RAPID ANALYSIS TECHNIQUES
IN FOOD MICROBIOLOGY

Rapid Analysis Techniques in Food Microbiology

Edited by

P.D. PATEL
Leatherhead Food Research Association
Surrey

BLACKIE ACADEMIC & PROFESSIONAL
An Imprint of Chapman & Hall
London · Glasgow · Weinheim · New York · Tokyo · Melbourne · Madras

Published by
Blackie Academic & Professional, an imprint of Chapman & Hall,
Wester Cleddens Road, Bishopbriggs, Glasgow G64 2NZ

Chapman & Hall, 2—6 Boundary Row, London SE1 8HN, UK

Blackie Academic & Professional, Wester Cleddens Road, Bishopbriggs, Glasgow G64 2NZ, UK

Chapman & Hall GmbH, Pappelallee 3, 69469 Weinheim, Germany

Chapman & Hall USA, 115 Fifth Avenue, Fourth Floor, New York, NY 10003, USA

Chapman & Hall Japan, ITP-Japan, Kyowa Building, 3F, 2-2-1 Hirakawacho, Chiyoda-ku, Tokyo 102, Japan

DA Book (Aust.) Pty Ltd, 648 Whitehorse Road, Mitcham 3132, Victoria, Australia

Chapman & Hall India, R. Seshadri, 32 Second Main Road, CIT East, Madras 600 035, India

First edition 1994
Reprinted 1995

Typeset in 10/12pt Times by Greenshires Icon, Exeter, Devon
Printed in Great Britain by St Edmundsbury Press, Bury St Edmunds, Suffolk

ISBN 0 7514 0030 0

A catalogue record for this book is available from the British Library
Library of Congress Catalog Card Number: 94-70712

∞ Printed on acid-free text paper, manufactured in accordance with ANSI/NISO Z39.48-1992 (Permanence of Paper)

RAPID ANALYSIS TECHNIQUES IN FOOD MICROBIOLOGY

Edited by P. Patel

BLACKIE ACADEMIC & PROFESSIONAL
An Imprint of Chapman & Hall

Preface

The food industry, with its diverse range of products (e.g. short shelf-life foods, modified atmosphere packaged products and minimally processed products) is governed by strict food legislation, and microbiological safety has become a key issue. Legally required to demonstrate 'due diligence', food manufacturers are demanding analytical techniques that are simple to use, cost effective, robust, reliable and can provide results in 'real time'.

The majority of current microbiological techniques (classical or rapid), particularly for the analysis of foodborne pathogens, give results that are only of retrospective value and do not allow proactive or reactive measures to be implemented during modern food production. Rapid methods for microbial analysis need to be considered in the context of modern Quality Assurance (QA) systems.

This book addresses microbiologists, biochemists and immunologists in the food industry, the public health sector, academic and research institutes, and manufacturers of kits and instruments. This volume is an up-to-date account of recent developments in rapid food microbiological analysis, current approaches and problems, rapid methods in relation to QA systems, and future perspectives in an intensely active field.

P.D.P.

Contributors

F.J. Bolton Public Health Laboratory, Royal Preston Hospital, PO Box 202, Sharoe Green Lane North, Preston PR2 4HG, UK.

D. M. Gibson Ministry of Agriculture, Fisheries and Food, Torry Research Station, 135 Abbey Road, Aberdeen AB9 8DG, Scotland.

P.A. Hall Microbiology and Food Safety, Kraft General Foods, 801 Waukegan Road, Glenview, Illinois 60025, USA.

W.E. Hill Seafood Products Research Center, Seattle District Office, Food and Drug Administration, Bothell, Washington 98041-3012, USA.

A.D. Hocking CSIRO Food Research Laboratory Division of Food Science and Technology, PO Box 52, North Ryde, New South Wales 2113, Australia.

A.L. Kyriakides J. Sainsbury plc, Scientific Services Division, Stamford House, Stamford Street, London SE1 9LL, UK.

