

Exhibit 1057
Mullins Decl. Appendix M-1
(Midura from WTS)

4TH
EDITION

COMPENDIUM OF METHODS FOR THE
MICROBIOLOGICAL EXAMINATION OF FOODS

COMPENDIUM OF METHODS FOR THE MICROBIOLOGICAL EXAMINATION OF FOODS

FOURTH EDITION



EDITED BY:
FRANCES POUCH DOWNES
KEITH ITO

AMERICAN PUBLIC HEALTH ASSOCIATION

QR
115
A5
2001

STEEN

Contents

COMPENDIUM OF METHODS FOR THE MICROBIOLOGICAL EXAMINATION OF FOODS

FOURTH EDITION

EDITED BY
FRANCES POUCH DOWNES
KEITH ITO



AMERICAN PUBLIC HEALTH ASSOCIATION

General Library System
University of Wisconsin - Madison
728 State Street
Madison, WI 53706-1494
U.S.A.

Steenbock Memorial Library
University of Wisconsin - Madison
550 Babcock Drive
Madison, WI 53706-1293

The American Public Health Association is an Association of individuals and organizations working to improve the public's health. It promotes the scientific and professional foundation of public health practice and policy, advocates the conditions for a healthy global society, emphasizes prevention, and enhances the ability of members to promote and protect environmental and community health.

American Public Health Association
800 I St., NW
Washington, DC 20001-3710

Mohammad N. Akhter, MD, MPH
Executive Vice President

© 2001 by the American Public Health Association

All rights reserved. This book is protected by copyright. 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, scanning, or otherwise, except as permitted under Sections 107 or 108 of the 1976 United States Copyright Act, without either the prior written permission of the Publisher or authorization through payment of the appropriate per-copy fee to the Copyright Clearance Center, 222 Rosewood Drive, Danvers, MA 01923, (978) 750-8400, fax (978) 750-4744. Requests to the Publisher for permission should be addressed to the Permissions Department, American Public Health Association, 800 I Street, NW, Washington, DC 20001-3710, fax (202) 777-2531, e-mail: <permissions@apha.org>.

10 M 3/01
Library of Congress Catalog Card Number: 99-073697

ISBN: 0-87553-175-x

Printed and bound in the United States of America.

Cover Design: Sam Dixon Design

Typesetting: Susan Westrate, Joseph R. Loehle

Set in: Palatino and Universe

Printing and Binding: Sheridan Books, Inc.

NOTE: Any discussion of medical or legal issues in this publication is being provided for informational purposes only. Nothing in this publication is intended to constitute medical or legal advice, and it should not be construed as such. This book is not intended to be used as a substitute for specific medical or legal advice, since medical and legal opinions may only be given in response to inquiries regarding specific factual situations. If medical or legal advice is desired by the reader of this book, a medical doctor or attorney should be consulted.

The use of trade names and commercial sources by authors in certain chapters of this compendium does not imply endorsement by either the APHA or the editorial board of this volume.

Contents

About the Editors	xiv
Editorial Committee	xiv
Authors	xv
Reviewers	xix
Preface	xxi
Chapter 1 Laboratory Quality Assurance	1
1.1 Introduction	1
1.2 Management's Role In Laboratory Quality Assurance	1
1.3 General Laboratory Operations	2
1.4 Personnel	3
1.5 Facilities	4
1.6 Equipment and Instrumentation	5
1.7 Laboratory Glassware and Plasticware	6
1.8 Media and Reagents	7
1.9 Record Keeping	9
1.10 Accreditation of Testing Laboratories	10
1.11 References	10
Chapter 2 Sampling Plans, Sample Collection, Shipment, and Preparation for Analysis	13
2.1 Introduction	13
2.2 General Considerations	13
2.3 Equipment, Materials, and Reagents	13
2.4 Precautions	14
2.5 Procedures	14
2.6 References	23
Chapter 3 Microbiological Monitoring of the Food Processing Environment	25
3.1 Introduction	25
3.2 Environmental Sampling Strategies	25
3.3 Sampling of Surfaces—Equipment and Physical Plant	26
3.4 Rinse Solution Method for Sampling Containers and Processing Equipment Systems	27
3.5 Surface Contact Methods	27
3.6 Microbiological Air-Sampling Strategies	30
3.7 Air-Sampling Methods	30
3.8 Aerosol Sampling and Measurement Guidelines	34
3.9 References	34
Chapter 4 Microscopic Methods	37
4.1 Introduction	37
4.2 General Procedures	37
4.3 Fluorescence Microscopy	37
4.4 Stains, Probes, and Indicators	38
4.5 Quantitation	40
4.6 References	42
Chapter 5 Cultural Methods for the Enrichment and Isolation of Microorganisms	45
5.1 Introduction	45
5.2 Enrichment Methods	45
5.3 Preenrichment	45
5.4 Selective Enrichment	46
5.5 Quality Assurance of Enrichment and Isolation Method	49

5.6	References	50
Chapter 6	Culture Methods for Enumeration of Microorganisms	53
6.1	Introduction	53
6.2	Dilutions	53
6.3	Plating Techniques	54
6.4	Most Probable Number Techniques	58
6.5	Membrane Filtration	61
6.6	Other Methods	61
6.7	Anaerobic or Other Atmospheres	61
6.8	References	62
Chapter 7	Aerobic Plate Count	63
7.1	Introduction	63
7.2	Microbiological Specifications	63
7.3	General Considerations	63
7.4	Equipment, Materials and Reagents	64
7.5	Precautions and Limitations	64
7.6	Standard Procedures	64
7.7	Alternative Methods	65
7.8	References	67
Chapter 8	<i>Enterobacteriaceae</i>, Coliforms, and <i>Escherichia coli</i> as Quality and Safety Indicators	69
8.1	Introduction	69
8.2	Definitions	70
8.3	Precautions	71
8.4	Equipment, Materials and Reagents	73
8.5	Sample Preparation	73
8.6	The <i>Enterobacteriaceae</i>	74
8.7	Coliforms	74
8.8	Fecal Coliform Group	76
8.9	<i>Escherichia coli</i>	76
8.10	Interpretation of Data: The Value of <i>Enterobacteriaceae</i> , Coliforms, and <i>E. coli</i> as Indicators of Quality and Indexes of Pathogens	79
8.11	Acknowledgments	80
8.12	References	80
Chapter 9	Enterococci	83
9.1	Introduction	83
9.2	General Considerations	83
9.3	Equipment, Materials, and Reagents	84
9.4	Precautions	84
9.5	Enumeration of Enterococci	85
9.6	Confirmation of Enterococci	85
9.7	References	86
Chapter 10	Rapid Methods for Detection, Identification, and Enumeration	89
10.1	Introduction	89
10.2	Antibody-Based Methods	89
10.21	Introduction	89
10.22	Immunofluorescence	89
10.23	Latex Agglutination	90
10.24	Immunoimmobilization	90
10.25	Enzyme immunoassay	90
10.26	Immunoprecipitate Detection	90
10.27	Immunoaffinity Chromatography and Immunomagnetic Separation	91
10.28	References	91
10.3	Nucleic Acid-Based Methods	93
10.31	Introduction	93
10.32	Hybridization	93
10.33	PCR	94
10.34	References	95
10.4	Biochemical and Enzymatic Methods	96
10.41	Introduction	96
10.42	Miniaturized Microbiological Methods	96
10.43	Commercial Miniaturized Diagnostic Kits ("Minikits")	96

10.44	Chromogenic Media	99
10.45	Quantitative Enzymatic Methods	99
10.46	References	100
10.5	Membrane Filtration	100
10.51	Hydrophobic Grid Membrane Filter	100
10.52	References	103
10.53	Commercial References	105
10.6	Impedance, Conductance, and Capacitance	105
10.61	Theory	105
10.62	Commercial Systems	105
10.63	General Considerations and Precautions	106
10.64	Typical Applications	107
10.65	Interpretation of Results	109
10.66	References	109
10.67	Commercial References	111
10.7	Phage Probes	111
10.71	Introduction to Bacterial Viruses	111
10.72	Bacteriophage-Based Detection Methods	113
10.73	Conclusions	122
10.74	References	122
Chapter 11	Molecular Typing and Differentiation	127
11.1	Introduction	127
11.2	Cellular Fatty Acid Analysis	127
11.21	Introduction	127
11.22	Principle	130
11.23	Apparatus	130
11.24	Reagents	130
11.25	General Instructions	130
11.26	Limitations	131
11.27	Library	131
11.28	Similarity Index	132
11.29	References	132
11.3	Multilocus Enzyme Electrophoresis	132
11.31	Introduction	132
11.32	General Description of the Method	133
11.33	Interpretation of the Gels	135
11.34	References	135
11.4	Ribotyping	136
11.41	Introduction	136
11.42	Reagent Preparation	136
11.43	Lysis of Bacteria and DNA Extraction	137
11.44	DNA Digestion and Electrophoresis	137
11.45	Electrophoretic Transfer, Denaturation, and UV Cross-Linking	137
11.46	Hybridization and Detection	137
11.47	Patterns: Data Processing	137
11.48	Automated Ribotyping	137
11.49	References	138
11.5	Restriction Fragment Length Polymorphism Fingerprinting	138
11.51	Introduction	138
11.52	Genomic Target	138
11.53	Restriction Enzyme	139
11.54	Combining Assays	139
11.55	Other Considerations	139
11.56	References	140
11.6	Rapid PCR-Based Methods	140
11.61	Introduction	140
11.62	Preparation of Genomic DNA from Bacteria for Use with Rapid Methods ..	145
11.63	Notes	147
11.64	Preparation, Amplification, Detection and Analysis of PCR for Rapid Methods	147
11.65	Notes	149
11.66	References	151

11.7	Pulsed Field Gel Electrophoresis	154
11.71	Introduction	154
11.72	Utility of PFGE in Foodborne Outbreak Investigations	154
11.73	Interpretation of PFGE Results	154
11.74	Choosing a PFGE Protocol	154
11.75	Standard PFGE Protocol for Subtyping <i>E. coli</i> O157:H7	156
11.76	References	157
Chapter 12	Labor Savings and Automation	157
12.1	Introduction	157
12.2	Solid Samples	158
12.4	Liquid Samples	158
12.5	References	159
Chapter 13	Psychrotrophic Microorganisms	159
13.1	Introduction	160
13.2	Review of Methods Used to Enumerate Psychrotrophs in Foods	161
13.3	General Recommendations	162
13.4	Sample Preparation	162
13.5	Equipment, Media, Materials, and Reagents	162
13.6	Procedures for Enumeration of Psychrotrophs	162
13.7	Precautions	162
13.8	Rapid Detection	163
13.9	Interpretation	164
13.10	References	167
Chapter 14	Thermotolerant Microorganisms and Heat Resistance Measurements	167
14.1	Introduction	168
14.2	Heat Resistance Measurements	169
14.3	Methods for the Determination of Heat Resistance	169
14.4	Equipment, Materials, and Procedures	172
14.5	Interpretation	172
14.6	References	175
Chapter 15	Lipolytic Microorganisms	175
15.1	Introduction	177
15.2	General Considerations	177
15.3	Equipment, Materials, and Reagents	178
15.4	Procedures	179
15.5	Precautions	180
15.6	References	183
Chapter 16	Proteolytic Microorganisms	183
16.1	Introduction	184
16.2	General Considerations	184
16.3	Equipment, Materials, and Reagents	184
16.4	Procedures	185
16.5	Precautions	185
16.6	Interpretation	185
16.7	References	187
Chapter 17	Halophilic and Osmophilic Microorganisms	187
17.1	Introduction	187
17.2	Halophilic Microorganisms	190
17.3	Osmophilic Microorganisms	192
17.4	References	195
Chapter 18	Pectinolytic and Pectolytic Microorganisms	195
18.1	Introduction	195
18.2	Detecting Pectinolytic and Pectolytic Organisms	197
18.3	Visualization and Assay of Pectic Enzymes	197
18.4	Sources of Pectic Enzymes	197
18.5	Equipment, Materials, and Reagents	198
18.6	Procedures	198
18.7	Interpretation	198
18.8	References	201
Chapter 19	Acid-Producing Microorganisms	201
19.1	Introduction	201
19.2	General Considerations	201

