * 0 1
J140  0 1 0 0 0 0 0 1 1 0 1 0 0 0 1 1 0 0 0 0 0 0 0 1 1 0 0 0 0 * * 1 1
J141  0 1 0 1 0 0 0 1 1 0 1 0 0 1 1 0 1 1 0 0 0 0 0 0 1 0 0 0 1 1 0 1 1
J142  0 1 0 0 0 0 0 0 1 0 1 0 0 1 0 0 1 1 0 0 0 0 0 0 1 0 0 0 1 0 * 1 1
J143  0 1 0 0 0 0 0 1 1 0 1 0 0 0 1 0 1 1 0 1 0 0 0 0 1 1 0 0 0 0 * * 1 1
J144  0 1 0 0 0 0 0 1 1 1 0 1 0 0 0 1 0 1 1 1 1 0 0 0 0 1 1 0 0 0 0 * * 1 1
J145  0 1 0 0 0 0 0 0 0 0 0 0 0 1 1 0 1 0 0 0 0 0 0 1 1 0 0 1 1 1 0 1 1
```

Table A2 (continued).

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| J146 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | * | * | 0 | 1 |
| J147 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 2 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | * | * | 0 | 0 |
| J148 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | * | * | 1 | 1 |
| J149 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | * | * | 1 | 1 |
| J150 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | * | * | 0 | 0 |
| J152 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | * | * | 0 | 0 |
| J153 | 0 | 0 | 0 | 1 | 1 | 2 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | * | * | 1 | 1 |
| J154 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | * | 0 | 1 | 1 | * | 1 | 1 |
| J158 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | * | 0 | 1 | 1 | * | 1 | 1 |
| J159 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | * | 0 | 0 | * | * | 1 | 0 |
| J161 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | * | 0 | 0 | * | * | 1 | 1 |
| J162 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | * | 0 | 1 | 0 | * | 1 | 1 |
| J165 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | * | 0 | 1 | 1 | * | 1 | 1 |
| J167 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | * | 1 | 1 |
| J168 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 |
| J169 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | * | 1 | 0 |
| J170 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | * | * | 0 | 1 |
| J171 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | * | 1 | 1 |
| J172 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | * | 1 | 1 |
| J173 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 |
| J174 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 |
| J175 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 |
| J176 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | * | 1 | 1 |
| J177 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 |
| J178 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 |
| J179 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | * | 0 | 1 | 0 | * | 1 | 1 |
| J180 | 0 | 1 | 0 | 0 | 0 | 2 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | * | 0 | 1 | 1 | * | 1 | 1 |
| J181 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | * | * | 1 | 0 |
| J182 | 0 | 0 | 0 | 1 | 1 | 2 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | * | * | 1 | 0 |
| J183 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | * | * | 1 | 0 |
| J186 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | * | * | 1 | 0 |
| J187 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | * | * | 1 | 0 |
| J188 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | * | * | 1 | 0 |
| J189 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | * | * | 1 | 0 |
| J190 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | * | * | 1 | 0 |
| J191 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | * | * | 1 | 0 |
| J192 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |

Table A2 (continued).

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| J193 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| J194 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| J195 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| J196 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| J197 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| J198 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| J199 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| J200 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| J201 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| J202 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| J203 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| J204 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| J205 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| J206 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| J207 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| J208 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| J209 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| J210 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 2 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | * | * | 1 | 1 |
| J211 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 |
| J212 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 |
| J214 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 |
| J215 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 |
| J216 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 |
| J217 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 |
| J218 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 |
| J219 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 |
| J220 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 |
| J221 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 |
| J222 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 |
| J223 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 |
| J224 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 |
| J225 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 |
| J226 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 |
| J227 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 |
| J228 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 |
| J229 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | * | * | 1 | 0 |
| J230 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | * | * | 1 | 0 |

Table A2 (continued).

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| J231 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | * | * | 1 | 0 |
| J232 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | * | * | 1 | 0 |
| J233 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | * | * | 1 | 0 |
| J234 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | * | * | 1 | 0 |
| J235 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | * | * | 1 | 0 |
| J236 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | * | * | 1 | 0 |
| J237 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | * | * | 1 | 0 |
| J238 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | * | * | 1 | 0 |
| J239 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | * | * | 1 | 0 |
| J240 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | * | * | 1 | 0 |
| J241 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| J242 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| J243 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| J244 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| J245 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 |
| J246 | 0 | 1 | 0 | 1 | 1 | 2 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | * | * | 1 | 0 |

Table A2 (continued).

| | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 | 49 | 50 | 51 | 52 | 53 | 54 | 55 | 56 | 57 | 58 | 59 | 60 | 61 | 62 | 63 | 64 | 65 | 66 | 67 | 68 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| J1 | 1 | 1 | 0 | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 3 | 3 | 1 | 0 | 2 | 1 | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J2 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 1 | 1 | 1 | 1 | 0 | 3 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | * |
| J3 | 0 | * | * | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 1 | 0 | 1 | 2 | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | * | 1 | 1 | 1 |
| J4 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 2 | 3 | 1 | 1 | 1 | 2 | 3 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J5 | 1 | 1 | 0 | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 2 | 3 | 3 | 1 | 3 | 2 | 3 | 1 | 1 | 0 | 0 | 2 | * | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| J6 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 2 | 1 | 1 | 2 | 2 | 3 | 1 | 1 | 1 | 0 | 3 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J7 | 1 | 0 | * | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 3 | 3 | 2 | 3 | 1 | 3 | 3 | 3 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J8 | 1 | 1 | 0 | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 2 | 2 | 2 | 2 | 3 | 3 | 1 | 2 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | * |
| J9 | 1 | 0 | * | * | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 2 | 3 | 3 | 2 | 1 | 2 | 1 | 3 | 3 | 3 | 1 | 0 | 3 | 0 | 1 | 1 | 1 | 1 | 0 | * | 1 | 0 | * |
| J10 | 1 | 0 | * | * | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 3 | 3 | 3 | 1 | 2 | 2 | 2 | 3 | 1 | 3 | 1 | 0 | 3 | 0 | 1 | 1 | 1 | 0 | * | * | 1 | 1 | 0 |
| J11 | 1 | 0 | * | * | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 3 | 3 | 3 | 1 | 1 | 2 | 2 | 2 | 1 | 2 | 1 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | * |
| J12 | 1 | 0 | * | * | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 2 | 2 | 1 | 1 | 2 | 2 | 2 | 1 | 1 | 0 | 0 | 2 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J13 | 1 | 0 | * | * | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 2 | 0 | 1 | 1 | 1 | 2 | 1 | 2 | 0 | 0 | 1 | * | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J17 | 0 | * | * | * | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 2 | 3 | 3 | 3 | 2 | 3 | 3 | 3 | 2 | 3 | 2 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | * | 0 | * | * |
| J18 | 1 | 0 | * | * | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 3 | 3 | 3 | 3 | 2 | 3 | 1 | 3 | 1 | 2 | 2 | 0 | 0 | * | 0 | 1 | 1 | 1 | 1 | 0 | 0 | * | * |
| J19 | * | * | * | * | 1 | 0 | 0 | 1 | 0 | 1 | 2 | 2 | 3 | 3 | 3 | 2 | 3 | 3 | 3 | 1 | 2 | 3 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | * |
| J20 | 0 | * | * | * | 1 | 0 | 1 | 1 | 0 | 1 | 2 | 2 | 3 | 3 | 2 | 1 | 2 | 2 | 3 | 1 | 2 | 3 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 0 | 0 | * | * |
| J21 | 0 | * | * | * | 1 | 0 | 1 | 1 | 1 | 1 | 2 | 2 | 3 | 3 | 3 | 2 | 3 | 3 | 3 | 1 | 2 | 3 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 0 | 0 | * | * |
| J23 | 1 | 0 | * | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 2 | 2 | 2 | 1 | 2 | 0 | 3 | 1 | 2 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| J24 | 1 | 0 | * | * | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 1 | 2 | 1 | 1 | 1 | 1 | 2 | 1 | 2 | 0 | 0 | 2 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J25 | 1 | 0 | * | * | 1 | 0 | 1 | 1 | 1 | 0 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 0 | 3 | 1 | 1 | 1 | 1 | 1 | 0 | * | * | 0 | * | * |
| J26 | 1 | 0 | * | * | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 1 | 2 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 0 | 0 | 2 | 0 | 1 | 1 | 1 | 0 | * | * | 1 | 0 | * |
| J27 | 1 | 0 | * | * | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 2 | 3 | 3 | 1 | 1 | 1 | 1 | 2 | 1 | 2 | 1 | 0 | 0 | * | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J28 | 1 | 1 | 0 | * | 0 | 0 | 1 | 1 | 1 | 1 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 0 | 2 | 1 | 2 | 1 | 0 | 2 | 0 | 1 | 1 | 1 | 0 | * | * | 1 | 1 | 0 |
| J29 | 1 | 0 | * | * | 0 | 0 | 1 | 1 | 1 | 1 | 2 | 2 | 3 | 3 | 1 | 2 | 2 | 0 | 2 | 1 | 3 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 |
| J30 | 1 | 0 | * | * | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 2 | 2 | 3 | 2 | 1 | 2 | 2 | 3 | 1 | 2 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| J31 | 1 | 1 | 0 | * | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 3 | 2 | 1 | 1 | 2 | 2 | 2 | 1 | 2 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| J32 | 1 | 0 | * | * | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 3 | 3 | 1 | 1 | 2 | 2 | 3 | 1 | 1 | 0 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J33 | 1 | 0 | * | * | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 1 | 2 | 1 | 2 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J34 | 1 | 0 | * | * | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 1 | 2 | 1 | 1 | 2 | 0 | 2 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J35 | 1 | 0 | * | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 1 | 3 | 2 | 1 | 2 | 0 | 3 | 1 | 3 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| J36 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 2 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 3 | 3 | 3 | 2 | 0 | 3 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| J37 | 1 | 0 | * | * | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 1 | 2 | 1 | 1 | 2 | 2 | 2 | 1 | 2 | 1 | 0 | 3 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| J38 | 1 | 0 | * | * | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 2 | 2 | 2 | 1 | 2 | 0 | 2 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| J39 | 1 | 0 | * | * | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 2 | 2 | 1 | 2 | 0 | 2 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J40 | 1 | 0 | * | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 3 | 3 | 1 | 2 | 0 | 2 | 1 | 2 | 1 | 0 | 0 | * | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| J41 | * | * | * | * | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 3 | 3 | 3 | 3 | 2 | 3 | 1 | 3 | 2 | 3 | 1 | 0 | 3 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |

Table A2 (continued).

| | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 | 49 | 50 | 51 | 52 | 53 | 54 | 55 | 56 | 57 | 58 | 59 | 60 | 61 | 62 | 63 | 64 | 65 | 66 | 67 | 68 |
|------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| J42 | * | * | * | * | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 0 | 3 | 3 | 3 | 3 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J43 | * | * | * | * | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 3 | 3 | 2 | 3 | 3 | 3 | 0 | 3 | 3 | 3 | 3 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J44 | * | * | * | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 3 | 3 | 3 | 0 | 3 | 0 | 3 | 3 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J45 | * | * | * | * | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 0 | 3 | 3 | 3 | 3 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J46 | * | * | * | * | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 0 | 3 | 3 | 3 | 3 | 0 | 3 | * | * | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J47 | * | * | * | * | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 0 | 3 | 3 | 3 | 3 | 0 | 3 | * | * | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J48 | 0 | * | * | * | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 0 | 3 | 3 | 3 | 3 | 0 | 3 | * | * | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J49 | * | * | * | * | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 0 | 3 | 3 | 3 | 3 | 0 | 3 | * | * | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J50 | * | * | * | * | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 0 | 3 | 3 | 3 | 3 | 0 | 3 | * | * | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J51 | * | * | * | * | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 3 | 3 | 3 | 1 | 3 | 3 | 0 | 3 | 2 | 3 | 3 | 0 | 3 | * | * | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J52 | * | * | * | * | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 0 | 3 | 2 | 3 | 3 | 0 | 3 | * | * | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J53 | 0 | * | * | * | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 0 | 3 | 3 | 3 | 3 | 0 | 3 | * | * | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J54 | 1 | 0 | * | * | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J55 | 1 | 0 | * | * | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J56 | 0 | * | * | * | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J57 | 0 | * | * | * | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J58 | 1 | 1 | 0 | * | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 3 | 3 | 2 | 1 | 1 | 0 | 3 | 1 | 3 | 0 | 0 | 3 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| J59 | 1 | 1 | 0 | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 2 | 2 | 1 | 1 | 0 | 3 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| J60 | 1 | 1 | 0 | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 3 | 2 | 1 | 1 | 0 | 3 | 1 | 2 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| J61 | 1 | 1 | 0 | * | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 3 | 3 | 2 | 2 | 2 | 2 | 1 | 3 | 1 | 2 | 0 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J62 | 1 | 1 | 0 | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 3 | 3 | 2 | 1 | 2 | 0 | 3 | 1 | 1 | 0 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J63 | 1 | 1 | 0 | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 2 | 2 | 2 | 2 | 0 | 3 | 1 | 2 | 0 | 0 | 3 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J64 | 1 | 1 | 0 | * | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 3 | 2 | 1 | 2 | 1 | 0 | 3 | 1 | 3 | 0 | 0 | 3 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J65 | 1 | 1 | 0 | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 3 | 2 | 1 | 1 | 1 | 0 | 3 | 1 | 2 | 0 | 0 | 3 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J66 | 1 | 1 | 0 | * | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 2 | 3 | 2 | 1 | 2 | 3 | 3 | 1 | 2 | 0 | 0 | 3 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| J67 | 1 | 0 | * | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 3 | 1 | 0 | 0 | 1 | 1 | 2 | 0 | 0 | 0 | 0 | 2 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 |
| J68 | 1 | 1 | 0 | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 2 | 2 | 1 | 1 | 3 | 3 | 1 | 2 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| J69 | 1 | 1 | 0 | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 2 | 1 | 1 | 1 | 3 | 1 | 2 | 1 | 0 | 3 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 |
| J70 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 3 | 1 | 1 | 2 | 1 | 2 | 1 | 0 | 1 | 0 | 2 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| J71 | 1 | 1 | 0 | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 3 | 3 | 2 | 2 | 2 | 0 | 3 | 1 | 1 | 1 | 0 | 0 | * | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J72 | 1 | 1 | 0 | * | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 3 | 2 | 1 | 2 | 2 | 3 | 2 | 1 | 3 | 0 | 0 | 3 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J73 | * | * | * | * | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 1 | 3 | 2 | 1 | 0 | 0 | * | * | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 |
| J74 | * | * | * | * | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 1 | 3 | 2 | 1 | 0 | 0 | * | * | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J75 | * | * | * | * | 1 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 3 | 2 | 3 | 1 | 0 | 3 | * | * | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 |
| J78 | * | * | * | * | 1 | 0 | 1 | 1 | 1 | 0 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 0 | 0 | * | * | 0 | 1 | 1 | 1 | 0 | 0 | * | * |
| J79 | * | * | * | * | 1 | 0 | 1 | 1 | 1 | 0 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 0 | 3 | * | * | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J80 | * | * | * | * | 0 | 1 | 1 | 0 | 0 | 1 | 3 | 3 | 3 | 2 | 2 | 1 | 3 | 0 | 3 | 2 | 2 | 3 | 2 | 0 | * | * | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |

Table A2 (continued).

| | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 | 49 | 50 | 51 | 52 | 53 | 54 | 55 | 56 | 57 | 58 | 59 | 60 | 61 | 62 | 63 | 64 | 65 | 66 | 67 | 68 |
|------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| J83 | * | * | * | * | 0 | 1 | 1 | 1 | 1 | 1 | 2 | 3 | 3 | 3 | 3 | 1 | 3 | 0 | 3 | 2 | 1 | 3 | 0 | 0 | * | * | 0 | 1 | 1 | 1 | 0 | 1 | 0 | * |
| J86 | * | * | * | * | 0 | 0 | 0 | 1 | 0 | 0 | 2 | 2 | 2 | 3 | 3 | 2 | 3 | 0 | 3 | 1 | 2 | 3 | 0 | 0 | * | * | 0 | 1 | 1 | 1 | 1 | 1 | 0 | * |
| J87 | * | * | * | * | 1 | 0 | 1 | 1 | 0 | 0 | 2 | 3 | 3 | 3 | 3 | 2 | 3 | 0 | 3 | 2 | 2 | 3 | 0 | 0 | * | * | 0 | 1 | 1 | 1 | 0 | 1 | 0 | * |
| J88 | * | * | * | * | 0 | 0 | 1 | 1 | 0 | 0 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 0 | 3 | 2 | 2 | 2 | 0 | 0 | * | * | 0 | 1 | 1 | 1 | 0 | 0 | * | * |
| J89 | * | * | * | * | 0 | 0 | 1 | 1 | 0 | 1 | 2 | 2 | 3 | 2 | 3 | 2 | 3 | 0 | 3 | 2 | 1 | 3 | 0 | 0 | * | * | 0 | 1 | 1 | 1 | 0 | 0 | * | * |
| J90 | * | * | * | * | 0 | 0 | 1 | 1 | 0 | 1 | 2 | 3 | 2 | 2 | 3 | 2 | 3 | 0 | 3 | 2 | 1 | 3 | 0 | 0 | * | * | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 |
| J91 | * | * | * | * | 0 | 0 | 1 | 1 | 0 | 0 | 2 | 2 | 3 | 2 | 3 | 2 | 3 | 0 | 2 | 1 | 1 | 3 | 0 | 0 | * | * | 0 | 1 | 1 | 1 | 0 | 0 | * | * |
| J92 | * | * | * | * | 0 | 0 | 1 | 1 | 1 | 0 | 2 | 2 | 3 | 2 | 2 | 2 | 3 | 0 | 3 | 2 | 2 | 3 | 0 | 0 | * | * | 0 | 1 | 1 | 1 | 0 | 0 | * | * |
| J93 | * | * | * | * | 0 | 0 | 1 | 1 | 1 | 0 | 3 | 2 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 2 | 3 | 3 | 0 | 1 | * | * | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J94 | 0 | * | * | * | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 3 | 0 | 2 | 3 | 3 | 3 | 3 | 3 | 1 | 3 | 3 | 0 | 0 | * | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| J95 | 0 | * | * | * | 0 | 0 | 0 | 1 | 1 | 0 | 3 | 2 | 3 | 3 | 2 | 1 | 2 | 0 | 3 | 1 | 2 | 3 | 0 | 0 | * | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| J99 | 0 | * | * | * | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 2 | 2 | 2 | 2 | 0 | 2 | 0 | 2 | 2 | 2 | 2 | 0 | 0 | * | * | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| J100 | * | * | * | * | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 3 | 3 | 3 | 2 | 0 | 0 | 3 | 2 | 2 | 3 | 0 | 0 | * | * | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J101 | * | * | * | * | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 3 | 3 | 2 | 1 | 2 | 0 | 3 | 1 | 1 | 3 | 0 | 0 | * | * | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J102 | 0 | * | * | * | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 3 | 2 | 1 | 3 | 1 | 2 | 1 | 2 | 3 | 0 | 0 | * | * | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| J103 | 0 | * | * | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 2 | 3 | 2 | 3 | 0 | 3 | 1 | 2 | 3 | 0 | 0 | * | * | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| J104 | 0 | * | * | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 2 | 3 | 0 | 3 | 0 | 2 | 1 | 2 | 3 | 0 | 0 | * | * | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| J108 | * | * | * | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 2 | 3 | 2 | 3 | 0 | 2 | 1 | 1 | 3 | 0 | 0 | * | * | 0 | 1 | 1 | 1 | 1 | 1 | 0 | * |
| J120 | * | * | * | * | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 3 | 3 | 3 | 0 | 3 | 3 | 2 | 2 | 1 | 3 | 0 | 0 | * | * | 0 | 1 | 1 | 1 | 0 | 0 | * | * |
| J121 | * | * | * | * | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 3 | 3 | 3 | 1 | 3 | 3 | 2 | 1 | 1 | 3 | 0 | 0 | * | * | 0 | 1 | 1 | 1 | 0 | 0 | * | * |
| J125 | * | * | * | * | 0 | 0 | 1 | 1 | 1 | 1 | 3 | 2 | 2 | 3 | 3 | 0 | 3 | 3 | 2 | 1 | 2 | 3 | 0 | 0 | * | * | * | 0 | 1 | 1 | 0 | 0 | * | * |
| J128 | 1 | 0 | * | * | 1 | 0 | 1 | 1 | 1 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | * | * | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J129 | 1 | 0 | * | * | 0 | 0 | 1 | 1 | 1 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | * | * | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J130 | 1 | 0 | * | * | 0 | 0 | 1 | 1 | 1 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 2 | * | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| J131 | 1 | 0 | * | * | 0 | 1 | 1 | 1 | 1 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 2 | * | 0 | 1 | 1 | 1 | 1 | 0 | 0 | * | * |
| J132 | 1 | 0 | * | * | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 0 | 1 | * | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| J133 | 1 | 0 | * | * | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 1 | 1 | 1 | 1 | 2 | 0 | 2 | 0 | 0 | 0 | * | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| J134 | 1 | 0 | * | * | 0 | 1 | 1 | 1 | 1 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 0 | 3 | 3 | 3 | 3 | 3 | 2 | 1 | * | 0 | 1 | 1 | 1 | 1 | 0 | 0 | * | * |
| J136 | * | * | * | * | 0 | 1 | 1 | 1 | 1 | 2 | 3 | 3 | 2 | 2 | 2 | 3 | 1 | 3 | 3 | 3 | 3 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J137 | 1 | 0 | * | * | 1 | 1 | 1 | 1 | 1 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 1 | 3 | * | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 |
| J139 | 1 | 0 | * | * | 1 | 1 | 1 | 1 | 1 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | * | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J140 | 0 | * | * | * | 1 | 0 | 1 | 1 | 1 | 1 | 3 | 2 | 3 | 2 | 2 | 2 | 2 | 0 | 3 | 2 | 3 | 2 | 0 | 1 | * | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| J141 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 2 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J142 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 3 | 2 | 2 | 2 | 3 | 2 | 3 | 1 | 2 | 1 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| J143 | 0 | * | * | * | 1 | 0 | 1 | 1 | 1 | 1 | 3 | 3 | 3 | 2 | 3 | 2 | 2 | 2 | 3 | 2 | 3 | 2 | 2 | 3 | * | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| J144 | 1 | 0 | * | * | 0 | 1 | 1 | 1 | 1 | 1 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 3 | 2 | 3 | 3 | 0 | 1 | * | 0 | 1 | 1 | 1 | 1 | 0 | 0 | * | * |
| J145 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 2 | 3 | 3 | 1 | 3 | 2 | 3 | 1 | 3 | 1 | 1 | 1 | 0 | 2 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |

Table A2 (continued).

| | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 | 49 | 50 | 51 | 52 | 53 | 54 | 55 | 56 | 57 | 58 | 59 | 60 | 61 | 62 | 63 | 64 | 65 | 66 | 67 | 68 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| J146 | 1 | 0 | * | * | 0 | 0 | 0 | 1 | 0 | 0 | 2 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 0 | 3 | * | * | 0 | 1 | 1 | 1 | 0 | 1 | 0 | * |
| J147 | * | * | * | * | 0 | 1 | 1 | 1 | 1 | 0 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 2 | 0 | 0 | * | 0 | 1 | 1 | 1 | 0 | * | 1 | 0 | * |
| J148 | 1 | 0 | * | * | 1 | 0 | 1 | 1 | 1 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 2 | 2 | 2 | * | 0 | 1 | 1 | 1 | 1 | 0 | 0 | * | * |
| J149 | 0 | * | * | * | 1 | 0 | 1 | 1 | 1 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 0 | 1 | * | 0 | 1 | 1 | 1 | 1 | 0 | 0 | * | * |
| J150 | * | * | * | * | 0 | 0 | 1 | 1 | 1 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 0 | 0 | * | * | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J152 | * | * | * | * | 0 | 1 | 1 | 1 | 0 | 0 | 2 | 3 | 3 | 3 | 2 | 1 | 3 | 3 | 3 | 2 | 3 | 3 | 1 | 3 | * | * | 0 | 1 | 1 | 1 | 1 | 1 | 0 | * |
| J153 | 1 | 0 | * | * | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 2 | 3 | 3 | 2 | 2 | 2 | 0 | 3 | 3 | 3 | 1 | 0 | 0 | * | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J154 | 0 | * | * | * | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 3 | 3 | 3 | 3 | 1 | 3 | 0 | 3 | 2 | 3 | 3 | 0 | 0 | * | * | 1 | 1 | 1 | 1 | * | * | * | * |
| J158 | 0 | * | * | * | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 0 | 3 | 1 | 0 | 2 | 0 | 0 | * | * | 1 | 1 | 1 | 1 | * | * | * | * |
| J159 | * | * | * | * | 0 | 0 | 0 | 1 | 0 | 0 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 0 | 3 | 3 | 0 | 2 | 0 | 0 | * | * | 0 | 1 | 1 | 1 | * | * | * | * |
| J161 | 0 | * | * | * | 0 | 0 | 1 | 1 | 1 | 0 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 0 | 3 | 3 | 0 | 2 | 0 | 0 | * | * | 1 | 1 | 1 | 1 | * | * | * | * |
| J162 | 0 | * | * | * | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 3 | 3 | 3 | 3 | 2 | 3 | 0 | 3 | 3 | 3 | 2 | 0 | 0 | * | * | 1 | 1 | 1 | 1 | * | * | * | * |
| J165 | 0 | * | * | * | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 3 | 3 | 3 | 3 | 1 | 3 | 2 | 3 | 0 | 2 | 2 | 0 | 0 | * | * | 1 | 1 | 1 | 1 | * | * | * | * |
| J167 | 0 | * | * | * | 0 | 0 | 1 | 0 | 1 | 1 | 2 | 2 | 3 | 3 | 3 | 2 | 3 | 0 | 3 | 2 | 2 | 2 | 0 | 0 | * | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J168 | 1 | 0 | * | * | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 2 | 2 | 1 | 2 | 0 | 3 | 1 | 1 | 1 | 0 | 3 | 0 | 1 | 1 | 1 | 1 | 0 | * | 1 | 0 | * |  |
| J169 | * | * | * | * | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 3 | 3 | 3 | 1 | 2 | 1 | 3 | 1 | 3 | 3 | 0 | 3 | * | * | 0 | 1 | 1 | 1 | 0 | 1 | 0 | * |
| J170 | 0 | * | * | * | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 2 | 3 | 3 | 2 | 2 | 2 | 0 | 2 | 1 | 2 | 3 | 0 | 3 | * | * | 0 | 1 | 1 | 0 | * | 0 | * | * |
| J171 | 0 | * | * | * | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 2 | 3 | 3 | 3 | 1 | 3 | 1 | 2 | 1 | 2 | 1 | 0 | 3 | * | * | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J172 | 1 | 0 | * | * | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 0 | 2 | 1 | 2 | 1 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J173 | 1 | 0 | * | * | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 3 | 3 | 3 | 0 | 3 | 0 | 3 | 2 | 0 | 2 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| J174 | 1 | 0 | * | * | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 2 | 3 | 3 | 3 | 1 | 3 | 1 | 3 | 1 | 0 | 1 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J175 | 1 | 0 | * | * | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 2 | 3 | 2 | 2 | 0 | 2 | 0 | 3 | 1 | 1 | 2 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J176 | 1 | 0 | * | * | 0 | 0 | 0 | 0 | 1 | 1 | 2 | 2 | 3 | 3 | 2 | 2 | 3 | 0 | 3 | 1 | 1 | 1 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J177 | 1 | 0 | * | * | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 2 | 2 | 2 | 0 | 2 | 0 | 3 | 1 | 1 | 2 | 0 | 0 | * | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J178 | 1 | 0 | * | * | 0 | 1 | 0 | 0 | 0 | 0 | 2 | 3 | 2 | 2 | 2 | 1 | 2 | 0 | 3 | 0 | 1 | 1 | 0 | 0 | * | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J179 | 0 | * | * | * | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 2 | 3 | 3 | 3 | 1 | 3 | 0 | 2 | 3 | 3 | 3 | 0 | 0 | * | * | 1 | 1 | 1 | 1 | * | * | * | * |
| J180 | 0 | * | * | * | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 3 | 3 | 2 | 3 | 3 | 0 | 2 | 1 | 3 | 0 | 1 | 0 | 0 | * | * | 1 | 1 | 1 | 1 | * | * | * | * |
| J181 | * | * | * | * | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 0 | 3 | 3 | 3 | 3 | 0 | 3 | * | * | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J182 | * | * | * | * | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 3 | 3 | 2 | 2 | 2 | 3 | 0 | 3 | 2 | 2 | 3 | 0 | 0 | * | * | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J183 | * | * | * | * | 0 | 0 | 1 | 1 | 0 | 1 | 2 | 2 | 3 | 1 | 1 | 2 | 2 | 0 | 2 | 2 | 2 | 2 | 0 | 0 | * | * | 0 | 1 | 1 | 1 | 0 | 0 | * | * |
| J186 | * | * | * | * | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 0 | 3 | 3 | 3 | 3 | 0 | 3 | * | * | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J187 | * | * | * | * | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 0 | 3 | 3 | 3 | 3 | 0 | 3 | * | * | 0 | 1 | 1 | 1 | 0 | 1 | 0 | * |
| J188 | * | * | * | * | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 3 | 3 | 2 | 3 | 3 | 3 | 0 | 3 | 3 | 3 | 3 | 0 | 3 | * | * | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J189 | * | * | * | * | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 0 | 3 | 3 | 3 | 3 | 0 | 3 | * | * | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J190 | * | * | * | * | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 3 | 3 | 2 | 3 | 3 | 3 | 0 | 3 | 3 | 3 | 3 | 0 | 3 | * | * | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J191 | * | * | * | * | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 3 | 3 | 2 | 3 | 3 | 3 | 0 | 3 | 3 | 3 | 3 | 0 | 3 | * | * | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J192 | 1 | 1 | 0 | * | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 3 | 2 | 2 | 1 | 2 | 0 | 3 | 1 | 3 | 0 | 0 | 3 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |

Table A2 (continued).

| | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 | 49 | 50 | 51 | 52 | 53 | 54 | 55 | 56 | 57 | 58 | 59 | 60 | 61 | 62 | 63 | 64 | 65 | 66 | 67 | 68 |
|------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| J193 | 1 | 1 | 0 | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 2 | 2 | 2 | 3 | 0 | 3 | 1 | 2 | 0 | 0 | 3 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J194 | 1 | 1 | 0 | * | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 3 | 2 | 3 | 2 | 3 | 3 | 3 | 1 | 2 | 1 | 0 | 3 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J195 | 1 | 1 | 0 | * | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 3 | 3 | 2 | 2 | 2 | 3 | 3 | 1 | 2 | 0 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J196 | 1 | 1 | 0 | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 3 | 2 | 2 | 3 | 0 | 3 | 1 | 2 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J197 | 1 | 1 | 0 | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 2 | 3 | 2 | 2 | 3 | 3 | 1 | 2 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J198 | 1 | 1 | 0 | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 2 | 1 | 1 | 1 | 3 | 2 | 1 | 1 | 1 | 0 | 0 | * | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J199 | 1 | 1 | 0 | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 2 | 1 | 1 | 1 | 0 | 2 | * | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J200 | 1 | 1 | 0 | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 3 | 1 | 2 | 1 | 0 | 2 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J201 | 1 | 1 | 0 | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 3 | 1 | 2 | 1 | 0 | 1 | * | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J202 | 1 | 1 | 0 | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 2 | 1 | 2 | 0 | 2 | 1 | 1 | 1 | 0 | 3 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| J203 | 1 | 1 | 0 | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 3 | 3 | 3 | 2 | 3 | 3 | 3 | 1 | 2 | 1 | 0 | 3 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| J204 | 1 | 1 | 0 | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 3 | 2 | 2 | 3 | 3 | 3 | 1 | 2 | 1 | 0 | 2 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J205 | 1 | 1 | 0 | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 3 | 2 | 2 | 3 | 3 | 2 | 1 | 1 | 1 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J206 | 1 | 1 | 0 | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 3 | 2 | 2 | 3 | 3 | 3 | 1 | 1 | 1 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J207 | 1 | 1 | 0 | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 2 | 2 | 3 | 3 | 1 | 2 | 1 | 0 | 3 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 |
| J208 | 1 | 1 | 0 | * | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 3 | 3 | 2 | 2 | 2 | 3 | 3 | 1 | 1 | 1 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J209 | 1 | 1 | 0 | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 2 | 2 | 2 | 2 | 3 | 3 | 1 | 2 | 0 | 0 | 3 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J210 | 1 | 0 | * | * | 0 | * | * | * | * | * | 0 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 0 | 0 | 3 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J211 | 0 | * | * | * | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 3 | 3 | 3 | 3 | 0 | 3 | 2 | 3 | 3 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J212 | 0 | * | * | * | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J214 | 0 | * | * | * | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J215 | 0 | * | * | * | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 3 | 3 | 3 | 3 | 2 | 3 | 2 | 3 | 3 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J216 | 0 | * | * | * | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 3 | 3 | 3 | 3 | 0 | 3 | 2 | 3 | 3 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J217 | 0 | * | * | * | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J218 | 0 | * | * | * | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J219 | 0 | * | * | * | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 3 | 3 | 3 | 3 | 1 | 3 | 2 | 3 | 3 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J220 | 0 | * | * | * | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J221 | 0 | * | * | * | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J222 | 0 | * | * | * | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J223 | 0 | * | * | * | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J224 | 0 | * | * | * | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 3 | 3 | 3 | 3 | 1 | 3 | 2 | 3 | 3 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J225 | 0 | * | * | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J226 | 0 | * | * | * | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 3 | 3 | 3 | 3 | 1 | 3 | 2 | 3 | 3 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J227 | 0 | * | * | * | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J228 | 0 | * | * | * | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J229 | * | * | * | * | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 3 | 3 | 2 | 3 | 3 | 3 | 0 | 3 | 3 | 3 | 3 | 0 | 3 | * | * | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J230 | * | * | * | * | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 3 | 3 | 2 | 3 | 3 | 3 | 0 | 3 | 3 | 3 | 3 | 0 | 3 | * | * | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |

Table A2 (continued).

| | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 | 49 | 50 | 51 | 52 | 53 | 54 | 55 | 56 | 57 | 58 | 59 | 60 | 61 | 62 | 63 | 64 | 65 | 66 | 67 | 68 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| J231 | * | * | * | * | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 3 | 1 | 3 | 3 | 3 | 0 | 3 | 3 | 3 | 3 | 0 | 3 | * | * | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J232 | * | * | * | * | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 0 | 3 | * | * | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J233 | * | * | * | * | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 3 | 3 | 2 | 3 | 3 | 3 | 0 | 3 | 3 | 3 | 3 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J234 | * | * | * | * | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 3 | 3 | 2 | 3 | 3 | 3 | 0 | 3 | 3 | 3 | 3 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J235 | * | * | * | * | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 3 | 3 | 2 | 3 | 3 | 3 | 0 | 3 | 3 | 3 | 3 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J236 | * | * | * | * | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 2 | 3 | 2 | 3 | 3 | 3 | 0 | 3 | 3 | 3 | 3 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J237 | * | * | * | * | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 3 | 3 | 2 | 3 | 3 | 3 | 0 | 3 | 3 | 3 | 3 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J238 | * | * | * | * | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 0 | 3 | 3 | 3 | 3 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J239 | * | * | * | * | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 3 | 3 | 2 | 3 | 3 | 3 | 0 | 3 | 3 | 3 | 3 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J240 | * | * | * | * | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 0 | 3 | 3 | 3 | 3 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J241 | 1 | 1 | 0 | * | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 3 | 3 | 2 | 2 | 2 | 1 | 3 | 1 | 1 | 1 | 0 | 3 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J242 | 1 | 1 | 0 | * | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 3 | 3 | 1 | 1 | 2 | 0 | 1 | 1 | 2 | 0 | 0 | 3 | * | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| J243 | 1 | 1 | 0 | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 3 | 2 | 2 | 2 | 3 | 2 | 1 | 2 | 1 | 0 | 3 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| J244 | 1 | 1 | 0 | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 3 | 2 | 2 | 2 | 3 | 1 | 1 | 1 | 1 | 0 | 3 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| J245 | 0 | * | * | * | 1 | * | * | * | * | * | 3 | 2 | 3 | 3 | 2 | 2 | 1 | * | 3 | 1 | 3 | 1 | 0 | 3 | * | 0 | 1 | 1 | 1 | 0 | * | 0 | * | * |
| J246 | * | * | * | * | 1 | * | * | * | * | * | 3 | 3 | 3 | 3 | 3 | 2 | 2 | 3 | 3 | 3 | 3 | 3 | 0 | 3 | * | * | 0 | 1 | 1 | 1 | 0 | 1 | 0 | * |

Table A2 (continued).

| | 69 | 70 | 71 | 72 | 73 | 74 | 75 | 76 | 77 | 78 | 79 | 80 | 81 | 82 | 83 | 84 | 85 | 86 | 87 | 88 | 89 | 90 | 91 | 92 | 93 | 94 | 95 | 96 | 97 | 98 | 99 | 100 | 101 | 102 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| J1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| J2 | * | 1 | 1 | 0 | * | * | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| J3 | 1 | 1 | 0 | * | * | * | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| J4 | 1 | 1 | 1 | 0 | * | * | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| J5 | 1 | 1 | 0 | * | * | * | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| J6 | 0 | 1 | 1 | 0 | * | * | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| J7 | 1 | 1 | 0 | * | * | * | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| J8 | * | 1 | 0 | * | * | * | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| J9 | * | 0 | * | * | * | * | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J10 | * | 1 | 0 | * | * | * | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| J11 | * | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J12 | 1 | 1 | 1 | 0 | * | * | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 |
| J13 | 0 | 1 | 1 | 0 | * | * | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 |
| J17 | * | 1 | 1 | 0 | * | * | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| J18 | * | 1 | 0 | * | * | * | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| J19 | * | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J20 | * | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J21 | * | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J23 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| J24 | 1 | 1 | 1 | 0 | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| J25 | * | 1 | 1 | 0 | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| J26 | * | 1 | 0 | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | * | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| J27 | 1 | 1 | 1 | 0 | * | * | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J28 | * | 1 | 0 | * | * | * | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| J29 | * | 1 | 1 | 1 | 0 | * | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| J30 | 1 | 1 | 1 | 0 | * | * | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| J31 | 1 | 1 | 0 | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J32 | 1 | 1 | 1 | 0 | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 |
| J33 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| J34 | 1 | 1 | 1 | 0 | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| J35 | 1 | 1 | 1 | 0 | * | * | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| J36 | 1 | 1 | 1 | 0 | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| J37 | 1 | 1 | 0 | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| J38 | 1 | 1 | 1 | 0 | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| J39 | 1 | 1 | 1 | 0 | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| J40 | 1 | 1 | 0 | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| J41 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 |

Table A2 (continued).

| | 69 | 70 | 71 | 72 | 73 | 74 | 75 | 76 | 77 | 78 | 79 | 80 | 81 | 82 | 83 | 84 | 85 | 86 | 87 | 88 | 89 | 90 | 91 | 92 | 93 | 94 | 95 | 96 | 97 | 98 | 99 | 100 | 101 | 102 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| J42 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J43 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J44 | 1 | 0 | * | * | * | * | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| J45 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J46 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| J47 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| J48 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| J49 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J50 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J51 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| J52 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J53 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| J54 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J55 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J56 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J57 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | |
| J58 | 1 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| J59 | 1 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| J60 | 1 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| J61 | 1 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| J62 | 1 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| J63 | 1 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| J64 | 1 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| J65 | 1 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| J66 | 1 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| J67 | 1 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J68 | 1 | 1 | 0 | * | * | * | * | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | |
| J69 | 1 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | |
| J70 | 1 | 1 | 0 | * | * | * | * | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | |
| J71 | 1 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | |
| J72 | 1 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | |
| J73 | * | 0 | * | * | * | * | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | * | 0 |
| J74 | 0 | 0 | * | * | * | * | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | * | 0 | |
| J75 | * | 0 | * | * | * | * | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J78 | * | 0 | * | * | * | * | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | * | 0 | |
| J79 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | * | 0 | |
| J80 | 0 | 0 | * | * | * | * | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | * | 0 |

Table A2 (continued).

| | 69 | 70 | 71 | 72 | 73 | 74 | 75 | 76 | 77 | 78 | 79 | 80 | 81 | 82 | 83 | 84 | 85 | 86 | 87 | 88 | 89 | 90 | 91 | 92 | 93 | 94 | 95 | 96 | 97 | 98 | 99 | 100 | 101 | 102 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| J83 | * | 0 | * | * | * | * | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| J86 | * | 0 | * | * | * | * | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| J87 | * | 0 | * | * | * | * | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | * | 0 |
| J88 | * | 0 | * | * | * | * | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J89 | * | 0 | * | * | * | * | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J90 | * | 0 | * | * | * | * | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J91 | * | 0 | * | * | * | * | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | * | 0 |
| J92 | * | 0 | * | * | * | * | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | * | 0 |
| J93 | 0 | 0 | * | * | * | * | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J94 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| J95 | * | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| J99 | 0 | 0 | * | * | * | * | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J100 | 0 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J101 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| J102 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | * | 0 |
| J103 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | * | 0 |
| J104 | * | 0 | * | * | * | * | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| J108 | * | 0 | * | * | * | * | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| J120 | * | 0 | * | * | * | * | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J121 | * | 0 | * | * | * | * | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J125 | * | 0 | * | * | * | * | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | * | 0 |
| J128 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J129 | 1 | 0 | * | * | * | * | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J130 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J131 | * | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J132 | 1 | 1 | 1 | 0 | * | * | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J133 | 1 | 1 | 1 | 0 | * | * | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J134 | * | 0 | * | * | * | * | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J136 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J137 | * | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J139 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J140 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J141 | 1 | 1 | 0 | * | * | * | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | |
| J142 | 1 | 1 | 0 | * | * | * | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 |
| J143 | * | 0 | * | * | * | * | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 |
| J144 | * | 1 | 0 | * | * | * | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| J145 | 1 | 1 | 0 | * | * | * | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 |

Table A2 (continued).

