Laboratory Methods in Food Microbiology

THIRD EDITION

Wilkie F. Harrigan

ACADEMIC PRESS

Laboratory Methods in Food Microbiology

3rd edition

by

W. F. Harrigan

San Diego London Boston
New York Sydney Tokyo Toronto

This book is printed on acid-free paper.

Academic Press
525 B Street, Suite 1900, San Diego, California 92101-4495, USA
http://www.apnet.com

Academic Press Limited
24–28 Oval Road, London NW1 7DX, UK
http://www.hbuk.co.uk/ap/

ISBN 0–12–326043–4

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

Typeset by LaserScript, Mitcham, Surrey
Printed in Great Britain by WBC Book Manufacturers, Bridgend, Mid-Glamorgan

98 99 00 01 02 03 WB 9 8 7 6 5 4 3 2 1

Laboratory Methods in Food Microbiology

3rd edition

This book is dedicated to my wife, Rita

Preface

This third edition continues to be written for a dual audience – first, food microbiologists working in the food industry and in microbiological quality assurance laboratories, and, second, final-year students taking degree and diploma courses and postgraduate students, in food science, food technology and allied subjects. Since the publication of the second edition, there has been great attention paid to quality management systems in general, and to Hazard Analysis Critical Control Point systems in particular. The subject of microbiological quality in this context has been discussed in another text (*Making Safe Food* by Harrigan and Park). In a company practising HACCP, many of the modern analytical methods could provide an opportunity for reasonably rapid monitoring. Nevertheless, there are still no microbiological methods that will permit on-line monitoring with feed-back control of the critical control points (CCPs) in a food processing line. However, microbiological monitoring of the effectiveness of the CCPs is essential for verification and validation, and many of the methods described in this edition are suitable for this purpose.

There has been increasing attention paid to the need to achieve international agreement on methodology and criteria for food quality. In food microbiology this started in earnest with the setting up of the International Committee (now Commission) on Microbiological Specifications for Foods and the start in the 1960s of publications by this organization. The uniting role of the ICMSF has made easier the task of the International Organization for Standardization in developing international standard methods (ISO standards). Nevertheless, many countries also use national standard methods that may or may not be in accord with ISO methods. On an international scale, in most quality assurance laboratories in the food industry and in government control agency laboratories, analyses still predominantly rely on the use of conventional methods for determining microbiological quality. With this edition, I am trying to encourage the use of ISO methods where appropriate. However, for many microbiological analyses an ISO method has not been agreed, and in these cases I have attempted to present the currently preferred method. Sometimes a national standard method is totally different from the ISO method, and occasionally an ISO method still requires modification and improvement. Where I have considered it necessary I have discussed the differences between ISO methods and standard methods of other origin (for example the methods of the Association of Official Analytical Chemists of the US).

In many quality assurance laboratories, rapid and instrumental methods are used – for example, electrometric methods, immunomagnetic capture, DNA probes and polymerase chain reaction (PCR) techniques. These methods are outlined in this edition. However, from my work in a number of countries (for example in Southeast Asia and South America), in both industrial consultancy and in presenting short courses for food microbiologists from industry and government regulatory agencies, it is obvious that in many countries such methodologies, especially those that involve either very expensive capital equipment (e.g., electrometric methods) or expensive diagnostic reagents (e.g., gene probe techniques) cannot be routinely applied, so that conventional methods will continue to be used for some time. International quality criteria will need to reflect this in the methods that are described within specifications. This edition, therefore, continues to describe the conventional methods of microbiological analysis, but easily adopted rapid diagnostic methods have also been included.

The book is intended to be capable of use as a bench-top reference for the vast majority of microbiological analyses, with adequate detail presented in Parts I to IV, and detailed recipes for reagents and media in Appendix 1.

Part I on 'Basic Techniques' includes sections outlining the management and safe operation of the food microbiology laboratory; the fundamentals of microscopy and staining; culture techniques; viable and total counts, including DEFT and its use with membrane filtration, ATP measurement, and impedance/conductance measurements; biochemical and physiological tests; serological methods including ELISA and magnetic immunocapture.

Part II provides the general (horizontal) techniques used to examine foods. The sections on detection and enumeration of pathogenic and toxigenic organisms have been extended to include, for example, the foodborne pathogenic vibrios, *Campylobacter jejuni*, *Listeria monocytogenes*, *Escherichia coli* O157, *Yersinia enterocolitica*.