19.3	Limitations	202
19.4	Equipment, Materials, and Reagents	202
19.5	Procedures	203
19.6	References	206
Chapter 20	Yeasts and Molds	209
20.1	Introduction	209
20.2	General Considerations	209
20.3	Precautions	210
20.4	Equipment, Materials, and Reagents	210
20.5	Procedures	210
20.6	Interpretation	211
20.7	New Methods under Development	211
20.8	References	213
Chapter 21	Detection and Enumeration of Heat-Resistant Molds	217
21.1	Introduction	217
21.2	General Considerations	217
21.3	Equipment, Materials, and Reagents	217
21.4	Procedures	218
21.5	Precautions	219
21.6	Interpretation	219
21.7	Taxonomy of Important Heat-Resistant Molds	219
21.8	References	221
Chapter 22	Mesophilic Aerobic Sporeformers	223
22.1	Introduction	223
22.2	Special Considerations	224
22.3	Sources of Error	224
22.4	Equipment, Materials, and Media	225
22.5	Procedures	225
22.6	Modifications	226
22.7	Interpretation	226
22.8	References	226
Chapter 23	Mesophilic Anaerobic Sporeformers	229
23.1	Introduction	229
23.2	General Considerations	231
23.3	Equipment, Materials, and Media	232
23.4	Precautions	232
23.5	Procedures	232
23.6	Interpretation of Results	235
23.7	References	235
Chapter 24	Aciduric Flat Sour Sporeformers	239
24.1	Introduction	239
24.2	General Considerations	240
24.3	Equipment, Materials, and Reagents	241
24.4	Procedures	241
24.5	Maintenance of Cultures	243
24.6	Precautions and Limitations of the Procedures	243
24.7	Interpretation	243
24.8	References	243
Chapter 25	Thermophilic Flat Sour Sporeformers	245
25.1	Introduction	245
25.2	General Considerations	245
25.3	Equipment, Materials, and Reagents	246
25.4	Precautions	246
25.5	Procedure	246
25.6	Interpretation of Results	247
25.7	References	248
Chapter 26	Thermophilic Anaerobic Sporeformers	249
26.1	Introduction	249
26.2	General Considerations	249
26.3	Equipment, Materials, and Solutions	249
26.4	Precautions	250
26.5	Procedure	250

26.6	Interpretation	251
26.7	References	251
Chapter 27	Sulfide Spoilage Sporeformers	253
27.1	Introduction	253
27.2	General Considerations	253
27.3	Equipment, Materials, and Reagents	254
27.4	Precautions and Limitations of Procedure	254
27.5	Procedure	254
27.6	Interpretation	255
27.7	References	255
Chapter 28	Investigation of Foodborne Illness Outbreaks	257
28.1	Introduction	257
28.2	Purpose of Investigation	257
28.3	Theory of Investigation	258
28.4	Consideration of Samples	258
28.5	Equipment, Materials, and Solutions	258
28.6	Investigative Procedures	262
28.7	Analysis and Conclusion	263
28.8	Molecular Subtyping Techniques—Bacteria	264
28.9	Molecular Subtyping Techniques—Viruses	265
28.10	Conclusion	265
28.11	References	265
Chapter 29	Microbial Food Safety Risk Assessment	267
29.1	Introduction	267
29.2	Risk Assessment: A Tool for Decision-Making	267
29.3	Approaches to Microbial Food Safety Risk Assessment	268
29.4	Steps of Risk Assessment	270
29.5	Mathematical Approaches to Risk Assessment	272
29.6	Monte Carlo Analysis	274
29.7	Uncertainty & Variability	275
29.8	Applications of Risk Assessment	276
29.9	Summary	276
29.10	References	277
	Appendix 1: Common Distributions	280
Chapter 30	<i>Aeromonas</i>, <i>Arcobacter</i>, and <i>Plesiomonas</i>	283
30.1	<i>Aeromonas Hydrophila</i>	283
30.11	General Characteristic of the Genus	283
30.12	Ecology	283
30.13	Epidemiology	283
30.14	Isolation Media	285
30.15	Characterization and Speciation in the Genus <i>Aeromonas</i>	286
30.16	Handling of Samples	287
30.17	Equipment, Materials, and Reagents	287
30.18	Procedures	287
30.19	Interpretations	288
30.110	Media, Reagents, and Stains	288
30.111	References	288
30.2	<i>Arcobacter</i>	290
30.21	Introduction	290
30.22	General Considerations	292
30.23	Media and Reagents	292
30.24	Procedures	292
30.25	Identification Tests	293
30.26	References	294
30.3	<i>Plesiomonas Shigelloides</i>	296
30.31	Introduction	296
30.32	Ecology	296
30.33	Isolation and Identification	297
30.34	Equipment, Materials, and Solutions	297
30.35	Procedure	297
30.36	References	298

Chapter 31	<i>Campylobacter</i>	301
31.1	Introduction	301
31.2	General Considerations	302
31.3	Media and Reagents	302
31.4	Procedures	303
31.5	Identification Tests	304
31.6	Interpretation	307
31.7	References	307
Chapter 32	<i>Bacillus cereus</i>	311
32.1	Introduction	311
32.2	Procedure	313
32.3	Interpretation of Data	315
32.4	References	315
Chapter 33	<i>Clostridium botulinum</i> and Its Toxins	317
33.1	Introduction	317
33.2	Treatment of Specimens	318
33.3	Special Equipment and Supplies	318
33.4	Media and Reagents	319
33.5	Precautions and Limitations of Methods	319
33.6	Procedure for Identifying Viable <i>C. botulinum</i>	319
33.7	Identifying Botulinum Toxin in Foods	320
33.8	Identifying Botulinum Toxin in Clinical Specimens	321
33.9	Interpreting Data	322
33.10	References	323
Chapter 34	<i>Clostridium perfringens</i>	325
34.1	Introduction	325
34.2	Sampling	326
34.3	Equipment and Supplies	326
34.4	Special Reagents and Media	326
34.5	Recommended Controls	327
34.6	Precautions and Limitations of Methods	327
34.7	Procedure	327
34.8	Interpretation of Data	329
34.9	References	329
Chapter 35	Pathogenic <i>Escherichia coli</i>	331
35.1	Introduction	331
35.2	Conventional Isolation Procedures	332
35.3	Rapid Methods	335
35.4	Serotyping	336
35.5	Pathogenicity Testing	336
35.6	References	338
Chapter 36	<i>Listeria</i>	343
36.1	Introduction	343
36.2	General Considerations	346
36.3	Media, Reagents, and Equipment	346
36.4	Precautions and Limitations of the Methods	347
36.5	Procedures	348
36.6	Interpretation of Data	353
36.7	References	353
Chapter 37	<i>Salmonella</i>	357
37.1	General Basis of Methods	357
37.2	Treatment of Sample	364
37.3	Equipment and Supplies	364
37.4	Precautions and Limitations of Reference and Rapid Methods	365
37.5	Reference Method	367
37.6	Interpretation of Data—Reference Method	372
37.7	Rapid Screening Tests	372
37.8	AOAC-RI Performance Tested Methods	375
37.9	References	376
Chapter 38	<i>Shigella</i>	381
38.1	Introduction	381
38.2	Recommended Procedures	382

38.3	Precautions	384
38.4	References	385
Chapter 39	<i>Staphylococcus aureus</i> and Staphylococcal Enterotoxins	387
39.1	Introduction	387
39.2	General Considerations for Isolation of <i>S. aureus</i>	388
39.3	Precautions and Limitations of Methods	389
39.4	Equipment, Reagents and Media	390
39.5	Procedures	390
39.6	Additional Tests	392
39.7	General Considerations for Detection of Staphylococcal Enterotoxins	392
39.8	Handling of Samples	393
39.9	Examining Staphylococcal Isolates for Enterotoxin Production	393
39.10	Enterotoxin Identification in Foods	397
39.11	References	400
Chapter 40	<i>Vibrio</i>	405
40.1	Introduction	405
40.2	General Considerations	407
40.3	Equipment, Materials, and Reagents	408
40.4	Precautions	409
40.5	Procedures	409
40.6	Interpretation	416
40.7	Acknowledgment	416
40.8	References	416
Chapter 41	<i>Yersinia</i>	421
41.1	Introduction	421
41.2	Analysis for <i>Yersinia</i> in foods	422
41.3	<i>Yersinia</i> Identification Tests	426
41.4	References	426
Chapter 42	Waterborne and Foodborne Parasites	429
42.1	Introduction	429
42.2	Waterborne Parasites: Prevalence, Disease, Transmission, and Methods of Identification	431
42.3	Foodborne Parasites: Prevalence, Disease, Transmission, and Methods of Identification	434
42.4	Emerging Methods	437
42.5	References	437
Chapter 43	Toxigenic Fungi and Fungal Toxins	439
43.1	Introduction	439
43.2	Mycotoxins	439
43.3	Mycotoxicoses	441
43.4	Mycotoxin Producing Molds	443
43.5	Equipment, Material, and Reagents	444
43.6	Precautions	444
43.7	Media and Procedures	444
43.8	Reviews and Compendia	445
43.9	References	445
Chapter 44	Foodborne Viruses	447
44.1	Introduction	447
44.2	Foods To Be Tested	449
44.3	Detecting Viruses in Foods	449
44.4	Equipment, Materials and Reagents	452
44.5	Sample Processing Procedures	452
44.6	Cell Culture-Based Virus Assays	455
44.7	Virus Identification	456
44.8	Interpretation of Results	457
44.9	References	457
Chapter 45	Meat and Poultry Products	463
45.1	Introduction	463
45.2	Raw Meat and Poultry Products	464
45.3	Ready-to-Eat Meat and Poultry Products	466
45.4	Pathogens	469
45.5	Recommended Methods	469

45.6	References	469
Chapter 46	Eggs and Egg Products	473
46.1	Introduction	473
46.2	General Considerations	474
46.3	Normal Flora	474
46.4	Flora Changes in Spoilage	474
46.5	Pathogens of Concern	475
46.6	Indicators of Lack of Sanitation	476
46.7	Recommended Methods	476
46.8	Interpretation of Data	478
46.9	References	479
Chapter 47	Milk and Milk Products	483
47.1	Introduction	483
47.2	Raw Milk	483
47.3	Pasteurized Milk	484
47.4	Dried Products	486
47.5	Butter	486
47.6	Frozen Dairy Products	487
47.7	Concentrated Products	487
47.8	Fermented Dairy Products	488
47.9	Acid-Producing Bacteria in Dairy Foods	490
47.10	Additional Reading	493
47.11	References	493
Chapter 48	Fish, Crustaceans, and Precooked Seafoods	497
48.1	Introduction	497
48.2	Fresh and Frozen Fish and Crustaceans	497
48.3	Cooked Crustacean Products	499
48.4	Breaded and Prepared Seafood Products	500
48.5	Salted and Smoked Products	500
48.6	Other Seafood Products	501
48.7	Modified-Atmosphere Packaging and Storage	502
48.8	Recommended Methods	503
48.9	Interpretation of Results	503
48.10	References	504
Chapter 49	Molluscan Shellfish: Oysters, Mussels, and Clams	507
49.1	Introduction	507
49.2	Examination of Shellfish	508
49.3	Preparation of Sample for Examining Shellfish in the Shell	509
49.4	Microbiological Procedures	511
49.5	Interpretation of Data	511
49.6	Rapid Methods	512
49.7	International Microbiological Standards	512
49.8	References	513
Chapter 50	Fruits and Vegetables	515
50.1	Introduction	515
50.2	Fresh Produce	515
50.3	Processed Fruits and Vegetables	516
50.4	Recommended Methods	518
50.5	Interpretation of Data	518
50.6	References	518
Chapter 51	Fermented and Acidified Vegetables	521
51.1	Introduction	521
51.2	Normal Flora	522
51.3	Flora Changes in Spoilage	522
51.4	Pathogenic Microorganisms	524
51.5	Recommended Methods	524
51.6	Interpretation of Data	528
51.7	References	529
Chapter 52	Gums and Spices	533
52.1	Introduction	533
52.2	General Considerations	533
52.3	Normal Flora	534