C.M.L. Marengo Joint Research Centre of the European Community, Ispra, Italy.

D.A.A. Mossel Eijkman Foundation, Utrecht University, PO Box 6024, 3503 PA Utrecht, The Netherlands.

S.H. Myint Department of Microbiology, University of Leicester, Leicester LE1 9HN, UK.

Ø. Olsvik Norwegian College of Veterinary Medicine, Oslo, Norway.

P.D. Patel Rapid Methods Section, Leatherhead Food Research Association, Randalls Road, Leatherhead, Surrey KT22 7RY, UK.

J.I. Pitt CSIRO Food Research Laboratory, Division of Food Science and Technology, PO Box 52, North Ryde, New South Wales 2113, Australia.

A.N. Sharpe Bureau of Microbial Hazards, Food Directorate, Health Protection Branch, Health and Welfare Canada, Ottawa, Ontario K1A 0LZ, Canada.

C.B. Struijk Eijkman Foundation, Utrecht University, PO Box 6024, 3503 PA Utrecht, The Netherlands.

D. W. Williams Rapid Methods Section, Leatherhead Food Research Association, Randalls Road, Leatherhead, Surrey KT22 7RY, UK.

Contents

1 History of and prospects for rapid and instrumental methodology for the microbiological examination of foods **1**
D.A.A. MOSSEL, C.M.L. MARENGO and C.B. STRUIJK

1.1 Introduction 1
1.2 The contemporary role and the character of microbiological examination of food samples 4
1.2.1 Principles 4
1.2.2 The part to be played by 'rapid' methods – semantics 5
1.3 Pitfalls in introducing 'rapid' methods 6
1.4 An overall appraisal of analytical principles suggested 9
1.5 Suitability of the most promising, available principles 14
1.5.1 Enumeration 14
1.5.2 Presence-or-absence tests 15
1.5.3 Identification 16
1.6 Achievements and prospects 1994 16
1.6.1 Overview 16
1.6.2 A truly real time check on adequate 'sanitation' 17
1.6.3 Theorising on a *Quo vadis* – a presumptuous exercise 18
1.7 Impact for education at undergraduate and postgraduate level 19
References 20

2 Development and evaluation of membrane filtration techniques in microbial analysis **29**
A.N. SHARPE

2.1 Brief history of membrane filters 29
2.2 Early uses of membrane filters 30
2.3 Inter-brand variability of membrane filters 36
2.4 Current status of membrane filter methods 36
2.5 Analyses based on membrane filters 37
2.5.1 *Campylobacter* species 37
2.5.2 *Escherichia coli* biotype 1 count 38
2.5.3 *Pediococcus* species 41
2.5.4 Epifluorescence microscopy 41
2.6 HGMF 43
2.6.1 Numerical range and accuracy of the HGMF 45
2.6.2 Counting and scoring HGMFs 46
2.6.3 Some well-established HGMF-based techniques 46
2.6.4 HGMFs in very practical HACCP situations 50
2.7 Millipore samplers 51
References 54

3 Evaluation of commercial kits and instruments for the detection of foodborne bacterial pathogens and toxins **61**
P.D. PATEL and D.W. WILLIAMS

3.1 Introduction 61

3.2 Detection of foodborne pathogens 62
3.2.1 *Salmonella* and *Listeria* 63
3.2.2 *Campylobacter* 79
3.2.3 *Escherichia coli* 81
3.2.4 *Yersinia enterocolitica* 85
3.3 Detection of bacterial toxins 88
3.3.1 Staphylococcal enterotoxins 89
3.3.2 Enterotoxin kits for enteric pathogens 92
3.4 Future perspectives 96
References 97