Part III discusses the microbial ecology of different types of food, and the specific methodologies required by those different food commodity types. The examination of starting materials and end-products to establish conformance to specification is addressed.

Part IV provides schemes for the identification of bacteria, yeasts and moulds isolated from foods using non-selective media. When selective media are used to detect particular organisms, such as *Escherichia coli* or *Salmonella*, a shorter series of tests will often permit identification of the target organism; such identification procedures are described in the appropriate sections of Part II.

The Appendix of recipes for reagents and culture media will permit the food microbiologist to prepare most media and reagents from their basic ingredients.

I am indebted to my wife Rita, not only for her tremendous encouragement and assistance, but also for the patience shown as our house became knee-deep in paper, journals and reference books.

Contents

PART II: Techniques for the Microbiological Examination of Foods

PART III: Microbiological Examination of Specific Foods

PART IV: Schemes for the Identification of Microorganisms

PART I

Basic Methods

1

Introduction

As long ago as 1955 Wilson observed that 'it is far more important to lay down a strict code for the preparation or processing of food and see that it is carried out properly than to rely on bacteriological sampling of the finished product.'

Microbiological examinations in the laboratory must be seen in the wider context of the entire quality management system. This, in relation to the microbiological quality of foods, is the subject of *Making Safe Food* by Harrigan and Park (1991). Consequently it is suggested that the reader and user of this present book needs to set the scene and perform those microbiological examinations which can throw light on the efficacy of the quality management and hygienic management of the food production–storage–distribution system. Only in end-product examinations being performed to assess batches of food solely against microbiological specifications will the laboratory work be dissociated from a study of the plant operations.

1.1 THE ROLE OF THE MICROBIOLOGIST IN THE FOOD INDUSTRY

There are a number of aspects to the job of a food microbiologist. Some will be undertaken as teamwork with, for example, marketing personnel, chemists, engineers and so on:

1. Training factory personnel in the need for hygienic practices and proper procedures for cleaning and disinfection.
2. Production of relevant 'in-house' codes of practice and hygiene manuals, involving as necessary the assessment of detergent–disinfectants and sanitizers.
3. Involvement in new product development and in the development of new and modified production processes.
4. Surveying the microbiological condition of (i) raw materials and (ii) water.
5. Process control; also quality control on the finished products (i.e. control of distribution, if possible), or quality assurance on the finished products.
6. Provision of detailed storage instructions for the wholesaler and retailer, and of detailed storage and/or cooking instructions for the consumer.
7. Investigation of customer complaints of a microbiological nature.

Additionally, the food microbiologist may find himself/herself involved in:

(a) maintenance and preparation of microbial cultures used in the production of fermented foods;
(b) microbiological assay of vitamins, amino acids and protein quality, or of antimicrobial constituents, additives and contaminants;
(c) examination and control of factory effluent quality.

It can be seen from this list that a number of these duties require microbiological analysis of foods or components. At present microbiological analyses are destructive, so that 100% inspection of a batch is impossible. Analysis of casually taken samples will provide information about the microbiological condition of the samples themselves, but it will be difficult to draw conclusions from such results about the condition of the unexamined items of the same batches. Sampling procedures should be designed statistically; only then can the results of microbiological analysis provide a basis for statistically valid conclusions about the microbiological quality of the batches of foods from which the samples were drawn.

1.1.1 Microbiological quality criteria

These may be applied for the purposes of:

1. An assessment of spoilage potential and keeping quality/shelf-life of a food.
2. An assessment of the public health hazard of a food in terms of the presence of pathogens and also the presence of toxins or of toxigenic organisms.
3. Microbiological counts which can be related to the hygienic standards of production, and/or which may be considered in an aesthetic sense to be undesirable in the food.
4. Microbiological quality that relates to the food conforming to legal standards or specifications.

A consideration of any specific quality criterion in the first two categories above will show that the value taken as representing the boundary between an acceptable and an unacceptable microbial content depends not only on the past history of the foodstuff but also on assumptions concerning the likely handling and treatment of the rest of the batch in the future. Thus, for example, the tolerance for a population concentration of a given pathogen will be related to:

1. The minimum effective dose required to establish an infection. This depends on the susceptibility of the individual consumer: certain sectors of the community may be more readily infected than others (e.g. babies, old people, people recently under antibiotic therapy, and immunodeficient or immunocompromised people).
2. The amount of foodstuff normally consumed.
3. Whether the pathogen dies, survives or multiplies in the foodstuff.