

# Table of contents

<b>Preface</b>	<b>XVII</b>
<b>About the authors</b>	<b>XIX</b>
<b>List of tables</b>	<b>XXI</b>
<b>List of figures</b>	<b>XXV</b>
<b>1 Sampling, transport and storage of samples for analysis</b>	<b>1</b>
1.1 Introduction	1
1.1.1 Lot	1
1.1.2 Lot sample and sample unit	1
1.1.3 Lot sampling plans	1
1.1.3.1 The two-class sampling plan	2
1.1.3.2 The three-class sampling plan	2
1.1.4 Analytical unit	2
1.2 Collecting samples for analysis	2
1.2.1 Selection and preparation of containers for the sampling of foods contained in non-individual packages	3
1.2.2 Procedures for the sampling of foods contained in non-individual packages	3
1.2.3 Sampling of foods involved in foodborne diseases	4
1.2.4 Sampling of water	4
1.3 Transportation and storage of samples until analysis	5
1.3.1 Foods with low water activity	5
1.3.2 Frozen foods	5
1.3.3 Refrigerated foods	5
1.3.4 Commercially sterile foods in sealed packages	6
1.3.5 Water samples	6
1.4 References	7
<b>2 Preparation of sample for analysis</b>	<b>9</b>
2.1 Introduction	9
2.2 Homogenization of samples and withdrawal of the analytical unit	10
2.2.1 Procedure for homogenization and withdrawal of analytical units from liquid products	10
2.2.2 Procedure for homogenization and withdrawal of analytical units from solid or concentrated liquid products	11
2.2.3 Procedure for withdrawing the analytical unit using the surface swabbing technique	11
2.2.3.1 Swab sampling	11
2.2.3.2 Sponge sampling	12
2.2.4 Procedure for withdrawing the analytical unit using the surface washing technique	12
2.2.4.1 Procedure for washing poultry carcasses	12
2.2.4.2 Procedure for washing other foods	14
2.2.4.3 Procedure for washing packages	14
2.2.5 Keeping of counter-samples	14
2.3 Preparation of the first dilution of the analytical unit	15
2.3.1 Diluents for presence/absence tests	15
2.3.2 Diluents for tests requiring differentiated handling of the sample	15

## VI Table of contents

2.3.3	Diluents for general quantification tests	15
2.3.4	How to prepare an initial 1:10 ( $10^{-1}$ ) dilution	15
2.3.5	How to prepare an initial dilution different from 1:10	15
2.3.6	Procedure for the preparation of the first dilution of liquid samples	15
2.3.7	Procedure for the preparation of the first dilution of solid or concentrated liquid samples	16
2.3.8	Procedure for the preparation of the first dilution of samples obtained by surface swabbing or surface washing	16
2.4	Serial decimal dilution of the sample	16
2.5	References	17
Annex 2.1 –	Procedures for the homogenization of the content and withdrawal of the analytical unit of different types of foods	18
Annex 2.2 –	Special cases in which there are variations in the analytical unit and/or dilution and/or diluents recommended for the preparation of the first dilution of samples of different types of foods	19
<b>3</b>	<b>Basic plate count techniques for the enumeration of microorganisms</b>	<b>23</b>
3.1	Introduction	23
3.2	Pour plate technique	23
3.2.1	Material required for the analyses	24
3.2.2	Procedure	24
3.3	Spread plate technique	25
3.3.1	Material required for the analyses	26
3.3.2	Procedure	26
3.4	Drop plate technique	27
3.4.1	Material required for the analyses	27
3.4.2	Procedure	27
3.5	Membrane filtration	27
3.5.1	Material required for the analyses	28
3.5.2	Procedure	28
3.6	Counting colonies and calculating results	29
3.6.1	Pour plate calculations	29
3.6.1.1	Calculating the pour plate results in the standard situation	30
3.6.1.2	Calculating the pour plates results for samples prepared by the surface swabbing technique (swabs or sponges)	33
3.6.1.3	Calculating the pour plate results for samples prepared by the surface washing technique	34
3.6.2	Spread plate calculations	34
3.6.3	Drop plate calculations	34
3.6.4	Membrane filtration calculations	35
3.7	Counting colonies and calculating results according to ISO 7218:2007	35
3.8	References	37
<b>4</b>	<b>Basic techniques for microbial enumeration by the most probable number method (MPN)</b>	<b>39</b>
4.1	Introduction	39
4.2	Multiple dilution test	40
4.2.1	Material required for the analyses	41
4.2.2	Procedure	41
4.3	Single dilution test	42
4.3.1	Material required for the analyses	42