4 Microbiological applications of immunomagnetic techniques 104
P.D. PATEL

4.1 Introduction 104
4.2 ImmunoDynabeads for separation and concentration of *Salmonella* 105
4.2.1 Development of *Salmonella* immunoDynabead particles 105
4.2.2 Evaluation of *Salmonella* immunoDynabeads in food enrichment broths 106
4.2.3 *Salmonella* immunoDynabeads and alternative detection systems 113
4.3 Potential of novel colloidal magnetic fluids in microbial analysis 118
4.3.1 Antibody-based systems for *Salmonella* and *Listeria* 119
4.3.2 Lectin-based systems for food-spoilage yeasts 119
4.4 Immunomagnetic detection of bacterial toxins 124
4.4.1 Staphylococcal enterotoxins (SE) 124
4.4.2 *Clostridium perfringens* enterotoxin 125
4.5 Future perspectives 127
Acknowledgements 128
References 128

5 Automated electrical techniques in microbiological analysis 131
F.J. BOLTON and D.M. GIBSON

5.1 Introduction 131
5.2 What do the instruments measure? 131
5.3 When are measurements made? 132
5.4 Are assays faster than those by conventional tests? 132
5.5 Current instrumentation and systems 133
5.5.1 Bactometer 134
5.5.2 BacTrac 136
5.5.3 Malthus 136
5.5.4 RABIT 137
5.6 Instrument outputs 138
5.6.1 Data 138
5.6.2 Computers and software 140
5.7 Spoilage assays 140
5.7.1 Total viable flora 140
5.8 Detection and enumeration of indicator organisms 142
5.8.1 Enterobacteriaceae 143
5.8.2 Coliforms 145
5.8.3 *Escherichia coli* 147
5.8.4. Enterococci 149
5.9 Detection of pathogens 149
5.9.1 *Salmonella* spp. 149
5.9.2 *Listeria* spp. 156
5.9.3 *Campylobacter* spp. 160
5.9.4 Other pathogens 162
5.10 Indirect conductimetry 163

5.10.1 Principle 163
5.10.2 Detection of yeasts and moulds 164
5.10.3 Other applications 166
5.11 Future trends 166
Acknowledgements 167
References 167

6 Modern methods for the detection of viruses in foods 170
S. H. MYINT

6.1 Introduction 170
6.2 Background 170
6.3 Preparation of samples 173
6.3.1 General principles 173
6.3.2 Methods 173
6.3.3 Immunomagnetic separation 180
6.4 Cell culture 180
6.5 Immunoassays 181
6.6 Nucleic acid hybridisation 183
6.6.1 Principles 183
6.6.2 Methods applied to foods 184
6.7 Gene amplification 186
6.7.1 Target amplification 186
6.7.2 Probe amplification 188
6.7.3 Applications of PCR to foods 189
6.8 Other methods 192
6.9 Why look for viruses in foods? 192
6.10 Future prospects 193
References 193

7 Luminescent techniques for microbiological analysis of foods 196
A.L. KYRIAKIDES and P.D. PATEL

7.1 Introduction 196
7.2 ATP bioluminescence 197
7.2.1 ATP bioluminescence applications in modern food hygiene 198
7.2.2 ATP bioluminescence for raw material testing 206
7.2.3 ATP bioluminescence for end-product testing 215
7.2.4 Other applications 221
7.3 Bacteriophage bioluminescence 223
7.3.1 Detection of pathogens and indicator microorganisms 224
7.3.2 Detection of inhibitory substances 225
7.4 Future trends 226
References 227

8 Modern methods for detecting and enumerating foodborne fungi 232
J.I. PITT and A.D. HOCKING

8.1 Introduction 232
8.2 Cultural methods 232
8.2.1 Direct plating 232
8.2.2 Dilution plating 233
8.2.3 Modern media 234
8.2.4 Special groups 240
8.3 Rapid methods 243
8.3.1 Detection of secondary metabolites 243

8.3.2 Estimation of fungal biomass 245
8.3.3 Molecular methods 250
References 250

9 Scope for rapid microbiological methods in modern food production 255
P.A. HALL

9.1 Introduction 255
9.2 Traditional approach to microbiological control 256
9.3 HACCP approach to microbiological control 256
9.4 Regulatory perspectives 259
9.5 Future directions 262
References 266