52.4	Post-Harvest Changes	535
52.5	Sampling and Preparation for Analysis	536
52.6	Methods	537
52.7	Interpretation of Data	537
52.8	References	538
Chapter 53	Salad Dressings	541
53.1	Introduction	541
53.2	General Considerations	541
53.3	Normal Flora	541
53.4	Floral Change in Spoilage	542
53.5	Human Disease Microorganisms	542
53.6	Microbial Indicators of Poor Sanitation	542
53.7	Equipment, Materials, and Solutions	543
53.8	Recommended Methods	543
53.9	Interpretation of Data	544
53.10	References	544
Chapter 54	Sweeteners and Starches	545
54.1	Introduction	545
54.2	General Considerations	545
54.3	Normal and Indicator Flora	546
54.4	Spoilage Organisms	546
54.5	Pathogens	547
54.6	Recommended Methods	547
54.7	Interpretation of Data	547
54.8	References	547
Chapter 55	Cereal and Cereal Products	549
55.1	Introduction	549
55.2	Factors Affecting Bioload	549
55.3	Methods	552
55.4	References	553
Chapter 56	Confectionery Products	555
56.1	Introduction	555
56.2	General Considerations	555
56.3	Spoilage of Confectionery	556
56.4	Pathogens	556
56.5	Microbial Control Procedures	556
56.6	Recommended Methods	557
56.7	Interpretation of Data	557
56.8	References	558
Chapter 57	Nut Meats	561
57.1	Introduction	561
57.2	General Considerations	561
57.3	Microflora	562
57.4	Pathogens	562
57.5	Molds and Mycotoxins	562
57.6	Laboratory Tests	562
57.7	References	563
Chapter 58	Fruit Beverages	565
58.1	Introduction	565
58.2	Normal Microflora	565
58.3	Pathogens	566
58.4	Spoilage	566
58.5	Recommended Methods	566
58.6	Interpretation of Data	567
58.7	References	567
Chapter 59	Soft Drinks	569
59.1	Introduction	569
59.2	Microbiology	569
59.3	Sanitation	570
59.4	Equipment, Media, and Reagents	570
59.5	Recommended Methods	570
59.6	Interpretation of Data	571

59.7	References	571
Chapter 60	Bottled Water	573
60.1	Introduction	573
60.2	Types of Microorganisms	574
60.3	Equipment, Media, and Reagents	574
60.4	Recommended Methods	574
60.5	Interpretation of Data	575
60.6	Acknowledgments	576
60.7	References	576
Chapter 61	Canned Foods—Tests for Commercial Sterility	577
61.1	Introduction	577
61.2	General Considerations	578
61.3	Equipment, Materials, and Reagents	578
61.4	Precautions	579
61.5	Procedure	579
61.6	Interpretation of Data	581
61.7	References	582
Chapter 62	Canned Foods—Tests for Cause of Spoilage	583
62.1	Introduction	583
62.2	Microbiology of Canned Foods	583
62.3	Examination Methods for Diagnosing Spoilage Conditions in Canned Foods	586
62.4	Keys to Probable Cause of Spoilage	596
62.5	Addresses for Some Firms Listed in References	596
62.6	References	596
Chapter 63	Media, Reagents, and Stains	601
63.1	Culture Media, Introduction	601
63.2	Equipment	605
63.3	Alphabetical Listing of Culture Media	607
63.4	Alphabetical Listing of Reagents, Diluents, and Indicators	636
63.5	Stains, Introduction	646
63.6	Alphabetical Listing of Stains	646
63.7	References	648
63.8	Acknowledgments	648
Chapter 64	Measurement of Water Activity (a_w), Acidity, and Brix	649
64.1	Measurement of a_w	649
64.2	Equipment	650
64.3	Calibration	652
64.4	Measurement of Samples	653
64.5	Precautions and Limitations	653
64.6	Measurement of Acidity	654
64.7	pH Measurements	654
64.8	Titrateable Acidity	656
64.9	Brix	656
64.10	References	657
Index		659

Sampling Plans, Sample Collection, Shipment, and Preparation for Analysis

Thaddeus F. Midura and Raymond G. Bryant

2.1 INTRODUCTION

The objective of this chapter is to enable the user to obtain representative samples of a food lot, submit the samples to the laboratory in a condition that is unchanged microbially from the time of sampling, and prepare the samples for analysis. The procedures described in this chapter apply generally to collecting, labeling, transporting, storing, and preparing samples for analysis. For specific information and discussion about sampling and analytical procedures for canned foods, see Chapters 61 and 62. Other foods also may require special sampling and preparation procedures depending upon the specific microorganisms involved. For such procedures, see the chapters covering the relevant food types and specific microorganisms.

2.2 GENERAL CONSIDERATIONS

A representative sample that is appropriately transported to the laboratory, and prepared for examination, is the first priority in the microbiological examination of any food product. Laboratory results and their interpretation are valid only when appropriate samples are examined. Samples must be representative of the entire lot of material under evaluation, must be the proper type for the determination to be made, and must be protected against extraneous contamination and improper handling, especially at temperatures that may significantly alter the microflora. Refrigeration often must be provided to prevent destruction or growth of organisms in a sample. Unfrozen samples must be refrigerated, preferably at 0° to 4.4°C, from the time of collection until receipt at the laboratory. To avoid contact of the product with melting ice, a sealed eutectic coolant is preferable for use in the shipment container. Samples collected while frozen should be kept solidly frozen. When dry ice is used, the containers should have tight closures to prevent pH changes in the sample caused by the absorption of carbon dioxide. As a general rule, samples should be examined within 36 hr after sampling. Perishable items that cannot be analyzed within 36 hr should be frozen or retained at refrigerated temperatures, depending upon the type of product, reason for analysis, and type of microorganisms sought. Non-frozen samples of shellfish should be examined within 6 hr after collection; they cannot be frozen.³

Samples must be clearly and completely identified with the following information: sample description, collector's name, name and address of the manufacturer, lot number, dealer or dis-

tributor, and date, place, and time of collection. Frequently the temperature at the time of collection is also useful to the laboratory for the interpretation of results. Further, it is often desirable that the reason for testing be given, e.g., samples may be collected as part of a quality control or surveillance program, as official samples to determine conformity to regulatory standards, or as part of a foodborne disease investigation.

2.3 EQUIPMENT, MATERIALS, AND REAGENTS

1. **Instruments for opening containers.** Sterile scissors, knives, scalpels, can openers, or other hand tools as required.
2. **Sample transfer instruments.** Sterile multiple- or single-use spatulas, scoops, spoons, triers, forceps, knives, scissors, tongue depressors, drills and auger bits, corers, dippers, metal tubes, and swabs as required.
3. **Sample containers.** Sterile multiple-use containers, either large or small-mouthed designs, nontoxic, leakproof, and presterilized polyethylene bags or other suitable sterile, nontoxic containers as appropriate.

Nonsterile, nontoxic, single-service vials, polyethylene bags, or bottles that are clean and dry and do not have a viable bacterial count in rinse tests in excess of one organism per mL of capacity are acceptable transport containers. Sterile, evacuated sampling equipment also can be used. Sterile glass containers usually are not desirable because of possible breakage and consequential glass contamination of the sampling environment.

4. **Thermometers.** Thermometers that measure -20 to 100°C with graduation intervals not exceeding 1°C. A metal dial type or digital electronic unit is preferred due to the risk of glass thermometers breaking. Thermometers should be sanitized by dipping in a hypochlorite solution not less than 100 mg/L, or other equivalent microbicide, for at least 30 sec before being inserted into foods.
5. **Microbicide.** Medium strength (100 mg/L) hypochlorite solution or other approved disinfectant.
6. **Labeling supplies.** Pressure-sensitive tapes and labels, tags of adequate size to hold sample information, indelible marking pens.
7. **Sample shipping containers.** For frozen or refrigerated samples, rigid metal or plastic containers that are insulated and equipped with a tight-fitting cover. Each container

should have ample space for the refrigerant so that samples will remain at the desired temperature until arrival at the laboratory.

Containers for nonperishable samples should be made of sturdy corrugated cardboard or other material capable of withstanding abusive shipping conditions.

Refrigerant or dry ice is to be added as needed for perishable samples.

8. **Balance.** A balance with 2000 g capacity having a sensitivity of 0.1 g with a 200 g load is acceptable.
9. **Blenders and mixers.** Mechanical blenders with several operating speeds or rheostat speed control with sterile glass or metal blending jars and covers or a Stomacher (Tekmar Company, Cincinnati, Ohio) are examples of acceptable devices.
10. **Diluents.** Sterile Butterfield's phosphate buffer (Chapter 63). Sterile 0.1% peptone water (Chapter 63). Sterile sodium chloride solutions (Chapter 63). Special diluents required for specific microorganisms and special analytical conditions (specific chapters).

2.4 PRECAUTIONS

Adequate precautions should be taken to prevent microbial contamination of samples from external sources, including the person taking the samples, air, sample containers, sampling devices, and the shipping vehicle. When foods are packaged in small, sealed containers, collect the unopened containers rather than portions from each container.

The sampling operation should be organized in advance with all the needed equipment and sterile containers at hand. For collecting samples, use an instrument appropriate to the physical state of the food.

Protect sampling instruments from exposure and contamination before and during use. When removing sampling instruments from the food container to the sample container, do not pass them over the remaining presterilized instruments. When opening the sterile sampling container, open it sufficiently to admit the sample, then close and seal it immediately. Do not touch the inside of the sterile container lip or lid. Do not allow the open lid to become contaminated. Do not hold or fill a sampling container over the top of a bulk food container when transferring a sample. Fill the sample container not more than 3/4 full to prevent overflow and to allow proper mixing of sample in the laboratory. Do not expel air when folding or whirling plastic sample bags. Submit an empty sterile sampling container similarly opened and closed as a control.

The sample collector must keep their hands away from the mouth, nose, eyes, and face. Hands must be washed immediately before beginning the sampling and during sampling if the hands become contaminated. Sterile plastic gloves may be useful to limit contamination during the procedure.

Contaminated sampling equipment must be placed into proper containers for ultimate disposal and/or sterilization. Labels must never be moistened with the tongue. Use direct labeling of the container or pressure-sensitive labels.

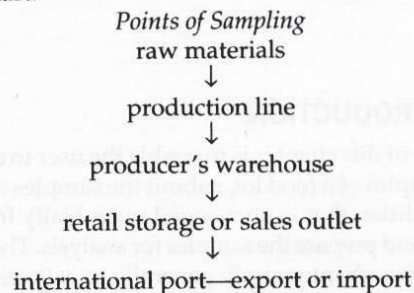
Chapter 1 gives additional safety precautions to ensure proper handling and preparation of samples for microbiological analysis. Food samples may contain infectious microorganisms or toxic materials that may be potentially hazardous. The best protection against hazards is the use of good sampling techniques and treating each sample as if it were contaminated.