4.3.2	Procedure	42
4.4	Calculation of the results	42
4.4.1	Calculating the results of the multiple dilution test	43
4.4.1.1	Calculation using the MPN tables (for decimal dilutions)	43
4.4.1.2	Calculating using the Thomas formula (for non-decimal dilutions)	44
4.4.1.3	Calculating the results for samples prepared by the surface swabbing or surface washing techniques	44
4.4.2	Calculating the results of the single dilution test	44
4.4.2.1	Rules for calculations performed using the MPN-3 Table	45
4.4.2.2	Calculation for samples prepared by the surface swabbing or surface washing techniques	45
4.5	References	45
	Annex 4.1 – MPN tables	46
<b>5</b>	<b>Basic techniques for the detection of the presence/absence of microorganisms</b>	<b>49</b>
5.1	Introduction	49
5.1.1	Enrichment	49
5.1.1.1	Pre-enrichment	49
5.1.1.2	Selective enrichment	50
5.1.2	Isolation in solid media (selective differential plating)	50
5.1.3	Confirmation	50
5.1.3.1	Catalase test	51
5.1.3.2	Citrate test	51
5.1.3.3	Amino acid decarboxylation tests	51
5.1.3.4	Phenylalanine deaminase test	51
5.1.3.5	Carbohydrate fermentation tests	51
5.1.3.6	Indole test	52
5.1.3.7	Malonate test	52
5.1.3.8	Oxidation/Fermentation test (O/F)	52
5.1.3.9	Oxidase test	52
5.1.3.10	Nitrate reduction test	53
5.1.3.11	Urease test	53
5.1.3.12	Methyl Red test (MR)	53
5.1.3.13	Voges-Proskauer test (VP)	53
5.2	Material required for the analyses	54
5.3	Procedure	54
5.3.1	Pre-enrichment	54
5.3.2	Selective enrichment	54
5.3.3	Selective differential plating	54
5.3.3.1	Streak plating technique for obtaining pure cultures	54
5.3.4	Selection of colonies and subculturing of cultures for confirmation	55
5.3.4.1	Technique for the subculturing of pure cultures starting from colonies isolated from plates	55
5.3.5	Confirmation tests	55
5.3.5.1	Gram-staining (Hucker's method)	55
5.3.5.2	Spore-staining (Schaeffer-Fulton's method)	56
5.3.5.3	Spore-staining (Ashby's method)	56
5.3.5.4	Wet mounts for direct (fresh) microscopic observation	56
5.4	References	56