| | 69 | 70 | 71 | 72 | 73 | 74 | 75 | 76 | 77 | 78 | 79 | 80 | 81 | 82 | 83 | 84 | 85 | 86 | 87 | 88 | 89 | 90 | 91 | 92 | 93 | 94 | 95 | 96 | 97 | 98 | 99 | 100 | 101 | 102 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| J146 | * | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J147 | * | 0 | * | * | * | * | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J148 | * | 0 | * | * | * | * | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 |
| J149 | * | 1 | 0 | * | * | * | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| J150 | 1 | 0 | * | * | * | * | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| J152 | * | 0 | * | * | * | * | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| J153 | 1 | 1 | 0 | * | * | * | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 |
| J154 | * | 1 | 1 | 0 | * | * | * | * | * | * | * | 1 | 1 | 1 | 0 | 0 | * | 0 | 0 | 0 | * | * | * | 1 | 1 | 1 | 0 | * | * | * | 0 | * | * | * |
| J158 | * | 1 | 0 | * | * | * | * | * | * | * | * | 1 | 1 | 1 | 1 | 0 | * | 0 | 0 | 0 | * | * | * | 1 | 1 | 1 | 1 | * | * | * | 0 | * | * | * |
| J159 | * | 0 | * | * | * | * | * | * | * | * | * | 1 | 1 | 1 | 1 | 0 | * | 0 | 0 | 0 | * | * | * | 1 | 1 | 1 | 1 | * | * | * | 0 | * | * | * |
| J161 | * | 1 | 0 | * | * | * | * | * | * | * | * | 1 | 1 | 0 | 0 | 0 | * | 0 | 0 | 0 | * | * | * | 1 | 1 | 1 | 1 | * | * | * | 0 | * | * | * |
| J162 | * | 0 | * | * | * | * | * | * | * | * | * | 1 | 1 | 1 | 1 | 0 | * | 0 | 0 | 0 | * | * | * | 1 | 1 | 1 | 1 | * | * | * | 0 | * | * | * |
| J165 | * | 0 | * | * | * | * | * | * | * | * | * | 1 | 1 | 1 | 0 | 0 | * | 0 | 0 | 0 | * | * | * | 1 | 1 | 1 | 1 | * | * | * | 0 | * | * | * |
| J167 | 0 | 1 | 0 | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| J168 | * | 1 | 0 | * | * | * | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| J169 | * | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J170 | * | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J171 | 0 | 1 | 0 | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J172 | 1 | 1 | 0 | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J173 | 1 | 0 | * | * | * | * | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| J174 | 0 | 0 | * | * | * | * | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| J175 | 1 | 1 | 0 | * | * | * | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 |
| J176 | 1 | 1 | 0 | * | * | * | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 |
| J177 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| J178 | 0 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| J179 | * | 0 | * | * | * | * | * | * | * | * | * | 0 | 0 | 0 | 1 | 1 | * | 0 | 0 | 0 | * | * | * | 1 | 0 | 0 | 0 | * | * | * | 0 | * | * | * |
| J180 | * | 1 | 1 | 0 | * | * | * | * | * | * | * | 0 | 0 | 0 | 0 | 0 | * | 0 | 0 | 0 | * | * | * | 1 | 0 | 0 | 0 | * | * | * | 0 | * | * | * |
| J181 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J182 | 1 | 1 | 0 | * | * | * | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | * | 0 |
| J183 | * | 0 | * | * | * | * | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | * | 0 |
| J186 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| J187 | * | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J188 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| J189 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| J190 | 0 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J191 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J192 | 1 | 1 | 0 | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | |

Table A2 (continued).

| | 69 | 70 | 71 | 72 | 73 | 74 | 75 | 76 | 77 | 78 | 79 | 80 | 81 | 82 | 83 | 84 | 85 | 86 | 87 | 88 | 89 | 90 | 91 | 92 | 93 | 94 | 95 | 96 | 97 | 98 | 99 | 100 | 101 | 102 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| J193 | 1 | 1 | 0 | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| J194 | 1 | 1 | 0 | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| J195 | 1 | 1 | 0 | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| J196 | 1 | 1 | 0 | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| J197 | 1 | 1 | 0 | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| J198 | 1 | 1 | 0 | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| J199 | 1 | 1 | 0 | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| J200 | 1 | 1 | 0 | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| J201 | 1 | 1 | 0 | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| J202 | 1 | 1 | 0 | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| J203 | 1 | 1 | 0 | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| J204 | 1 | 1 | 0 | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| J205 | 1 | 1 | 0 | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| J206 | 1 | 1 | 0 | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| J207 | 1 | 1 | 0 | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| J208 | 1 | 1 | 0 | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| J209 | 1 | 1 | 0 | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| J210 | 1 | 1 | 0 | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J211 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| J212 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| J214 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| J215 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| J216 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| J217 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| J218 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| J219 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| J220 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J221 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| J222 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| J223 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J224 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J225 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| J226 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| J227 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| J228 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| J229 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| J230 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |

Table A2 (continued).

| | 69 | 70 | 71 | 72 | 73 | 74 | 75 | 76 | 77 | 78 | 79 | 80 | 81 | 82 | 83 | 84 | 85 | 86 | 87 | 88 | 89 | 90 | 91 | 92 | 93 | 94 | 95 | 96 | 97 | 98 | 99 | 100 | 101 | 102 |
|------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| J231 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J232 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J233 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J234 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J235 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| J236 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| J237 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| J238 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| J239 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| J240 | 1 | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| J241 | 1 | 1 | 0 | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| J242 | 1 | 1 | 0 | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| J243 | 1 | 1 | 0 | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| J244 | 1 | 1 | 0 | * | * | * | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| J245 | * | 1 | 0 | * | * | * | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| J246 | * | 0 | * | * | * | * | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Table A2 (continued).

| | 103 | 104 | 105 | 106 | 107 | 108 | 109 | 110 | 111 | 112 | 113 | 114 | 115 | 116 | 117 | 118 | 119 | 120 | 121 | 122 | 123 | 124 | 125 | 126 | 127 | 128 | 129 | 130 | 131 | 132 | 133 | 134 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| J1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J2 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J3 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J4 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J5 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J6 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J7 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J9 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J10 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J11 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J12 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J13 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J17 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 |
| J18 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 |
| J19 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| J20 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| J21 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| J23 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| J24 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| J25 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 |
| J26 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J27 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J28 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| J29 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J31 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J32 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| J33 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J34 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 |
| J35 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 |
| J36 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 |
| J37 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| J38 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| J39 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 |
| J40 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| J41 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Table A2 (continued).

| | 103 | 104 | 105 | 106 | 107 | 108 | 109 | 110 | 111 | 112 | 113 | 114 | 115 | 116 | 117 | 118 | 119 | 120 | 121 | 122 | 123 | 124 | 125 | 126 | 127 | 128 | 129 | 130 | 131 | 132 | 133 | 134 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| J42 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J43 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J44 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J45 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J46 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J47 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J48 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J49 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J51 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J52 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J53 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J54 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J55 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J56 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J57 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J58 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J59 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J60 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J61 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J62 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J63 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J64 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J65 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J66 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J67 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J68 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J69 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J70 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J71 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J72 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J73 | 0 | 0 | 0 | 0 | 1 | 1 | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| J74 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| J75 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 1 |
| J78 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| J79 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 |
| J80 | 0 | 0 | 0 | 0 | 1 | 1 | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 |

Table A2 (continued).

| | 103 | 104 | 105 | 106 | 107 | 108 | 109 | 110 | 111 | 112 | 113 | 114 | 115 | 116 | 117 | 118 | 119 | 120 | 121 | 122 | 123 | 124 | 125 | 126 | 127 | 128 | 129 | 130 | 131 | 132 | 133 | 134 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| J83 | 0 | 0 | 0 | 0 | 1 | 1 | * | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| J86 | 0 | 0 | 0 | 0 | 1 | 1 | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| J87 | 0 | 0 | 0 | 0 | 1 | 1 | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| J88 | 0 | 0 | 0 | 0 | 1 | 1 | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| J89 | 0 | 0 | 0 | 0 | 1 | 1 | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| J90 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 |
| J91 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 |
| J92 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| J93 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| J94 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| J95 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| J99 | 0 | 0 | 0 | 0 | 1 | 1 | * | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 |
| J100 | 0 | 0 | 0 | 0 | 1 | 1 | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 |
| J101 | 0 | 0 | 0 | 0 | 1 | 1 | * | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 |
| J102 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 |
| J103 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| J104 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 |
| J108 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 |
| J120 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 |
| J121 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 1 |
| J125 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| J128 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J129 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J130 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 |
| J131 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J132 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J133 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J134 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J136 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J137 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J139 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 |
| J140 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J141 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J142 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J143 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J144 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J145 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Table A2 (continued).

| | 103 | 104 | 105 | 106 | 107 | 108 | 109 | 110 | 111 | 112 | 113 | 114 | 115 | 116 | 117 | 118 | 119 | 120 | 121 | 122 | 123 | 124 | 125 | 126 | 127 | 128 | 129 | 130 | 131 | 132 | 133 | 134 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| J146 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J147 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J148 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J149 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J150 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 |
| J152 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 |
| J153 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 |
| J154 | * | * | * | * | 0 | 0 | 1 | 0 | 0 | 1 | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * |
| J158 | * | * | * | * | 0 | 0 | 1 | 0 | 0 | 1 | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * |
| J159 | * | * | * | * | 0 | 0 | 1 | 0 | 0 | 1 | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * |
| J161 | * | * | * | * | 0 | 0 | 1 | 0 | 0 | 1 | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * |
| J162 | * | * | * | * | 0 | 0 | 1 | 0 | 0 | 1 | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * |
| J165 | * | * | * | * | 0 | 0 | 1 | 0 | 0 | 1 | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * |
| J167 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 |
| J168 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 1 |
| J169 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 |
| J170 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 |
| J171 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 |
| J172 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 |
| J173 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 1 |
| J174 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 1 |
| J175 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 1 |
| J176 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 1 |
| J177 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 |
| J178 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 |
| J179 | * | * | * | * | 1 | 0 | 1 | 0 | 0 | 1 | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * |
| J180 | * | * | * | * | 1 | 0 | 1 | 0 | 0 | 1 | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * |
| J181 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J182 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 |
| J183 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 |
| J186 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J187 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J188 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J189 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J190 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J191 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J192 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Table A2 (continued).

| | 103 | 104 | 105 | 106 | 107 | 108 | 109 | 110 | 111 | 112 | 113 | 114 | 115 | 116 | 117 | 118 | 119 | 120 | 121 | 122 | 123 | 124 | 125 | 126 | 127 | 128 | 129 | 130 | 131 | 132 | 133 | 134 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| J193 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J194 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J195 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J196 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J197 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J198 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J199 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J200 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J201 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J202 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J203 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J204 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J205 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J206 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J207 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J208 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J209 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J210 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | * | * | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| J211 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J212 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J214 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J215 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J216 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J217 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J218 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J219 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J220 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J221 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J222 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J223 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J224 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J225 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J226 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J227 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J228 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J229 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J230 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Table A2 (continued).

| | 103 | 104 | 105 | 106 | 107 | 108 | 109 | 110 | 111 | 112 | 113 | 114 | 115 | 116 | 117 | 118 | 119 | 120 | 121 | 122 | 123 | 124 | 125 | 126 | 127 | 128 | 129 | 130 | 131 | 132 | 133 | 134 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| J231 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J232 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J233 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J234 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J235 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J236 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J237 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J238 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J239 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J240 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J241 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J242 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J243 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J244 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| J245 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 |
| J246 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 |  |

Key:  The character numbers correspond to those shown in table A1.

The key to the coding is also given at the end of table A1.

\*    indicates a result not compared.  This is explained in introduction to Appendix 3.

APPENDIX 4

Abbreviated data matrix

Table A3    Abbreviated data matrix.

Clusters (columns):
ALCALIGENES, BRONCHISEPTICA, PERTUSSIS, PARAPERTUSSIS, MOPPII, ANITRATUS, M-N I, M-N II, M-N III, BRUCELLA, ACHROMOBACTER, FRANCISELLA, ACTINOBACILLUS, PASTEURELLA, YERSINIA, HAIM I, HAIM II, HAIM III, STRAINS NOT CLUSTERED (Bordetella, J 172, J 130, J 139, J 150, J 152, J 25, J 210, J 36, J 28, J 33, J 41, J 47, J 26, J 30, J 31, J 44)

Characters (rows):
- Number of strains
- Character
- Gram reaction
- retention of methyl violet
- chains or filaments
- pleomorphism
- cell shape
- colony viscid
- pigment diffusible
- pigment colour
- pigment from tyrosine
- growth anaerobically
- growth anaerobically + nitrate
- CO2 requirement
- X factor requirement
- V factor requirement
- XeV factor requirement
- growth on nutrient agar
- growth on blood agar
- haemolysis of horse red cells
- haemolysis of sheep red cells
- motility
- growth on 5% bile salts
- 10% bile salts
- 40% bile salts
- growth on 0% NaCl
- 3% NaCl
- 4.5% NaCl
- 6% NaCl
- 7.5% NaCl
- 9% NaCl
- sensitivity to 0/129
- sensitivity to thionine
- basic fuchsin
- methyl violet
- safranine 0
- pyronin G

Table A3 (continued).

