
Maintenance of Microorganisms and Cultured Cells

A Manual of Laboratory Methods



Edited by
B E KIRSOP & A DOYLE

SECOND EDITION

Maintenance of Microorganisms and Cultured Cells

A Manual of Laboratory Methods
Second Edition

Edited by

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PREFACE TO THE SECOND EDITION

Microbiology and cell biology have always depended upon the maintenance of biological material to provide viable and stable cultures. The increasing use of organisms for industrial environmental purposes reinforces this need and makes effective culture maintenance an essential prerequisite.

The first edition of *Maintenance of Microorganisms* filled a gap in the scientific literature by bringing together in one volume *methods that had been developed and used successfully by those experienced in culture maintenance across a broad spectrum of biological material*. It included chapters on bacteria (of general, medical and industrial importance; anaerobes, methanogens and leptospira), fungi (including yeasts), algae and protozoa.

As predicted in the first edition, the second edition up-dates preservation methodology in the light of new research and developments and also expands the coverage. Most chapters have been substantially revised; new sections have been added to include contributions on the preservation of further groups of sensitive bacteria and on the use of a number of simple methods that can be carried out with inexpensive and readily available equipment. Sections on the maintenance of plasmid-bearing strains of bacteria and yeasts have been added, with particular attention being paid to genetic stability. New chapters on animal cells and plant tissue cultures, which are increasingly important in research and industrial manufacturing processes, have been included. To encompass this new material, the title of the book has been amended to *Maintenance of Microorganisms and Cultured Cells*.

As in the first edition, some repetition has been deliberately accepted in the interests of ease of use, but with increased coverage it is now even more important to recognize that successful techniques applied to one group of microorganism or cell type may well be applicable to other groups. Although cross-referencing has been made to other relevant sections, readers are advised not to confine their attention only to the contributions relating to the organisms of interest. Indeed, added value may well be gained from the cross-fertilization of ideas between specialist microbiologists that a compilation such as this allows.

As before, the volume remains a practical guide. In some chapters, where technology is still experimental and precise methodology for a wide range of species cannot yet be recommended, more background discussion is included. The chapters do not, therefore, all conform to an exact format and the editors

believe that, in the present state of the art, flexibility of this kind is necessary.

We thank the contributors who have written and revised the material and who through their willing support have made the compilation of this volume a pleasure.

B. E. Kirsop
A. Doyle

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1. INTRODUCTION

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The ability to preserve successfully a wide range of microorganisms and cell cultures has been a major achievement in biology over the last century that many have taken for granted. The need to conserve biological material such as blood cells, animal semen and starter cultures provided a strong stimulus to research, and the basic principles affecting cell survival and cell death have come nearer to being understood at the molecular level. As a result, culture preservation has reached a high level of success at a time when the needs of biotechnologists and environmental biologists have become more demanding.

Although a number of excellent preservation techniques have now been developed, they must be correctly applied if contamination, loss of viability and genetic change are to be prevented. Reliable stock cultures are needed for a variety of reasons. Teaching laboratories need a library of cultures that exhibit typical reactions; industrial laboratories need to maintain production strains, in addition to a bank of biological material for screening purposes; medical laboratories need reference strains both for routine testing in pathology laboratories and for research; taxonomists must maintain large numbers of cultures for comparative studies; and research laboratories need pure cultures for a wide range of purposes.

Despite the fundamental importance of a reliable supply of pure and stable cultures, culture preservation is often afforded low priority and is often carried out with inadequate staffing levels and inferior equipment. The importance of culture preservation and the value of investing in staff and equipment for this purpose is often only recognized when valuable or unique

strains are lost. Industrial, research or teaching collections are often housed in laboratories where culture maintenance is not a primary function and little time is available to develop or assess different preservation methods. The service culture collections, however, have a full commitment to the preservation of cultures and their accumulated expertise is of great value to others.

Information on preservation methods is thinly distributed through the literature and often not readily available; this manual assembles in one volume a collection of reliable preservation methods for a diverse range of organisms. Guidance is provided on the selection of the most appropriate method for particular circumstances. Technical details of methods are presented, and their reliability with regard to expected survival levels, shelf-life and stability is assessed. The relative costs of different methods, sources of equipment and materials, and the relevant literature references are given.

It must be stressed that there is no universal method for the successful preservation of all microorganisms and cell cultures. Different taxonomic groups, and even strains within a species, exhibit significant differences in response to the stresses imposed by culture preservation and resuscitation. The methods described in this manual, when used as described and for the defined group of organisms, will generally prove successful, but occasions will arise when further investigation will be needed to achieve complete success.

The manual is practical in nature and aims to provide details which the authors have found to work well. Each chapter covers a number of methods that have been found successful for a particular group of microorganisms or cell cultures. These methods may, however, prove successful for other cell types, and this should be borne in mind when using the book.

Repetition of detail occurs in a number of chapters, but since the book will be used primarily as a practical manual these have been deliberately retained to avoid the need for repeated reference to other sections to obtain essential methodology. The names and addresses of a number of suppliers of equipment quoted in the text are supplied in Appendix II. Although this list is not exhaustive, and is uneven in coverage of different geographical regions, it nevertheless provides a lead to possible suppliers. Further information can also be obtained by contacting any of the service collections listed in Chapter 2.

