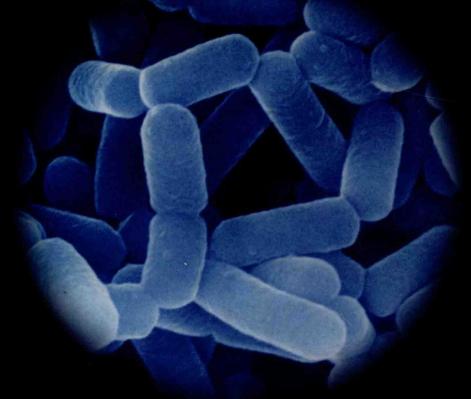
The Microbiology of Safe Food

Second Edition



Stephen J. Forsythe

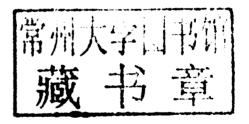


The Microbiology of Safe Food

Second edition

Stephen J. Forsythe

School of Science and Technology, Nottingham Trent University



This edition first published 2010

© 2010 by Blackwell Publishing Ltd

Blackwell Publishing was acquired by John Wiley & Sons in February 2007. Blackwell's publishing programme has been merged with Wiley's global Scientific, Technical, and Medical business to form Wiley-Blackwell.

Registered office

John Wiley & Sons Ltd, The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, United Kingdom

9600 Garsington Road, Oxford, OX4 2DO, United Kingdom

2121 State Avenue, Ames, Iowa 50014-8300, USA

For details of our global editorial offices, for customer services and for information about how to apply for permission to reuse the copyright material in this book please see our website at www.wiley.com/wiley-blackwell.

The right of the author to be identified as the author of this work has been asserted in accordance with the UK Copyright, Designs and Patents Act 1988.

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, except as permitted by the UK Copyright, Designs and Patents Act 1988, without the prior permission of the publisher.

Wiley also publishes its books in a variety of electronic formats. Some content that appears in print may not be available in electronic books.

Designations used by companies to distinguish their products are often claimed as trademarks. All brand names and product names used in this book are trade names, service marks, trademarks or registered trademarks of their respective owners. The publisher is not associated with any product or vendor mentioned in this book. This publication is designed to provide accurate and authoritative information in regard to the subject matter covered. It is sold on the understanding that the publisher is not engaged in rendering professional services. If professional advice or other expert assistance is required, the services of a competent professional should be sought.

Library of Congress Cataloging-in-Publication Data

Forsythe, S. J. (Steve J.)

The microbiology of safe food / Stephen J. Forsythe. – 2nd ed.

Includes bibliographical references and index.

ISBN 978-1-4051-4005-8 (pbk.: alk. paper) 1. Food-Microbiology. I. Title.

[DNLM: 1. Food Microbiology. 2. Food Poisoning-microbiology. 3. Legislation, Food.

4. Risk Assessment. 5. Safety Management. QW 85 F735m 2010]

OR115.F675 2010 664.001'579-dc22

2009037331

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

Set in 10/12 pt Minion by Aptara® Inc., New Delhi, India Printed and bound in Malaysia by KHL Printing Co Sdn Bhd

1 2010

The Microbiology of Safe Food

Preface to second edition

Although I was pleased with the first edition of this book (MoSF), I nevertheless felt that it was not complete. This new edition tries to address this by including new sections on bioinformatics, biothreats and personnel, as well as updating many other sections. Since 2000, the topic of microbiological risk assessment has increased, and subsequently, I have incorporated parts of my other Blackwell's book *Microbiological Risk Assessment of Food* (2002) into Chapter 10 as it was a substantial improvement on the first MoSF edition's few pages. My appreciation is due to Simon Illingworth (LabM, Bury, UK) for reviewing Chapter 5 on detection methods for me.

