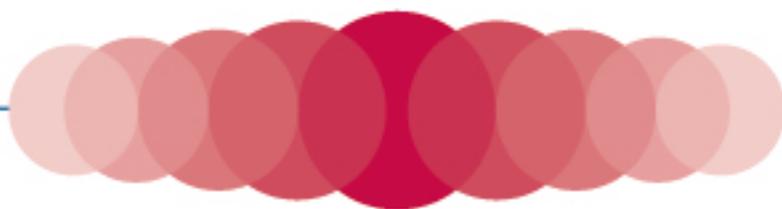


ENCYCLOPEDIA  
OF  
RAPID  
MICROBIOLOGICAL  
METHODS

VOLUME 4



Michael J. Miller  
Editor

# **Encyclopedia of Rapid Microbiological Methods**

## **Volume 4**

Michael J. Miller  
Editor

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*This volume is dedicated to the love of my life  
and my childhood sweetheart, Christine,  
whose inspiring support throughout the  
years has provided me with the courage  
and desire to follow my dreams,  
both professionally and personally.*



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# FOREWORD

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Most of the history of microbiology as we know it has relied on the ability to visualize microorganisms following cultivation. Typically, cultivation has been done in a liquid medium such as a broth, or on a solid or semi-solid surface such as an agar plate (or in earlier times a potato slice). Although the ability to grow microorganisms is still an important aspect of microbiology, the time required for visible microbial growth (i.e., turbidity in liquid medium or a colony on solid medium) can be a hindrance in some circumstances.

The need for rapid results from microbiological tests has long been recognized by microbiologists in the clinical lab and the food industry. Rapid identification methods are now standard in the clinical microbiology lab. These methods range from automated variations on the traditional biochemical and phenotypic microbial identification tests to other rapid tests such as nucleic acid based methods (e.g., sequencing or microarrays) or Matrix-Assisted Laser Desorption/Ionization-Time of Flight (MALDI/TOF). Other rapid clinical tests utilize immunologic reagents to test for the presence of specific pathogenic microorganisms in patient samples. Some of these rapid tests are even used at the patient's bedside (or in the exam room), thus saving the time needed to transport the sample to the lab. The use of rapid microbiological methods has transformed clinical microbiology and improved patient care.

The food industry has also had a strong incentive for rapid microbial results. Screening tests for food borne pathogens must provide rapid results to ensure the safety of perishable fresh food. Waiting for potentially pathogenic microorganisms to grow on conventional media might not provide results quick enough to be truly useful. Rapid methods similar to those used in clinical microbiology are a mainstay in food microbiology and provide valuable information to protect consumers.

The pharmaceutical industry, in general, has not been as pro-active as the clinical and food sectors when it comes to rapid microbiological methods. The microbiological testing paradigm in the pharmaceutical industry has taken two pathways. The first of these has involved the microbiological release test (e.g., sterility or microbial limits). Although sterility tests take at least two weeks for final results, for most drug products it is not considered a problem to wait this long to release a batch of a sterile drug product. However, for some drug products, waiting several weeks for a traditional sterility test may not be practical. For example, for products with very short shelf lives (e.g., blood products, cell therapy products, and radiopharmaceuticals) two weeks might be most or even all of their useful life. Additionally, for some products manufactured in high volumes, the storage of product while waiting for sterility test results can be a significant expense and shortening that storage time could be economically advantageous to their manufacturer.

The other arm of the pharmaceutical microbiology testing approach involves in-process tests. This category of samples would include drug components, bulk drug product, environmental monitoring and water. All of these types of samples are currently tested for bioburden using traditional microbiological methods (typically plate counts) but the results are not available for at least several days. Meanwhile, manufacturing has moved on (perhaps all the way to a finished product) before the results of the microbiological tests are known. If the results of these tests are acceptable, this is a tolerable situation and the process is considered to be in a state of microbiological control. However, if any of these samples return results that exceed an alert or action level, then decisions must be made regarding the significance of these results and what affect they may have on product disposition. For a component like Water for Injection (WFI), a bioburden action level may affect multiple drug products produced using that WFI.