| Character | ALCALIGENES | BRONCHISEPTICA | PERTUSSIS | PARAPERTUSSIS | LWOFFII | ANITRATUS | M-R I | M-R II | M-R III | BRUCELLA | ACHROMOBACTERIUM | FRANCISELLA | ACTINOBACILLUS | PASTEURELLA | YERSINIA | HAIN I | HAIN II | HAIN III | STRAINS NOT CLUSTERED |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| growth on 0.1% phenol agar | | | | | | | | | | | | | | | | | | | |
| 0.2% phenol agar | | | | | | | | | | | | | | | | | | | |
| 0.3% phenol agar | | | | | | | | | | | | | | | | | | | |
| 0.4% phenol agar | | | | | | | | | | | | | | | | | | | |
| 0.5% phenol agar | | | | | | | | | | | | | | | | | | | |
| sensitivity to penicillin | | | | | | | | | | | | | | | | | | | |
| streptomycin | | | | | | | | | | | | | | | | | | | |
| chloramphenicol | | | | | | | | | | | | | | | | | | | |
| aureomycin | | | | | | | | | | | | | | | | | | | |
| terramycin | | | | | | | | | | | | | | | | | | | |
| erythromycin | | | | | | | | | | | | | | | | | | | |
| tetracycline | | | | | | | | | | | | | | | | | | | |
| sulphonamide | | | | | | | | | | | | | | | | | | | |
| neomycin | | | | | | | | | | | | | | | | | | | |
| novobiocin | | | | | | | | | | | | | | | | | | | |
| oleandomycin | | | | | | | | | | | | | | | | | | | |
| nitrofurantoin | | | | | | | | | | | | | | | | | | | |
| bacitracin | | | | | | | | | | | | | | | | | | | |
| Marin Roche | | | | | | | | | | | | | | | | | | | |
| potassium tellurite (growth) | | | | | | | | | | | | | | | | | | | |
| sodium azide (growth) | | | | | | | | | | | | | | | | | | | |
| thallous acetate (growth) | | | | | | | | | | | | | | | | | | | |
| M. and R. 936 (growth) | | | | | | | | | | | | | | | | | | | |
| growth at 5°C. | | | | | | | | | | | | | | | | | | | |
| 10°C. | | | | | | | | | | | | | | | | | | | |
| 15°C. | | | | | | | | | | | | | | | | | | | |
| 25°C. | | | | | | | | | | | | | | | | | | | |
| 35°C. | | | | | | | | | | | | | | | | | | | |
| 37°C. | | | | | | | | | | | | | | | | | | | |
| 44°C. | | | | | | | | | | | | | | | | | | | |
| catalase | | | | | | | | | | | | | | | | | | | |
| peroxidase | | | | | | | | | | | | | | | | | | | |
| Kova's oxidase | | | | | | | | | | | | | | | | | | | |
| hydrogen sulphide production | | | | | | | | | | | | | | | | | | | |
| phosphatase | | | | | | | | | | | | | | | | | | | |
| gelatin liquefaction | | | | | | | | | | | | | | | | | | | |
| starch hydrolysis | | | | | | | | | | | | | | | | | | | |
| egg yolk reaction | | | | | | | | | | | | | | | | | | | |
| casein hydrolysis | | | | | | | | | | | | | | | | | | | |
| aesculin hydrolysis | | | | | | | | | | | | | | | | | | | |

Table A3 (continued)

| CLUSTERS / Character | ALCALIGENES | BRONCHISEPTICA | PERTUSSIS | PARAPERTUSSIS | TROFFII | ANITRATUS | M-R I | M-R II | M-R III | BRUCELLA | AGROBACTERIUM | FRANCISELLA | ACTINOBACILLUS | PASTEURELLA | YERSINIA | MAYR I | MAYR II | MAYR III | STRAINS NOT CLUSTERED | Legionella |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ammonia from peptone | | | | | | | | | | | | | | | | | | | | |
| urease | | | | | | | | | | | | | | | | | | | | |
| arginine hydrolysis | | | | | | | | | | | | | | | | | | | | |
| nitrate reduction | | | | | | | | | | | | | | | | | | | | |
| deoxyribonuclease | | | | | | | | | | | | | | | | | | | | |
| methyl red | | | | | | | | | | | | | | | | | | | | |
| Voges Proskauer | | | | | | | | | | | | | | | | | | | | |
| tyrosine hydrolysis | | | | | | | | | | | | | | | | | | | | |
| xanthine hydrolysis | | | | | | | | | | | | | | | | | | | | |
| phenylalanine deaminase | | | | | | | | | | | | | | | | | | | | |
| tween 20 hydrolysis | | | | | | | | | | | | | | | | | | | | |
| tween 40 hydrolysis | | | | | | | | | | | | | | | | | | | | |
| tween 60 hydrolysis | | | | | | | | | | | | | | | | | | | | |
| tween 80 hydrolysis | | | | | | | | | | | | | | | | | | | | |
| clearing of tween 80 | | | | | | | | | | | | | | | | | | | | |
| acid from 1% glucose, AO2. | | | | | | | | | | | | | | | | | | | | |
| 1% glucose, AnO2. | | | | | | | | | | | | | | | | | | | | |
| 10% glucose | | | | | | | | | | | | | | | | | | | | |
| 1% lactose | | | | | | | | | | | | | | | | | | | | |
| 10% lactose | | | | | | | | | | | | | | | | | | | | |
| alkali from 1% lactose | | | | | | | | | | | | | | | | | | | | |
| acid from xylose | | | | | | | | | | | | | | | | | | | | |
| melizin | | | | | | | | | | | | | | | | | | | | |
| raffinose | | | | | | | | | | | | | | | | | | | | |
| methyl d glucoside | | | | | | | | | | | | | | | | | | | | |
| ribose | | | | | | | | | | | | | | | | | | | | |
| rhamnose | | | | | | | | | | | | | | | | | | | | |
| mannose | | | | | | | | | | | | | | | | | | | | |
| inositol | | | | | | | | | | | | | | | | | | | | |
| cellobiose | | | | | | | | | | | | | | | | | | | | |
| fructose | | | | | | | | | | | | | | | | | | | | |
| dextrin | | | | | | | | | | | | | | | | | | | | |
| arabinose | | | | | | | | | | | | | | | | | | | | |
| sorbitol | | | | | | | | | | | | | | | | | | | | |
| galactose | | | | | | | | | | | | | | | | | | | | |
| maltose | | | | | | | | | | | | | | | | | | | | |
| sucrose | | | | | | | | | | | | | | | | | | | | |
| mannitol | | | | | | | | | | | | | | | | | | | | |
| glycerol | | | | | | | | | | | | | | | | | | | | |
| glucosamine hydrochloride | | | | | | | | | | | | | | | | | | | | |

Key: each result is accompanied by the number of strains of each cluster
producing the indicated reaction.

| | | | |
|------|----------------|-----|----------------------|
| – | negative, | y | yellow, |
| ± | weak positive, | c | coccus, |
| + | positive, | cb | coccobacillus, |
| * | no result, | sr | short rod, |
| AO2 | aerobic, | mr | medium rod, |
| AnO2 | anaerobic, | r | medium to long rod. |

## Acknowledgements

I wish to express my sincere gratitude to my supervisor Professor P.H.A. Sneath, without whose help and guidance this thesis would never have been completed. I would also like to take this opportunity to thank Dr. Dorothy Jones of the Microbial Systematics Research Unit for many fruitful discussions, and for the great encouragement received from her. My thanks are also offered to Mr. Michael Stevens, F.I.M.L.T. for many useful discussions and for strains received; to Mrs. Jane Campbell-Kelly and Mr. Michael Sackin for running the computer data; to Mr. G. Asquith for help with the illustrations and diagrams and to Miss Susan N. Dilks and Mrs. J. Skinner for correcting and typing the thesis.

Finally, I would also like to thank many other people not mentioned by name, who have provided cultures or have given advice and encouragement.

Bibliography

REFERENCES

Affanassjew, M.J. (1887). Aetiologie und klinische bacteriologie des keuchhustens. St. Petersb. Med. Wschr. 12 (N.F.4), 323, 331, 339, 347.

Ahvonen, P. and Sievers, K. (1969). Yersinia enterocolitica infection associated with Brucella agglutinins. Acta. Med. Scand. 185, 121.

Ambler, R.P. (1968). Species differences in the amino acid sequences of bacterial proteins. In J.G. Hawkes (ed.), Chemotaxonomy and Serotaxonomy, Symposium of the Systematics Association, P.57. Academic Press, London, 299.pp.

American Type Culture Collection (1970). Catalogue of Strains. 9th Ed.

Andersen, E.K. (1953). Serological studies on H.pertussis, H.parapertussis, and H.bronchisepticus. Acta Pathol. Microbiol. Scand. 33, 202.

Arnheim, G. (1909). Keuchhustenuntersuchungen. Arch. für kinderheilkunde 50, 296.

Bacon, M.F., Overend, W.G., Lloyd, P.H. and Peacocke, A.R. (1967). The isolation, composition and physiological properties of DNA from B.pertussis. Arch. biochem. biophys. 118, 352.

Bailey, J.H. (1933). A medium for the isolation of Bacillus pertussis. J. infect. Dis. 52, 94.

Banerjea, A. and Munoz, J. (1962). Antigens of Bordetella pertussis. II Purification of Heat-Labile Toxin. J. Bact. 84, 269.

Baptist, J.N., Shaw, C.R., and Mandel, M. (1969). Zone electrophoresis of enzymes in bacterial taxonomy. J. Bact. 99, 180.

Baumann, P., Doudoroff, M., and Stanier, R.Y. (1968a). A study of the Moraxella group. I The Genus Moraxella and the Neisseria catarrhalis group. J. Bact. 95, 58.

Baumann, P., Doudoroff, M., and Stanier, R.Y. (1968b). A study of the Moraxella Group. II Oxidative-negative species (Genus Acinetobacter). J. Bact. 95, 1520.

Beer, J. (1960). Zur differenzierung von Bordetella. (B) bronchiseptica unter besondarer berücksichtigung der abgrenzung von Alcaligenes faecalis. Zbl. Bakt., I. Abt. Orig. 177, 196.

Bell, E.J. and Marus, A. (1966). Carbohydrate catabolism of Mima polymorpha. I Supplemental energy from glucose added to a growth medium. J. Bact. 91, 2223.

Bergey, D.H., Breed, R.S., Hammer, B.W., Huntoon, F.M., Murray,E.G.D. and Harrison, F.C. (1934). Bergey's Manual of Determinative Bacteriology. 4th. ed. The Williams and Wilkins Co., Baltimore, pp. 1-664.

Bergey, D.H., Harrison, F.C., Breed, R.S., Hammer, B.W., and Huntoon, F.M. (1923). Bergey's Manual of Determinative Bacteriology. 1st ed. The Williams and Wilkens Co., pp. 1-442.

Bergey, D.H., Harrison, F.C., Breed, R.S., Hammer, B.W., and Huntoon, F.M. (1925). Bergey's Manual of Determinative Bacteriology. 2nd. ed. The Williams and Wilkins Co., Baltimore. pp. 1-462.

Biberstein, E.L. and Cameron, H.S. (1961). The family Brucellaceae in veterinary research. Ann. rev. Microbiol. 15, 93.

Biberstein, E.L., and White, D.C. (1969). A proposal for the establishment of two new Haemophilus species. J. Med. Microbiol. 2, 75.

Biberstein, E.L., and Zinnemann, K. (1971). Report (1966-1970) of the Subcommittee on the Taxonomy of Haemophilus to the International Committee on Nomenclature of Bacteria. Int. J. System. Bact. 21, 133.

Billaudelle, H., Edebo, L., Hammarsten, E., Heden, C.-G., Malmgren, B., and Palmstierna, H. (1960). Studies on the chemical and immunological structure of Bordet ella pertussis. Acta. Path. Microbiol. Scand. 50, 208.

Bordet, J., and Gengou, O. (1906). Le microbe de la coqueluche. Ann. Inst. Pasteur 20, 731.

Bordet, J., and Gengou, O. (1907). Le microbe de la coqueluche. Ann. Inst. Pasteur 21, 733.

Bordet, J., and Gengou, O. (1909). Etiologie de la coqueluche. Etat actual de la question. Zbl. Bakt. I. Abt. Ref., 43, 273.

Bordet, J., and Sleeswyk, (1910). Sérodiagnostic et variabilité des microbes suivant le milieu de culture. Ann. Inst. Pasteur, 24, 476.

Bovre, K. (1967). Transformation and DNA base composition in Taxonomy, with special reference to recent studies in Moraxella and Neisseria. Acta Path. Microbiol. Scand. 69, 123.

Bovre, K., Fiandt, M., and Szybalski, W. (1969). DNA base composition of Neisseria, Moraxella, and Acinetobacter. Canad. J. Microbiol. 15, 335.

Boyce, J.M.H., Frazer, J., and Zinnemann, K. (1969). The growth requirements of Haemophilus aphrophilus. J. med. Microbiol. 2, 55.

Breed, R.S., Murray, E.G.D., and Smith, N.R. (1957). Bergey's Manual of Determinative Bacteriology. 7th. ed. Bailliere, Tindall and Cox Ltd., London. pp.1-1094.

Brinley Morgan, W.J., and Gower, S.G.M. (1966). Techniques in the identification and classification of Brucella. In Identification Methods for Microbiologists ed. Gibbs, B.M., and Skinner, F.A. Part A. Academic Press, London.

Brisou, J. (1953). Essai sur la systématique due genre Achromobacter. Annls. Inst. Pasteur, Paris, 84, 812.

Brisou, J. (1957a). Contribution a l'étude des Pseudomonadaceae. Précisions taxonomiques sur le genre Acinetobacter. Annls. Inst. Pasteur, Paris, 92, 134.

Brisou, J. (1957b). Contribution a l'étude de la systématique des Pseudomonadaceae. Annls. Inst. Pasteur, Paris, 93, 397.

Brisou, J., and Prévot, A-R. (1954). Etudes de systematique bactérienne X. Révision des espèces réunies dans le genre Achromobacter. Annls. Inst. Pasteur, Paris, 86, 722.

Bruckner, I.E., and Evans, D.G. (1939). The toxin of

B.parapertussis and the relationship of this organism to H.pertussis

and Br.bronchiseptica. J. Path. Bact., 49, 563.


Brumfitt, W. (1959). Some growth requirements of Haemophilus

influenzae and Haemophilus pertussis. J. Path. Bact., 77, 95.


Buchanan, R.E., Holt, J.G., and Lessel, Jr., E.F. (1966). Index

Bergeyana. Livingstone Ltd., Edinburgh. pp. 1-1472.


Burger, C. (1883). Der keuchhustenpilz. Berl. Klin. Wschr. 20, 7.


Butler, O.L. (1962). A defined medium for Haemophilus influenzae

and Haemophilus parainfluenzae. J. gen. Microbiol. 27, 51.


Cain, A.J., and Harrison, G.A. (1960). The phenetic affinity of

some hominoid skulls. Proc. Soc. Study Human Bid., 27 May, 1960.


Carmichael, J.W., and Sneath, P.H.A. (1969). Taxometric maps.

Systematic Zool. 18, 402.


Castellani, A., and Chalmers, A.J. (1919). Manual of Tropical

Medicine. 3rd. ed. William Wood and Co., New York. pp. 1-2436.