10 Detection and identification of foodborne microbial pathogens by the polymerase chain reaction: food safety applications 268
W.E. HILL and Ø. OLSVIK

10.1 Introduction 268
10.2 Foodborne diseases 269
10.3 PCR fundamentals 269
10.4 PCR: some practical considerations 272
10.4.1 Samples 272
10.4.2 Primers 272
10.4.3 Reaction conditions 272
10.5 PCR format variations and applications 273
10.5.1 Multiplex PCR 273
10.5.2 Nested PCR 273
10.5.3 Reverse transcription PCR 273
10.5.4 Ligase chain reaction (LCR) 273
10.5.5 Detection of immobilised amplified nucleic acid (DIANA) 274
10.6 Immunomagnetic separation and PCR 274
10.7 PCR detection and identification of foodborne microorganisms: bacteria 275
10.7.1 *Campylobacter* 276
10.7.2 *Clostridium botulinum* 276
10.7.3 *Escherichia coli* and *Shigella* spp. 277
10.7.4 *Listeria monocytogenes* 278
10.7.5 *Salmonella* 278
10.7.6 *Staphylococcus aureus* 279
10.7.7 *Vibrio* spp. 279
10.7.8 *Yersinia enterocolitica* and *Y. pseudotuberculosis* 280
10.8 PCR detection and identification of foodborne microorganisms: viruses 281
10.8.1 Norwalk virus 281
10.8.2 Rotavirus 281
10.8.3 Hepatitis A virus (HAV) 282
10.8.4 Enteroviruses 282
10.9 PCR detection and identification of foodborne microorganisms: parasites 282
10.9.1 Giardia 283
10.9.2 Entamoeba 283
10.10 Summary 283
Acknowledgements 283
References 284

Index 291

1 History of and prospects for rapid and instrumental methodology for the microbiological examination of foods

D.A.A. MOSSEL, C.M.L. MARENGO and C.B. STRUIJK

1.1 Introduction

The modest start, made in the 1920s, with microbiological safety assurance – initially of milk and dairy products, later, to a lesser extent, of other foods – was modelled after the well-established matrix of ensuring the chemical integrity of food products. This originated from a branch of science termed 'bromatology'. It attempted to attain two main purposes: (i) to avoid the ingestion of foods contaminated with toxic elements including arsenic, mercury and lead; and (ii) to control the nutritive value of staple foods by detecting elevated water content or increasing the weight by adulteration, i.e. the addition of non-nutritive materials. The public was protected against such frauds by monitoring the food supply at points of sale. If a contaminant or untoward stretching was observed, the food was eliminated from the trade. This strategy was quite successful in assuring the chemical safety and quality of foods for two reasons: (i) the unwanted constituents were rather homogeneously distributed in the food, so that any sample of sufficient size drawn from a consignment for analysis represented the lot; and (ii) the concentration of the analytes sought was fairly constant in time, further contributing to the reliability of data obtained on samples (Mossel *et al.*, 1994).

It could have been anticipated, right from the beginning, that this scenario could not even be *expected* to be applicable to ensuring microbiological food safety. First and foremost none of the circumstances identified above as contributory factors to the efficacy of the retrospective approach apply in microbiology. In the vast majority of foods, microorganisms are erratically distributed, depriving negative results of tests of *any* significance (Mossel and Drion, 1954; Habraken *et al.*, 1986). Moreover, except for endospores of bacteria and ascospores of moulds and yeasts, microbial populations of foods bear an outspoken dynamic character: as a rule they either decrease or increase in numbers of viable cells during storage and distribution (Mossel and Struijk, 1992), making the prediction of the microbiological condition of foods at the moment of ingestion quite insecure. This awkward situation was compounded by a tremendous shortage of readily available reliable analytical techniques (Mossel, 1987). Whereas bromatological examination of foods dated back to early in the 19th century, selective-diagnostic methods required in the microbio-