A major change is the complementing websites at http://www.wiley.com/go/forsythe. This was available with the first edition, but was an afterthought and so unfortunately was not fully utilised. In fact, it was one of the first web-based supported books by Blackwells, and the listing of URL in the Appendix was considered a 'novelty'! How much has changed since 2000. I am using the web for two main purposes. Firstly to keep some chapters up-to-date, and secondly to offer various data exercises which are not in keeping with the book format. One aspect which I have been wanting to expand and encourage 'younger' readers to explore is the application of genomics, post-genomics and bioinformatics to food microbiology. Again, the first edition included microarrays but not the tools for one to investigate microbial genomes for oneself. In fact, 2000, the year MoSF was published, was also the year when the first version of the Campylobacter jejuni genome was released, and since the MoSF text was written in 1999, the whole topic of microbial genomics was not even on the radar. The fact that genomes are sequenced faster than they can be fully annotated means that one can quickly discover something which no one else has even known before, and I hope the bioinformatics aspects will enable and encourage readers to try in silico research. One topic which was gaining increasing public attention in 1999–2000 was BSE-vCJD. It appears in the intervening years that we have possibly passed the peak incidence, fortunately. However, over the same period, the spectre of bioterrorism has arisen and so this issue is addressed in a new section of this edition.

One thing which has not changed between these two editions is the unacceptable high incidence of foodborne disease. Even more alarming is that we still are only aware of the 'tip of the iceberg' with regard to its true incidence. When one considers that it has been estimated that in the United States, 3400 deaths are due to unknown foodborne agents (Frenzen *et al.* 2005), then there is evidently a considerable amount of research and investment still to be undertaken.

Confession time, it was my full intention to complete this new edition for publication in 2005. However, our intensive research into *Cronobacter* spp. (*Enterobacter sakazakii*) and related organisms have taken up more of my time than the hours in the day can permit. This emergent pathogen, sadly, can infect neonates causing severe illness, and even death. In order not to unbalance this book by excessive reference to this organism of my own personal interest,

readers should consult the 2008 ASM Press book Enterobacter sakazakii, edited by myself and Jeff Farber (Health Canada) as well as my homepage (see http://www.wiley.com/go/forsythe).

As always, my thanks and appreciation go to Nigel Balmforth, David McDade and especially Katy Loftus at Wiley-Blackwell for their patience as the deadlines made a whooshing sound as they went by (frequently). Finally, a special thanks to my forever supportive wife Debbie, my children James and Rachel, and my parents - without whom none of this would have been possible.

> Steve Forsythe Professor of Microbiology Nottingham Trent University

Preface to first edition

Throughout the world, food production has become more complex. Frequently, raw materials are sourced globally and the food is processed through an increasing variety of techniques. No longer does the local farm serve the local community through a local shop; nowadays, there are international corporations adhering to national and international regimes. Therefore, approaches to safe food production are being assessed on an expanding platform from national, European, trans-Atlantic and beyond. Against this backdrop, there have been numerous highly publicised food safety issues such as BSE and *E. coli* O157:H7 which have caused the general public to become more cautious of vociferous concerning food issues. The controversy in Europe over genetically modified foods is perceived by the general public within the context of 'food poisoning'.

This book aims to review the production of food and the level of micro-organisms which humans ingest. Certain circumstances require zero tolerances for pathogens, whereas more frequently, there are acceptable limits set, albeit with statistical accuracy or inaccuracy depending upon whether you subsequently suffer from food poisoning. Microbes are traditionally ingested in fermented foods and this has developed into the subject of pre- and probiotics with refuted health benefits. Whether engineered 'functional foods' will be able to attain consumer acceptance remains to be seen.

Food microbiology covers both food pathogens and food spoilage organisms. This book aims to cover the wide range of micro-organisms occurring in food, both as contaminants and deliberate inoculation. Due to the heightened public awareness over food poisoning, it is important that all companies in the food chain maintain high hygienic standards and assure the public of the safety of the produce. Obviously, over time, there are technological changes in production methods and methods of microbiological analysis. Therefore, the food microbiologist needs to know the effect of processing changes (pH, temperature, etc.) on the microbial load. To this end, this book reviews the dominant foodborne micro-organisms, the means of their detection, microbiological criteria as the numerical means of interpreting end-product testing, predictive microbiology as a tool to understand the consequences of processing changes, the role of 'Hazard Analysis Critical Control Point' (HACCP) and the objectives of Microbial Risk Assessment (MRA) and the setting of Food Safety Objectives which have recently become a focus of attention. In recent years, the web has become an invaluable source of information and to reflect this a range of useful food safety resource sites are given in the back to encourage the reader to boldly go and surf. Although primarily aimed for undergraduate and postgraduate courses, I hope the book will also be of use to those working in industry.