## VIII Table of contents

<b>6 Aerobic plate count</b>	<b>57</b>
6.1 Introduction	57
6.1.1 The importance and significance of the total aerobic mesophilic count	57
6.1.2 Definition of psychrotrophics	58
6.1.3 Methods of analysis	58
6.2 Plate count method APHA 2001 for aerobic mesophilic bacteria in foods and water	59
6.2.1 Material required for analysis	60
6.2.2 Procedure	60
6.2.2.1 Pour plate technique	60
6.2.2.2 Spread plate technique	61
6.2.2.3 Membrane filtration technique	62
6.3 Petrifilm™ AOAC official methods 990.12 - 989.10 - 986.33 for aerobic mesophilic bacteria in foods	63
6.3.1 Material required for analysis	63
6.3.2 Procedure	63
6.4 Plate count method APHA 2001 for aerobic psychrotrophic bacteria in foods	64
6.4.1 Material required for analysis	64
6.4.2 Procedure	64
6.5 References	65
<b>7 Yeasts and molds</b>	<b>67</b>
7.1 Introduction	67
7.1.1 Yeasts and molds in foods	67
7.1.2 Methods of analysis for total yeast and mold counts	68
7.1.3 Psychrotrophic fungi	68
7.1.4 Heat-resistant molds	68
7.1.5 Preservative-resistant yeasts (PRY)	69
7.1.5.1 <i>Zygosaccharomyces bailii</i> (Lindner) Guilliermond 1912	69
7.1.5.2 <i>Zygosaccharomyces bisporus</i> (Naganishi) Lodder and Kreger 1952	70
7.1.5.3 <i>Schizosaccharomyces pombe</i> Lindner 1893	70
7.1.5.4 <i>Candida krusei</i> (Castellani) Berkhoult 1923	70
7.1.5.5 <i>Pichia membranaefaciens</i> Hansen 1904	70
7.1.6 Osmophilic yeasts	71
7.1.6.1 <i>Zygosaccharomyces rouxii</i> (Boutroux) Yarrow	71
7.2 Plate count method APHA 2001 for yeasts and molds in foods	71
7.2.1 Material required for analysis	72
7.2.2 Procedure	72
7.3 Plate count method APHA 2001 for psychrotrophic fungi in foods	74
7.3.1 Material required for analysis	74
7.3.2 Procedure	74
7.4 Plate count method APHA 2001 for heat-resistant molds in foods	76
7.4.1 Material required for analysis	76
7.4.2 Procedure	76
7.5 Presence/absence method Pitt and Hocking 2009 and Plate count method Pitt and Hocking 2009 for preservative-resistant yeasts in foods	78
7.5.1 Material required for analysis	78
7.5.2 Procedure	78
7.6 Membrane filtration method APHA 2001 and Plate count method APHA 2001 for osmophilic yeasts in foods	80
7.6.1 Material required for analysis	80
7.6.2 Procedure	80
7.7 References	81

<b>8 Enterobacteriaceae</b>	<b>83</b>
8.1 Introduction	83
8.1.1 Taxonomy	83
8.1.2 Methods of analysis	84
8.2 Plate count method APHA 2001 for <i>Enterobacteriaceae</i> in foods	84
8.2.1 Material required for analysis	84
8.2.2 Procedure	84
8.3 Most probable number (MPN) method APHA 2001 for <i>Enterobacteriaceae</i> in foods	85
8.3.1 Material required for analysis	85
8.3.2 Procedure	85
8.4 Petrifilm™ AOAC official method 2003.1 for <i>Enterobacteriaceae</i> in selected foods	87
8.4.1 Material required for analysis	87
8.4.2 Procedure	87
8.5 References	88
<b>9 Total and thermotolerant coliforms and <i>Escherichia coli</i></b>	<b>89</b>
9.1 Introduction	89
9.1.1 Definition of total coliforms	89
9.1.2 Definition of thermotolerant coliforms	89
9.1.3 <i>Escherichia coli</i>	89
9.1.4 Use as indicators	90
9.1.5 Methods of analysis	90
9.2 Most probable number (MPN) method APHA 2001 for total coliforms, thermotolerant coliforms and <i>E. coli</i> in foods	92
9.2.1 Material required for analysis	92
9.2.2 Procedure	93
9.3 Most probable number (MPN) methods ISO 4831:2006 and ISO 7251:2005 for total coliforms and presumptive <i>E. coli</i> in foods	97
9.3.1 Material required for analysis	97
9.3.2 Procedure	97
9.4 Most probable number (MPN) method APHA/AWWA/WEF 2005 for total and thermotolerant coliforms and <i>E. coli</i> in water	99
9.4.1 Material required for analysis	99
9.4.2 Procedure	101
9.5 Plate count method APHA 2001 for total coliforms in foods	102
9.5.1 Material required for analysis	102
9.5.2 Procedure	102
9.6 References	103
<b>10 <i>Staphylococcus aureus</i></b>	<b>105</b>
10.1 Introduction	105
10.1.1 Taxonomy	105
10.1.1.1 The genus <i>Staphylococcus</i>	105
10.1.1.2 The coagulase positive staphylococci	105
10.1.1.3 <i>Staphylococcus aureus</i>	106
10.1.2 Pathogenicity	107
10.1.2.1 <i>Staphylococcus aureus</i> enterotoxins	107
10.1.2.2 Staphylococcal food poisoning	108
10.1.3 Methods of analysis	108
10.2 Plate count method APHA 2001 for coagulase positive staphylococci and <i>S. aureus</i> in foods	109
10.2.1 Material required for analysis	110
10.2.2 Procedure	110