logical monitoring of foods had to be borrowed from clinical microbiology until about 1960 – and still partly have to. Finally, while in bromatology, as well as clinical microbiology, one discipline, i.e chemistry and medicine respectively, was responsible for scientific progress and strategic decisions, food microbiology was practised by six different professional groups. These include food science, veterinary medicine, pharmacy, biology, agricultural sciences and, to a lesser extent, medicine, which markedly hampered progress and above all the elaboration of effective management policies (Mossel, 1991a).

It was therefore not at all surprising that the transmission of foodborne diseases with a microbial aetiology was far from being brought under control (Mossel, 1989; Bean and Griffin, 1990; Skjerve and Johnson, 1991; Bautista *et al.*, 1992; Du Pont, 1992). On the contrary, intoxinations provoked by *Staphylococcus aureus*, *Bacillus cereus* and a few allied bacilli and a scala of pressor-amine-producing bacteria (Mossel *et al.*, 1994), but particularly the incidence of the most prevalent food-transmitted infectious enteric disease – salmonellosis – increased rather than decreased (Hedberg *et al.*, 1991; Tauxe, 1991; Luby *et al.*, 1993). Meanwhile, the aggressive serotype *Salmonella enteritidis* came to the fore (Hedberg *et al.*, 1991; Barnes and Edwards, 1992; Van der Giessen *et al.*, 1992; Vugia *et al.*, 1993). It was joined by a multitude of enteropathogenic agents whose aetiological role was identified, or that re-emerged, since the early 1960s. A selection of the most striking examples is collected in Table 1.1. This dim picture was aggravated by the identification of a broad spectrum of systemic complications, often very serious, occurring as a sequel to

Table 1.1 A few enteropathogenic agents transmitted by foods, identified after about 1960, when salmonellae, *Staphylococcus aureus*, *Clostridium perfringens* and *Clostridium botulinum* had been well established as foodborne pathogens

Pathogen	Main source of transmission	Reference
Adenovirus 40, 41	Faecal contamination	Jarecki-Khan *et al.* (1993)
Aeromonas hydrophila	Waterborne contamination	Thomas *et al.* (1990)
Astrovirus	Faecal contamination	Lew *et al.* (1991)
Campylobacter spp.	Chicken and pork	Nachamkin *et al.* (1992)
Citrobacter spp.	Faecal contamination	Schmidt *et al.* (1992)
Cryptosporidium parvum	Calf, lamb, poultry, pig; waterborne contamination	Gatti *et al.* (1993)
Cyclospora cayetanensis	Not yet clearly established	Bean and Griffin (1990); Long *et al.* (1991)
E. coli, enterohaemorrhagic pathotype(s)	Beef	Le Saux *et al.* (1993)
Hafnia alvei	Not yet clearly established	Westblom and Milligan (1992); Albert *et al.* (1992a); Reina *et al.* (1993)
Listeria monocytogenes, serotype 4b	Ubiquitous in the farm environment	Amgar (1991); Goulet *et al.* (1993)
Norwalk virus group	Faecal contamination	Kapikian (1993)
Providencia spp.	Faecal contamination	Albert *et al.* (1992b)
Shigella spp.	Faecal contamination	Hedberg *et al.* (1992b)
Toxocara canis	Animal environment	Salem and Schantz (1992)
Vibrio vulnificus	Waterborne contamination	Wachsmuth *et al.* (1993)

Table 1.2 Recommendations for microbiological safety assurance of foods, relying on intervention, made since about 1930