The majority of this book was written during the last months of 1999, a time when France was being taken to the European Court over its refusal to sell British beef due to BSE/nvCJD and there had been riots in Seattle concerning the World Trade Organisation. Whilst large organisations

were wondering about the impact of the millennium bug, in the United Kingdom, the public were waiting to see the impact of the BSE 'bug' (a few hundred or a few thousand cases?).

As usual, no book can be achieved without assistance, and special thanks are due to Phil Vosey concerning MRA, Ming Lo for considerable help with the computer packages, Alison at Oxoid Ltd for the invaluable information on microbiological testing procedures around the world, Pete Silley and Andrew Pridmore at Don Whitley Scientific Ltd for the RABIT diagrams, and Garth Lang at Biotrace Ltd for the ATP bioluminescence data. Not forgetting of course Debbie and Cathy for reading through the draft copy, nevertheless all mistakes are the author's fault.

This book is especially dedicated to Debbie, James and Rachel, Mum and Dad for their patience whilst I have been burning the midnight oil.

Dr Steve Forsythe on 6 January, 2000

Contents

			dedition	XIV
Pr	eface to	o first e	dition	XV
•	F	II	Information and to a contract	
1			infections and intoxications	1
	1.1	_	s of safe food production	1
	1.2		orne illness	2 7
			of foodborne illness	
			perception of safe food	8
			elated issues	15
			ne hypothesis	17
	1.7		ze of the foodborne illness problem	17
			ic sequelae following foodborne illness	19
			es in antibiotic resistance	21
	1.10		st of foodborne diseases	22
	1.11		ol of foodborne pathogens	26
			Example 1 – the control of <i>Salmonella</i> serovars in poultry	26
			Example 2 – control of <i>E. coli</i> and <i>Salmonella</i> in fresh produce	28
	1.12		lance programmes	29
			International Food Safety Authorities Network (INFOSAN)	30
			FoodNet in the United States	31
		1.12.3	PulseNet: US E. coli O157:H7, Salmonella and Shigella detection	
			network	32
		1.12.4	European Centre for Disease Prevention and Control (ECDC) and	
			Enter-Net; European surveillance network for salmonellosis and	
			shigatoxic E. coli (STEC)	32
			Foodborne viruses in Europe network	33
		1.12.6	Rapid Alert System for Food and Feed (RASFF)	34
		1.12.7	Global Salm-Surv (GSS)	34
		1.12.8	Surveillance of ready-to-eat foods in the United Kingdom	34
	1.13	Outbre	eak investigations	36
		1.13.1	Preliminary outbreak investigation	37
		1.13.2	Case definition and data collection	40
		1.13.3	Data collation and interpretation	41
	1.14	Food to	errorism and biocrimes	47
1.15 Food safety following natural disasters, and conflict				

vi Contents

2	Basic	Basic aspects			
	2.1	The m	icrobial world	52	
	2.2	Bacteri	ial cell structure	55	
		2.2.1	Morphology	55	
		2.2.2	Cell membrane structure and the Gram stain	55	
		2.2.3	Lipopolysaccharide, O antigen	57	
		2.2.4	Flagella (H antigen)	57	
			Capsule (Vi antigen)	58	
	2.3		ial toxins and other virulence determinants	.58	
			Bacterial toxins	58	
			Pathogenicity islands	63	
			Bacterial toxins encoded in bacteriophages	64	
			pial growth cycle	65	
	2.5		kinetics	66	
		2.5.1	Expressions	66	
			The second secon	66	
	2.6		s affecting microbial growth	71	
		2.6.1	Intrinsic and extrinsic factors affecting microbial	71	
		262	growth	71	
			Water activity	72	
			pH Temperature	73 75	
		2.6.5	_	75 75	
	2.7		Interplay of factors affecting microbial growth in foods bial response to stress	75	
	4.7	2.7.1	General stress response (GSR)	77	
				78	
			Heat shock	78	
			Cold shock	79	
			Osmotic shock	80	
	2.8		tive modelling	80	
		2.8.1	Predicting modelling development	81	
		2.8.2	Primary models and the Gompertz and Baranyi		
			equations	81	
		2.8.3	Secondary models	84	
			Tertiary models	84	
		2.8.5	Application of predictive microbial modelling	85	
	2.9	Bioinfo	ormatic studies	87	
		2.9.1	Bioinformatics and genomes	87	
		2.9.2	16S rRNA gene sequence and denaturing gradient gel		
			electrophoresis (DGGE)	90	
		2.9.3	Campylobacter jejuni and Campylobacter coli genome		
			sequence	91	
		2.9.4	Salmonella evolution and PAIs	92	
		2.9.5	E. coli O157:H7 genome sequence	93	
		2.9.6	The diversity of the lactic acid bacteria and bifidobacteria	95	
		2.9.7	Listeria species genome sequence analysis	98	
		2.9.8	Staphylococcus aureus enterotoxin phylogenetic analysis	100	

