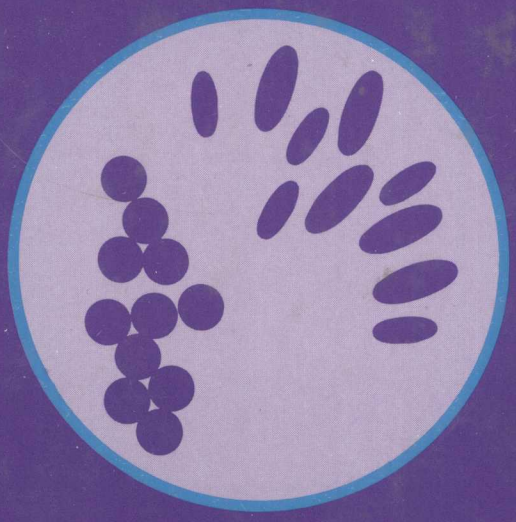
Cowan and Steel's Manual for the Identification of Medical Bacteria

Third Edition

Edited by
G.I. Barrow and R.K.A. Feltham



COWAN AND STEEL'S
Manual for the identification of medical bacteria

THIRD EDITION
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CAMBRIDGE
UNIVERSITY PRESS

Published by the Press Syndicate of the University of Cambridge,
The Pitt Building, Trumpington Street, Cambridge CB2 1RP
40 West 20th Street, New York, NY 10011–4211, USA
10 Stamford Road, Oakleigh, Victoria 3116, Australia

First published 1965
Second edition 1974
Third edition 1993

Printed in Great Britain at the University Press, Cambridge

A catalogue record for this book is available from the British Library

Library of Congress cataloguing in publication data
Cowan and Steel's manual for identification of medical bacteria –
3rd ed. / edited and rev. by G.I. Barrow and R.K.A. Feltham.
p. cm.
Includes bibliographical references.
Includes index.
ISBN 0 521 32611 7
1. Diagnostic bacteriology – Handbooks, manuals, etc. I. Cowan, S.T. (Samuel Tertius) II. Steel, K.J. (Kenneth John)
III. Barrow, G.I. IV. Feltham, R.K.A. V. Title: Manual for the identification of medical bacteria.
[DNLM: 1. Bacteria – classification.
QW 15 C874]
QR67.2.C68 1993
616.014 – dc20 90-2511 CIP

ISBN 0 521 32611 7 hardback

DG/GO

This standard and internationally known reference manual for the identification of medically important bacteria, *Cowan and Steel*, occupies an essential place at the bench of all medical microbiologists. The material in this new edition, which follows the successful pattern of previous editions, has been extensively revised, and is suitable for use in all medical bacteriology laboratories using traditional diagnostic methods.

The core of the manual is the series of diagnostic tables which, with the accompanying descriptive text and definitions, give the characteristics of all bacteria likely to be encountered in public health laboratories, and in medical and veterinary practice. This edition contains new sections on rapid and mechanized test methods and on the laboratory applications of computers to the identification of bacteria. The importance of laboratory quality control and proficiency procedures is emphasized throughout.

The Appendices give details of laboratory methods and media for all the recommended diagnostic tests, and provide abstracts of the official guidelines for bacterial nomenclature. As in previous editions, the text contains comprehensive and up to date references.

Cowan and Steel's manual for the identification of medical bacteria

The following titles, originally published by the Public Health Laboratory Service, might also be of interest and these are now available from Cambridge University Press

Quality Control – Principles and Practice in the Microbiology Laboratory (1991) Edited by J. J. S. Snell, I. D. Farrell & C. Roberts

Current Topics in Clinical Virology (1991) Edited by P. Morgan-Capner

Multipoint Methods in the Clinical Laboratory (1991) M. Faiers, R. George, J. Jolly & P. Wheat

ELISA in the Clinical Microbiology Laboratory (1990) Edited by T. G. Wreghitt & P. Morgan-Capner

Anaerobic Infections: Clinical and Laboratory Practice (1988) A. Trevor Willis & Kenneth D. Phillips

Making Monoclonals: A Practical Beginners' Guide to the Production and Characterisation of Monoclonal Antibodies Against Bacteria and Viruses (1988) D. G. Newell, B. W. McBride and S. A. Clark

Foreword

For over 25 years now, medical bacteriologists all over the world have turned to 'Cowan and Steel' as their first reference book when they encountered an unfamiliar bacterial isolate. A generation of laboratory workers has grown up with it. They turned to it not only because there was clear information on how to examine isolates, with concise details of culture media and test methods that were applicable to the great majority of bacteria of medical importance, but also because of the famous successive tables that led from genera with their minidefinitions to species with their characters. These were combined with practical hints on where one might go wrong, and succinct information on the pathogenic species. The tables contained carefully chosen data in just the right amount for a useful laboratory manual on the identification of medically important bacteria.