2.5 PROCEDURES

2.51 Sampling Plans

Sampling plans were first developed in 1923 by engineers at the Western Electric Company, but were not immediately adopted.²¹ After World War II the Department of Defense developed Military Standards for attribute and variables sampling plans^{21,22,23,24} (definitions in Section 2.511). These plans considered single and multiple sampling based on the history of the producer performance. Levels of sampling (i.e., tightened, normal, and reduced) that reflected prior producer performance were established for these plans. Special categories of sampling plans have been adapted to agricultural products.^{5,6,14,19,20,25}

A comprehensive set of single sampling attribute plans has been published for food microbiology.^{7,12} Typical points of sampling where sampling plans may be applied are shown in the following chart:



This discussion of sampling plans will be clarified if certain basic concepts are stated at the outset. First, the lot to be sampled must be defined. When a given quantity of product is surveyed and units or portions of this lot are taken for examination (including microbiological analyses), the procedure is called "sampling." Sampling can be applied in a way that ensures that results obtained will be statistically valid. In this case, a "sampling plan" is developed, where selection of samples taken from a lot is carried out in a manner that ensures that each sample has the same chance of being chosen. For example, assume that a lot defined as "1000 packages in a warehouse" is to be sampled. Each package is assigned a number. If the 1000 items are stacked equally in 10 rows, then 1 to 100 would be assigned to the units in the first row, 101 to 200 to the second, and so on until all packages are numbered. A random number table is used to choose the required numbered containers for sampling.^{12,17} In this example, if we select five as the required number of packages, then five typical random numbers (lot size being 1000) could be 586, 973, 99, 838, and 737. In addition to random sample selection, a sampling plan will also contain instructions that specify the number of packages to be taken and the basis for accepting or rejecting the lot. Thus, a sampling plan as employed in this context states the number of units required to be randomly collected from a lot and lists the acceptance and rejection criteria. Before using a sampling plan, it is usually prudent to consult a professional statistician to ascertain that the lot of food to be sampled meets the criteria required by that particular sampling plan.

2.511 Definitions Used in Sampling Plans

The following statistical terms are frequently used in the sampling literature.^{9,12,13,24,25}

1. **Acceptance number(s)**—The maximum number(s) of defectives in an attribute sampling plan for which a lot will be accepted.

2. **Acceptance quality level (AQL)**—The maximum percent defective (or the maximum number of defects per 100 units) that for purposes of sampling inspection can be considered satisfactory as a process average.²³
3. **Analytical unit**—The amount of sample actually analyzed.
4. **Attribute**—A qualitative characteristic of a sample unit, e.g., the results of an analysis are positive or negative for *Salmonella*.
5. **Average outgoing quality limit (AOQL)**—The maximum possible percent defective that will result after a rectifying inspection of the lot has been performed. In such an inspection, 100% of rejected lots are screened to remove defective items, provided such screening is 100% effective.²⁴ Typically, AOQL values do not exceed 5%.
6. **Binomial distribution**—Distribution of a population in which a proportion (p) of the units in the population has a certain characteristic, and a proportion ($q = 1 - p$) does not. Thus, each individual unit falls into one of only two classes, either p or q .
7. **Consumer protection**—The ability of a sampling plan to identify unacceptable lots. This is measured as the complement of the probability of acceptance for Limiting Quality (LQ) lots.²⁵ The consumer protection is often set at 90%.
8. **Consumer's risk**—The risk a consumer takes that a lot will be accepted by a sampling plan even though the lot does not conform to requirements.²⁵ This risk is known as a Type I risk, and in many standards^{8,21,23,25} this risk is nominally set at 10%. A second type is a consumer beta risk, which is the risk that the lot will be rejected even though it is actually satisfactory.
9. **Continuous distribution**—A distribution of a population of measures that take on a continuum of values. A variables sampling (Section 2.515) plan may be applicable.
10. **Control chart**—A graphic device that can be used to monitor repeated sampling from a manufacturing or measurement process.
11. **Destructive testing**—A testing process that results in destruction of the unit or sample under test.
12. **Discrete variate**—A random variable consisting of isolated points that can have a finite or infinite number of values. These values can be used in an attribute sampling plan. (Sections 2.512, 2.513, 2.514).
13. **Estimate**—Any value computed from sample data and used to infer a corresponding population (lot) value, e.g., the sample mean (average).
14. **Estimated process average**—The average percent defective or average number of defects per 100 units of product found at the time of original inspection.²⁴
15. **Frequency distribution**—The mathematical description of the way members of a population are distributed. The information about the distribution is used to calculate the probability of lot acceptance or rejection. The discrete random variable takes on a countable number of values, and the probability distribution is defined by a probability mass function. A continuous random variable is defined by a density function.
16. **Homogeneity of variances**—The equality of variances among populations, which must sometimes be tested.
17. **Homogeneous**—A product having a uniform texture or content.
18. **Limiting Quality (LQ)**—Percent defective or defects per 100 units.²⁵ A lot having a 10% probability of acceptance is referred to under many common standards^{8,21,23,25} as a lot having a quality level equal to LQ.
19. **Lot**—The number of sample units produced in one batch or in some specified period of time such that the units will be about the same quality. Each lot or batch should consist of units of product of a single type, grade, class, size, and composition. The lot should be manufactured under the same conditions and at essentially the same time.
20. **Lot inspection by attributes**—Inspection whereby either the sample unit is classified as defective or nondefective with respect to a requirement or set of requirements (when on a "defective" basis), or inspection whereby defects in each sample unit are counted with respect to a requirement or set of requirements (when on a "defect" basis).²⁵
21. **Lot quality**—A measure of the characteristic being controlled. The results of lot inspection are often expressed as percent defective units (e.g., a unit containing *Salmonella* is defective). Less frequently, the quality will be expressed in terms of the variable measured (weight/unit, coliform/g).
22. **Operating characteristic (OC) curve**—A graphical representation of the relation of the probability of lot acceptance to lot quality (usually expressed as percent defective units). This curve will depend on the number of units required in the sampling plan and the acceptance number. The curve also shows the lot quality associated with the consumer's risk and the producer's risk. It thus describes the consequences of the sampling plan (decision rule) for accepting lots of different quality.
23. **Population**—Any finite or infinite collection of individuals (samples or units) on which decisions are to be made.
24. **Probability**—An estimate of the frequency of occurrence of an event, e.g., probability of n sample units out of a population being positive for *Salmonella*, expressed as a value from 0 to 1. For a sampling plan, assumption of a particular probability distribution (i.e., binomial or Poisson) allows the estimation of the computation of the probability of lot acceptance versus lot quality in an operating characteristic curve.
25. **Producer's risk**—The risk that a producer takes that a lot will be rejected by a sampling plan even though the lot conforms to requirements.²⁵ This is called an alpha risk, and in many plans^{8,21,23,25} this risk is set at 5%. Another type is the producer's Type I risk, which is the risk that the lot will be accepted even though it does not conform to requirements.
26. **Proportion defective units (P)**—The number of defective units divided by total units in a lot. Proportion defectives P or percent defectives (100P) are often plotted as the abscissa on an OC curve.
27. **Random sample**—A sample that was chosen in such a way that every sample or unit in the lot had equal chance of being selected. This is often achieved with the aid of a random number table.
28. **Representative sample**—In the widest sense, a sample which is representative of a population, and not merely a portion of it. Some confusion arises if "representative" is regarded as meaning "selected by some process that gives all samples an equal chance of appearing to represent the population." In fact, a representative sample is one that is considered typical of a population in respect of certain characteristics, however chosen. To obtain a truly representative sample, there are four requirements: (a) determine the location of sampling points critical to the population; (b) establish a sampling method representing the population characteristics; (c) select the sample size; and (d) specify the frequency of sampling.

29. **Sample unit**—The smallest definable part of a lot, also called a unit. For example, a singleton or a package is a sample unit. When lots are bulk packaged in bins, barrels, bags, etc., then the sample unit is arbitrary and may depend on the sampling device. The use of the word in this context should be differentiated from the analytical sample unit specified by the analytical method.
30. **Sampling plan**—A design that indicates the number of units to be collected from each lot and the criteria to be applied in accepting or rejecting the lot. A *single sampling plan* requires the lot to be judged on the basis of one set of sample units (Section 2.512). A *double sampling plan* (Section 2.513) is a sampling inspection in which the inspection of the first sample leads to a decision to accept, to reject, or to take a second sample. The inspection of a second sample, when required, then leads to a decision to accept or reject. Two or more such collections would mean that a multiple sampling plan was being used. Another procedure where units are drawn one by one (or in groups) and drawing at any stage determines whether to accept, reject, or continue sampling is called a sequential sampling plan.
31. **Sample size**—The number of samples selected from a lot. The sample size should accurately describe the population, and should be the most economical possible to reach a certain level of accuracy. If the testing method is nondestructive, it may be possible to conduct sampling of 100% of the units in a lot. Typically, microbiological testing destroys product, and thus 100% sampling is not possible. Sample size can be determined by using sampling plan master tables, such as those found in Military Standard 105D, published by the American National Standards Institute.²³
32. **Stratified random sampling**—A procedure for sampling where the lot is divided into parts or strata that differ with respect to the characteristics under study. In some cases, this is a way of improving the estimate of lot quality.
33. **Zero defective tolerance**—An evaluation system implying that a lot must be free of the undesirable characteristic or defect. All units in the lot must be tested in order to ensure zero defectives. This can be performed only where the test is nondestructive. The requirement of zero defects is definitely not possible in microbiological sampling plans since these cannot be applied to all units in a test lot.

2.512 Single Sampling (Two-Class) Attribute Plans

Single sampling procedures are useful in food inspection since, in many production processes, the lot can be sampled and tested only once. In addition, the results of microbiological tests are clearly defined as attributes. For example, an attribute such as the presence or absence of a microorganism, e.g. *Salmonella*, is frequently reported. In other instances, a certain level of organisms may be acceptable. For example, a unit may be acceptable if it has less than 3 *E. coli* colonies /g of sample.

Single sampling attribute plans also have the advantage that the true distribution of the variable in question (e.g., *E. coli*/g) is not required. Single sample plans and the multiple sample plans in the next section (2.513) can be evaluated by using the hypergeometric, binomial, or Poisson distributions. The choice of distributions used to compute the probability relationships^{8,9} depends on the number of units (N) in a lot. When N is large relative to the sample size (n), or when the number of defective samples in a lot is small, a Poisson distribution can be assumed, serving as an approximation of the binomial distribution. The type of lots

acceptable for single sample procedures are assumed to be large, of homogeneous quality, and to satisfy the conditions above.

Steps for choosing and applying an attribute sampling plan are as follows:

- Select the measurements of interest.
- Define the sampling units that constitute a lot.
- Determine a value of consumer's and/or producer's risk to ensure the lot quality (LQ or AQL) desired.
- Obtain an estimate of process average.
- Compute or select a plan that meets the risk and lot quality requirements.
- Calculate the OC curve.
- Apply the plan on a group of randomly selected units from a lot.
- Maintain records on the process average (if you are a producer) and make changes in the plan as necessary.

Figure 1 presents the operating characteristics (OC) curves for seven single sampling plans. These OC curves for the sampling plans will be used to help illustrate the process of choosing a plan. The reader should refer to Dodge and Romig,⁸ Duncan,⁹ and International Commission on Microbiological Specifications for Foods¹² for presentations of complete sets of plans where OC curves for additional values of n or c are given. Figure 1 has an acceptance number (c) of zero, or zero tolerance, which means that any positive results on a test lot will result in the rejection of that specific lot.