## X Table of contents

10.3	Most probable number (MPN) method APHA 2001 for coagulase positive staphylococci and <i>S. aureus</i> in foods	113
10.3.1	Material required for analysis	113
10.3.2	Procedure	113
10.4	Presence/absence method APHA 2001 for coagulase positive staphylococci and <i>S. aureus</i> in foods	115
10.4.1	Material required for analysis	115
10.4.2	Procedure	115
10.5.	References	115
<b>11</b>	<b><i>Bacillus cereus</i></b>	<b>119</b>
11.1	Introduction	119
11.1.1	<i>B. cereus</i> Group	119
11.1.2	Main characteristics of <i>B. cereus</i>	120
11.1.3	Methods of analysis	121
11.2	Plate count method APHA 2001 for <i>Bacillus cereus</i> in foods	121
11.2.1	Material required for analysis	122
11.2.2	Procedure	122
11.3	Most probable number (MPN) method APHA 2001 for <i>Bacillus cereus</i> in foods	126
11.3.1	Material required for analysis	126
11.3.2	Procedure	126
11.4	References	126
<b>12</b>	<b><i>Clostridium perfringens</i></b>	<b>129</b>
12.1	Introduction	129
12.1.1	Main characteristics of <i>C. perfringens</i>	129
12.1.2	Epidemiology	130
12.1.2.1	<i>C. perfringens</i> type A food poisoning	130
12.1.2.2	<i>C. perfringens</i> type C necrotic enteritis	130
12.1.3	Methods of analysis	130
12.2	Plate count method APHA 2001 for <i>Clostridium perfringens</i> in foods	131
12.2.1	Material required for analysis	131
12.2.2	Procedure	132
12.3	Presence/absence method APHA 2001 for <i>Clostridium perfringens</i> in foods	134
12.3.1	Material required for analysis	134
12.3.2	Procedure	134
12.4	References	136
<b>13</b>	<b>Enterococci</b>	<b>137</b>
13.1	Introduction	137
13.1.1	Enterococci	139
13.1.1.1	Species of intestinal origin	139
13.1.1.2	Species found in plants, soil and water	140
13.1.1.3	Species found in foods	140
13.1.1.4	Biochemical characteristics of the genus <i>Enterococcus</i>	141
13.1.2	Fecal streptococci	141
13.1.2.1	Biochemical characteristics of the genus <i>Streptococcus</i>	142
13.1.3	Differentiation of enterococci from fecal streptococci	142
13.1.4	Methods of analysis	142