Catlin, B.W., and Cunningham, L.S. (1964). Transforming activities

and base composition of deoxyribonucleates from strains of Moraxella

and Mima. J. gen. Microbiol. 37, 353.

Cohen, S.M., and Wheeler, M.W. (1946). Pertussis vaccine
prepared with phase-I cultures grown in fluid medium.
Amer. J. publ. Hlth. 36, 371.


Colwell, R.R., and Mandel, M. (1964). Adansonian analysis and
DNA base composition of some Gram-negative bacteria.
J. Bact. 87, 1412.


Committee: Society of American Bacteriologists (1917). The
families and genera of the Bacteria. J. Bact. 2, 552.


Conn, H.J. (1942). Validity of the genus Alcaligenes.
J. Bact. 44, 353.


Conn, H.J., Wolfe, G.E., and Ford, M. (1940). Taxonomic
relationships of Alcaligenes spp. to certain soil sap rophytes
and plant parasites. J. Bact. 39, 207.


Controni, G., Ballard, S., and Griffith, M.A. (1964). I. A review
of the literature on classification of the Mima-Morexella.
Amer. J. Med. Tech. 30, 257.


Cowan, S.T. (1956). Taxonomic rank of Enterobacteriaceae 'Groups'.
J. gen. Microbiol. 15, 345.


Cowan, S.T. (1965). Development of coding schemes for microbial
taxonomy. Advances in Applied Microbiology 7, 139.

Cowan, S.T. (1971). Sense and nonsense in bacterial Taxonomy. J. gen. Microbiol. 67, 1.

Craigie, J. (1931). Studies on the serological reactions of the flagella of B.typhosus. J. Immunol. 21, 417.

Cruickshank, R. (1965). Medical Microbiology. E. and S. Livingstone Limited, Edinburgh. pp.1-1067.

Dawson, B., Farnworth, E.H., McLeod, J.W.,and Nicholson, D.E. (1951). Observations on the value of the Bordet-Gengou medium for the cultivation of Haemophilus pertussis. J. gen. Microbiol. 5, 408.

De Ley, J. (1968). DNA base composition and taxonomy of some Acinetobacter strains. Antonie v. Leeuwenhoek, 34, 109.

De Ley, J. (1969). Compositional nucleotide distribution and the theoretical prediction of homology in bacterial DNA. J. Theoret. Biol. 22, 89.

De Ley, J. (1970). Re-examination of the association between melting point, buoyant density, and chemical base composition of deoxyribonucleic acid. J. Bact. 101, 738.

De Ley, J., Bain, N., and Shewan, J.M. (1967). Taxonomy of the Achromobacter and allied species. Nature, Lond. 214, 1037.

De Ley, J., Kersters, K., Khan-Matsubara, J., and Shewan, J.M. (1970). Comparative D-gluconate metabolism and DNA base composition in Achromobacter and Alcaligenes. Antonie v. Leeuwenkoek 36, 193.

Edmunds, P.N. (1962). The biochemical, serological and haemagglittinating reactions of "Haemophilus vaginalis". J. Path. Bact. 83, 411.

Ehrenberg, C.G. (1828). Symbolae physicae sen icones et descript-iones animalium evertebratorum sepositis insectis quae ex itinere per Africum Borealum et Asaim Occidentalem. iv. Evertebrata. Berlin.

Eldering, G. (1942). A study of the antigenic properties of Haemophilus pertussis and related organisms. II Protection tests in mice. Amer. J. Hyg. 36, 294.

Eldering, G., Hornbeck, C., and Baker, J. (1957). Serological study of Bordetella pertussis and related species. J. Bact. 74, 133.

Eldering, G., and Kendrick, P. (1937). A group of cultures resembling both Bacillus pertussis and Bacillus bronchisepticus but identical with neither. J. Bact. 33, 71.

Eldering, G., and Kendrick, P. (1938). Bacillus para-pertussis: a species resembling both Bacillus pertussis and Bacillus bronchisepticus but identical with neither. J. Bact. 35, 561.

Ensminger, P.W. (1953). Pigment production by Haemophilus parapertussis. J. Bact. 65, 509.

Evans, A.C. (1918). Further studies on Bacterium abortus and related bacteria. II A comparison of Bacterium abortus with Bacterium bronchisepticus and with the organism which causes Malta Fever. J. infect. Dis. 22, 580.

Evans, D.G., and Maitland, H.B. (1937). Preparation of toxin of H.pertussis: the properties and relation to immunity. J. Path. Bact. 45, 715.

Evans, D.G., and Maitland, H.B. (1939). The toxin of Br. bronchi-septica and the relationship of this organism to H.pertussis. J. Path. Bact. 48, 67.

Farkas-Himsley, H. (1963). Differentiation of Pasteurella pseudotuberculosis and Bordetella bronchiseptica by simple biochemical tests. Amer. J. Vet. Res. 24, 871.

Ferry, N.S. (1910). A preliminary report of the bacterial findings in canine distemper. Amer. Vet. Rev. 37, 499.

Ferry, N.S. (1911). Aetiology of canine distemper. J. infect. Dis. 8, 399.

Ferry, N.S. (1912). Further studies on Bacillus bronchicanis, the cause of canine distemper. Vet. J. 68, 376.

Ferry, N.S., and Noble, A. (1918). Studies relative to the apparent close relationship between Bact. pertussis and B.bronchisepticus. I. Cultural, aggluttination and absorption reactions. J. Bact. 3, 193.

Fewson, C.A. (1967a). The growth and metabolic versatility of the Gram-negative bacterium NCIB 8250. ("Vibrio 01"). J. gen. Microbiol. 46, 255.

Fewson, C.A. (1967b). The identity of the Gram-negative bacterium NCIB 8250 ("vibrio 01"). J. gen. Microbiol. 48, 107.

Fildes, P. (1923). The classification of haemoglobinohilic bacteria based upon their relation to blood pigment and to the 'vitamine' factor. Brit. J. exp. Path. 4, 265.

Flosdorf, E.W., Dozois, T.F., and Kinball, A.C. (1941). Studies with H.pertussis. v. Agglutinogenic relationships of the phases. J. Bact. 41, 457.

Fraenkel, C. (1908). Untersuchungen zur Entstehung des Keuchhustens. Münchener med. Wochenschr., 55, 1683.

Fujita, A., and Kodama, T. (1934). Untersuchungen über Atmung und Garung pathogener Bakterien. III. Mitteilung Über Cytochrom und das sauerstoffünbertragende Ferment, sourie die Atmungschemmung der pathogen Bakterien durch CO. und HCN. Biochem. Z. 273, 186.

Fukumi, H., Sayama, E., Tomizawa, J. and Uchida, T. (1953). Nutritional requirements and respiratory pattern of pertussis - parapertussis - bronchisepticus group of micro-organisms. Jap. J. med. Sci. Biol. 6, 587.

Galarneault, T.P., and Leifson, E. (1956). Taxonomy of Lophomonas n. gen. Can. J. Microbiol. 2, 102.

Gerhadt, P., and Wilson, I.B. (1948). The nutrition of brucellae: Growth in simple chemically defined media. J. Bact. 56, 17.

Gilardi, G.L. (1967). Achromobacter and Moraxella (Tribe Mimeae): A review and new studies. Amer. J. Med. Tech. 33, 201.

Goldner, M., Jakus, C.M., Rhodes, H.K. and Wilson, R.J. (1966). The amino acid utilisation by phase I Bordetella pertussis in a chemically defined medium. J. gen. Microbiol. 44, 439.

Goldner, M., Jakus, C.M. and Wilson, R.J. (1962). Amino acid utilisation by Bordetella pertussis. VIIIth. Inst. Cong. for Microbiology. Abstr. Montreal, Quebec, Canada. No. E 34. 7, 118.

Gordon, R.E. and Smith, M.M. (1955). Rapidly growing, acid fast bacteria. II. Species description of Mycobacterium fortuitum Cruz. J. Bact. 69, 502.

Gottlieb, D. (1961). An evaluation of criteria and procedures used in the description and characterisation of the streptomycetes. A cooperative study. Appl. Microbiol. 9, 55.

Gottlieb, D. and Hepden, P.M. (1966). The electrophoretic movement of proteins of various Streptomyces species as a taxonomic criterion J. gen. Microbiol. 44, 95.

Griffiths, B.W. and Mason, M.A. (1964). Immunochemical studies on the antigens of Bordetella pertussis. Canad. J. Microbiol. 10, 123.

Haupt, H. (1935). Zur systematik der bakterien. Die für mensch und tier pathogenen gramnegativen alkali-bildenden stäbchenbakterien (Aerobactereae Pribram 1929 em.). Ergebn. Hyg. Bakt. 17, 175.

Henderson, A. (1965). The Moraxella lwoffii group of bacteria; a review. Antonie van Leeuwenhoek 31, 395.

Henderson, A. (1967). The saccharolytic activity of Acinetobacter lwoffii and A. anitratus. J. gen. Microbiol. 49, 487.

Hendrie, M.S., Hodgkiss, W. and Shewan, J.M. (1964). Considerations on organisms of the Achromobacter-Alcaligenes group. Annls. Inst. Pasteur, Lille 15, 43.

Henriksen, S.D., and Bøvre, K. (1968a). Moraxella kingii sp. nov., a haemolytic, saccharolytic species of the genus Moraxella. J. gen. Microbiol. 51, 377.

Henriksen, S.D., and Bøvre, K. (1968b). The taxonomy of the genera Moraxella and Neisseria. J. gen. Microbiol. 51, 387.

Hill, L.R. (1966). An index to deoxyribonucleic acid base compositions of bacterial species. J. gen. Microbiol. 44, 419.

Hill, L.R. (1968). The determination of deoxyribonucleic acid base compositions and its application to bacterial taxonomy. In Identification Methods for Microbiologists, Part B, Academic Press, London, p.177.

Holding, A.J. (1960). The properties and classification of the predominant Gram-negative bacteria occurring in soil. J. appl. Bact. 23, 515.

Holland, D. (1920). Generic index of the commoner forms of bacteria. J. Bact. 5, 215.

Hornibrook, J.W. (1939). Cultivation of phase I H.pertussis in a semi-synthetic liquid medium. Publ. Hlth.Rep. (Wash). 54, 1847.

Hoyer, B.H., and McCullough, N.B. (1968). Homologies of Deoxyribonucleic acids from Brucella ovis, canine abortion organisms, and other Brucella species. J. Bact. 96, 1783.

Hugh, R. (1953). The genus Alcaligenes and related organisms. Ph.D. thesis. Loyola University.

Inamdar, A.N., Bundeally, A.E. and Ganapathi, K. (1965a). Biochemistry of Bordetella pertussis: Part I - metabolism of some sugars, Krebs cycle intermediates, purines and pyrimidines. Indian J. Biochem. 2, 22.

Inamdar, A.N., Bundeally, A.E., and Ganapathi, K. (1965b).
Biochemistry of Bordetella pertussis: Part II - Metabolism of
some amino acids. Indian J. Biochem. 2, 25.

International Code of Nomenclature of Bacteria (1966).
Int. J. System. Bact. 16, 459.

James, A.M. (1949a). Growth requirements of virulent and avirulent
strains of H.pertussis in a semisynthetic medium. J. gen. Microbiol. 3, i.

James, A.M. (1949b). The utilisation of starch by virulent and
avirulent strains of Haemophilus pertussis. J. gen. Microbiol. 3, xxv.

Jebb, W.H.H., and Tomlinson, A.H. (1951). The catabolic activity of
washed suspensions of Haemophilus pertussis. J. gen. Microbiol. 5, 951.

Jebb, W.H.H., and Tomlinson, A.H. (1955). The nutritional requirements
of Haemophilus pert ussis. J. gen. Microbiol. 13, 1.

Jebb, W.H.H., and Tomlinson, A.H. (1957). The minimal amino acid
requirements of Haemophilus pertussis. J. gen. Microbiol. 17, 59.

Jochmann, G., and Krause, P. (1901). Zur aetiologie des Keuchhustens.
Z. Hyg. Infekt. Kr. 36, 193.

Jochmann, G., and Krause, P. (1902). Zur aetiologie des keuchhustens.
Centralbl. f. Bakt. 32, 21.

Johnson, B.L., and Thein, M.M. (1970). Assessment of evolutionary
affinities in Gossypium by protein electrophoresis. Amer. J. Bot. 57, 1081.

Johnson, J.L., Anderson, R.S., and Ordal, E.J. (1970). Nucleic acid
homologies among oxidase-negative Moraxella species. J. Bact. 101, 568.

Jones, D., and Sneath, P.H.A. (1970). Genetic transfer and Bacterial taxonomy. Bact. Rev. 34, 40.

Jones, D.M. (1962). A Pasteurella-like organism from the human respiratory tract. J. Path. Bact. 83, 143.

Jones, L.M. (1960). Comparison of phage typing with standard methods of species differentiation in brucellae. Bull. WHO. 23, 120.

Jones, L.M. (1967). Report to the International Committee on Nomenclature of Bacteria by the Subcommittee on Taxonomy of Brucellae. Minutes of meeting, July 1966. Int. J. System. Bact. 17, 371.

Jones, L.M. (1968). Taxonomic position in the genus Brucella of the causative organism of canine abortion. J. Bact. 95, 625.

Kaltschmidt, E. and Wittmann, H.G. (1970). Ribosomal proteins. VII Two-dimensional polyacrylamide gel electrophoresis for fingerprinting of ribosomal proteins. Analyt. Biochem. 36, 401.

Kasuga, T. (1957). Relations between antigenic components and protective potency of Hemophilus pertussis. Jap. J. Microbiol. 1, 139.

Kasuga, T., and Nakase, Y. (1960). Serological relationship between H.pertussis, H.parapertussis, and H.bronchisepticus. Jap. J. Microbiol. 4, 283.

Kasuga, T., Ukishima, K., and Nakase, Y. (1958). Antigenic structure and phases of Hemophilus parapertussis. Jap. J. Microbiol. 2, 231.

Kendrick, P.L., Nadolski, E.B., Eldering, G., and Baker, J. (1953). Antigenic relationships of Haemophilus pertussis, the parapertussis bacillus and Brucella bronchiseptica as shown by cross-protection tests in mice. J. Bact. 66, 166.