As an example, consider that a lot N > 1000 units is to be analyzed for coliform MPN/g and a unit is to be called defective if it

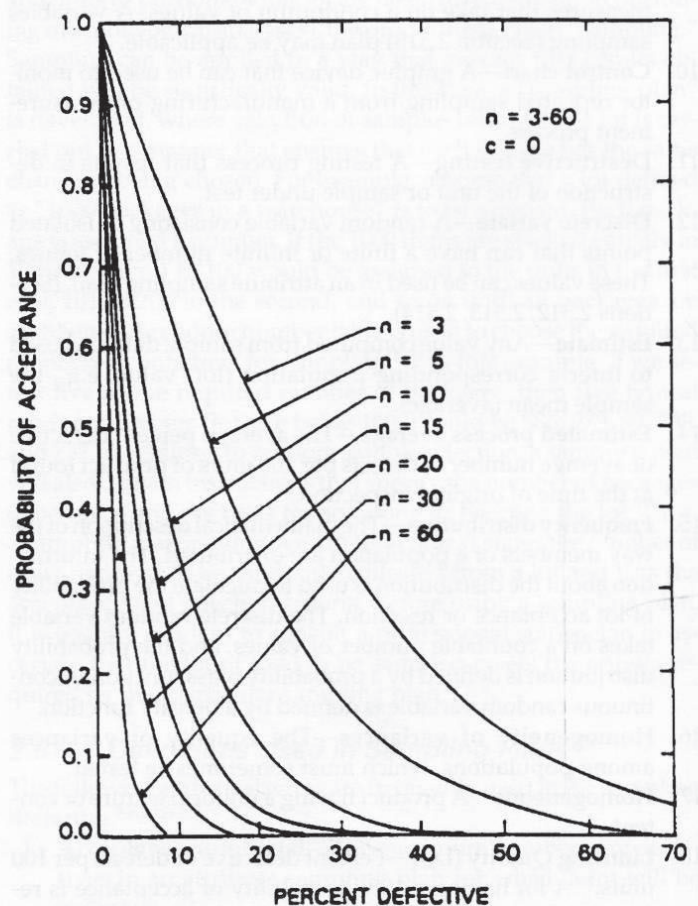


Figure 1. Single sampling (two-class) attribute plans for sample sizes $n = 3, 5, 10, 15, 20, 30, 60$, and $c = 0$.

has MPN $\geq 100/\text{g}$, and to be called acceptable or nondefective if it has an MPN $< 100/\text{g}$. A sampling plan is wanted to define the number of units (n) to sample where the number of defectives (c) equals zero and the probability (consumer's risk) of accepting lots with 8% or more defective units is 10%. Using these criteria, the sampling plan to be chosen (Figure 1) is $n = 30$ and $c = 0$.

Since microbiological tests are destructive (the sample unit cannot be used after testing), cost of sampling may be balanced with the cost of the risk. For example, a plan may be designed to inspect incoming raw materials and, on the other hand, may be designed to sample consumer products before releasing lots for sale. The measurement (i.e., coliform MPN/g) may be the same, but the choice of plan will be affected by other factors such as (a) processing conditions, (b) potential health hazard of the products, (c) persistence of organisms under different storage conditions, and (d) type of plan used by regulatory agencies to inspect the same lots. The $n = 15, 20, 30$, and 60 with $c = 0$ plans in Figure 1 are in common use^{11,12} to test for *Salmonella*. The $n = 3$ and 5 with $c = 0$ plans could be used for screening raw materials.

Some analytical procedures are sufficiently sensitive to measure the presence of a single organism when the sampling units are pooled. A positive test indicates that one or more of the units was positive from n pooled units. This produces the same decision for a plan (e.g., $n = 5, c = 0$) as if the units had been analyzed separately. Pooling of sampling units cannot be done when $c > 0$ or when a positive sample is defined quantitatively (e.g., *E. coli* colonies/g). Calculations with OC curves are found in the references.^{8,9}

It should be noted that as sample size n increases, the OC curve becomes idealized, with a probability of acceptance being 100% with 0 defects, and falling to a probability of 0% when the fraction of defects is > 0 . Also, as the acceptance number c decreases, the OC curve shifts to the left, evidence that with a given number of defective units, the probability of accepting the lot decreases.

2.513 Multiple Sampling (2-Class) Attribute Plans

Sampling plans of this type are designed to inspect lots based on multiple samplings. The lot is accepted or rejected based on sequential decisions. As an example, consider a double sampling plan (Figure 2) of $n_1 = 10, c_1 = 0$ and $n_2 = 6, c_2 = 1$. This plan requires that 10 units be analyzed and the lot be rejected if any unit is defective. If only 1 of the 10 units sampled from the same lot is defective, then 6 additional units are tested. If one or more of the 6 units are defective, then the lot is rejected; otherwise it is accepted. The operating characteristic curve for this plan is given in Figure 2. In this example, if there were 24% defectives in the lot, the plan would accept 10 of 100 lots and the average outgoing quality would be 5% defectives.

Figure 2 shows the operating characteristic curve for the double sampling plan and one for a single sampling plan ($n = 7, c = 0$) that have the same average (5%) outgoing quality limit (AOQL). Although the AOQLs are equal in the two plans, it can be seen that the probability of accepting a defective lot is lower for the single sampling plan. In addition, the choice of taking 15 units ($c = 0$) for a single sampling results in a lowering of the AOQL from 5% to 2.5%. Thus, in choosing sampling plans, one must consider the probability of acceptance of a defective lot that will be tolerated.

A double or other multiple sampling plan requires that the lot of units be available if extended sampling is necessary. This is not practical in many types of foods. However, the advantage of

multiple sampling plans is that lots of good quality (i.e., low coliform count) will on average require fewer samples to be tested than if tested by a single sample plan giving equal protection. For example, a two-stage or double sample plan of $n_1 = 10, n_2 = 6$ might require that just the first 10 units be analyzed. Thus, the average sample number tested would be decreased.

Further information on the design of multiple sampling plans can be found in Dodge and Romig⁸ and Duncan.⁹ A plan can be devised if the desired average quality limit (LQ), consumer (AQL) and/or producer's risk has been selected. Dodge and Romig⁸ present the operating characteristic curves for double sampling plans and several outgoing quality limits (0.1 to 10%, p. 112). The figures allow a quick assessment of risks for each plan. The diagram shown in Figure 3 is an interpretation of double sampling plans.⁸

2.514 Three-Class Attribute Plans

Three-class attribute plans were developed by Bray et al.⁷ in conjunction with the ICMSF¹² to be used with methods recommended by Thatcher and Clark¹⁷ who stated¹² that the "test is concerned primarily with plans that may be applied to lots presented for acceptance at ports or similar points of entry." It was assumed that very little, if any, information would be known about the quality of lots and, thus, attribute plans, two-class plans (Section 2.513), and three-class plans would be applicable. These plans are

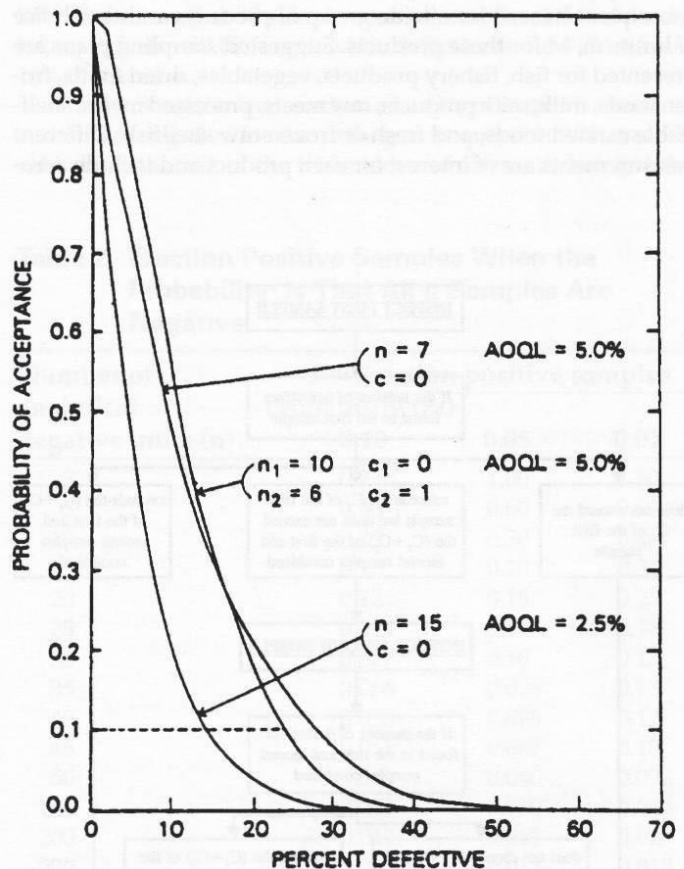


Figure 2. Operating characteristic curves for two single and one double sampling plan with average outgoing lot quality limit 2.5 and 5.0%. The $c = 0$ for a single sampling plan indicates that a lot will be rejected if a single unit of the sample is positive. Rejection of the lot will occur if two or more units of the first or second sample are positive for the double sampling plan.

also useful for inspecting lots within a country or corporation where more information is known about a lot.

Three-class attribute plans differ from those described in Section 2.513 by having two microbiological limits that create three classes of product. Bray et al.⁷ noted that the choice of limits (e.g., for coliform MPN, a quality or sanitation indicator) is difficult. Nonetheless they state that "most scientists can provide two numbers: one below which they have little or no concern and a higher value above which they clearly begin to have critical concern. If we denote the lower level by m and the larger one by M , then the set of values in the range $[m, M]$ can be considered safe." Observations falling in the regions defined by the two limits are called acceptable, marginal, and unacceptable. The acceptable results have values equal to or less than m ($\leq m$). Marginal observations fall between greater than m and equal to or less than M (e.g., $[m, M]$). Unacceptable results are greater than M . Three-class attribute plans can be specified by a sample size n , the number of units allowed (c_1) between limits $\geq m$ and $\leq M$, and the number of units allowed (c_2) equal to or above M . It is assumed that all units $\leq m$ are acceptable. However, as stated above, the value of M is the decision point for this type of sampling plan. The value of $c_2 = 0$ was set in all plans discussed in *Microorganisms in Foods* 2¹² and in the discussion below. Thus, the sampling plans are noted as n, c where c is the number of marginal samples allowed. The three-class attribute sampling plans found in *Microorganisms in Foods* 2¹² present a detailed description of the type of microbiological methods to be recommended, potential risk of contamination, and subsequent hazard for a wide group of products and the choice of limits m, M for these products. Suggested sampling plans are presented for fish, fishery products, vegetables, dried foods, frozen foods, milk, milk products, raw meats, processed meats, shelf-stable canned foods, and fresh or frozen raw shellfish. Different measurements are of interest for each product and include aero-

bic plate count, coliforms, fecal coliforms, *Salmonella*, *V. parahaemolyticus*, *Staphylococcus aureus*, *B. cereus*, *C. perfringens*, and *C. botulinum*. Limits $[m, M]$ were chosen for product-measurement combination and plans were established based on risk.

The ICMSF¹² categorized the types of microbiological hazards and conditions to which a lot of food would be exposed. This categorization is an aid to the microbiologist when considering a choice of sampling plan. These hazards were defined as follows:

1. No direct health hazard, utility (e.g., general contamination, reduced shelf life, and spoilage).
2. Health hazard low, indirect (indicator).
3. Health hazard moderate, direct, limited spread.
4. Health hazard moderate, direct, potentially extensive spread.
5. Health hazard severe, direct.

These hazards are linked with three conditions of risk (reduction, no change, or increase) that reflect how a food was expected to be handled and consumed after sampling. The combination of the types of hazards and conditions of risk yields 15 cases where suggestions of sampling plans and limits could be presented for products, although only a few cases would be realistically applicable for a given product and measurement.

Sampling plans for hazards 1, 2, and 3 are three-class plans in which $n = 10, c = 1$; $n = 5, c = 3$; $n = 5, c = 2$; and $n = 5, c = 1$. In these cases (of the 15 defined cases) limits $[m, M]$ are set to define marginal quality. When the hazards are more severe (i.e., 4 and 5) two-class plans in which $n = 60, c = 0$; $n = 30, c = 0$; $n = 20, c = 0$; $n = 15, c = 0$; $n = 10, c = 0$; and $n = 5, c = 0$ are suggested. As noted in Section 2.513, the two-class plans above mean that n units are analyzed and the lot is accepted only if all units are negative for some characteristic (e.g., *Salmonella*). The operating characteristic curves are given for the two-class plans above in Figure 1.

The approach of the ICMSF¹² on three-class plans was to define risks, suggest limits for a wide variety of specific cases, and suggest plans to be employed. This approach differs from other texts on sampling plans in that the mathematical tools for computing and comparing plans are presented, and the reader must make a choice of various conditions. Those desiring to compute their own three-class plan should consult Bray et al.⁷

Operating characteristic contours presented as two-way tables are used rather than operating characteristic curves for three-class attribute plans. These contours reflect the fact that the true marginal percent can vary from 0 to 100% at the same time the proportion of defective units (value $\geq M$) varies from 0 to 100% with the obvious restriction that the sum of percent good units, percent marginal units and percent bad units must be 100%.