Modern approaches to process control (including Process Analytical Technology) require the availability of results in real-time (or at least close to real-time) to enable the operator to use the test results to make process decisions and adjustments. Although real-time results are only currently available for a limited category of microbiological tests, there are many microbiological

methods that are significantly more rapid than the traditional test methods. The rapid methods available today vary a great deal in their mechanisms of operation. Some of these methods still rely on a period of microbial growth using traditional media but reduce their time to result by using an alternate method of microbial detection. Other rapid methods do away with growth entirely and utilize a stain or inherent microbial auto fluorescence to detect microorganisms even down to the level of a single microbial cell. Some of the available methods are quantitative, some are qualitative, and they vary in their time to result (from real-time to several days) but all of these methods seem to have found a niche in the pharmaceutical microbiologist's arsenal. These current rapid microbiological test methods are now able to start providing some of the advantages (from a process control and economic return standpoint) long enjoyed by our colleagues in the clinical and food microbiology labs. Pharmaceutical microbiologists would be well served by considering which of their samples would provide a benefit with a more rapid result and then assessing the current alternate microbiological methods to see if any of them are a good fit for their needs. This *Encyclopedia of Rapid Microbiological Methods* will be an excellent resource to start that assessment.



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# CONTENTS

<b>Foreword</b>		<b>v</b>
	<i>Bryan S. Riley</i>	
<b>1. The Application of Modern Microbial Methods to the Quality Control Testing of Probiotics</b>		<b>1</b>
	<i>Anthony M. Cundell</i>	
Introduction		1
Quality Control Testing of Probiotics		2
Master and Working Cell Bank		5
<i>Release and Stability Testing</i>		5
<i>Opportunities for the Application of Rapid Microbial Methods</i>		5
<i>Viable Cell Count</i>		6
<i>Identification and Strain Typing</i>		7
<i>Absence of Bacterial Pathogens</i>		10
<i>Antibiotic Resistance</i>		10
<i>Adherence to the Intestinal Wall</i>		11
<i>Acid and Bile Resistance</i>		11
Conclusions		12
References		12
About the Author		17
<b>2. Considerations for Choosing a Rapid Microbiological Method: Aligning Your Needs with Available Technology</b>		<b>19</b>
	<i>Julie Schwedock</i>	
Introduction		19
Drivers for Rapid Methods		20
<i>Will One Method Cover All Testing Needs?</i>		20
<i>What is the Minimum Time Savings Needed to Create Value?</i>		
<i>Are Same Day Results Useful/Necessary?</i>		20
		<b>ix</b>

<i>Is Your Sample Compatible with the Rapid Method?</i>	21
<i>Does the RMM Add Labor or Reduce Labor?</i>	22
<i>Will Automation Help Me by Reducing Human Error?</i>	22
<i>Does the RMM Provide a Count or a Presence/Absence (+/-, or Qualitative) Result?</i>	23
<i>If My Counts are Usually Zero, Can I Get Away with Using a Qualitative RMM as a Screen?</i>	23
<i>How Easy or Difficult is it to Validate the RMM?</i>	24
<i>Is the Count in Real CFU, or Estimated from Another Parameter, such as Relative Fluorescent Units?</i>	25
<i>Do the Microorganisms Survive the RMM, Such that They are Available for Identification?</i>	25
<i>Will the RMM Easily Integrate with the LIMS (Laboratory Information Management System) or Other Data Management Platform?</i>	25
<i>Does the RMM Provide Reporting?</i>	26
<i>How Much Reporting Do I Need?</i>	26
<i>What is the RMM's Record with False Positives?</i>	26
<i>What is the Impact to Me When I Get a False Positive?</i>	26
<i>What is the RMM's Record with False Negatives?</i>	27
<i>What is the LOD (Limit of Detection)?</i>	27
Conclusion	27
Disclaimer	27
Acknowledgement	27
References	28
About the Author	29