Keogh, E.V., North, E.A., and Warburton, M.F. (1947). Haemagglut-
inins of the Haemophilus group. Nature, Lond. 160, 63.

Klimenko, W.N. (1909). Morphologie und Biologie des
Keuchhustenbacillus. Zbl. Bakt. I. Abt. Orig. 50, 305.

Koplik, H. (1897). The bacteriology of Pertussis. Brit. Med. J.
ii. 1051.

Kovacs, N. (1956). Identification of Pseudomonas pyocyanea by
the oxidase reaction. Nature, Lond. 178, 703.

Kreig, R.E., and Lockhart, W.R. (1966). Classification of entero-
bacteria based on overall similarity. J. Bact. 92, 1275.

Kristensen, M. (1922). Investigations into the occurrence and
classification of the Haemoglobinophilic Bacteria. Levin and
Munksgaard, Coperhagen. pp. 1-272.

Krumweide, C., Mishulow, L., and Oldenbusch, C. (1923). The
existence of more than one immunologic type of B.pert ussis.
J. infect. Dis. 32, 22.

Kumajima, Y., Matsui, T., and Asano, A. (1955). Production of
toxin of phase I Hemophilus pertussis in the medium added with
anion exchange resin and mouse protection test of culture
supermate. Yokohama. med. Bull. 6, 375.

Kuwajima, Y., Matsui, T. and Kishigami, M. (1967). The growth-
supporting effect of some anion exchange resins for phase I
Haemophilus pertussis. Jap. J. Microbiol. 1, 375.

Lacey, B.W. (1951). Antigenic modulation of Haemophilus pertussis.
J. gen. Microbiol. 5, xxi.

Lacey, B.W. (1953a). Three dimensional patterns of antigenic modulation of Haemophilus pertussis, H.parapertussis,and H.bronchisepticus. J. gen. Microbiol. 8, iii.

Lacey, B.W. (1953b). The influence of growth conditions on the antigenic structure of Haemophilus pertussis, parapertussis and bronchisepticus. Atti del Congresso Internazionale di microbiologica 1, vi-vii, 524.

Lacey, B.W. (1953c). On the serology and taxonomy of Haemophilus pertussis, parapertussis, and bronchisepticus. Atti del Congresso Internazionale di microbiologica, 1, vi-vii, 526.

Lacey, B.W. (1960). Antigenic modulation of Bordetella pertussis. J. Hyg (Lond.). 58, 57.

Lacey, B.W. (1961). Non-genetic variation of surface antigens in Bordetella and other micro-organisms. Symp. Soc. gen. Microbiol. xi, 343.

Lapage, S.P., Bascomb, S., Willcox, W.R., and Curtis, M.A. (1970). Computer identification of bacteria. In automation, mechanisation and Data Handling in Microbiology. Ed. by A. Baillie and R.J. Gilbert. pp. 1-22. London: Academic Press.

Lautrop, H. (1954). Parapertussis. Thesis. University of Copenhagen.

Lautrop, H. (1960). Laboratory diagnosis of whooping cough or Bordetella infections. Bull. Wld. Hlth. Org. 23, 15.

Lawton, W.D., Morris, B.C., and Burrows, T.W. (1968). Gene transfer in strains of Pasteurella pseudotuberculosis. J. gen. Microbiol. 52, 25.

Lazarus, A.S., and Gunnison, J.B. (1947). The action of Pasteurella pestis bacteriophage on strains of Pasteurella, Salmonella and Shigella. J. Bact. 53, 705.

Lelliot, R.A. (1966). The plant pathogenic coryneforms. J. appl. Bact. 29, 114.

Leslie, P.H., and Gardner, A.D. (1931). The phase of H.pertussis J. Hyg. 31, 423.

Lessel, E.F. (1970). Judicial Commission of the International Committee on Nomenclature of Bacteria. Minute 48. Int. J. System. Bact. 20, 1.

Lessel, E.F. (1971). International Committee on Nomenclature of Bacteria. Subcommittee on the Taxonomy of Moraxella and Allied Bacteria. Int. J. System. Bact. 21, 213.

Liston, J., Weibe, W., and Colwell, R.R. (1963). Quantitative approach to the study of bacterial species. J. Bact. 85, 1061.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951). Protein measurement with Folin-phenol reagent. J. Biol. Chem. 193-265.

Lund, B.M. (1965). A comparison by the use of gel electrophoresis of soluble protein components and esterase enzymes of some group D streptococci. J. gen. Microbiol. 40, 413.

Maclennan, A.P. (1960). Specific lipopolysaccharides of Bordetella. Biochem. J. 74, 398.

Madsen, T. (1937). Whooping cough. Its bacteriology, diagnosis, prevention and treatment. Boston Med. and Surg. J. 192, 50.

Maitland, H.B., and Guérault, A. (1958). Some surface components of H. pertussis: immunising antigen, histamine-sensitising factor and agglutinogen. J. Path. Bact. 76, 257.

Mandel, M., Igambi, L., Bergendahl, J., Dodson, Jr. M.L., and Scheltgen, E. (1970). Correlation of melting temperature and caesium chloride buoyant density of bacterial deoxyribonucleic acid. J. Bact. 101, 333.

Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from micro-organisms. J. Mol. Biol. 3, 208.

Marmur, J. and Doty, P. (1959). Heterogeneity in deoxyribonucleic acids. I. Dependence on composition of the configurational stability of deoxyribonucleic acids. Nature, Lond. 183, 1427.

Marmur, J. and Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. J. Mol. biol. 5, 109.

Martin, G. and Jacob, F. (1962). Transfert de L'episome sexuel d'Escherichia coli à Pasteurella pestis. C.r. hebd. Séanc. Acad. Sci., Paris 254, 3589.

Martinevskij, I.L. (1968). Taxonomie du genre Yersinia et nomenclature de ses representants. In International Symposium on Pseudotuberculosis, Paris 1967; Symp. Series immunobiol. Standard, vol. 9, pp. 361-368. Karger. Basel/New York.

Marus, A., and Bell, E.J. (1966). Carbohydrate catabolism of Mima polymorpha. II. Abortive catabolism of glucose. J. Bact. 91, 2229.

Matosuoka, Y., Yamamura, Y., and Kitagawa, M. (1965). Relationship between protective antigen and cellular components of Haemophilus pertussis. Jap. J. Med. Sci. Biol. 18, 172.

Meyer, K.F., and Shaw, E.G. (1920). A comparison of the morphologic, cultural and biochemical characteristics of B. abortus and B. melitensis. Studies on the genus Brucella Nov. Gen. 1. J. infect. Dis. 27, 173.

Meyer, M.E., and Cameron, H.S. (1958). Species metabolic patterns within the genus Brucella. Amer. J. Vet. Res. 19, 754.

Migula, W. (1900). System der Bakterien. 2 Gustav Fischer, Jena. pp. 1-1068.

Miller, J.J., Saito, T.M., and Silverberg, R.J. (1941). Parapertussis. Clinical and serologic observations. J. Pediat. 19, 229.

Mollaret, H.H., and Knapp, W. (1971). Report (1966-1970) of the Subcommittee on Pasteurella, Yersinia and Pasteurella to the International Committee on Nomenclature of Bacteria. Int. J. System. Bact. 21, 157.

Moore, H.B., and Pickett, M.J. (1960). Organisms resembling Alcaligenes faecalis. Can. J. Microbiol. 6, 35.

Moreno Lopez, M. (1952). El genero Bordetella. Microbiol. esp. 5, 177.

Mraz, O. (1964). Concerning some relations (morphological, cultural, biochemical and biological) between the genera Actinobacillus and Pasteurella. Spisy Prirodovedecke Fak. Univ. Brne. 458, 519.

Mraz, O. (1968). Reevaluation of original strains Actinobacillus suis and haemolytic strains Actinobacillus lignieresii ATCC isolated from organs of diseased pigs. Sbornik VŠZ v Brně, Spisy Fak. Vet. 37, 277.

Mraz, O. (1969a). Vergleichende studie der arten Actinobacillus lignieresii und Pasteurella haemolytica. I Actinobacillus lignierisii. Brumpt, 1910; emend. Zbl. Bakt., I. Abt. Orig. 209, 212.

Mraz, O. (1969b). Vergleichende studie der arten Actinobacillus lignieresii und Pasteurella haemolytica. II Pasteurella haemolytica Newsom und Cross, 1932. Zbl. Bakt., I. Abt. Orig. 209, 336.

Mraz, O. (1969c). Vergleichende studie der arten Actinobacillus lignieresii und Pasteurella haemolytica. III Actinobacillus haemolyticus ( Newsom und Cross, 1932) comb. nov. Zbl. Bakt., I. Abt. Orig. 209, 349.

Munoz, J.J. (1963). Immunological and other biological activities of Bordetella pertussis antigens. Bact. Rev. 27, 325.

Munoz, J.J., Ribi, E. and Larson, C.(1959). Antigens of B. pertussis. I. Activities of cell walls and protoplasm. J. Immunol. 83, 496.

Nakase, Y. (1957a). Studies on Hemophilus bronchisepticus. I. The antigenic structures of H. bronchisepticus from guinea pig. Kitasato Arch. Exp. Med. 30, 57.

Nakase, Y. (1957b). Studies on Hemophilus bronchisepticus. II. Phase variation of H. bronchisepticus. Kitasato Arch. Exp. Med. 30, 73.

Nakase, Y. (1957c), Studies on Hemophilus bronchisepticus. III. Differences of biological properties between phase I and phase III of H. bronchisepticus. Kitasato Arch. Exp. Med. 30, 79.

Nakase, Y. (1957d). Studies on Hemophilus bronchisepticus. IV. Serological relation of H. bronchisepticus from guinea pig, dog and human. Kitasato Arch. Exp. Med. 30, 85.

Nakase, Y., Tateisi, M., Sekiya, K. and Kasuga, T. (1970). Chemical and biological properties of the purified O antigen of Bordetella pertussis. Jap. J. Microbiol. 14, 1.

Nicholson, D. E. and Turner, G.C. (1954). A simple selective medium for the primary isolation of Haemophilus pertussis and parapertussis. J. gen. Microbiol. 10, 1.

Norris, J.R. (1968). The application of gel electrophoresis to the classification of microorganisms. In Systematics Association Special Vol. No. 2. " Chemotaxonomy and Serotaxonomy ". ed. J.G. Hawkes. Academic press. p.49.

Odaira. (1911). Beiträge zur kenntnis der hämoglobinophilen bacillen, mit besonderer berucksichtigung des Bordetschen bacillus. Cent. f. Bakt. Abt. Orig. 61, 289.

Olitsky, P.K., Syverton, J.T. and Tyler, J.R. (1934). Studies on the etiology of spontaneous conjunctival folliculosis of rabbits. J. exp. Med. 60, 375.

Onoue, K., Kitagawa, M.and Yamamura, Y. (1961). Chemical studies on cellular components of Bordetella pertussis. I. Purification and properties of agglutinogen. J. Bact. 82, 648.

Opinion 4 (1954). Rejection of the generic name Bacterium. Int. Bull. Bact. Nomen. and Taxon. 4, 141.

Opinion 32 (1970). Conservation of the specific epithet rhusiopathiae in the scientific name of the organism known as Erysipelothrix rhusiopathiae (Migula 1900) Buchanan 1918. Int. J. System. Bact. 20, 9.

Petruschky, J. (1896). Bacillus faecalis alcaligenes (n. sp.). Zbl. Bakt., Abt. I, 19, 187.

Pickett, M.J. (1955). Fermentation tests for the identification of brucellae. Amer. J. Med. Tech. 21, 166.

Pickett, M.J., Nelson, E.L., Hoyt, R.E. and Eisenstein, B.E. (1952). Speciation within the genus Brucella. I. Dye sensitivity of smooth Brucella. J. Lab. and Clin. Med. 40, 2.

Pickett, M.J. and Pedersen, M.M. (1970). Salient features of nonsaccharolytic and weakly saccharolytic nonfermentative rods. Canad. J. Microbiol. 16, 401.

Piéchaud, M. et Szturm-Rubinstein (1965). De quelques caratères différentiels entre Bordetella bronchiseptica and Bordetella parapertussis. Ann. Inst. Past. Paris. 108, 391.

Pintér, M. and Bende, I. (1967). Computer analysis of Acinetobacter lwoffii ( Moraxella lwoffii ) and Acinetobacter anitratus ( Moraxella glucidolytica ) strains. J. gen Microbiol. 46, 267.

Pintér, M. and Bende, I. (1968). Biochemical similarity of Acinetobacter lwoffii and Acinetobacter anitratus. Path. Microbiol. 31, 41.

Pittman, M. (1955). The genus Bordetella Moreno-Lopez 1952. In Breed, R.S. et al. (1955). Symposium on Taxonomy. Bact. Rev. 19, 273.

Pollock, M.R. (1947). The growth of H. pertussis on media without blood. Brit. J.exp. Path. 28, 295.

Portwood, L.M. (1946). Catalase activity of Hemophilus pertussis. J. Bact. 51, 265.

Preston, N.W. and Te Punga, W.A. (1959). The relation between agglutinin production by pertussis vaccines and their immunising potency in mice. J. Path. Bact. 78, 209.

Pribram, E. (1933). Klassification der Schizomyceten. F. Deuticke, Leipzig. pp. 1-143.

Proom, H. (1955). The minimal nutritional requirements of organisms of the genus Bordetella López. J. gen Microbiol. 12, 63.

Razin, S. (1968). Mycoplasma taxonomy studied by electrophoresis of cell proteins. J. Bact. 96, 687.

Reeves, P. (1965). The bacteriocins. Bact. Rev. 29, 24.

Ritter, D.B. and Gerloff, R.K. (1966). Deoxyribonucleic acid hybridisation among some species of the genus Pasteurella. J. Bact. 92, 1838.

Rockwood, S.W. (1962). Comparative taxonomic studies of members of the tribe Mimeae. Ph.D. thesis. University of Cincinnati.

Rogul, M., Brendle, J.J., Haapala, D.K. and Alexander, A.D. (1970). Nucleic acid similarities among Pseudomonas pseudomallei, Pseudomonas multivorans and Actinobacillus mallei. J. Bact. 101, 827.