3	The	microb	ial flora of food and its preservation	101
	3.1	Spoilag	ge micro-organisms	101
		3.1.1	Spoilage micro-organisms	103
			Spoilage of diary products	105
			Spoilage of meat and poultry products	105
			Fish spoilage	106
			Egg spoilage	106
	3.2		fe indicators	106
	3.3		ds of preservation and shelf-life extension	109
	3.4		rdle concept	110
	3.5	Preserv		110
			Organic acids	110
			Hydrogen peroxide and lactoperoxidase system	111
			Chelators	113
			Natural antimicrobials	113
			Non-acidic preservatives	113
	26		Preservation due to weak acids and low pH	115
	3.6		Il methods of preservation	116
			Preservation by heat treatment High-pressure treatment	116
			Ohmic heating and radio frequency	117
			Pulsed electric fields	118
			Ultrasound	119
			Intense light pulse	119 119
			Food irradiation	119
			Reduced oxygen packaging, modified atmosphere	113
		3.0.0	packaging and active packaging	123
	3.7	Fermen	nted foods	125
	0.7		Lactic acid bacteria and their metabolism	126
			Fermented milk products	130
			Fermented meat products	133
			Fermented vegetables	134
			Fermented protein foods; shoyu and miso	134
			Future use of the lactic acid bacteria	134
	3.8		onal foods; prebiotics, probiotics and synbiotics	136
			Functional foods	136
		3.8.2	Claims of probiotics	137
			Probiotic studies	138
	3.9	Nanote	echnology and food preservation	139
4	Food	lborne	pathogens	141
		Introdu		141
		4.1.1	The human intestinal tract	141
		4.1.2	Host resistance to foodborne infections	144
			The normal human intestinal flora	147
	4.2		or organisms	151

	4.2.1	Coliforms	152
	4.2.2	Enterobacteriaceae	152
	4.2.3	Enterococci	152
	4.2.4	Bacteriophage	153
4.3	Foodbo	orne pathogens, bacteria	153
	4.3.1	C. jejuni, Campylobacter coli and Campylobacter lari	153
	4.3.2	Salmonella species	157
	4.3.3	Pathogenic E. coli	163
	4.3.4	Sh. dysenteriae and Sh. sonnei	174
	4.3.5	Listeria monocytogenes	176
	4.3.6	Y. enterocolitica	180
	4.3.7	St. aureus	182
	4.3.8	Cl. perfringens	184
	4.3.9	Cl. botulinum	186
	4.3.10	B. cereus	187
	4.3.11	Vibrio cholerae, V. parahaemolyticus and V. vulnificus	190
	4.3.12	Brucella melitensis, Br. abortus and Br. suis	192
	4.3.13	Aeromonas hydrophila, A. caviae and A. sobria	193
	4.3.14	Plesiomonas shigelloides	194
	4.3.15	Streptococcus and Enterococcus species	195
4.4		orne pathogens, viruses	197
	4.4.1	Norovirus (formerly known as Norwalk-like viruses and small	
		round structured viruses)	199
		Hepatitis A	202
		Hepatitis E	204
		Rotaviruses	204
	4.4.5	Small round viruses, astroviruses, SLVs, adenoviruses and	
		parvoviruses	205
		Human enteroviruses	206
4.5		d and shellfish poisoning	207
		Ciguatera poisoning	208
		Scombroid poisoning	208
		Paralytic shellfish poisoning	209
		Diarrhoeic shellfish poisoning	209
		Neurotoxic shellfish poisoning	210
		Amnesic shellfish poisoning	210
4.6		orne pathogens: eucaryotes	210
		Cyclospora cayetanensis	211
	4.6.2	71 1	211
	4.6.3		211
	4.6.4	Taenia saginata and Taenia solium	212
	4.6.5	Toxoplasma gondii	213
4.7	4.6.6	Trichinella spiralis	214
4.7	Mycoto		214
		Aflatoxins Ochratoxins	216
		Fumonisins	217 217
	7./)	E000000000000	/. /