**3. Looking to the Future: Rapid and Automated Microbial Identification Technologies** **31**

*Michael J. Miller, Ph.D.*

Introduction	31
A Brief History Lesson	32
<i>Rapid and Automated Microbiological Technologies</i>	33
Growth-Based ID and Presence/Absence Technologies	35
<i>Utilization of Biochemical and Carbohydrate Substrates for Microbial Identification</i>	35
<i>Use of Selective Media for the Rapid and Automated Detection of Specific Microorganisms</i>	37

Cellular Component-Based ID and Presence/Absence Technologies	37
<i>Fatty Acid Analysis for Microbial Identification</i>	37
<i>MALDI-TOF Mass Spectrometry for Microbial Identification</i>	37
<i>SELDI-TOF Mass Spectrometry for Microbial Identification</i>	38
<i>Fourier Transform-Infrared (FT-IR) Spectrometry for Microbial Identification</i>	38
Optical Spectroscopic-Based ID and Presence/Absence Technologies	39
<i>Elastic Scattering for the Detection of Specific Microorganisms</i>	39
<i>Inelastic Scattering for the Detection of Specific Microorganisms</i>	40
Nucleic Acid Amplification-Based ID and Presence/Absence Technologies	41
<i>Ribotyping for Bacterial Identification and Strain Differentiation</i>	42
<i>PCR for the Detection of Specific Microorganisms</i>	42
<i>SYBR Green and Taqman Probes</i>	44
<i>MALDI-TOF Mass Spectrometry of PCR Products for Microbial Identification</i>	46
MEMS-Based ID and Presence/Absence Technologies	49
<i>Microfluidics or Lab-on-a-Chip Systems for Microbial Identification</i>	49
<i>Microarrays for Microbial Identification of Mycoplasma</i>	50
Summary	51
About the Author	52
<b>4. Use of MALDI-TOF Mass Spectrometry for Microbiological Identification in the Pharmaceutical Industry</b>	<b>53</b>
<i>Lothar Bomblies</i>	
Introduction	53
Method	54
Sample Preparation	55
Data Evaluation	55
Species and Strain Identification	56
MALDI-TOF MS in GMP Environments	57
Validation for Actual Use	57
Operational Qualification (OQ)	58
Performance Qualification (PQ) – Accuracy	59

Performance Qualification (PQ) – Precision	60
Performance Qualification (PQ) – Robustness	62
Computer Validation	63
Mixed Cultures	63
Investigation of Broth Cultures	63
Experiences from Routine Investigations	64
Summary	64
Conclusion	65
About the Author	66
<b>5. Implementation of a Genotypic Method for Microbial Identification</b>	<b>67</b>
<i>Sara Gamberini and Emiliano Toso</i>	
Introduction	67
<i>Overview of Traditional Methods</i>	69
<i>Overview of Alternative Methods</i>	70
<i>Advantages of Genotypic Microbiological Methods</i>	72
<i>How to Choose a Method for Microorganism Identification</i>	74
<i>Validation of Alternative Microbiological Methods</i>	75
<i>A Case Study from a Pharmaceutical Company:     Implementation of a Genotypic Method for Microbial     Identification</i>	75
<i>Shipment of Microbial Samples and DNA Extraction</i>	76
<i>Amplification of Microbial DNA</i>	77
<i>Increasing Speed of Identification</i>	78
<i>Automation</i>	78
<i>Validation of the Method</i>	78
<i>Setup and Robustness of the Method</i>	79
<i>Specificity of the Method</i>	79
<i>Accuracy of the Method</i>	79
<i>Precision of the Method</i>	80
<i>Compliance with GMP Principles</i>	80
Conclusions	81
References	81
About the Authors	82