The choice of an ICMSF sampling plan is straightforward. First, specify the food and measurement (e.g., freshwater fish and fecal coliforms). Second, specify the risk. ICMSF¹² suggests case 4 in this example, which reflects low indirect health hazard at reduced risk. The sampling plan is $n = 5, c = 3$. The last step is to choose limits m and M for the product to be sampled. ICMSF¹² recommends $m = 4$ and $M = 400$ for freshwater fish. The complete plan is listed (e.g., $n = 5, c = 3; m = 4, M = 400$), once the product and measurement are specified.

To derive a three-class plan, one must consider (a) the assignment of risk to the product, (b) the choice of sampling plan based on a comparison of probability of acceptance versus percent marginal units (assuming we will not accept any bad units), and (c) the values of m and M for the product. The choice of risk and the setting of m and M may differ depending on the purpose of the sampling. One set of FDA sampling plans for *Salmonella* has values of $n = 15, c = 0$; $n = 30, c = 0$; $n = 60, c = 0$.¹¹

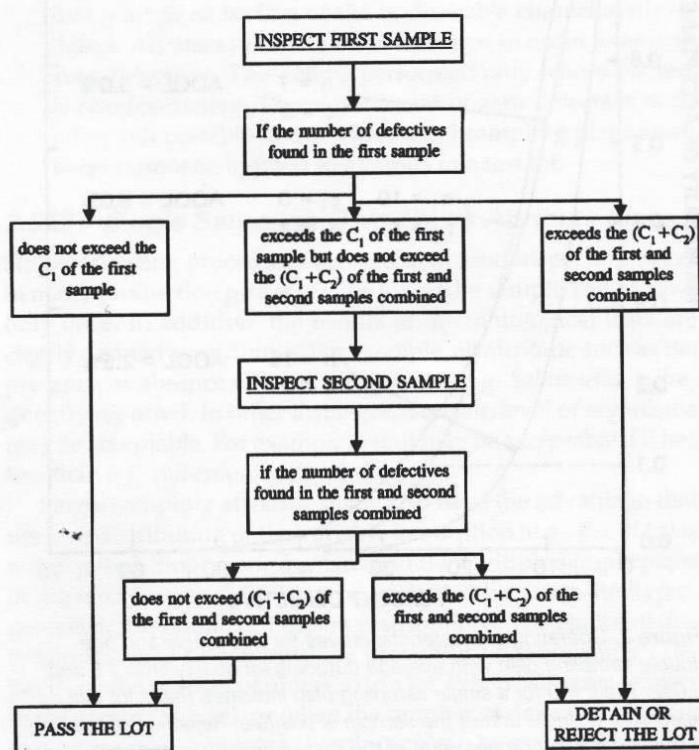


Figure 3. Interpretation of a double sampling plan.

2.515 Variables Sample Plans

A variables plan can be used when the probability density function of a measurement is known, and a majority of published plans are computed when the distribution of the variable or its transformation is normal (Gaussian). An advantage of the variables plans over single sampling plans is that fewer samples are required, resulting in a lower cost to achieve the same protection as a single attribute plan. Disadvantages include the calculations involved in evaluating a lot, the different calculations required for each variable (coliform, *E. coli*, etc.), and the requirement that the probability distribution must be known or assumed for each measurement. The last two requirements may prove to be the most inconvenient. A series of three or four variable (e.g., aerobic plate count, coliform, *E. coli*, and *S. aureus*) measurements may be required on a product. The transformation that ensures a normal distribution may not be known, and the estimates of variance are often different for each measurement. For these reasons, variables sampling plans are not widely used in the food industry for microbiological measurements. For further information about derivation of variables sampling plans, refer to Duncan,⁹ Kramer,¹⁴ and MIL-STD 414.²²

2.516 Sampling Procedures for Low Contamination Levels

The microbiologist often encounters a situation where production lots of a product with low incidence of a pathogen (low number of contaminated units) must be sampled (e.g., 1 positive

sample unit in 500). How many sample units should be taken to have a high probability of detecting the pathogen? Suppose 200 sample units have been analyzed and all are negative. What can be reported about the lot? Clearly, the whole lot may not be free of the pathogen. The same situation also arises in sterility testing. In both of these situations, it is assumed that the pathogen or contaminant can be detected if present in an analytical sample (e.g., 25 g) and the results will be reported as positive or negative. Other necessary assumptions are given in the discussion of single sampling attribute plans (Section 2.512).

Table 1 lists the number of sample units (*n*) needed to detect a positive result at a given level of fraction positive analytical units in a lot. This table can be used to determine how many units should be analyzed in order to detect a positive at a given fraction positive level. Suppose a regulator knows that the fraction positive of a certain pathogen is 0.04. How many sample units should be obtained in order to find a positive unit with probability 0.95? In Table 1, we go down the left hand (fraction positive samples) to 0.04 and over two columns to $(1-Pr) = 0.95$. *Pr* is the probability that all *n* units are negative. The required sample size is 75.

Let us examine the situation from another point of view. Suppose a producer knows that pathogens can be found in his product with a frequency of 6 in 1,000 units. The regulator takes 30 sample units per lot. What is the probability (*Pr*) that all 30 samples will be negative? The answer is $Pr = e^{-(30)(0.006)} = 0.84$. Thus, the regulator has a 0.16 chance of detecting a positive from a lot with a frequency of defective units of 0.006. Simply stated, 84% of the time that 30 samples are collected from this defective lot, no defects will be found, while defective units will be found 16% of the time.

Another situation arises when a sample of *n* units is examined and all are negative for the pathogen in question. What can be said about the rest of the lot in terms of any units being defec-

Table 1. Number of Samples Needed to Detect a Fraction Positive^a with Probability 0.90, 0.95, and 0.99 Where at Least One Positive Result Occurs

Fraction positive ^b samples (<i>P</i>)	Number of analytical units to be tested (<i>n</i>)		
	Probability (1- <i>Pr</i>) 0.90	0.95	0.99
1.0	3	4	4
0.9	3	4	5
0.8	3	4	6
0.7	4	5	7
0.6	4	5	8
0.5	5	6	9
0.4	6	8	12
0.3	10	10	16
0.2	12	15	23
0.1	23	30	46
0.09	26	34	51
0.08	29	38	58
0.07	33	43	66
0.06	39	50	77
0.05	46	60	92
0.04	58	75	115
0.03	77	100	154
0.02	115	150	230
0.01	230	299	461
0.001	2,303	2,996	4,605
0.0001	23,026	29,963	46,052

^a Can be computed from Thorndike Table, p. 35 of Dodge and Romig.⁸

^b The fraction of positive units (e.g., 90 positives in 100 analytical units).

Table 2. Fraction Positive Samples When the Probability^a Is That All *n* Samples Are Negative

Number of analytical negative units (<i>n</i>)	P = Fraction positive samples Probability (<i>Pr</i>)		
	0.10	0.05	0.01
3	0.77 ^b	1.00	1.50
5	0.46	0.60	0.92
10	0.23	0.30	0.46
15	0.15	0.20	0.31
20	0.12	0.15	0.23
25	0.092	0.12	0.18
30	0.077	0.10	0.15
35	0.066	0.086	0.13
40	0.058	0.075	0.12
45	0.051	0.067	0.10
50	0.046	0.060	0.092
100	0.023	0.030	0.046
200	0.012	0.015	0.023
300	0.0077	0.012	0.015
400	0.0058	0.0075	0.012
500	0.0046	0.0060	0.0092
1000	0.0023	0.0030	0.0046

^a Can be computed from Thorndike Table, p. 35 of Dodge and Romig.⁸

^b Rounded to two significant digits.

tive? Table 2 shows one way of expressing the result. The fraction positive (defective units) per lot can be related to the probability, Pr , when all n sample units examined are negative. If 100 sample units were negative, then there is only 1 chance in 10 ($Pr = 0.10$) that the fraction positive exceeds 0.023 per lot. Conversely, the probability is 9 chances in 10 that the fraction positive is 0.023 or lower. Stated yet another way, there is a probability of 10% (at most) that 230 sample units in 10,000 have a pathogen. So even in a lot size of 10,000, there is some chance (although only 10%) that a positive unit will reach a consumer. There is an additional, though small, probability that the consumers will receive the unit. This is because the distribution of microbial contaminants in the lot is not uniform. The fact that contaminants are not evenly distributed throughout the lot increases the chances of not detecting them, and thus increases the probability of accepting the defective lot.

If the samples were homogeneous and the organisms could be assumed to be randomly distributed throughout the lot, then an estimate of concentration could be obtained (Chapter 6). For example, assuming that one *Listeria monocytogenes* organism occurs in each 10 mL of milk, if n samples of milk are taken from a well-agitated bulk milk tank, then the concentration of pathogen could be estimated. If the analytical sample is 1 mL, how many sample units are required to obtain at least one positive sample with probability 0.95? From Table 1 we see that 30 samples should be examined.

Let us further suppose that 2 of the 30 results are positive upon examination. Based on the results of a single dilution, the estimate of concentration of *L. monocytogenes*/mL = $\ln(n/\# \text{ negatives}) = \ln(30/28) = 0.069$. Thus, sampling plans are useful not only for lot inspection, but in determining risk and in predicting a specified level of organisms associated with a given probability, although this is limited to lots where the organism is evenly distributed. In addition, the concentration can also be estimated in some restricted cases.

2.517 Summary

The discussion of sampling plans in this section has been modified for application to microbiological measurements. A general survey of plans is presented, but some of these procedures do not lend themselves directly to microbiological sampling in foods. The brief discussion of multiple sampling plans does not mean that these procedures may not be found useful. The sampling for microorganisms is destructive, the results may be delayed for several days because of incubation requirements, and the foods may be perishable. Thus, single sampling plans are generally best suited to most situations.

Variables sampling plans are subject to additional disadvantages beyond those stated in Section 2.515. The general level of statistical knowledge in the typical food facility may prevent the use of these plans. Calculations needed to choose and apply the variables plans may prove burdensome. The two- and three-class attribute plans are easy to apply and thus should aid in the spread of statistics in microbiological sampling of foods. Additionally, the ICMSF¹² has presented extensive suggestions of specific plans and microbial criteria to be used on a wide variety of products. This reduces the amount of work necessary in calculations and time spent in plan selection. Thus, the single sampling two-class and three-class attribute plans have the most utility in sampling of foods for microbiological analysis. Individuals who wish to derive their own sampling plans are advised to use Dodge and Romig,⁸ Duncan,⁹ and MIL-STD 105.²³

2.52 Sampling Procedures

It is necessary to consider the physical state of the product to be sampled (e.g., dry, semisolid, viscous) and the reason for sampling and testing before taking samples so that the number of units will be representative and/or statistically significant for the intended use.¹² The sampling procedures described in this section are those that generally apply. Unusual sampling procedures that may be required for specific foods are given in the chapters dealing with particular microorganisms or commodities.

2.521 Finished Products

Consumer packages of foods should be sampled from original unopened containers of the same processing lot. Record processing information and product code of samples on forms submitted with samples. The practice of submitting unopened containers prevents contamination that might be introduced by opening at the sampling location. In addition, it allows laboratory examinations to be performed on products and packages or containers as they are offered to the public.

2.522 Bulk Liquid Material

If the products are in bulk form or in containers of a size impractical for submission, aseptically transfer a representative sample portion to a sterile, leakproof container. Before drawing a sample, aseptically mix the food mass to ensure that the sample is as homogeneous as possible. If adequate mixing or agitation of the bulk product is not possible, multiple samples should be drawn from the bulk container. Do not fill sample containers over bulk containers of food. Care must be taken to select sample containers with enough capacity to accommodate the needed sample volume when the container is 3/4 full. Avoid the use of glass sample containers. Thermometers used in bulk food containers should be sanitized before use.⁴ Cool perishable samples to 0° to 4.4°C quickly if they are not already refrigerated. Metal thermometers are preferred since breakage of a mercury thermometer would contaminate the product.