			Co	ntents	ix
		4.7.4	Zearalenone		217
			Trichothecenes		218
	4.8		ing and uncommon foodborne pathogens		218
			Prions		219
			Cronobacter spp.		220
		4.8.3	**		
			pathogen?	_	222
		4.8.4	Arcobacter genus		222
		4.8.5	Nanobacteria		223
5	Met	hods of	f detection and characterisation	1	224
_	5.1	Prolog			224
			ntional methods		229
	5.2		Culture media		230
			Sublethally injured cells		232
			Viable but non-culturable bacteria (VNC)		233
	5.3		methods		234
		_	Sample preparation		234
			Separation and concentration of target		234
	5.4		end-detection methods		238
		-	ELISA and antibody-based detection systems		238
			Reversed passive latex agglutination (RPLA)		239
			Impedance (conductance) microbiology		239
			ATP bioluminescence techniques and hygiene monitoring		240
		5.4.5	Protein detection		241
		5.4.6	Flow cytometry		242
		5.4.7	Nucleic acid probes and the polymerase chain reaction (PCR)		243
		5.4.8	Microarrays		245
		5.4.9			246
	5.5		ular typing methods		248
			Pulsed-field gel electrophoresis (PFGE)		248
			Restriction fragment length polymorphism (RFLP)		249
			Multiple-locus variable-number tandem-repeats (MLVA)		249
		5.5.4	VI Comment		249
	5.6	•	c detection procedures		250
		5.6.1	Aerobic plate count (APC)		250
		5.6.2			250
		5.6.3 5.6.4	* 2		252253
		5.6.5			255
		5.6.6			255
			Cronobacter spp.		256
		5.6.8			258
		5.6.9	, 8		260
		5.6.10			261
			Bacillus cereus, B. subtilis and B. licheniformis		262

x Contents

		5.6.12	Mycotoxins	263
		5.6.13 V	Viruses	263
	5.7	Accredit	ation schemes	265
6	Micr	obiologi	ical criteria	266
	6.1	Backgrou	und to microbiological criteria and end-product testing	266
	6.2	Internati	ional Commission on Microbiological Specifications for Foods	
		(ICMSF)		266
	6.3		limentarius principles for the establishment and application of	
			ological criteria	267
	6.4	Sampling		269
		Variables		271
	6.6		es sampling plan	274
			Two-class plan	274
	13.14		Γhree-class plan	275
	6.7	Principle		275
			Defining a 'lot' of food	275
			Sample unit number	275
			Operating characteristic curve	276
			Producer risk and consumer risk	279
			Stringency of two- and three-class plans, setting <i>n</i> and <i>c</i>	279
	(0		Setting the values for <i>m</i> and <i>M</i>	281
	6.8		ological limits	281
			Definitions	281
	6.0		Limitations of microbiological testing	282
	6.9		es of sampling plans	282
			Egg products	282
			Milk and milk products Processed meats	282
			Cereals and cereal products	283 284
			Cook-chill and cook-freeze products	284
			Seafoods	284
	6.10		ented microbiological criteria	286
	0.10		Microbiological criteria in the European Union	286
			EU directives specifying microbiological standards for foods	288
	6.11		elines for ready-to-eat foods	288
7	Hygi	enic pro	duction practices	289
	7.1	-	ution of food handlers to foodborne illness	289
	7.2		hygiene and training	289
	7.3	Cleaning	1.5	292
	7.4		nts and disinfectants	294
	7.5		al biofilms	295
			ent of cleaning and disinfection efficiency	299
8	Food	safety ı	management tools	301
	8.1	The man	nufacture of hygienic food	301
	8.2	Microbio	ological safety of food in world trade	307