<b>6. Microbial Identification Using the bioMérieux VITEK® 2 System – An Update</b>	<b>85</b>
<i>David H. Pincus</i>	
Introduction	85
Objective	86
Principles	86
<i>VITEK® 2 Compact</i>	87
<i>VITEK® 2 and VITEK® 2 XL</i>	87
<i>Reagent Cards</i>	87
Workflow	87
<i>Reagent Card Types</i>	89
<i>Culture Requirements</i>	89
<i>Suspension Preparation</i>	89
<i>Inoculation</i>	89
<i>Optical System</i>	91
<i>Database Development</i>	91
<i>Analytical Techniques</i>	91
Results	91
<i>Test Reactions</i>	92
<i>Identification Levels</i>	92
<i>Mixed Taxa Identifications</i>	93
<i>Supplemental Testing</i>	93
<i>Non-Reactive Biopattern</i>	93
Applications	93
<i>GN Card</i>	93
<i>GP Card</i>	98
<i>YST Card</i>	102
<i>BCL Card</i>	107
<i>NH Card</i>	107
<i>ANC Card</i>	112
<i>CBC Card</i>	114
<i>Validation Procedure</i>	119
Conclusion	119
References	119
About the Author	121

<b>7. Case Study of a New Growth-Based Rapid Microbiological Method that Detects the Presence of Specific Organisms and Provides an Estimation of Viable Cell Count</b>	<b>123</b>
<i>Ruth Eden and Roger Brideau</i>	
Introduction	123
<i>Industry Needs</i>	123
<i>Limitations of the Traditional Methods</i>	125
<i>Validation Requirements for Change</i>	125
The BioLumix® System	126
<i>Technology</i>	126
<i>Dilute to Spec</i>	126
<i>How Microorganism Change Optical Characteristics</i>	127
<i>Instrumentation</i>	128
<i>Software</i>	129
<i>Disposable Vials</i>	129
Methods	130
<i>General Sample Preparation</i>	130
<i>BioLumix Vial Assay</i>	130
<i>Objectionable Organisms (Absent in 10 Grams)</i>	131
<i>E. coli (EC) Testing</i>	131
<i>Staphylococcus aureus (SA) Testing</i>	131
<i>Pseudomonas aeruginosa (PSE) Testing</i>	132
<i>Salmonella (SAL) Testing</i>	132
<i>Plate Count Method</i>	132
<i>LOD Studies</i>	132
<i>Specificity Studies</i>	133
<i>Repeatability Studies or Precision Testing</i>	133
<i>Robustness</i>	133
<i>Ruggedness</i>	133
Results	134
<i>Comparison to USP &lt;61 &gt;</i>	134
<i>Total Aerobic Count</i>	134
<i>Yeast and Molds</i>	135
<i>Gram-Negative Bile Tolerant (Enterobacterial Counts)</i>	135
<i>Specificity (Inclusivity and Exclusivity)</i>	135
<i>Total Aerobic Count</i>	136

<i>Yeast and Molds</i>	136
<i>Gram-Negative Bile Tolerant (Enterobacterial Counts)</i>	136
<i>Detection Limit</i>	136
<i>Total Count</i>	137
<i>Yeast and Molds</i>	139
<i>Gram-Negative Bile Tolerant (Enterobacterial Count)</i>	139
<i>Precision and Repeatability of Data</i>	140
<i>Yeast and Molds</i>	141
<i>Gram-Negative Bile Tolerant</i>	141
<i>Robustness</i>	142
<i>Statistical Analysis</i>	142
<i>Effect of Temperature</i>	143
<i>Effect of Sample Size</i>	144
<i>Effect of Medium Volume</i>	145
<i>Ruggedness</i>	146
<i>Effect of Analyst</i>	146
<i>Effect of Different BioLumix Units</i>	147
<i>Effect of Reagent Lots (Vial Lots)</i>	148
<i>Objectionable Organisms</i>	148
Summary	150
References	150
About the Authors	152