13.2	Plate count method APHA 2001 for enterococci and fecal streptococci in foods	143
13.2.1	Material required for analysis	143
13.2.2	Procedure	144
13.3	Most probable number (MPN) method APHA 2001 for enterococci and fecal streptococci in foods	145
13.3.1	Material required for analysis	145
13.3.2	Procedure	145
13.4	Membrane filtration method APHA/AWWA/WEF 2005 for enterococci and fecal streptococci in water	145
13.4.1	Material required for analysis	145
13.4.2	Procedure	146
13.5	Membrane filtration method ISO 7899-2:2000 for intestinal enterococci in water	148
13.5.1	Material required for analysis	148
13.5.2	Procedure	148
13.6	References	149
<b>14</b>	<b>Lactic acid bacteria</b>	<b>151</b>
14.1	Introduction	151
14.1.1	<i>Carnobacterium</i> Collins <i>et al.</i> 1987	151
14.1.2	<i>Enterococcus</i> (ex Thiercelin & Jouhaud 1903) Schleifer & Kilpper-Bälz 1984	153
14.1.3	<i>Fructobacillus</i> Endo and Okada 2008	153
14.1.4	<i>Lactobacillus</i> Beijerinck 1901 emend. Haakensen <i>et al.</i> 2009	154
14.1.5	<i>Lactococcus</i> Schleifer <i>et al.</i> 1986	154
14.1.6	<i>Leuconostoc</i> van Tieghem 1878	155
14.1.7	<i>Oenococcus</i> Dicks <i>et al.</i> 1995 emend. Endo and Okada 2006	155
14.1.8	<i>Pediococcus</i> Balcke 1884	156
14.1.9	<i>Streptococcus</i> Rosenbach 1884	156
14.1.10	<i>Tetragenococcus</i> Collins <i>et al.</i> 1993	156
14.1.11	<i>Weissella</i> Collins <i>et al.</i> 1994	157
14.1.12	Methods of analysis	157
14.2	Plate count method APHA 2001 for lactic acid bacteria in foods	160
14.2.1	Material required for analysis	160
14.2.2	Procedure	160
14.3	Most probable number (MPN) methods APHA 2001 for lactic acid bacteria in foods	162
14.3.1	Material required for analysis	162
14.3.2	Procedure using the MRS broth	162
14.3.3	Procedure using the Rogosa SL Broth	162
14.4	References	165
<b>15</b>	<b>Campylobacter</b>	<b>167</b>
15.1	Introduction	167
15.1.1	Taxonomy	167
15.1.2	Epidemiology	169
15.2	Presence/absence method ISO 10272-1:2006 for thermotolerant <i>Campylobacter</i> in foods	169
15.2.1	Material required for analysis	170
15.2.2	Procedure	170
15.3	References	173
<b>16</b>	<b>Cronobacter</b>	<b>175</b>
16.1	Introduction	175
16.1.1	Taxonomy	175

16.1.1.1	<i>Cronobacter</i> Iversen <i>et al.</i> 2008, gen. nov.	175
16.1.2	Epidemiology	175
16.1.3	Codex Alimentarius microbiological criteria for <i>Cronobacter</i> spp. in powdered infant formulae	176
16.2	Presence/absence method ISO 22964:2006 for <i>Cronobacter</i> [ <i>Enterobacter sakazakii</i> ] in milk powder and powdered infant formula	177
16.2.1	Material required for analysis	177
16.2.2	Procedure	178
16.3	References	180
<b>17</b>	<b><i>Pseudomonas</i></b>	<b>181</b>
17.1	Introduction	181
17.1.1	Taxonomy	181
17.1.1.1	<i>Pseudomonas</i> Migula 1894	181
17.1.1.2	<i>Shewanella</i> MacDonell & Colwell 1986	184
17.1.1.3	<i>Janthinobacterium</i> De Ley <i>et al.</i> 1978 emend. Lincoln <i>et al.</i> 1999	185
17.1.1.4	<i>Stenotrophomonas</i> Palleroni & Bradbury 1993	186
17.2	Most probable number (MPN) method APHA/AWWA/WEF 2005 for <i>Pseudomonas aeruginosa</i> in water	186
17.2.1	Material required for analysis	186
17.2.2	Procedure	187
17.3	Membrane filtration method ISO 16266:2006 for <i>Pseudomonas aeruginosa</i> in water	188
17.3.1	Material required for analysis	188
17.3.2	Procedure	188
17.4	Plate count method ISO 13720:2010 for presumptive <i>Pseudomonas</i> spp. in meat and meat products	190
17.4.1	Material required for analysis	191
17.4.2	Procedure	191
17.5	Plate count method ISO 11059:2009 IDF/RM 225:2009 for <i>Pseudomonas</i> spp. in milk and milk products	193
17.5.1	Material required for analysis	194
17.5.2	Procedure	194
17.6	References	196
<b>18</b>	<b><i>Listeria monocytogenes</i></b>	<b>197</b>
18.1	Introduction	197
18.1.1	Taxonomy	197
18.1.2	Epidemiology	198
18.1.3	Methods of analysis	199
18.2	Presence/absence method BAM/FDA 2011 for <i>Listeria monocytogenes</i> in foods	200
18.2.1	Material required for analysis	200
18.2.2	Procedure	200
18.3	Presence/absence method MLG/FSIS/USDA 2009 for <i>Listeria monocytogenes</i> in foods	204
18.3.1	Material required for analysis	204
18.3.2	Procedure	206
18.4	Plate count method ISO 11290-2:1998 Amendment 1:2004 for <i>Listeria monocytogenes</i> in foods	207
18.4.1	Material required for analysis	207
18.4.2	Procedure	209
18.5	Presence/absence method ISO 11290-1:1996 Amendment 1:2004 for <i>Listeria monocytogenes</i> in foods	212
18.5.1	Material required for analysis	212
18.5.2	Procedure	212
18.6	References	214