			mer pressure effect on food processing	308
	8.4		anagement of hazards in food which is in international trade	309
	8.5	HACC		309
	8.6		uisite programme	310
	8.7		e of HACCP	312
			Food hazards	312
			Preparation for HACCP	312
			Principle 1: hazard analysis	313
			Principle 2: critical control points	314
			Principle 3: critical limits	316
			Principle 4: CCP monitoring	316
			Principle 5: corrective actions	316
			Principle 6: verification	316
			Principle 7: record keeping	317
	8.8		piological criteria and HACCP	317
	8.9		piological hazards and their control	319
			Sources of microbiological hazards	319
			Temperature control of microbiological hazards	320
			Non-temperature control of microbiological hazards	321
	8.10		P plans	321
			Production of pasteurised milk	321
			Swine slaughter in the abattoir	322
			Chilled food manufacture	322
			Generic models	323
	8.11		and GHP	327
	8.12		y systems	336
	8.13	TQM		336
9	Mics	obiolo	gical risk assessment	338
7			—»	338
	9.1		nalysis and microbiological risk assessment	339
	9.2	-	of microbiological risk assessment	342
	9.3		biological risk assessment – an overview	344
	9.4		biological risk assessment – structure	344
			Risk assessment Risk management	347
			Risk communication	348
	0.5			348
	9.3		ssessment Statement of purpose	349
		9.5.1		349
		9.5.2		350
		9.5.4		354
		9.5.4		356
			Dose–response models	358
			Dose and infection	362
		9.5.8		365
			Production of a formal report	367
			Triangular distributions and Monte Carlo simulation	367

Contents xi

xii Contents

9.6	6 Risk management		368
	9.6.1 Risk assess	sment policy	372
	9.6.2 Risk profi	ling	372
9.7	Food safety object	tives	373
9.8	Risk communicati	ion	374
9.9	Future developme	ents in microbiological risk assessment	376
	9.9.1 Internatio	onal methodology and guidelines	376
	9.9.2 Data		376
	9.9.3 Training of	courses and use of resources	377
10 App	ication of micro	biological risk assessment	378
10.1	Salmonella risk ass	sessments	378
		idis in shell eggs and egg products	378
		entification and hazard characterisation of Salmonella	
	in broilers		381
		assessment of Salmonella spp. in broilers	384
		a spp. in cooked chicken	385
		a spp. in cooked patty	386
	10.1.6 Poultry FA		387
		and sporadic human salmonellosis	388
10.2	Campylobacter ris		388
		risk from fresh chicken	388
		le for pathogenic species of Campylobacter in Denmark	390
		sment of <i>C. jejuni</i> in broilers	391
		pacter fluoroquinolone resistance	392
10.3	L. monocytogenes		394
	,	vtogenes hazard identification and hazard characterisation	
		co-eat foods	394
	10.3.2 L. monocy	vtogenes exposure assessment in RTE foods	396
	10.3.3 Relative ri	isk of L. monocytogenes in selected RTE foods	398
	10.3.4 L. monocy	vtogenes in EU trade	400
	10.3.5 L. monocy	vtogenes in meat balls	401
		s from RTE meat products	402
10.4	E. coli O157 risk a	ssessment	403
	10.4.1 E. coli O1	57:H7 in ground beef	403
10.5	Bacillus cereus risl	k assessment	406
	10.5.1 B. cereus r	risk assessment	406
10.6	Vibrio parahaemo	olyticus risk assessment	407
	10.6.1 Public hea	alth impact of V. parahaemolyticus in raw molluscan	
	shellfish		407
10.7	Cronobacter spp. ((Enterobacter sakazakii) and Salmonella in powdered	
	infant formula	•	409
10.8	Viral risk assessm	ents	410
	10.8.1 Viral cont	tamination of shellfish and coastal waters	410

	rnational control of microbiological hazards in foods: regulations authorities	412	
	World Health Organisation, global food security from accidental and		
	deliberation contamination	412	
11.2	The foodborne disease burden epidemiology reference group (FERG)	415	
11.3	Regulations in international trade of food	416	
11.4	Codex Alimentarius Commission	417	
11.5	Sanitary and phytosanitary measures (SPS), technical barriers to trade (TBT)		
	and WHO	417	
11.6	European Union legislation	419	
	11.6.1 Food hygiene directive (93/43/EEC)	420	
11.7	Food safety agencies	420	
	11.7.1 Food authorities in the United States	421	
Glossary	y of terms	423	
List of al	List of abbreviations		
Food saf	Food safety resources on the world wide web		
Reference	es	435	
Index		461	

A colour plate section is found facing page 222

See the supporting companion website for this book: http://www.wiley.com/go/forsythe