



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.

Copyright © 1998 by W.F. Harrigan

All Rights Reserved.

No part of this publication may be reproduced or transmitted in any form or by any means electronic or mechanical, including photocopy, recording, or any information storage and retrieval system, without permission in writing from the publisher.

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

Preface

xi

PART I: Basic Methods

1	Introduction	3
1.1	The role of the microbiologist in the food industry	3
1.2	Microbiological specifications for foods	5
1.3	Microbiological analyses in the monitoring of quality management and of HACCP systems	6
1.4	Laboratory reports	6
2	Management and Operation of the Microbiological Laboratory	8
2.1	Safety precautions in the microbiological laboratory	8
2.2	Cleaning of glassware and apparatus	15
2.3	Sterilization of glassware, culture media, etc.	17
2.4	Evaluation of disinfectants	22
2.5	Quality control and quality assurance in the laboratory	26
3	Basic Microscopic Techniques	31
3.1	Use of the optical microscope	31
3.2	Staining methods	33
3.3	Examination of cultures for motility by 'hanging drop' preparations	39
3.4	Examination of microbial colonies	40
3.5	Fluorescence microscopy	41
4	Cultivation of Microorganisms	43
4.1	Types of culture	43
4.2	Method of inoculation: aseptic technique	44
4.3	Incubation of cultures	45
4.4	Maintenance of pure cultures in the laboratory	45
4.5	Plate cultures	47
4.6	Description of the morphological and cultural characteristics of microorganisms	49

5	Determination of the Number, and Detection, of Viable Microorganisms in a Sample	52
5.1	Colony count methods	53
5.2	Membrane filtration	61
5.3	Most probable number (MPN) counts	63
5.4	Dye reduction methods	67
5.5	Electrometric methods	68
5.6	Nucleic acid probes and the polymerase chain reaction	69
6	Determination of the Total Number of Microorganisms in a Sample	71
6.1	The Breed's smear method for direct microscopic counts	71
6.2	Direct microscopic counts by membrane filtration	73
6.3	Direct epifluorescent filter technique (DEFT)	74
6.4	Flow cytometry	74
6.5	ATP determination by bioluminescence	75
6.6	Turbidimetric methods	76
7	Composition of Culture Media	79
7.1	Introduction	79
7.2	Dehydrated media	80
7.3	Determination and adjustment of the pH of culture media	80
7.4	Examples of non-selective culture media	81
7.5	Separation of mixed cultures: enrichment procedures, elective and selective media	83
7.6	Examples of selective culture media	85
7.7	Heat sterilization of media and diluents	87
8	Sampling Methods for the Selection and Examination of Microbial Colonies	89
8.1	Non-selective media	89
8.2	Selective and differential media	90
9	Methods of Anaerobic Culture	92
9.1	Robertson's cooked meat medium	92
9.2	Shake cultures	93
9.3	Semi-solid media	93
9.4	Vaseline, paraffin wax and agar seals	94
9.5	The anaerobic jar	94
10	Cultivation in Microaerobic and Carbon Dioxide-enriched Atmospheres	98
10.1	Cultivation in microaerobic atmospheres	98
10.2	Cultivation in a carbon dioxide-enriched atmosphere	99
11	Biochemical Tests for Identification of Microorganisms	100
11.1	Reactions involving protein, amino acids and other nitrogen compounds, including tests for proteolytic activity	100
11.2	Reactions involving carbohydrate and other carbon compounds	107
11.3	Reactions involving lipids, phospholipids and related substances	111
11.4	Tests for the presence of active enzymes	113
11.5	Miscellaneous tests	115
12	Physiological Tests	119
12.1	Introduction	119

12.2	Growth rate determinations on pure cultures	119
12.3	Temperature	120
12.4	Effect of heat on microorganisms: the determination of decimal reduction times (D values) and <i>z</i> values	123
12.5	pH tolerance	127
13	Serological Methods	130
13.1	Introduction	130
13.2	Slide agglutination tests	131
13.3	The milk ring test for <i>Brucella abortus</i> and <i>Br. melitensis</i>	133
13.4	Enzyme-linked immunosorbent assays	134
13.5	Concentration of microorganisms by immunocapture	134
13.6	The precipitin test	135
14	Moulds and Yeasts	137
14.1	General conditions for the growth of moulds and yeasts	137
14.2	Media for the growth of moulds and yeasts	137
14.3	Examination of moulds	138
14.4	Examination of yeasts	139
14.5	Identification of moulds and yeasts	139

PART II: Techniques for the Microbiological Examination of Foods

15	Introduction	143
16	Methods of Sampling and Investigation	147
16.1	Liquid samples	147
16.2	Solid samples	147
16.3	Sampling of surfaces	148
16.4	Sampling for anaerobic bacteria	151
16.5	Attributes sampling plans	152
16.6	Choice of samples on a non-random basis	154
16.7	Transport and storage of samples	155
17	Preparation of Dilutions	156
17.1	Choice of diluent	156
17.2	Liquid samples	158
17.3	Fine particulate solid samples	158
17.4	Other solid samples	158
18	General Viable Counts	160
18.1	Aerobic mesophilic counts	162
18.2	Yeast and mould counts	162
19	Psychrotrophic, Psychrophilic and Thermophilic Counts	163
20	Detection and Enumeration of Indicator and Index Organisms	164
20.1	'Total Enterobacteriaceae', coliform organisms and <i>Escherichia coli</i>	165
20.2	<i>Enterococcus</i> ('faecal streptococci')	171
20.3	<i>Bacillus</i> as indicator organisms	173