<b>19</b>	<b><i>Salmonella</i></b>	<b>217</b>
19.1	Introduction	217
19.1.1	Taxonomic classification of <i>Salmonella</i>	217
19.1.2	Serological classification of <i>Salmonella</i>	219
19.1.3	Biochemical characteristics of <i>Salmonella</i>	221
19.1.4	Epidemiology	221
19.1.5	Traditional methods used for the examination of <i>Salmonella</i>	223
19.1.6	Alternative methods for the analysis of <i>Salmonella</i>	225
19.1.7	Composite samples for analysis	225
19.2	Presence/absence method ISO 6579:2002 Amendment 1:2007 for <i>Salmonella</i> in foods	227
19.2.1	Material required for analysis	227
19.2.2	Procedure	227
19.3	Presence/absence method BAM/FDA 2011 for <i>Salmonella</i> in foods	232
19.3.1	Material required for analysis	232
19.3.2	Procedure	232
19.4	Presence/absence method MLG/FSIS/USDA 2011 for <i>Salmonella</i> in foods	242
19.4.1	Material required for analysis	242
19.4.2	Procedure	242
19.5	References	247
<b>20</b>	<b><i>Vibrio cholerae</i> and <i>Vibrio parahaemolyticus</i></b>	<b>249</b>
20.1	Introduction	249
20.1.1	Taxonomy	249
20.1.2	Epidemiology	253
20.1.2.1	<i>V. cholerae</i>	254
20.1.2.2	<i>V. parahaemolyticus</i>	254
20.1.2.3	<i>V. vulnificus</i>	254
20.1.3	Methods of analysis	255
20.2	Presence/absence method APHA 2001 and BAM/FDA 2004 for <i>Vibrio cholerae</i> in foods	255
20.2.1	Material required for analysis	256
20.2.2	Procedure	256
20.3	Most probable number (MPN) method APHA 2001 and BAM/FDA 2004 for <i>Vibrio parahaemolyticus</i> in foods	258
20.3.1	Material required for analysis	258
20.3.2	Procedure	259
20.4	Presence/absence method ISO 21872-1:2007 for presumptive enteropathogenic <i>Vibrio cholerae</i> and <i>Vibrio parahaemolyticus</i> in foods	261
20.4.1	Material required for analysis	261
20.4.2	Procedure	261
20.5	References	265
<b>21</b>	<b><i>Yersinia enterocolitica</i></b>	<b>267</b>
21.1	Introduction	267
21.1.1	Taxonomy	267
21.1.2	Epidemiology	270
21.2	Presence/absence method ISO 10273:2003 for presumptive pathogenic <i>Yersinia enterocolitica</i> in foods	270
21.2.1	Material required for analysis	271
21.2.2	Procedure	271
21.3	References	275