In the years since the last edition, test methods and the variety of bacteria of medical interest have both grown considerably. Not only have poorly studied areas like the 'diphtheroids' been much clarified, but a number of newly recognized pathogens such as legionellae have become important. Medical and other workers will therefore welcome this new edition, which follows closely the emphasis and style of its predecessors. The contributors and editors are to be congratulated on their labours in bringing a complex field to the concise summary that is contained here, often in the face of difficulties in finding convenient diagnostic tests for the newer taxa.

Some new features in this edition will greatly help users. The chapters on theory and practice in bacterial taxonomy, on computer identification and on bacterial nomenclature will be especially welcomed. The further emphasis on quality control and proficiency assessment procedures, both within laboratories and between them, will also be most useful. It is a pleasure to be able to recommend this *Manual* wholeheartedly to those concerned with medical and associated bacteriology everywhere.

Leicester *P.H.A. Sneath*

Preface to the first edition

Our 'Diagnostic Tables for the Common Medical Bacteria' were originally published in the *Journal of Hygiene*. The tables seemed to fill a need and the demand for reprints was so great that Cambridge University Press reprinted them in pamphlet form.

Many inquired about the technical methods, and there were constant complaints that the methods were not described and that the text lacked details of the taxonomic problems. We resolved, therefore, to expand the original paper and to prepare a book which would give sufficient detail of media and methods to justify its description as a laboratory manual.

Although designed for medical workers we hope that others will use it.

The value of a laboratory manual was impressed on one of us in 1935 at the British Postgraduate Medical School. Dr A. A. Miles had prepared a loose-leaf mimeographed manual to supplement (and improve on) a popular laboratory handbook. With this example in mind a manual suited to the special needs of the National Collection of Type Cultures was prepared, and contributions were made by other members of the Collection staff, particularly Mrs P. H. Clarke, Miss H. E. Ross, Miss C. Shaw, and Mr C. S. Brindle. The National Collection Manual in turn became the basis for the appendices to the present *Manual*.

In compiling the tables we sought information from various sources, including authoritative works such as the Reports of the Enterobacteriaceae Subcommittee of the International Committee on Bacteriological Nomenclature, and monographs such as Kauffmann's (1954) *Enterobacteriaceae*, Edwards & Ewing's *Identification of Enterobacteriaceae* (1962), and Smith, Gordon & Clark's (1952) *Aerobic Sporeforming Bacteria*. We found large gaps in published works, and in many instances our own data have been the only source of information. While we have taken great care in compiling and checking the tables, we are sure that the *Manual* is unlikely to be free from error. When such errors are detected we hope that the finders will let us know. We will also welcome data to fill up the few gaps in the tables.

It is with pleasure that we acknowledge our indebtedness to many friends and colleagues at home and abroad for facts and discussions that have helped to clarify ideas. It is impossible to name them all, but we could not have planned or written the *Manual* without the help of Dr R. E. Gordon, Dr P. R. Edwards, Dr W. H. Ewing, Dr T. Gibson, Dr Joan Taylor, Mrs P. H. Clarke, Miss C. Shaw, and Miss H. E. Ross. We also wish to thank Miss B. H. Whyte and Miss A. Bowman, the Colindale librarians, Miss M. I. Hammond who dealt skilfully with the manuscript, and Mr W. Clifford who made the figures.

London S.T.C.
1964 K.J.S.

POSTSCRIPT

My colleague, Dr K. J. Steel, died suddenly on 25 September 1964, between the completion of the manuscript and the proof stage of the book. His death at the age of 34 is a great loss for he seemed destined to reach the highest branches of bacteriology. In this *Manual* he was responsible for the whole of Appendices A to D and F and for much of Chapter 3; and he played a big part in revising and recasting the tables that form the heart of our work. I hope that the book will serve as a fitting memorial to a great collaborator and friend.