21	Detection and Enumeration of Pathogenic and Toxigenic Organisms	175
21.1	Introduction	175
21.2	Quantification of selective isolation techniques	179
21.3	<i>Salmonella</i> and <i>Shigella</i>	180
21.4	Pathogenic <i>Escherichia coli</i>	186
21.5	<i>Yersinia enterocolitica</i>	189
21.6	<i>Vibrio</i>	191
21.7	<i>Clostridium perfringens</i>	194
21.8	<i>Campylobacter jejuni</i>	195
21.9	<i>Listeria monocytogenes</i>	198
21.10	<i>Staphylococcus aureus</i>	201
21.11	<i>Bacillus cereus</i>	202
21.12	<i>Clostridium botulinum</i>	204
21.13	Other pathogenic and toxigenic bacteria	205
21.14	Toxigenic microfungi and their mycotoxins	207
21.15	Protozoan and helminthic parasites	208
21.16	<i>Brucella</i>	209
21.17	<i>Mycobacterium bovis</i>	210

PART III: Microbiological Examination of Specific Foods

22	Introduction: Effect of the Food Environment on Constituent Microflora	213
23	Raw Meat and Raw Meat Products	214
23.1	Fresh, chilled and frozen raw meats	214
23.2	Raw sausages, burgers, ground beef and other comminuted raw meat products	216
23.3	Meats and meat products preserved by curing and pickling	217
24	Cooked Meat Products	220
24.1	Sliced cooked meats	220
24.2	Cooked meat pies, meat casseroles, etc.	220
25	Poultry and Poultry Products	222
25.1	Refrigerated poultry	222
25.2	'New York' dressed poultry and game	223
25.3	Cured and smoked poultry products, poultry sausages, etc.	224
25.4	Microbiological examinations	224
26	Eggs and Egg Products	225
26.1	Introduction	225
26.2	Shell eggs	225
26.3	Frozen whole egg	226
26.4	Dried egg	226
26.5	Frozen, dried and flake albumen	227

27	Fish, Shellfish and Crustacea	228
27.1	Introduction	228
27.2	Salt-water fish	228
27.3	Freshwater fish	229
27.4	Smoked, marinated and pickled fish	230
27.5	Shellfish and crustacea	230
27.6	Recommended specifications	232
28	Milk and Milk Products	234
28.1	Liquid milk	234
28.2	Milk powder	250
28.3	Canned, concentrated milk	252
28.4	Cream	254
28.5	Dairy starter cultures	255
28.6	Fermented milks	260
28.7	Cheese	263
28.8	Butter	269
29	Ice-cream and Frozen Desserts	272
29.1	Collection of samples	272
29.2	Treatment of samples	273
29.3	Cultural examination	273
30	Fruits, Nuts and Vegetables	275
30.1	Introduction	275
30.2	Vegetables	276
30.3	Nuts	278
30.4	Fruit	279
30.5	Sugars and sugar syrups	282
30.6	Chocolate and chocolate confectionery, sugar confectionery	283
30.7	Salted, pickled and fermented vegetables	283
30.8	Spices and condiments	284
31	Alcoholic Beverages	285
32	Breads, Cakes and Bakery Goods	287
32.1	Examination for moulds	288
32.2	Examination of compressed bakers' yeast	288
32.3	Examination of stored cereal grains	289
32.4	Examination of flour	290
32.5	Pasta	291
33	Convenience Meals	292
33.1	Introduction	292
33.2	Microbiological examinations	293
33.3	Microbiological specifications	293
34	Canned Foods	294
34.1	Introduction	294
34.2	Procedure for microbiological examination	295
34.3	Direct determination of <i>F</i> values	296

35	Water	298
35.1	Introduction	298
35.2	Hazards from water used for consumption or for the preparation and production of food	299
35.3	Bottled mineral waters	300
35.4	Sampling procedure for water from taps, stand-pipes, etc.	301
35.5	Cultural examinations	302
35.6	Membrane filtration methods	304
35.7	Microbiological specifications	304
35.8	Special requirements for food manufacture	304
36	Examination of Food Processing Plant	306
36.1	Introduction	306
36.2	Examination of processing plant, equipment, working surfaces, etc.	307
36.3	Examination of washed bottles and food containers	309
36.4	Air	311
36.5	Detection of <i>Salmonella</i> in processing plant effluents	312

PART IV: Schemes for the Identification of Microorganisms

37	Introduction	315
38	A Scheme for the Identification of Gram-negative Bacteria	317
38.1	Gram-negative bacteria that can grow on nutrient agar or plate count agar	317
38.2	Gram-negative bacteria that grow on glucose yeast extract agar, wort agar, malt extract agar or similar media	331
38.3	Bacteria requiring at least 12% sodium chloride for growth	332
39	A Scheme for the Identification of Gram-positive Bacteria	333
40	A Scheme for the Identification of Yeasts and Moulds	349
40.1	Identification of yeasts	350
40.2	Identification of filamentous microfungi	359
	Appendix 1: Recipes for Stains, Reagents and Media	377
	Appendix 2: Probability Tables for the Estimation of Microbial Numbers by the Multiple Tube Technique	479
	ISO Standards for Microbiological Methods	486
	References	491
	Subject index	519

PART I

Basic Methods

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.