The support available to the scientific community from the service collections is not always recognized, and Chapter 2 provides a description of the role they play in microbiology. Further information on services in Europe can be obtained from the European Culture Collection Information Centre; world-wide information is available from the World Data Center on

Collections of Microorganisms or the Microbial Strain Data Network. Contact information on these centres is listed in Chapter 2.

The methods described in this volume provide reliable information on how to preserve cultures successfully; nevertheless, continued monitoring of viability levels, genetic stability and purity must be carried out for the cultures to serve as completely reliable inocula for experimental, industrial or educational purposes. Guidance is given in Appendix I on documentation that could be adopted for maintaining proper records.

It is the aim of this manual to raise the quality of the biological material provided by collections and, in turn, the level of scientific activity. It is hoped that the first edition has gone some way towards achieving this aim and that the additional information contained in this second edition will continue the process.

2. SERVICE COLLECTIONS: THEIR FUNCTIONS

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The main function of service culture collections is to act as depositories for all kinds of microorganisms that are of past, present or potential importance, so that resource and information centres for the general support of microbiology are established. The basic function of culture collections, therefore, is the collection, maintenance and supply of cultures.

The organisms collected reflect the interests of the collection and are obtained in one of several ways. Following searches through the scientific literature, direct application may be made to authors to deposit strains; alternatively, microbiologists may themselves make approaches to appropriate collections. Again, collections themselves may deposit cultures that have formed part of their own taxonomic or identification activities. Deposited cultures are checked for purity and authenticity before accession by the collection.

The selection of maintenance methods that produce maximum survival levels and strain stability is of fundamental importance, since strain drift in stocks maintained in service collections is unacceptable. Assessments of survival are made immediately after processing and at intervals during storage, so that fall-off in viability is monitored and an effective maintenance programme established. In order to assure a reliable service for the supply of cultures, quality control measures are carried out routinely. In addition, appropriate stock-holding levels are determined and administrative arrangements are established for handling orders and invoicing customers; postal regulations are followed for the despatch of cultures at home and overseas.

These activities, common to all service culture collections, generate a quantity of essential information that must be systematically recorded. Many collections are now using computers for this purpose. They are ideally suited to the storage and management of both customer records and stock supplies, and

greatly streamline the business administration of collections. Again, after appropriate coding, strain data is generally stored in a computer, allowing rapid searching and retrieval of scientific information, and establishing a database that may be used subsequently for computer identification. Computers are increasingly being used for the storage of literature references and are equally appropriate for the preparation of catalogues. An up-to-date catalogue is an essential requirement for the effective functioning of a service collection and, in the past, its preparation was an onerous and lengthy job. The transfer of catalogue data to a computer, however, allows ready updating for future editions and provides copy that can be printed directly from disk or tape, thus eliminating the need for conventional proof-reading.

In addition to the three basic activities (the collection, maintenance and supply of cultures, and the record-keeping associated with them), culture collections provide a number of other services. As a result of the expertise developed by collection staff and the close contacts established with microbiologists using their strains, collections become information centres for all matters relating to the microorganisms they hold. Many collections are now collaborating in the development of on-line databases and networks (see Table I) in order to make the information they hold readily available to the scientific community. In addition to supplying answers to written and telephoned enquiries, staff are called upon to lecture, help on courses and contribute to scientific journals and books. Much of the expertise existing in the collections is of a taxonomic nature and most service collections are able to provide an identification service. This is particularly valuable for microbiologists, since identification may be lengthy, expensive, demand sophisticated techniques, and yet only be required intermittently.

A further service of particular interest to industry is the provision of 'safe-deposit' facilities. Several collections are able to maintain important strains under optimum conditions, while providing access only to the depositor. This enables industry to depend on a reliable back-up for the preservation of strains that may be of crucial importance to production. Again, collections may provide a culture preservation service in which strains are expertly preserved and returned to the customer for storage. This may be useful both to industry, which is assured of a reliable supply of a 'standard inoculum' for production purpose, or to research in which certain experiments may require the use of aliquots of a single population of cells.

With the present rapid developments in biotechnology there is an increasing need to patent processes or, more recently, genetically engineered strains, and there is a need for recognized

Table I: Information centres.

*Regional***ICECC — for all information on the services provided by the European Culture Collections**

Information Center for European Culture Collections
Mascheroder Weg 1
4400 — Braunschweig
Germany

Telephone: +39-531-618715
Fax: +39-531-618718
Telex: 531104
Electronic mail: Telecom Gold 75: DBI0274

*International***MSDN — for international communications and links to databases related to microbiology and biotechnology**

Microbial Strain Data Network
Institute of Biotechnology
Cambridge University
307 Huntingdon Road
Cambridge CB3 0JX, UK

Telephone: +44-223-276622
Fax: +44-223-277605
Telex: 81240 Camspl G
Electronic mail: Telecom Gold 75: DBI0001

WDC — for a Directory of Culture Collections worldwide and other associated databases

World Data Center for Collections of Cultures of Microorganisms
RIKEN
2-1 Hirosawa, Wako
Saitama 351-01
Japan

Telephone: +81-484-62-1111
Fax: +81-484-64-5651
Electronic Mail: BT TYMNET 42: CDT0007

depositories. Many culture collections have now become accepted as International Depository Authorities and are able to provide the legal requirements for the purpose. Acceptance of collections for this role demands certain assurances regarding permanence,