London S.T.C.
1965

Preface to the second edition

The first edition of this *Manual*, judged by its spread around the world, seems to have been useful to hospital bacteriologists. It was translated into Japanese by Dr Riichi Sakazaki, who will also translate this edition.

It has not been easy to prepare a worthy successor; not only have I been unable to discuss and argue every sentence with my colleague, but I have missed the ready access to libraries that one has when working in a large research institution. However, I have been greatly helped by the Librarians at Colindale (Miss B. H. Whyte) and the Royal Society of Medicine (Mr P. Wade) and their staffs.

In this edition Chapter 2 and Appendices A, B, C and E, originally written mainly by Dr Steel, are little changed; most of the other chapters have been completely rewritten. Chapters 8 and 9 are entirely new, as are Appendices D, F, G and H. I must thank Mr A. Waltho, of the Medical Research Council's Central Store, who gave me great help in preparing the list of firms which supply media and chemicals (Appendix H) and, together with Dr O. M. Lidwell, suggested and drafted what became Table 2.1.

I am also grateful to many other colleagues who gave me information and advice; while it is impossible to mention all by name, I am particularly indebted to G. I. Barrow, W. B. Cherry, E. A. Dawes, N. E. Gibbons, R. E. Gordon, R. M. Keddie, S. P. Lapage, H. Lautrop, J. Midgeley, M. J. Pickett, R. Sakazaki, R. Whittenbury and S. A. Wright.

On behalf of the Executive Committee of the International Association of Microbiological Societies (IAMS), Dr N. E. Gibbons gave permission for the reproduction of the Introduction to the proposed revision of the Bacteriological Code (Appendix G), and I should like to express my thanks to the IAMS Executive.

In a book with so many tables and cross-references it is inevitable that some errors and inconsistencies are still undetected; I hope that these will be drawn to my attention so that corrections can be made in later impressions.

For the proof reading I am grateful for help from former colleagues, Miss H. E. Ross, Dr G. I. Barrow and Dr A. F. B. Standfast. Checking the numerous and large tables in the manuscript and proof stages has been an onerous task which I could not have done without the co-operation of my wife, who also helped to check the references, which must now number about a thousand.

With all this help, I hope the book will continue to be a worthy memorial to my much missed young colleague, Dr K. J. Steel.

Queen Camel S.T.C.
1973

Preface to the third edition

The demand for a new edition of this *Manual* has been enormous. We hope that we have done justice to it and that it will prove a fitting tribute to the late Sam Cowan. He not only obtained every paper he cited in the references but personally perused and annotated each one. We cannot alas say the same. It is now beyond the scope of one person or even of two persons to cover the entire and seemingly ever-changing fields of bacterial classification, nomenclature and taxonomy, especially with the range of 'medical' bacteria expanding with the advancement of biotechnology and modern medicine to include many environmental organisms. For this third edition we have therefore sought the help of the experts listed on pages xv and xvi for various groups of organisms and we gratefully acknowledge all their contributions to this *Manual*. The opinions expressed are mostly theirs though the final responsibility is ours. We hope that together they will provide enlightenment and understanding of a subject which, though not everyone's 'cup of tea', is nevertheless at the heart of diagnostic medical bacteriology.

In outline, this edition follows that of the two previous ones. We have received numerous suggestions for change but have resisted many of them, preferring to regard continuity as more important. We have also retained references to some methods and equipment which may be regarded as 'old-fashioned' or not quite reaching the current acme of absolute safety, but we are conscious that not all diagnostic laboratories are equally endowed; we know for example that, despite their limitations, manually operated autoclaves are still used frequently and apparently satisfactorily throughout the world. Apart from extensive updating of the text, tables and appendices, we have added new Sections: on rapid methods and test kits; on the theory and use of computers for bacterial identification; on the principles of the Bacteriological Code and the *Approved Lists of Bacterial Names*; and on the reconciliation of different approaches to bacterial systematics. Unlike previous editions, we have not listed 'sources of information' separately in Chapters 6 and 7 but have included all references in the text. Also, in this edition we have omitted the Appendix listing some of the manufacturers of media, reagents and other laboratory supplies as the international names are now well known and we think it would be invidious to select arbitrarily from the many others. For reference purposes we have included the type strain of the type species in the minidefinitions, but we emphasize that they should not be regarded as necessarily 'typical' in all respects of the species.