**8. Evaluation of the Millipore Milliflex® Quantum Rapid Detection System: An Internal Study of a Novel Rapid Method for Microbial Detection in Traditional Membrane Filtration Bioburden Assays** **153**

*Eric J. Ward*

Abstract	153
Introduction	154
System Overview	154
1. Filter and Incubate	155
2. Fluorescent Staining Reagent	155
3. Quantum Reader	156
4. Enumeration	156
5. Re-incubation	157

Evaluation Parameters	157
<i>Time-to-Detection</i>	157
<i>Comparability</i>	157
<i>Microorganism Viability</i>	158
<i>Additional Benefits</i>	158
Time-to-Detection	159
Comparability	161
Microorganism Viability	163
Microorganism Identification	164
Additional Benefits	165
<i>Mammalian Cell Culture Samples</i>	167
<i>Operational Notes</i>	167
<i>Training</i>	168
<i>Low Initial Investment</i>	168
<i>Cost Per Test</i>	168
<i>Work Flow</i>	168
<i>Camera and Software</i>	169
<i>Validation Requirements</i>	169
Potential Applications	169
<i>Mammalian Cell Culture Samples</i>	170
<i>Investigational Testing</i>	170
Conclusions	170
Acknowledgements	170
References	171
About the Author	171
<b>9. Application of USP &lt; 1223 &gt; and Other Guidelines to Comprehensively Assess an Environmental Monitoring RMM for Validation</b>	<b>173</b>
<i>Scott Morris</i>	
Abstract	173
RMM Overview: The IMD-A System	174
RMM Validation: Supplier and End-User Context	176
RMM Validation: Guidance and Interpretation	179
RMM Validation: Experimental Design	181
RMM Validation: Statistical Design	186

RMM Validation: Test Metrics	188
<i>Accuracy</i>	189
<i>Precision</i>	191
<i>Limit of Detection (LOD)</i>	192
<i>Limit of Quantification (LOQ)</i>	193
<i>Linearity</i>	194
<i>Operational Range</i>	196
<i>Ruggedness</i>	197
<i>Robustness</i>	199
<i>Specificity</i>	200
RMM Validation: Data and Analysis	202
RMM Validation: Additional Efforts	204
<i>Summary of RMM Validation</i>	205
Conclusion	206
Acknowledgements	207
References	207
About the Author	211

**10. Evaluation of the BioVigilant IMD-A™, A Novel Optical Spectroscopy Technology for the Continuous and Real-time Environmental Monitoring of Viable and Nonviable Particles.**

<b>Part 1. Review of the Technology and Comparative Studies with Conventional Methods</b>	<b>213</b>
<i>Michael J. Miller, Horatio Lindsay, Rene Valverde-Ventura, and Michael J. O’Conner</i>	
Abstract	213
Introduction	214
<i>Opportunities for Using a Rapid Microbiological Method for Environmental Monitoring</i>	216
Materials	218
Methods	220
<i>Data Analysis for Comparative Studies</i>	222
<i>Evaluation of Three Instruments Under Laboratory Conditions</i>	223
Results and Discussion	224
<i>Evaluation of Three Instruments Under Laboratory Conditions</i>	228
Summary	229
References	231