When appropriate, a temperature control sample should be prepared and submitted with the samples to be tested. If a temperature control sample is needed for frozen samples, use a container of ethylene glycol. The sample should be sealed in its container so that the container will not break or leak and introduce extraneous contamination. If the sample is to be examined for a regulatory purpose, the sample container must be sealed so that it cannot be opened without breaking the seal. In addition, an empty sterile sample container should be submitted as a container control.

2.523 Bulk Solids or Semisolids

Sampling of dry or semisolid foods should be done with sterile triers, spoons, or spatulas. Sterile tongue depressors may be substituted for spatulas. Aliquots from several areas of the food under examination should be taken to ensure a representative sample. Care should be taken to protect this type of sample from excess humidity.

2.524 Frozen Bulk Materials

Frozen bulk foods may be sampled with sterile corers, auger bits, and other sharp sampling instruments. A presterilized auger bit or hollow tube may be used to obtain enough material for analysis. Frozen samples should be kept frozen until arrival at the laboratory. Thawing and refreezing of samples must be avoided.

A suitable procedure for obtaining test units of frozen foods (particularly from larger samples) is to use an electric drill combined with a funnel.¹ The sterile auger bit is inserted through a sterile plastic funnel (which has been cut off so the hole is just slightly larger than the bit) held against the frozen sample (Figure 4). The frozen shavings are conveyed to the surface and collected in the funnel. The shavings then can be placed in a sterile sample container. For larger solid food samples—frozen or unfrozen, test units should be taken aseptically from several areas using sterile knives and forceps. These portions should be mixed as a composite to provide a sample representative of the food to be evaluated.

2.525 Line samples (in-process samples)

1. **Liquids.** Sterile metal tubes or dippers may be suitable sampling instruments at certain plant locations for liquid food samples. Disposable plastic transfer pipets can also be used. A special line sampling technique involves the use of a disposable sterile hypodermic needle and syringe. The needle is inserted into a rubber closure of a stainless steel nipple. The nipple can be clamped on or permanently located at any desired spot.¹⁰

Sampling cocks on holding tanks and product pipelines may be used, but disinfection and material flow-through of the sampling cock must be assured before collecting the sample.

2. **Solids.** Sampling of solid line samples may be accomplished using the same equipment and procedures as would be used for bulk solid or semisolid products. Automatic sampling devices are available for powdered products and other solid products not requiring refrigeration. When automatic samplers are used, the manufacturer's directions must be strictly followed.
3. **Special purpose samples (foodborne outbreaks, consumer complaints).** In some instances samples are tested as part of a foodborne disease outbreak investigation or on the basis of a consumer complaint. Consult a clinical or public health laboratory. Such situations may be the subject of legal proceedings, and laboratory personnel may be required to testify concerning the results of their examinations. If the record of sample collection and handling is incomplete, or if samples are received in a partially decomposed state or having been

temperature abused, the laboratory results may be of little or no value.

In outbreak situations, collect all suspect foods. If there are no leftover foods, try to get samples of items prepared in a similar manner subsequent to the suspected food. Collect ingredients or raw items used in the suspect food, if available. All of these foods should be held under suitable conditions until an analysis of the attack rate data and other facts can define more accurately the suspect food(s).¹² The original containers in which the foods were found also should be collected, labeled, and submitted for examination. Other specimens as well as foods from outbreak investigations are essential. Human specimens may include stools, vomitus, and serum, depending upon the suspected cause of illness. These specimens should be collected in sterile leakproof containers and should be properly identified as to the patient's name, type of specimen, and date of collection. Transport media is sometimes indicated. Aseptic techniques always should be used to obtain samples even if the foods have been mishandled grossly.

4. **Samples for water activity measurements.** Collect samples intended for water activity in sealable, vapor tight containers. Obtain small, unopened, retail-size packages if possible. When sampling from bulk containers, work quickly to minimize changes in water content.
5. **Samples for pH measurements.** Samples to be tested for their pH and/or titratable acidity should be collected into tightly sealed containers. If the material to be tested is undergoing fermentation or some other gas producing reaction, use a vented container or flexible plastic bag with ample space for expansion.

2.526 Storage and Shipment of Samples

When it is necessary to store samples prior to shipment, a storage area for frozen (-20°C) and for refrigerated (0° to 4.4°C) samples should be available. Waterproof labels should be used on sample containers to prevent loss of labels. Direct labeling of containers is preferred.

Whenever possible, samples should be submitted to the laboratory in original unopened containers.

Samples should be delivered to the laboratory as rapidly as possible. The condition, time, and date of arrival at the laboratory should be recorded. The samples should be packed to prevent breakage, spillage, or change in temperature. Since laboratory examination of food samples requires preparatory work, the laboratory should be given advance notice, if possible, of the number and types of samples being submitted. If the product is in a dry condition or is canned (flat or normal), it need not be refrigerated for shipment. Swollen containers should be shipped with coolant. The product label may also indicate whether refrigeration is required. Samples not requiring refrigeration or freezing may be packed in a cardboard box using appropriate packing material to prevent breakage. Refrigerated products must be transported in an insulated shipping container with sufficient refrigerant to maintain the samples at 0° to 4.4°C until arrival at the laboratory. Water frozen in plastic containers or cold packs serves well for 0° to 4.4°C shipping and should last 48 hr under most conditions. Do not use loose ice as this may cause product contamination if the container breaks or leaks. Dry ice may be used for longer transit times if the sample is separated from the dry ice packing material to avoid freezing. Refrigerated products should not be frozen, as destruction of certain microorganisms

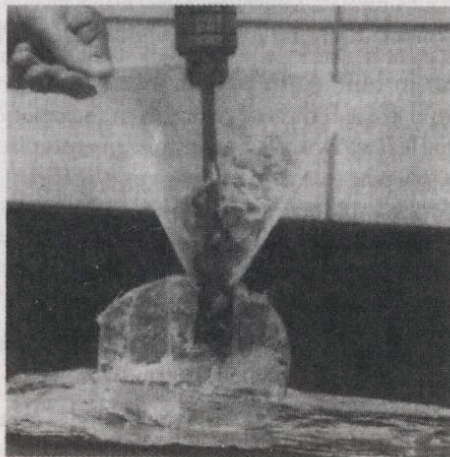


Figure 4. Funnel collection apparatus.

will occur. Frozen samples can be kept frozen by ensuring that the samples are shipped with dry ice. Frozen samples collected in plastic bags, however, must not come in direct contact with dry ice as the plastic bag will become brittle and subject to rupture from the extreme temperature. Use paper or another suitable material to protect the sample. Such samples should be transported to the laboratory by the fastest possible means. Mark the shipment of samples as "Perishable," "Packed in Dry Ice," "Refrigerated Biologic Material," or "Fragile," as appropriate. Label the shipment according to federal postal service rules and Department of Transportation regulations.^{26,27}

2.527 Preparation of Homogenates

Use aseptic technique. Prior to opening, swab the exterior area of the container with 70% ethanol to destroy microorganisms that might contaminate the sample.

Samples should be examined promptly. When initiation of analysis must be postponed, store frozen samples at -20°C until they are to be examined. Refrigerate unfrozen perishable samples at 0° to 4.4°C for not more than 36 hr.

Store nonperishable, canned (normal, flat), or low-moisture foods at room temperature until ready for analysis. Frozen samples should be thawed at refrigeration temperatures ($\leq 4.4^{\circ}\text{C}$) for no longer than 18 hr in the original container in which received.⁴ If the sample must be removed from the original container, it should be done aseptically. Alternatively, higher temperatures may be used for a short period of time, but the temperature must remain low to prevent destruction of microorganisms ($< 40^{\circ}\text{C}$ for ≤ 15 min). Frequent shaking of samples is necessary when samples are thawed by the alternate procedure. A thermostatically controlled water bath with agitator is recommended for the rapid thawing of samples.

Liquid or semiliquid samples in containers that have an airspace can be mixed by rapidly inverting the sample container 25 times. Sample containers that are $2/3$ to $3/4$ full should be shaken 25 times in 7 seconds over a 30 cm arc. The interval between mixing and removing the test portion should not exceed 3 min. To ensure a homogeneous sample when no airspace is present, aseptically open the container and pour the product from the filled container back and forth into a sterile container three times.

Dry samples should be aseptically stirred with a sterile spoon, spatula, or other utensil to ensure a homogeneous sample. Test portions of nonviscous liquid products (i.e., viscosity not greater than milk) may be measured volumetrically using a sterile pipette (11 mL into 99 mL, or 10 mL into 90 mL, or 50 mL into 450 mL). If the pipette becomes contaminated before completing the transfer, replace it with a sterile pipette. Do not insert the pipette more than 2.5 cm below the surface of the sample. The pipette should be emptied into the diluent by letting the column drain from the graduation mark to the rest point of the liquid in the tip of the pipette within 2 to 4 sec, then touch the lower edge of the pipette tip against the inside of the neck of the dilution container. Do not blow out the last drop or rinse the pipette in the dilution fluid.⁴ When measuring products having a viscosity similar to milk, the last drop should be blown from the pipette. For viscous liquid products, the test portion for the initial dilution should be aseptically weighed (11 ± 0.1 g) into a sterile 99 mL dilution blank (or 10 ± 0.1 g into 90 mL, or 50 ± 0.1 g into 450 mL). This provides a 1:10 dilution.

Test portions of solid or semisolid foods should be 50 ± 0.1 g. The 50 ± 0.1 g test portion should be weighed aseptically (using sterile forceps or spatulas) into a sterile tared blender cup^{11,12}; then

add 450 mL of sterile diluent. A variety of diluents may be used depending upon the nature of the product. Those most commonly used are Butterfield's phosphate buffer and 0.1% peptone water. When analyzing for specific organisms, other diluents may be appropriate, e.g., 3% NaCl for *Vibrio parahaemolyticus*. (Refer to the chapters on specific groups of organisms for other diluents.) When analyzing fatty foods or lump-forming powder, wetting agents such as Tergitol Anionic-7 (1% weight/volume) may be included in the diluent to promote emulsification. Blend for 2 min at low speed (approximately 8000 rpm) to disperse the material.¹¹ The blending time may vary depending on the type of food.¹² Some blenders may operate at speeds lower than 8000 rpm. It is preferable to use a higher speed for a few sec initially. No more than 15 min should elapse from the time the sample is blended until all dilutions are in appropriate media.

Optionally, if the entire food sample is less than 50 g, weigh to the nearest 0.1 g portion approximately one-half of the sample into a sterile tared blender cup. Add sufficient sterile diluent to make a 1:10 dilution (i.e., add an amount of diluent equal to nine times the weight of the test portion in the blender cup). The total volume in the blender cup must cover the blades completely. Blend as described above.

If the sample is not homogeneous, weigh 50 g from a representative portion of the package into a sterile, tared blender cup or analyze each portion of food separately. Proceed as described above. Caution should be exercised in the blending step to prevent excessive heating. The amount of heating may vary with foods of different consistencies and may be expected to increase if blending times greater than 2 min are required. Chilled diluent (i.e., tempered in an ice-water bath) may be employed to decrease the chances of excessive heating.

Stomaching is acceptable as an alternative to blending in preparing the food sample homogenate.¹⁶ In this procedure, the food sample with diluent is placed in a clean, preferably sterile plastic bag. The plastic bag is positioned within the stomacher and pummeled for 1 to 2 min. Because the sample is contained in a plastic bag, the developers recommend that samples with bones or other sharp or protruding objects not be prepared by stomaching. Thirty foods were evaluated using this procedure to determine the usefulness in a regulatory agency's laboratory. Results indicated that only certain food homogenates should be prepared using this procedure.²

In some solid food products, the microbial flora is restricted primarily to the surface area. More accurate enumeration of these microorganisms may be obtained by rinsing the sample with sterile diluent rather than by blending. This can be accomplished by placing the sample in a suitable sterile container (plastic bag or sealable bottle) and adding a volume of sterile diluent equal to the weight of the sample. The container is then shaken in a manner similar to that used for preparing an initial dilution of a liquid food sample. Each mL of "rinse" thus prepared represents 1 g of sample.