In a book such as this with so much material and so many cross-references we know that there are bound to be some errors and inconsistencies that we have missed; moreover with the increasing scope and application of genetic and other techniques, some of the taxonomic information will probably be out of date already. We should be glad therefore if readers would draw such occurrences to our attention for subsequent correction.

Professor P. H. A. Sneath, who has himself contributed much to bacterial systematics and taxonomy, has kindly written a Foreword. On behalf of the International Union of Microbiological Societies Professor S. W. Glover gave permission to reproduce the Introduction from the *International Code of Nomenclature of Bacteria* and from the *Approved Lists of Bacterial Names*, and also the text of the *Report of the Ad Hoc Committee on the Reconciliation of Approaches to Bacterial Systematics*. We wish to express our thanks to him and to the IUMS executive. For proof-reading and checking so many tables,

we express our sincere thanks to Dr Joan M. Davies and, in particular to Dr B. Holmes who also contributed a large part of Chapter 7 on the Gram-negative bacteria and made many useful suggestions as did Dr Dorothy Jones. We also thank our many other colleagues, too numerous to name, for their help in many if unspecified ways. As before, Dr R. Sakazaki will translate this edition into Japanese and we are grateful to him for this. Last but not least we thank our wives and the staff of Cambridge University Press for their forbearance and support in what proved to be a long and arduous task. We hope that this new edition will be at least as useful as the previous two seem to have been.

Salisbury G.I.B.
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1992

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Introduction

It is assumed that the reader of this *Manual* has some knowledge and experience of bacteriology and of elementary chemistry and that the basic principles including those of laboratory safety are understood. Thus, though many other essential details are given in the Appendices, how to determine the pH value of a medium, or how to make a normal or molar solution, is not described; nor are details given about how to use anaerobic jars or microscopes. Serology is not discussed but methods commonly used in the preparation of extracts for grouping streptococci are described as the Lancefield serological groups are referred to in Table 6.3*b*. Details of sterilization temperatures and times are also given as these so-called standard procedures still vary from one laboratory to another.

This *Manual* is intended to help those who have isolated a bacterium and want to identify it. The methods used by clinical bacteriologists to isolate organisms from specimens sent to the laboratory are not described as to do so would be to enter ever-changing fields, and our recommendations might well be out of date. We stress, however, that before identification of any organism is attempted, it must be obtained in pure culture. Some advice on how to recognize that a culture is impure, and on the steps to be taken to purify it, is therefore given.

The tables for identification of medical bacteria developed in phases: the original tables of Cowan & Steel (1961) were based mainly on the results of tests carried out on strains in the National Collection of Type Cultures (NCTC) between 1948 and 1960. For the tables in the first edition of this *Manual*, the NCTC information was supplemented by surveys of the literature up to 1963; the second edition included the results of further literature surveys up to 1972; and for the present (third) edition, the literature up to 1990 has been reviewed by individual experts for each of the principal bacterial groups; where possible, new genera subsequently accepted, such as *Enterococcus* and *Helicobacter*, have been included. Each expert was asked to provide identification tables and methods suitable for use in routine diagnostic laboratories. It follows that in this edition we are less often able to indicate the relative value of the different technical methods used to obtain the characters shown in the tables. Once again, discrepancies occurred between the results of different workers – probably due more often to differences in methods than to variation between strains of the same species. To try to cover every possibility in the tables would be self-defeating, for either we multiply the columns (species or varieties) or we increase the number of equivocal or doubtful entries (d or D) equivalent to words like 'often', 'some(times)', or 'not infrequently', so that a clear positive or negative character would become rare and the tables thus confusing and unhelpful. We were tempted to use the percentage of positive results, as utilized for computer-assisted identification, but after careful consideration we felt that this would complicate the tables unnecessarily and be helpful only to a minority of readers. We have tried therefore to be definite and have treated descriptions such as 'occasionally', 'occasional strains' and 'a few strains' as exceptions not worthy of note. The tables are therefore not perfect as there are exceptions to all rules: it is the user who must be realistic and bear in mind that the bacteria they are trying to identify may not conform to the expected norm.