<b>11. Evaluation of the BioVigilant IMD-A™, A Novel Optical Spectroscopy Technology for the Continuous and Real-time Environmental Monitoring of Viable and Nonviable Particles.</b>	
<b>Part 2. Case Studies in Environmental Monitoring During Aseptic Filling, Intervention Assessments, and Glove Integrity Testing in Manufacturing Isolators</b>	<b>235</b>
<i>Michael J. Miller, Michael R. Walsh, Jerry L. Shrake, Randall E. Dukes, and Daniel B. Hill</i>	
Abstract	236
Introduction	236
Materials and Methods	239
<i>Materials</i>	239
<i>Methods</i>	239
<i>Static Monitoring in Filling and Transfer Isolators</i>	240
<i>Transfer of Sterilized Components into the Filling Isolator</i>	241
<i>Dynamic Monitoring of the Filling Isolator During an Aseptic Fill</i>	243
<i>Monitoring During Interventions</i>	244
<i>Monitoring Isolator Exit Ports</i>	246
<i>Monitoring Glove Interventions and Loss of Glove Integrity</i>	246
<i>Monitoring Glove Integrity</i>	248
Results and Discussion	250
<i>Static Monitoring in Filling and Transfer Isolators</i>	250
<i>Transfer of Sterilized Components into the Filling Isolator</i>	254
<i>Dynamic Monitoring During an Aseptic Fill</i>	255
<i>Monitoring During Interventions</i>	256
<i>Monitoring Isolator Exit Ports</i>	257
<i>Monitoring Glove Integrity</i>	259
Summary	264
References	268
<b>12. A Rapid Microbiological Method for the Release Testing of Sterile And Non-Sterile Products</b>	<b>273</b>
<i>Alessio Fantuzzi</i>	
Overview	273
Introduction	274

Project Overview	275
<i>Selection of Technology – Feasibility Study</i>	276
<i>Selection of Key Application</i>	276
<i>Evaluation of Critical Issues</i>	277
<i>Methods Development and Validation</i>	280
<i>Implementation in the Laboratory</i>	286
Conclusion	288
References	288
About the Author	289
<b>13. Validation of the BacT/Alert® Microbial Detection System as an Alternate Rapid Sterility Test for Dendreon Active Cellular Immunotherapy Products</b>	<b>291</b>
<i>Timothy D. Wood</i>	
Introduction	291
Dendreon Activated Cellular Immunotherapy (ACI) Platform	292
Challenges with the Traditional Methods	293
Alternate Rapid Methods	294
BacT/Alert Detection Platform	295
<i>BacT/Alert Detection Principle</i>	295
<i>Culture Bottles</i>	295
Validation Approach	296
<i>Feasibility Study</i>	296
<i>Performance Qualification and Method Validation</i>	299
Regulatory Path for Commercial Approval	305
<i>Equivalence</i>	306
<i>Approval</i>	309
BacT/Alert Method Transfer	310
<i>Transfer to U.S. Commercial Facilities</i>	310
<i>Transfer to EU Contract Manufacturing</i>	310
Conclusions	311
Acknowledgements	311
References	312
About the Author	313

<b>14. Rapid Method for Microbiological Quality Control to Obtain Same Day Results: A Validation Approach</b>	<b>315</b>
<i>Ir Peter Cornelis</i>	
Introduction	315
Technology	316
Guidance on Validation Parameters	318
Specificity: Challenge to the System	319
Comparability: Accuracy and Precision	319
Limit of Detection	321
Robustness and Ruggedness	323
Considerations	323
<i>Accuracy of the Spike</i>	323
<i>Stressed Cells</i>	323
<i>Matrix Effects</i>	324
Conclusion	326
References	327
About the Author	327
<b>15. Feasibility Studies on Rapid Sterility Testing Using a Bioluminescence-based Method</b>	<b>329</b>
<i>Claudio Denoya, Jennifer Reyes, Maitry Ganatra, and Daniel Eshete</i>	
Introduction	329
Selection of a Rapid Microbiological Method Suitable for Sterility Testing	331
Detection of Microbial Contamination Using ATP Bioluminescence	332
The Pallchek™ Rapid Microbiology System	333
<i>Validation of an Alternative Microbiological Method</i>	333
<i>Challenge Microorganisms, Media and Growth Conditions</i>	334
<i>Components of the Pallchek Rapid Microbiology System</i>	335
<i>Bioluminescence Assay Requirements of Test Environment</i>	336
<i>Drug Product Sample</i>	336
<i>Presence-Absence Test with Enrichment</i>	336
<i>Validation Strategy of Rapid Sterility Test</i>	337
<i>System Suitability Testing</i>	337
<i>Establishment of Background Values</i>	337
<i>Initial Validation Parameters of the Qualitative Rapid Method</i>	338