<b>22 Bacterial spore count</b>	<b>277</b>
22.1 Introduction	277
22.1.1 The bacterial spore	277
22.1.1.1 Sequence of spore formation	277
22.1.1.2 Spore ultrastructure	277
22.1.1.3 Mechanisms of spore resistance	278
22.1.2 Taxonomy of sporeforming bacteria important in foods	278
22.1.2.1 <i>Aeribacillus</i> Miñana-Galbis <i>et al.</i> 2010	278
22.1.2.2 <i>Alicyclobacillus</i> Wisotzkey <i>et al.</i> 1992 emend. Goto <i>et al.</i> 2003 emend.	
Karavaiko <i>et al.</i> 2005	279
22.1.2.3 <i>Aneurinibacillus</i> Shida <i>et al.</i> 1996 emend. Heyndrickx <i>et al.</i> 1997	281
22.1.2.4 <i>Anoxybacillus</i> Pikuta <i>et al.</i> 2000 emend. Pikuta <i>et al.</i> 2003	281
22.1.2.5 <i>Bacillus</i> Cohn 1872	282
22.1.2.6 <i>Brevibacillus</i> Shida <i>et al.</i> 1996	283
22.1.2.7 <i>Clostridium</i> Prażmowski 1880	284
22.1.2.8 <i>Cohnella</i> Kämpfer <i>et al.</i> 2006	287
22.1.2.9 <i>Desulfotomaculum</i> Campbell and Postgate 1965	287
22.1.2.10 <i>Geobacillus</i> Nazina <i>et al.</i> 2001	287
22.1.2.11 <i>Jeotgalibacillus</i> Yoon <i>et al.</i> 2001 emend. Chen <i>et al.</i> 2010	288
22.1.2.12 <i>Lentibacillus</i> Yoon <i>et al.</i> 2002 emend. Jeon <i>et al.</i> 2005	288
22.1.2.13 <i>Lysinibacillus</i> Ahmed <i>et al.</i> 2007	289
22.1.2.14 <i>Moorella</i> Collins <i>et al.</i> 1994	289
22.1.2.15 <i>Oceanobacillus</i> Lu <i>et al.</i> 2002 emend. Lee <i>et al.</i> 2006	289
22.1.2.16 <i>Paenibacillus</i> Ash <i>et al.</i> 1994 emend. Shida <i>et al.</i> 1997	290
22.1.2.17 <i>Sporolactobacillus</i> Kitahara and Suzuki 1963	290
22.1.2.18 <i>Thermoanaerobacter</i> Wiegel and Ljungdahl 1982 emend. Lee <i>et al.</i> 2007	290
22.1.2.19 <i>Thermoanaerobacterium</i> Lee <i>et al.</i> 1993	291
22.1.2.20 <i>Virgibacillus</i> Heyndrickx <i>et al.</i> 1998 emend. Wainø <i>et al.</i> 1999 emend.	
Heyrman <i>et al.</i> 2003	291
22.2 Methods APHA 2001 for spores of total and “flat sour” thermophilic aerobic sporeformers in foods	292
22.2.1 Material required for analysis	292
22.2.2 Procedure for the analysis of sugar	292
22.2.3 Procedure for the analysis of starch	293
22.2.4 Procedure for the analysis of whole tomatoes, tomato pulp, tomato puree and concentrated milk	293
22.2.5 Procedure for the analysis of nonfat dry milk	294
22.2.6 Procedure for the analysis of milk cream	294
22.2.7 Procedure for the analysis of other foods and ingredients (general)	294
22.3 Methods APHA 2001 for spores of thermophilic anaerobic sporeformers in foods	296
22.3.1 Material required for analysis	296
22.3.2 Procedure for the analysis of sugar and powdered milk	296
22.3.3 Procedure for the analysis of starches and flours	297
22.3.4 Procedure for the analysis of cereals and alimentary pastes	297
22.3.5 Procedure for the analysis of fresh mushrooms	297
22.3.6 Procedure for the analysis of “in-process” products	297
22.4 Methods APHA 2001 for spores of sulfide spoilage anaerobic sporeformers in foods	298
22.4.1 Material required for analysis	298
22.4.2 Procedure for the analysis of sugar	298
22.4.3 Procedure for the analysis of starch and flour	298