Intelligent use of the tables demands technical skill and sensitive but specific methods for the individual tests. As in all determinative bacteriology, true identification must be based on careful work, and the tables will not help those who are in too much of a hurry to

carry out the basic tests needed, though this does not necessarily mean every test in a table. We considered cutting down the tables to show only those characters that had immediate value in distinguishing one species from others in the tables, but we decided against this because conditions vary considerably in different laboratories and in different countries. We do not expect all the tests in the tables to be carried out; each bacteriologist has individual preferences and dislikes; and not all laboratories are equally well equipped. For these reasons many more characters than are necessary for identification have been included. We considered indicating the more important characters in bold type, but as this would merely reflect our own preferences we decided against it. Bacteriologists must choose those tests that seem to them to be most discriminating and use those that can be performed with the equipment and media available. As the tables are constructed from information from many sources, particular methods are not stipulated, but those given in the Appendices should be satisfactory. In this edition we have omitted many of the micromethods described in the first edition; those retained are included in Appendix C, together with methods using larger volumes (and often taking a longer time).

Three points should be emphasized about the tables. (i) They should not be considered in isolation; other evidence that cannot be included in them such as colony form, experimental pathogenicity, chromatographic profiles, chemotaxonomy and DNA hybridization results should also be taken into account. (ii) The tables do not characterize an organism; they are intended to focus attention on tests and characters most valuable in differentiation. (iii) The tables do not form part of any classification system, but they may draw attention to bacterial similarities and relationships that are not otherwise apparent. We have not been able to avoid taxonomic terms completely but a brief glossary of those in current use is included at the end of this *Manual*; for further information, Cowan's *Dictionary of microbial taxonomy* (Hill, 1978) should be consulted.

Names of species are not shown in the table headings but as numbered footnotes, and these include common synonyms so that, with the Index, it should be possible to find the main characters of many named species. The definitive names given in the new *Approved Lists of Bacterial Names* (Skerman, McGowan & Sneath, 1980) including validated changes and additions subsequently published in the *International Journal of Systematic Bacteriology* (IJSB), are used throughout. The older generic name '*Bacterium*' is not included and the term *Bacillus* is restricted to aerobic spore-formers.

In general, the tables allow identification of species that can often be further differentiated into serotypes, biotypes or phage types. However, users of the *Manual* will seldom have all the sera needed for detailed antigenic analysis of the species they isolate; this is a task for a reference laboratory. Those who aspire to do such work themselves should consult the excellent practical manual by Edwards & Ewing (1962, 1972) which highlights the problems and, for the Enterobacteriaceae, gives the essential details.

The tables seldom mention sensitivity or resistance to antibiotics although sometimes such tests are of practical differential value. In the present era, however, apart from selection pressure, the genetic effects of antibiotic therapy and usage are known to affect bacterial characters, including sensitivity or resistance to antibiotics, by transfer of plasmids.

We have tried to refer readers to pertinent literature in which fuller details of methods are given; in this way we have kept the *Manual* free from unnecessary detail and from the more theoretical aspects of taxonomy and nomenclature. We have, however, tried to retain where possible some of the nomenclatural life stories of many organisms, lest they be forgotten.

In plan, the *Manual* falls into two parts: the first, divided into chapters, is discursive; the second, made up of appendices, is instructive and written tersely with free use of scientific abbreviations (Ellis, 1971), chemical formulae, and prescription-like recipes for media. The essence of the book is in Chapters 6 and 7, which comprise the diagnostic tables and notes on the different genera. In this edition we include more little-known genera, many of them incompletely defined. It may seem that we have sometimes strayed outside the medical field, but many environmental organisms are now becoming important as opportunist pathogens especially with the widespread use of immunosuppressive therapy and the advent of AIDS and the Human Immunodeficiency Virus.

A practical example is given of the application of the diagnostic tables to punched cards for easy

sorting and bacterial identification. Reference is also made to the use of computers for rapid comparison and identification of isolates. In addition, the *Manual* now includes a short chapter on the quality control of laboratory procedures and reagents as well as on quality assessment, both within and between laboratories, with simulated material of known but undisclosed content.

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