See Chapter 6 for preparation of further dilutions and plate count procedures. Use media recommended in the specific chapter for the organisms of interest.

2.528 Preparation of Samples for Water Activity Testing

The ideal preparation of samples for water activity measurements is to grind the material to a fine consistency before testing. It is important to avoid heat build-up in the sample during the grind-

ing process. Certain emulsions such as oil/water emulsions may be difficult to measure unless the water phase can be separated by low temperature cycling or centrifugation. Judgement must be used in evaluating the accuracy of measurements done on the water phase of an emulsion. In any case, the prepared sample should be added to the test chamber of the water activity meter quickly to avoid an exchange of moisture with the air. If prepared samples will not be tested promptly, store them in vapor tight, sealed containers. Avoid storage in high humidity environments.

When performing the analysis, transfer sample portions to the test instrument sample holder and follow the manufacturers instructions. A reliable reading may take from 5 minutes to several hours depending on the type of instrument in use. Proper maintenance and calibration of water activity instruments requires some skill and experience. High quality training and support is available from the instrument manufacturers. A complete description of water activity testing can be found in the work of Troller and Christian,¹⁸ and the symposium series by Rockland and Beuchat.¹⁵ See Chapter 64 for a further discussion of water activity measurement.

2.529 Preparation of Samples for pH Determinations

Many types of liquid samples require very little preparation for pH determination. Semisolid samples, mixtures of solids and liquids, emulsions, and various types of marinated foods in oil, require special preparation steps. Semisolid samples can be blended to a thick paste and a small amount (no more than 20 mL/100 g) of distilled water added to provide a more fluid test portion. Mixtures of solids and liquids can be tested either by blending the mixture to a paste and measuring directly or by separating the solid and liquid portions using a U.S. standard #8 sieve. For marinated products in oil, separate the oil from the product, blend the solids (adding a small amount of distilled water if necessary) and test the paste. When attempting to determine the pH of emulsions, it may be necessary to separate the water phase for testing by low temperature cycling or centrifugation.

Temperature effects on the pH electrodes and the actual hydrogen ion activity will modify the pH readings from electronic pH meters. Instruments that have temperature compensation adjust the response of the electrodes. The ionic activity of the sample, however, cannot be corrected for. For accurate results, the standardization and actual determinations should all be done at the same temperature and within the range of 20° to 30°C. See Chapter 64 for a further discussion of pH measurement.

2.6 REFERENCES

- Adams, D. M., and F. F. Busta. 1970. Simple method for collection of samples from a frozen food. *Appl. Microbiol.* 19:878.
- Andrews, W. H., C. R. Wilson, P. L. Poelma, A. Romero, R. A. Rude, A. P. Duran, F. D. McClure, and D. E. Gentile. 1978. Usefulness of the stomacher in a microbiological regulatory laboratory. *Appl. Environ. Microbiol.* 35:89-93.
- APHA. 1970. Recommended procedures for the examination of seawater and shellfish, 4th ed. Am. Public Health Assoc., Washington, D. C.
- APHA. 1985. Standard methods for the examination of dairy products, 15th ed. Am. Public Health Assoc., Washington, D. C.
- Bartlett, R. P., and H. H. Weatherspoon. 1964. USDA variables control chart plan applied to fill weights. *Food Technol.* 18:40-42, 44-45, 48.
- Bartlett, R. P., and J. B. Wegener. 1957. Sampling plans developed by United States Department of Agriculture for inspection of processed fruits and vegetables. *Food Technol.* 11:526-532.
- Bray, D. F., D. A. Lyon, and I. W. Burr. 1973. Three-class attributes plans in acceptance sampling. *Technometrics* 85:575.
- Dodge, H. F., and H. G. Romig. 1959. Sampling inspection tables, 2nd ed. John Wiley & Sons, Inc., New York.
- Duncan, A. J. 1959. Quality control and industrial statistics, rev. ed. Richard D. Irwin, Inc., Homewood, Ill.
- Elliker, P. R., E. L. Sing, L. J. Christensen, and W. E. Sandine. 1964. Psychrophilic bacteria and keeping quality of pasteurized milk. *J. Milk Food Technol.* 27:69-75.
- FDA. 1984. Bacteriological analytical manual, 6th ed. AOAC, Arlington, Va.
- ICMSF. 1986. Microorganisms in foods, vol. 2. Sampling for microbiological analysis: Principles and specific applications, 2nd ed. University of Toronto Press, Toronto, Canada.
- Kendall, M. G., and W. R. Buckland. 1960. A dictionary of statistical terms, 2nd ed. Hafner Publ. Co., New York.
- Kramer, A., and B. A. Twigg. 1966. Fundamentals of quality control for the food industry, 2nd ed. AVI Publishing Company, Westport, Conn.
- Rockland, L. B., and L. R. Beuchat. 1987. Water activity: Theory and applications to food. IFT Basic Symposium Series. Marcel Dekker, Inc., New York.
- Sharpe, A. N., and A. K. Jackson. 1972. Stomaching: A new concept in bacteriological sample preparation. *Appl. Microbiol.* 24:175-178.
- Thatcher, F. S., and D. S. Clark (ed.). 1968. Microorganisms in foods: Their significance and methods of enumeration. University of Toronto Press, Toronto, Canada.
- Troller, J. A., and J. H. B. Christian. 1978. Water activity and food. Academic Press, New York.
- U. S. Department of Agriculture, ARS. 1964. United States standards for sampling plans for inspection by attributes single and double sampling plans. U.S. Government Printing Office, Washington, D. C.
- U.S. Department of Agriculture. 1966. Accuracy of attribute sampling: A guide for inspection personnel. USDA Consumer and Market Service, Washington, D. C.
- U.S. Department of Defense. 1954. Administration of sampling procedures for acceptance inspection (H-105). U.S. Government Printing Office, Washington, D. C.
- U.S. Department of Defense. 1957. MIL-STD 414. Military standard, sampling procedures, and tables for inspection by variables for percent defective. U.S. Government Printing Office, Washington, D. C.
- U.S. Department of Defense. 1963. MIL-STD 105D. Military standard, sampling procedures, and tables for inspection by attributes. U.S. Government Printing Office, Washington, D. C.
- U.S. Department of Defense. 1969. MIL-STD 109B. Military standard quality assurance terms and definitions. U.S. Government Printing Office, Washington, D. C.
- U.S. Government. National Archives and Records Administration. 1988. CFR. Title 7. Part 43 - Standards for sampling plans, p. 320.
- U.S. Government. National Archives and Records Administration. 1997. CFR. Title 49. Part 172. Hazardous materials table, special provisions, hazardous materials communications, emergency response information, and training requirements. Subparts C-F, p. 286-343.
- U.S. Government. National Archives and Records Administration. 1997. CFR. Title 49. Part 173. Section 217 B Carbon dioxide, solid (dry ice), p. 487-488.

Exhibit 1057
Mullins Decl. Appendix M-2
(WorldCat Entry for Midura)



Items ▾

Search for books, articles, and more

[Home](#) [Libraries](#) [Topics](#) [Lists](#) [About](#) [For Librarians](#)



Compendium of methods for the microbiological examination of foods

Authors: [Frances Pouch Downes](#), [Keith Itō](#), [American Public Health Association](#)

Summary: "The Nordic Council and the Nordic Council of Ministers decided in 1994 to initiate and finance a comparative study to understand better the structure and development of

[Show more](#) ▾

Print Book, English, ©2001

Edition: 4th ed [View all formats and editions](#)

Publisher: American Public Health Association, Washington, DC, ©2001

Physical Description: xxi, 676 pages : illustrations ; 29 cm

ISBN: 9780875531755, 087553175X

OCLC Number / Unique Identifier: 46887688

Subjects:

[Aliments Microbiologie](#)
[Aliments Microbiologie Méthodologie](#)
[Aufsatzsammlung](#)
[Food Analysis methods](#)
[Food Contamination analysis](#)
[Food Microbiology](#)
[Food Microbiology Methodology](#)
[Food Microbiology Technique](#)
[Lebensmittel](#)
[Mikrobiologische Analyse](#)

4 editions in 294 libraries

Featured libraries

[All libraries](#)

Showing 206 libraries near [Wisconsin, United States](#)

☐ Only public libraries



Academic

University of Wisconsin - Madison General Library System



[58 miles](#) from your current location.

Memorial Library, 728 State Street, Madison, WI, 53706, United States

Borrow



Favorite Library

Exhibit 1057
Mullins Decl. Appendix M-3
(OPAC Record for Midura)



CATALOG SEARCH

Keywords

Books, music, movies, and more

Q

[Search](#) > [Catalog](#) > [Compendium of methods for the microbiological e...](#)



Books

Compendium of methods for the microbiological examination of foods

AVAILABLE ASPhysical

SUMMARY

"The Nordic Council and the Nordic Council of Ministers decided in 1994 to initiate and finance a comparative study to understand better the structure and development of poverty in five Nordic coun..."

Show More

Steenbock Library

Today's hours: 9 a.m.–11:30 p.m.

✓ Stacks

QR115 A5 2001

Publication Details

CREATOR	edited by Frances Pouch Downes, Keith Ito
FORMAT	Books
LANGUAGE	English
CONTRIBUTORS	Downes, Frances Pouch Itō, Keith American Public Health Association
PUBLICATION	Fourth edition Washington, DC : American Public Health Association, [2001] ©2001
PHYSICAL DETAILS	xxi, 676 pages : illustrations ; 29 cm
ISBNs	9780875531755, 087553175X
OCLC	ocm46887688

National Beef Ex. 1057

030 of 034

Subjects

Food Microbiology.

Medical Subject Heading

Food Analysis -- methods.

Medical Subject Heading

Food Contamination -- analysis.

Medical Subject Heading

Food -- Microbiology -- Methodology.

Food -- Microbiology -- Technique.

Exhibit 1057
Mullins Decl. Appendix M-4
(MARC Record for Midura)

Library Staff Details

IARC Bibliographic Record

LEADER	02021nam a2200445 a 4500
001	9954088163602122
005	20011129122040.0
008	010507t20012001dcua b 001 0 eng
010	\$a 99073697
016	7_ \$a 101097237 \$2 DNLM
020	\$a 087553175X
035	\$a (OCoLC)ocm46887688
035	\$a (WU)5408816-uwmadisondb
035	\$a (EXLNZ-01UWI_NETWORK)999917927602121
040	\$a NLM \$b eng \$c NLM \$d JSJ \$d NOH \$d CUY
042	\$a nlmcopyc
049	\$a GZMA
060	00 \$a 2001 G-403
060	10 \$a QW 85 \$b C737 2001
090	\$a QR115 \$b .A5 2001
245	00 \$a Compendium of methods for the microbiological examination of foods / \$c edited by Frances
246	30 \$a Microbiological examination of foods
250	\$a Fourth edition.
264	_1 \$a Washington, DC : \$b American Public Health Association, \$c [2001]
264	_4 \$c @2001
300	\$a xxi, 676 pages : \$b illustrations ; \$c 29 cm

336		\$a text \$b txt \$2 rda content
337		\$a unmediated \$b n \$2 rda media
338		\$a volume \$b nc \$2 rda carrier
504		\$a Includes bibliographical references and index.
505	0_	\$a 1. Introduction / Bjorn Gustafsson and Peder J. Pedersen -- 2. Low Incomes in Denmark 1980-1995 / Peder J.
520	1_	\$a "The Nordic Council and the Nordic Council of Ministers decided in 1994 to initiate and finance a comparat
650	12	\$a Food Microbiology.
650	22	\$a Food Analysis \$x methods.
650	22	\$a Food Contamination \$x analysis.
650	_0	\$a Food \$x Microbiology \$x Methodology.
650	_0	\$a Food \$x Microbiology \$x Technique.
700	1_	\$a Downes, Frances Pouch.
700	1_	\$a Itô, Keith.
710	2_	\$a American Public Health Association.
994		\$a X0 \$b GZM
997		\$a MARCIVE

MS IDs

Document ID: 999917927602121