Country and era	Reference	Pathogen to be brought under control	Food vehicle
USA 1920–1935	Meyer (1931)	*Clostridium botulinum*	Canned vegetables and cured meats
	Prescott (1920)	General	Dried foods
UK 1930–1935	Wilson (1955, 1964)	Enterobacteriaceae, *Mycobacterium bovis*, group A streptococci, *Corynebacterium diphtheriae* (*Listeria monocytogenes*)	Milk, ice cream, cheese, egg products
France 1953–1956	Buttiaux *et al.* (1956) Cheftel (1955)	*Clostridium botulinum*, *Staphylococcus aureus*, Enterobacteriaceae, *Clostridium perfringens*	Canned, large size hams, infant food
USA 1960	Dack (1956)	*Salmonella* spp. and allied enteropathogens	General
Netherlands 1975–1980	Kampelmacher (1983)	*Salmonella* spp.	Fresh and frozen poultry
USA	Roberts (1985)	*Campylobacter* spp.	
Netherlands 1978–1985	Van Netten *et al.* (1984) Smulders *et al.* (1986)	*Salmonella*, *Campylobacter*, *Yersinia* and enterovirulent *Escherichia coli*	Fresh meat and poultry

a primary spell of gastroenteritis, in itself of a relatively mild nature (Mossel, 1989; Mossel *et al.*, 1994).

There was, nonetheless, no shortage, in academic circles, of recognition of the futility of simply mimicking, in attempts to assure microbiological safety, what had ensured chemically sound food. As shown in Table 1.2, since about 1920 professorial ranks in the USA, the UK and France alike have emphasised that the retrospective approach had to be replaced by a prospective one (Mossel, 1989). Their messages were not heeded, however, until the 1970s. At that time Dr H. Bauman, chief microbiologist for a leading American food manufacturing company, suggested a complete change in course with respect to ensuring microbiological food safety (Bauman, 1974). Instead of relying on *post mortem* inspections of doubtful significance of samples of uncontrolled history, he advocated the introduction of a forward control strategy. Microbiological hazards had to be identified and faulty practices and procedures to be rectified before any monitoring would make sense. Bauman introduced the term 'hazard analysis and control of critical points', abbreviated to HACCP (Bauman, 1990). This strategy became extremely popular and is now, some 20 years after its introduction, generally accepted by professional circles (Amgar, 1992; Bryan, 1992; Mossel *et al.*, 1992; Pierson and Corlett, 1992; Shakespeare *et al.*, 1992; Macler and Regli, 1993). In Europe, Lord Hugo Plumb of Coleshill, a leading politician with an agricultural background, strongly recommended to *extend* HACCP from raw material to, and including, serving – 'from farm to fork' (Mossel, 1991b; Mossel and Struijk, 1992; Altekruse *et al.*, 1993). The term 'longitudinally integrated safety assurance', or LISA, had earlier been suggested for this most reasonable and effective strategy (Mossel, 1983; Jakobsen and Lillie, 1992).

1.2 The contemporary role and the character of microbiological examination of food samples

1.2.1 Principles

Substitution of the forward control approach for the ineffective retrospective scenario also completely changed the role of microbiological monitoring of foods. Had this previously and unsuccessfully been used to *attain* a safe food supply, it would henceforth serve to *assess* whether good manufacturing and distribution practices had been strictly followed. It would *inter alia* be utterly unwise to refrain from such validation steps within the HACCP framework. First and foremost, unfortunately, food manufacturers and caterers far too frequently fail to allow well established practice guidelines to guide practice *at all*. In many instances the LISA-maxim has indeed been adopted, but incidental breakdown of effective control may nonetheless sometimes occur, due to instrumental or human failure. Such hiatuses as a rule bring about only minor adverse effects,

but may sometimes entail dramatic consequences and most expensive recalls of distributed merchandise. The earlier reliable data, confirming or refuting adherence to safe practices, are obtained, the more rapidly rectification can be applied and, consequently, the more consistently will the public be protected against products that have lost their microbiological integrity.