Evaluation of the Rapid Bioluminescence Test in the Presence of Excipients	343
<i>Product Specific Feasibility Study</i>	344
Summary	345
Acknowledgments	348
References	349
About the Authors	350
<b>16. Statistics of Validating an Alternate Sterility Test: Limits of Detection and Other Problems</b>	<b>353</b>
<i>Julie Schwedock</i>	
Introduction	353
Probabilities and Multiplicity	354
Statistically Different vs. Statistically Equivalent	355
Multiplicity, Validation, and Controlling Risk	359
Limit of Detection	361
Conclusions	367
Acknowledgements	368
References	368
About the Author	369
<b>17. Validation of SECOND Generation ATP Monitoring Technology for Rapid Microbiological Quantification in Fluid Applications</b>	<b>371</b>
<i>David R. Tracey, Patrick A. Whalen, and James E. Cairns</i>	
Introduction and Purpose	371
Principle of the Method	372
<i>Measuring ATP</i>	373
<i>Methods for Detection of Total Microorganisms</i>	374
<i>Why Measure Total Microorganisms?</i>	374
<i>Technology Versatility</i>	376
<i>Limit of Detection</i>	377
<i>Enzyme Activity</i>	377
<i>Sample Volume</i>	378
<i>Strategy for Determining Low Detection Limit</i>	378

Method Validation Summary	379
<i>Potable and High-Purity Water</i>	379
<i>Biological Wastewater Treatment</i>	381
<i>Other Applications</i>	384
<i>Oil and Gas</i>	384
<i>Chemical Preservation</i>	385
Conclusions	386
References	386
About the Authors	388
<b>18. Detection of Specified Microorganisms in Drug Formulations Using A New Quantitative Polymerase Chain Reaction (qPCR) System with Preloaded Multi-well Discs</b>	<b>389</b>
<i>Daniel Eshete, Maitry Ganatra, and Claudio Denoya</i>	
Introduction	389
Basic Principles of qPCR	390
<i>The Cycling Process</i>	390
<i>Using PCR Assays in Manufacturing Quality Control</i>	393
Overview of the GeneDisc System	393
<i>GeneDisc Ultra-Lyser</i>	394
<i>GeneDisc Plates</i>	394
<i>GeneDisc Cyclers</i>	395
Data Analysis	395
Applications	396
<i>GeneDisc Assay for Pharmaceutical Applications</i>	396
<i>Assay Description</i>	397
<i>A Preliminary Study on Validation Parameters</i>	398
<i>Limit of Detection</i>	399
<i>Specificity (Exclusivity and Inclusivity)</i>	402
<i>Detection of Low-Level Contamination in Pharmaceutical Products</i>	405
Summary	408
References	408
About the Authors	410

<b>19. Mycoplasma Testing: Overview, Detection and Validation</b>	<b>413</b>
<i>Fabrizio Lecce, Fabio La Neve, Federica Gillone, and Emiliano Toso</i>	
Mycoplasma	413
<i>Characteristics of Mycoplasma</i>	413
<i>Mycoplasma as Cell Culture Contaminants</i>	415
<i>The Importance of Mycoplasma Detection in the     Biopharmaceutical Industry</i>	417
Traditional Methods to Detect Mycoplasma Contamination	418
<i>Overview</i>	418
Alternative Methods for Rapid Mycoplasma Detection	422
<i>Overview</i>	422
<i>Nucleic Acid Technique-based Methods (NAT)</i>	424
<i>Extraction Methods</i>	434
Validation of NAT Methods for Mycoplasma Detection	436
Overview	436
LOD	436
Specificity	438
Robustness	439
Accuracy	439
Precision	440
Comparability Study	441
Health Authorities Requirements for NAT Methods	442
Overview	442
Sample Volume	442
LOD	443
Specificity	445
In-process Laboratory Controls	446
Comparability Study	446
Conclusions	448
References	449
About the Authors	452