Rohlf, F.J. (1964). Methods for checking the results of a numerical taxonomic study. System. Zool. 13, 102.

Ross, R., Munoz, J. and Cameron, C. (1969). Histamine-sensitising Factor, Mouse-protective antigens, and other antigens of some members of the genus Bordetella. J. Bact. 99, 57.

Rosypal, S. and Rosypalova, A. (1966). Genetic, phylogenetic and taxonomic relationships among bacteria as determined by their deoxyribonucleic acid base composition. Folia Přírodovědecké Fakulty Univ. J.E. Purkyně v Brné. vii 14, 3.

Rosypal, S., Rosypalova, A. and Hořejš, J. (1966). The classification of micrococci and staphylococci based on their DNA base composition and Adansonian analysis. J. gen. Microbiol. 44, 281.

Rouatt, J.W., Skyring,G.W., Purkayastha, V. and Quadling, C. (1970). Soil bacteria: numerical analysis of electrophoretic protein patterns developed in acrylamide gels. Canad. J. Microbiol. 16, 202.

Rowatt, E. (1955). Amino acid metabolism in the genus Bordetella. J. gen. Microbiol. 13, 552.

Rowatt, E. (1957a). Some factors affecting the growth of Bordetella pertussis. J gen. Microbiol. 17, 279.

Rowatt, E. (1957b). The growth of Bordetella pertussis: a review. J. gen.Microbiol. 17, 297.

Sacks, T.G., Haas, H. and Razin, S. (1969). Polyacrylamide-gel electrophoresis of cell proteins of Enterobacteriaceae. Israel J. med. Sci. 5 49.

Sato, Y. and Nagase, K. (1965). Studies on protective antigen of Bordetella pertussis: isolation of protective antigen by sucrose density gradient centrifugation and some properties of the antigen. Jap. J. Med. Sci. Biol. 18, 173.

Schildkraut, C.L. and Lifson, S. (1965). Dependence of the melting temperature od DNA on salt concentration. Bipolymers 3, 195.

Schildkraut, C.L., Marmur, J. and Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its buoyant density in CsCl. J. Mol. Biol. 4, 430.

Schuchardt, L.F., Munoz, J., Verwey, W.F. and Sagin, J.F. (1963). The relationship of agglutinogen to other antigens of Bordetella pertussis. J. Immunol. 91, 107.

Seiffert, G. (1909). Ueber den Bordetschen keuchhustenbazillus. Munchener med. Wochenschr. January 131.

Silverthorne, N. and Cameron, C. (1942). Whooping cough. IV. Beef liver extract blood agar media for growth of H. pertussis. J.Pediat. 20, 16.

Smith, D. A. and Burrows, T.W. (1962). Phage and bacteriocin investigations with Pasteurella pestis and other bacteria. Nature, Lond. 193, 397.

Smith, G.R. (1961). The characteristics of two types of Pasteurella haemolytica associated with different pathological conditions in sheep. J. Path. Bact. 81, 431.

Smith, J.E. and Thal, E. (1965). A taxonomic study of the genus Pasteurella using a numerical technique. Acta Path. Microbiol. scand. 64, 213.

Smith, N.R., Gordon, R.E. and Clark, F.E. (1952). Aerobic sporeforming bacteria. U.S..Dep. Agric. Agriculture Monograph no. 16.

Sneath, P.H.A. (1956). Cultural and biochemical characteristics of the genus Chromobacterium. J.gen. Microbiol. 15, 70.

Sneath, P.H.A. (1957a). Some thoughts on bacterial classification. J. gen. Microbiol. 17, 184.

Sneath, P.H.A. (1957b). The application of computers to taxonomy. J. gen. Microbiol. 17, 201.

Sneath, P.H.A. (1962). The construction of taxonomic groups. In G.C. Ainsworth and P.H.A. Sneath (eds.), Microbial Classification, 12th. Symposium of the Society for General Microbiology, p. 289. Cambridge University Press, Cambridge.

Sneath, P.H.A. (1966). Identification methods applied to Chromobacterium. In Identification Methods for Microbiologists, Part A. eds. B.M. Gibbs and F.A. Skinner. Academic Press, London. p.15.

Sneath, P.H.A. (1968). Vigour and pattern in taxonomy. J. gen. Microbiol. 54, 1.

Sneath, P.H.A. (1971). Theoretical aspects of microbiological taxonomy. In Recent Advances in Microbiology, X International Congress for Microbiology. pp. 581-586.

Sneath, P.H.A. and Cowan, S.T. (1958). An electro-taxonomic survey of bacteria. J. gen. Microbiol. 19, 551.

Sneath, P.H.A. and Skerman, V.B.D. (1966). A new list of type and reference strains. Int. J. System. Bact. 16, 1.

Snell, J.J.S. and Lapage, S.P. (1971). Comparison of four methods for demonstrating glucose breakdown by bacteria. J. gen. Microbiol. 68, 221.

Sokal, R.R. and Michener, C.D. (1958). A statistical method for evaluating systematic relationships. Univ. Kansas Sci. Bull. 38, 1409.

Sokal, R.R. and Sneath, P.H.A. (1963). Principles of Numerical Taxonomy. W.H. Freeman, San Francisco. pp. 1-359.

Stableforth, A.W. and Jones, L.M. (1963). Report of the subcommittee on taxonomy of the genus Brucella. Intern. Bull. Bact. Nomen. Taxon. 13, 145.

Stainer, D.W. and Scholte, M.J. (1971). A simple chemically defined medium for the production of phase I Bordetella pertussis. J. gen. Microbiol. 63, 211

Stanier, R.Y., Doudoroff, M. and Adelberg, E.A. (1970). General Microbiology. Third Ed. pp. 1-873. MacMillan. London.

Steel, K.J. and Cowan, S.T. (1964). Le rattachement de Bacterium anitratum, Moraxella lwoffii, Bacillus mallei et Haemophilus parapertussis au genre Acinetobacter Brisou et Prévot. Annls. Inst. Pasteur. 106, 479.

Stenzel, W. and Mannheim, W. (1963). On the classification of some non-motile and coccoid diplobacteria exhibiting the properties of Achromobacteriaceae. Intern. Bull. Bact. Nomen. Taxon. 13, 195.

Stevens, M. (1969). Development and use of multi - inoculation test methods for a taxonomic study. J. med. Lab. Technol. 26, 253.

Stevenson, J.P. (1959). A note on the genus Aeromonas. J. gen. Microbiol. 21, 366.

Stillman, E.G. and Bourn, J.M. (1920). Biological study of the haemophilic bacilli. J. Exp. Med. 32, 665.

Sueoka, N. (1961). Variation and heterogeneity of base composition of deoxyribonucleic acids: a compilation of old and new data. J. Mol. Biol. 3, 31.

Sutherland, I.W. (1963). Two cytochromes isolated from the bacterial genus Bordetella. Biochim. biophys. Acta. 73, 162.

Sutherland, I.W. and Wilkinson, J.F. (1961). A new growth medium for virulent Bordetella pertussis. J. Path. Bact. 82, 431.

Sutherland, I.W. and Wilkinson, J.F. (1963). Azurin: a copper protein found in Bordetella. J. gen. Microbiol. 30, 105.

Szturm, S. and Bourdon, D. (1948). Bacterium alcaligenes fecalis et Hemophilus bronchisepticus caractères morphologiques et biochemiques. Ann. Inst. Pasteur, 75, 65.

Talbot, J.M. and Sneath, P.H.A. (1960). A taxonomic study of Pasteurella septica, especially of strains from human sources. J. gen. Microbiol. 22, 303.

Taylor, G.R., Guthrie,R.K. and Shirling, E.B. (1970). Serological characteristics of Streptomyces species using cell wall immunising antigens. Canad. J. Microbiol 16, 107.

Thal, E. (1954). Untersuchungen über Pasteurella pseudotuberculosis. Berlingska Boktryckeriet. Lund.

Thornley, M.J. (1960). The differentiation of _Pseudomonas_ from other Gram-negative bacteria on the basis of arginine metabolism. J. appl. Bact. _23_, 37.

Thornley, M.J. (1967). A taxonomic study of _Acinetobacter_ and related genera. J. gen. Microbiol. _49_, 211.

Tilden, E.B. and Tyler, J.R. (1930). _Bacterium granulosis_ in relation to trachoma: its recovery from experimentally infected monkeys and from human trachoma. J. exp. Med. _52_, 617.

Timofeeva, L. (1968). Criteres fondementeux de la differenciation de " _Pasteurella pestis_", " _Pasteurella pseudotuberculosis_ " et de quelques autres especes de microbes. In International Symposium on Pseudotuberculosis, Paris 1967; Symp. Series immunobiol. Standard vol.9, pp. 233-244. Karger, Basel/New York.

Toomey, J.A., Ranta, K., Robey, L. and McClelland, J.E. (1935). The phases and types of _H.pertussis_. J. Infect. Dis. _57_, 49.

Topley, W.W.C. and Wilson, G.S. (1929). The Principles of Bacteriology and Immunity. 1st. ed. Edward Arnold and Co., London. pp. 1-587.

Torrey, J.C. and Rahe, A.H. (1913). Studies in canine distemper. J. med. Res. _27_, 291.

Turner, G.C. (1961). Cultivation of _Bordetella pertussis_ on agar media. J. Path. Bact. _81_, 15.

Ulrich, J.A. and Needham, G.M. (1953). Differentiation of _Alcaligenes faecalis_ from _Brucella bronchisepticus_ by biochemical and nutritional methods. J. Bact. _65_, 210.

Ungar, J., James, A.M., Muggleton, P.W., Pegler, H.F. and Tomich, E.G. (1950). The cultivation of Haemophilus pertussis in partially defined liquid media. J. gen. Microbiol. 4, 345.

Ungar, J. and Stevens, W.K. (1951). Haemophilus pertussis as an homologous bacterial type. J. gen. Microbiol. 5, iv.

Vajdic, A.H., Goldner, M. and Wilson, R.J. (1964). The amino acid uptake of phase I Bord. pertussis cultivated in achemically defined medium. Canad. J. Publ. Hlth. 55, 35.

Vajdic, A.H., Goldner, M. and Wilson, R.J. (1966). The amino acid use in cultures of phase I Bordetella pertussis during growth in chemically defined media. J. gen. Microbiol. 44, 445.

Verwey, W.F., Thiele, E.H., Sage, D.N. and Schuchardt, L.F. (1949). A simplified liquid culture medium for the growth of Hemophilus pertussis. J. Bact. 58, 297.

Wetzler, T.F., Eitzen, H.E., Currie, J.A. and Marshall, J.D. (1968). Lipopolysaccharide-like antigens from Pasteurella pseudotuberculosis, shared by various genera of the Enterobacteriaceae as demonstrated by hemagglutination tests. International Symposium on Pseudotuberculosis, Paris 1967; Symp. Series immunobiol. Standard., vol 9, pp. 155-166. Karger, Basel/New York.

Wilson, R.J. (1963). Cultivation of Bordetella pertussis (phase I) in a chemically defined medium. Can J. Publ. Hlth. 54, 518.

Wilson, G.S. and Miles, A.A. (1946). Topley and Wilson's Principles of Bacteriology and Immunity. 3rd. ed. Edward Arnold and Co., London. pp. 1-970.

Wilson, G.S. and Miles, A.A. (1961). Topley and Wilson's Principles of Bacteriology and Immunity. 4th. ed. Edward Arnold and Co., London.

Wilson, G.S. and Miles, A.A. (1964). Topley and Wilson's Principles of Bacteriology and Immunity, 5th. ed. Edward Arnold and Co., London. pp. 1-2563.

Woiwod, A.J. (1954). The inhibition of bacterial growth by colloidal heavy metal sulphides and by colloidal sulphur. J. gen. Microbiol. 10, 509.

Wollstein, M. (1905). A study of the bacteriology of pertussis, with special reference to the agglutination of the patients blood. J. Exp. Med. 7, 335.

Zinnemann, K. (1967). Report of the Subcommittee on the Taxonomy of Haemophilus (1962-1966). Int. J. System. Bact. 17, 165.

Zinnemann, K., Rogers, K.B., Frazer, J. and Boyce, J.M.H. (1968). A new V-. dependent Haemophilus species preferring increased $CO_2$ tension for growth and named Haemophilus paraphrophilus, nov. sp. J. Path. Bact. 96, 413.

## SUMMARY

Two hundred and one strains of Gram-negative bacteria representing the genera of the family Brucellaceae, together with species of Neisseria, Achromobacter, Acinetobacter, Alcaligenes and Agrobacterium have been subjected to a taxonomic examination. A numerical taxonomic analysis has separated the strains into fifteen clusters. Two of the clusters consisted of composite groups each containing two genera which could not be clearly separated on the tests carried out. The homogeneity of the clusters was substantiated by results obtained on selected strains when examined for GC base ratios ( by thermal denaturation ) and similarity of protein patterns ( examined by poly-acrylamide gel electrophoresis ).

The family Brucellaceae Breed, Murray and Smith 1957 contains a heterogeneous collection of genera which have little in common apart from often requiring special media for growth and in being potentially pathogenic.

The results of this study suggest that the Brucellaceae should be sub-divided into two families,

1) Pasteurellaceae Pribram 1933 or Fam. nov. This would contain the genera Haemophilus, Pasteurella and Actinobacillus. The close similarity between Pasteurella and Actinobacillus is recognised and it is suggested that further work on these two genera may reveal that they can be combined in a single genus.

2) Brucellaceae Breed, Murray and Smith 1957 emend. This family would contain only three genera, Brucella, Bordetella and Alcaligenes.

Difficulties were found in separating oxidase-positive, motile Alcaligenes species from Bordetella bronchiseptica, although there is little evidence for separating B. bronchiseptica from the other species of Bordetella. Further work may suggest combining Alcaligenes and

Bordetella.

Oxidase-negative, non-motile Alcaligenes species should not be included in the genus Alcaligenes. Results from this study indicate that they should be transferred to the species Acinetobacter lwoffii.

Definitions for the families Pasteurellaceae and Brucellaceae are proposed.