This calls for the introduction of a few essential elements into microbiological inspection of food samples. First of all, examination of line specimens including the food production environment (Slade, 1992) has an absolute priority over analysing finished products. Moreover, data should become available as fast as possible, because it allows earliest corrective action to be taken against hiatuses. It is therefore not at all surprising that food microbiology, ever since the 1970s, has been challenged to achieve the same speed, reliability and facility that chemical examination of foods has displayed since the introduction of the first 'auto-analyser'.

In addition, it is worth noting that acceptance of, and adherence to, the HACCP/LISA strategy will ensure that the majority of the samples reaching the laboratory are of good microbiological quality. Consequently, a very minor fraction will be found contaminated or colonised at a high level: in popular laboratory jargon most specimens will give 'negative results'. Laboratory procedures have to be geared to this situation, which is essentially different from that prevailing in the pre-proactive scenario era, when many trade samples would, unfortunately, contain high levels of organisms of concern.

1.2.2 The part to be played by 'rapid' methods – semantics

This new situation entails two effects of a most important nature. On the one hand, it markedly facilitates routine monitoring. However, it calls for a substantially increased sensitivity of methodology, which, not infrequently, conflicts with the desired rapidity, as is elucidated in detail below. In view of the main subject of this presentation it seems therefore most desirable to define precisely what the popular, customary term 'rapid' methods really wishes to convey.

In fact, the analyst seeks at least five attributes in methods aiming at validating microbiological integrity of end-product samples or compliance with hygiene standards in line specimens. These include: (i) facility, (ii) rapidity, (iii) consistency, (iv) intrinsic guarantees for avoidance of errors, e.g. through the exclusive use of reagents or ingredients certified by the supplier, and (v) mechanisation, if not automation. The often-used term *instrumental* methods covers these requirements fairly well in that laborious and subjective elements of analytical methods have been eliminated; the designation does not, however, explicitly include rapidity. In this chapter, the term 'rapid' will be used to describe methods which have most of the advantages listed, though not necessarily all; and even not consistently extreme rapidity, i.e. having data available within an hour or so, if not instantaneously.

1.3 Pitfalls in introducing 'rapid' methods

Unfortunately cardinal differences between the mechanisms of loss of chemical integrity and microbial deterioration of foods interfere in the pursuit of elaboration of rapid methods. First, as emphasised previously, the pertinent levels of detection in foods processed for safety are often extremely low, e.g. 1 cfu kg^{-1}; but worse, these low concentrations have sometimes to be isolated amongst innocuous populations exceeding the target organism by a factor of up to 10^6. The combination of the required sensitivity and the necessary selectivity is of an order of magnitude of 10^{-9}, calling for extremely selective procedures. These include the following steps: (i) concentration of primary food macerates by centrifugation (Mossel and Visser, 1960; Hawa *et al.*, 1984; Van Netten *et al.*, 1987; Fleet *et al.*, 1991; Mossel *et al.*, 1991) or filtration (section 1.5.1); or else by the advanced technique of immunoabsorption onto magnetic beads (Skjerve *et al.*, 1990; Cudjoe *et al.*, 1991; Lund *et al.*, 1991; Vermunt *et al.*, 1992; Mansfield and Forsythe, 1993); and (ii) highly selective enrichment and isolation procedures which are not yet as perfect as one would wish or suppose.

Problems surrounding the latter methods are compounded by the observation, made, for the first time, by Eijkman (1908) that the majority of microorganisms of significance in foods have incurred sublethal lesions as a result of having been exposed to adverse external conditions. These are either directly injurious, like heating, or indirectly so, e.g. lowered food pH or a_w, and sometimes even both (Mossel and Van Netten, 1984; Ray, 1989; Turpin *et al.*, 1993). If highly selective procedures, including the use of particular antimicrobial agents or increased incubation temperature, are applied to such debilitated populations, the combined stress will result in cell death, causing erroneously low results (Sallam and Donnelly, 1992; Morinigo *et al.*, 1993). This would lead to failure to take corrective measures where these were required. Consequently, meticulously elaborated *resuscitation* procedures (Figures 1.1–1.3) are required to restore the viability and unlimited culturability (Roszak *et al.*, 1984; Jones *et al.*, 1991; Nilsson *et al.*, 1991; Saha *et al.*, 1991) of debilitated populations, ensuring their inclusion in colony counts or most probable number (MPN) determinations.