<b>20. A Microarray for Mycoplasma Detection and Identification: CytolInspect™</b>	<b>455</b>
<i>Annett Kilic and Jörg Stappert</i>	
Abstract	455
Mycoplasma	456
Regulatory Guidelines	458
Traditional Methods for the Detection of Mycoplasmas in Biopharmaceuticals	460
Selected Microscopic, Immunological and Biochemical Detection Methods	461
NAT-based Detection Methods	462
Microarrays/CytolInspect	463
<i>Detection Limit</i>	468
<i>Specificity</i>	468
<i>Robustness</i>	468
Conclusion	469
References	469
About the Authors	473
<b>21. Rapid Viral Methods (RVM) for Biological Quality Control (BQC)</b>	<b>475</b>
<i>Laura Barberis and Emiliano Toso</i>	
Viral Safety Overview	475
<i>Prospects for Viral Safety Testing</i>	478
<i>Viral Safety Testing</i>	484
<i>Classic Method Limitations and Molecular Biology Approach</i>	490
<i>Public Viral Incidents and Role of PCR-based Assays</i>	494
<i>MVM Experience</i>	496
<i>Vesivirus Experience</i>	497
<i>Molecular Biology Approach</i>	497
<i>Superseding In Vivo Tests: MAP/HAP by qPCR</i>	499
<i>Superseding Enzymatic RT Assays: Fluorescent Product Enhanced Reverse Transcriptase (FPERT) and Quantitative Real Time Product Enhanced Reverse Transcriptase (qPERT)</i>	500
<i>Starting Material Quality Control: Virus Stock Qualification</i>	502

Future Directions	504
MPS and TIGER	505
<i>PCR Using Degenerate Primers</i>	505
References	508
About the Authors	512
<b>22. Rapid Microbiological Methods and New Pharmaceutical Microbiology Curriculum</b>	<b>513</b>
<i>Claudio D. Denoya</i>	
Introduction	513
A “Rapid” History of Microbiology	514
Today’s Microbiology: the Fundamentals of Traditional Microbiology	516
The New Microbiological Technology Wave: Alternative and Rapid Microbiological Methods (ARMM) for the QC Laboratory	518
What are ARMM?	518
Metabolic or Growth-based Technologies	520
Viability-based Technologies	521
Technologies Based on Cell Component Analysis	522
ARMM and the Pharmaceutical Industry	523
AMM, PAT and Regulatory Status	524
Microbiology Curricula	525
The Skill Sets of a Pharmaceutical QC Microbiologist	526
Pharmaceutical Microbiologist Desirable Knowledge and Skill Sets	528
Curricula Surveys	530
The Disparity Between the Tertiary Microbiology Curricula and the Needs of the Pharmaceutical Industry QC Microbiologist	537
An Interesting Step Forward: The United States Professional Science Master’s Programs	538
Conclusions and Recommendations	539
Acknowledgments	540
References	540
About the Author	543

<b>23. Application of Rapid Microbiological Methods in BioProcessing and Regulatory Considerations</b>	<b>545</b>
<i>Anastasia G. Lolas</i>	
Introduction	545
The Age of Biotechnology	546
Manufacture of Biopharmaceuticals	548
Biopharmaceuticals and US FDA Regulation	551
Testing Requirements and Guidelines	552
Traditional Microbiological Test Methods Used in Biopharmaceuticals' Manufacture	554
Current Uses of Alternative and RMM	556
Contamination Events and Alternative and RMM	558
Potential Applications of Alternative and RMM	560
Regulatory Considerations for the Application of Alternative and RMM	562
Additional Thoughts Regarding the Use of Alternative and RMM	565
Summary	567
References	567
About the Author	574