22.4.4	Procedure for the analysis of skim milk powder	299
22.4.5	Procedure for the analysis of soy protein isolates	299
22.5	Methods APHA 2001 for spores of mesophilic aerobic sporeformers in foods	299
22.5.1	Material required for analysis	299
22.5.2	Procedure for foods in general	300
22.5.3	Procedure for the analysis of milk and dairy products	300
22.5.4	Procedure for the analysis of water	302
22.6	Methods APHA 2001 for spores of mesophilic anaerobic sporeformers in foods	302
22.6.1	Material required for analysis	302
22.6.2	Procedure for the analysis of sugar	302
22.6.3	Procedure for the analysis of starch, flours and other cereal products	303
22.6.4	Procedure for the analysis of dehydrated vegetables	303
22.6.5	Procedure for the analysis of seasonings and spices	303
22.6.6	Procedure for the analysis of egg powder, milk powder and other powdered dairy products	303
22.6.7	Procedure for the analysis of fluid milk and cheeses	304
22.6.8	Other procedures for mesophilic anaerobic sporeformers	304
22.7	Methods IFU 12:2007 for <i>Alicyclobacillus</i> in foods	304
22.7.1	Material required for analysis	305
22.7.2	Procedure for the analysis of raw material	305
22.7.3	Procedure for analysis of the finished product	306
22.7.4	Interpretation and calculation of the results	307
22.8	References	307
<b>23</b>	<b>Commercial sterility</b>	<b>311</b>
23.1	Introduction	311
23.1.1	Parameters for evaluating the heat resistance of microorganisms	312
23.1.1.1	Survival curve and decimal reduction time (D value)	312
23.1.1.2	Number of decimal reductions	312
23.1.1.3	Thermal destruction curve and temperature coefficient (z value)	312
23.1.2	D and z values of microorganisms of importance in foods	313
23.1.3	Dimensioning heat treatments and thermal processing	316
23.1.4	Microbial spoilage of canned foods	317
23.2	Method APHA 2001 for commercial sterility or cause of spoilage of low acid canned foods	318
23.2.1	Material required for analysis	319
23.2.2	Procedure	319
23.2.3	Interpretation of the results	323
23.3	Method APHA 2001 for commercial sterility or cause of spoilage of acid canned foods	326
23.3.1	Material required for analysis	326
23.3.2	Procedure	327
23.3.3	Interpretation of the results	330
23.4	References	332
<b>24</b>	<b>Guidelines on preparation of culture media</b>	<b>335</b>
24.1	Introduction	335
24.1.1	Ingredients used in the formulation of culture media	335
24.1.1.1	Water for preparing media and reagents	335
24.1.1.2	Nutrient sources for culture media	335
24.1.1.3	Selective agents	338
24.1.1.4	Differential agents	338

XVI *Table of contents*

24.1.1.5 Reducing agents	338
24.1.1.6 Buffering agents	339
24.1.1.7 Chromogenic and fluorogenic substrates	339
24.1.1.8 Agar	340
24.1.2 Types of culture media	340
24.2 Procedure for the preparation of culture media	341
24.2.1 Storing supplies and ingredients for preparation of culture media	341
24.2.2 Weighing and rehydration	341
24.2.3 Dissolution and dispersion	341
24.2.4 Verification and adjustment of the pH before sterilization	342
24.2.5 Distribution	342
24.2.6 Sterilization by moist heat	342
24.2.7 Sterilization by filtration	343
24.2.8 Verification after sterilization	344
24.2.9 Preparation of supplements for culture media	344
24.2.10 Storage of sterilized media until the moment of use	344
24.2.11 Preparation of the media at the moment of use	344
24.3 References	345
<b>Annex 1 – Preparation of media and reagents</b>	<b>347</b>
<b>Annex 2 – Sampling plans and microbiological limits recommended by ICMSF for foods</b>	<b>437</b>
<b>Subject index</b>	<b>445</b>