A third factor accounting in part for the slow progress made in introducing more substantial modernisation in analytical food microbiology and particularly with respect to the use of molecular microbiological methodology is related to the nature of foods themselves. Methods that work remarkably well with pure cultures of target organisms, like the polymerase chain reaction (PCR)-approach (Section 1.5.2) failed initially when applied to 'real world' specimens, e.g. chicken carcasses. This results from the presence in many foods of contaminating inhibitory material (De Leon *et al.*, 1992; Abbaszadigan, *et al.*, 1993; Payne *et al.*, 1993; Bej *et al.*, 1994). Such interferences were overcome by previous concentration and purification of target organisms, obviously at the expense of simplicity and rapidity. A remaining difficulty arises from the failure

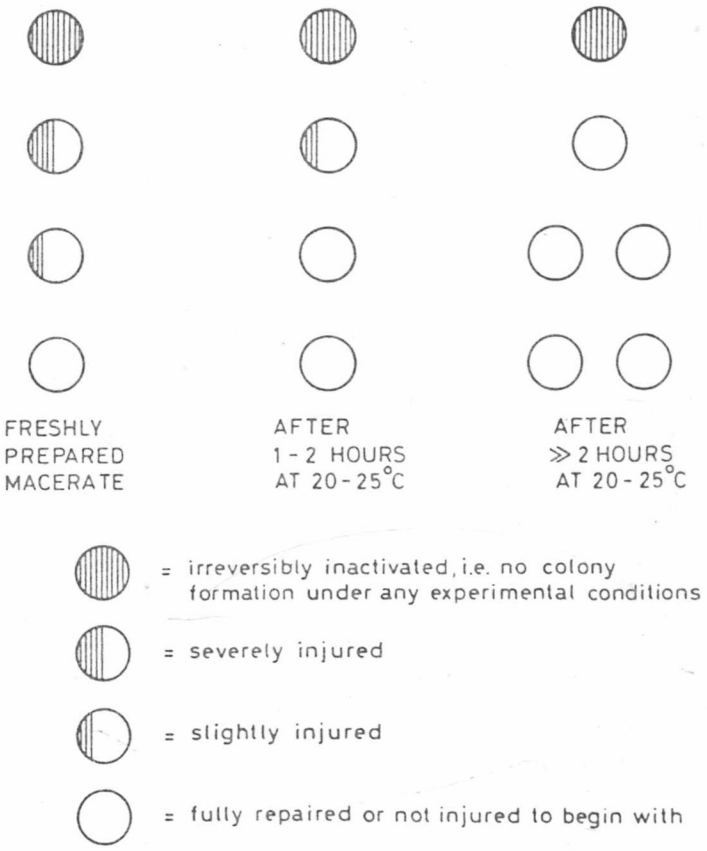

Figure 1.1 Repair versus proliferation as it occurs in various resuscitation procedures.

of PCR techniques to allow determination of viability of bacteria whose presence they visualize (Bej *et al.*, 1994).

A fourth hurdle is raised by rather successful novel rapid methods measuring parameters distinct from the classically accepted ones. This compounds the already, in general, most difficult problem of interpreting the results of microbiological examination of foods and particularly gauging analytical data against reference ranges, the much disputed microbiological specifications for foods (Mossel and Van Netten, 1991).

In essence, methods yielding non-conventional data may be very useful and should not, therefore, be rejected lightheartedly. They may provide most serviceable information of the *semaphora* ('traffic light') type. This indicates that a specimen belongs to one of the following three broad categories: pass ('green'), doubtful ('orange') or reject ('red').