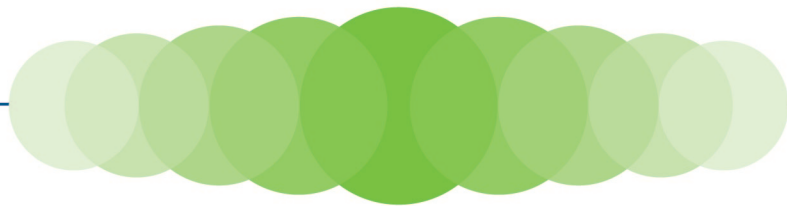


ENCYCLOPEDIA  
OF  
RAPID  
MICROBIOLOGICAL  
METHODS

VOLUME 2



Michael J. Miller  
Editor

---

---

# CONTENTS

<b>Preface</b>		<b>xix</b>
	<i>David Hussong</i>	
<b>Foreword</b>		<b>xxi</b>
	<i>Michael J. Miller</i>	
<b>1. Microbial Identification Using the bioMérieux VITEK® 2 System</b>		<b>1</b>
	<i>David H. Pincus</i>	
Objective		1
Principles		1
<i>VITEK 2 Compact</i>		2
<i>VITEK 2 and VITEK 2 XL</i>		2
<i>Reagent Cards</i>		2
<i>Culture Requirements</i>		3
<i>Suspension Preparation</i>		4
<i>Inoculation</i>		4
<i>Card Sealing and Incubation</i>		4
<i>Optical System</i>		6
<i>Test Reactions</i>		6
<i>Database Development</i>		6
<i>Analytical Techniques</i>		6
<i>Identification Levels</i>		6
<i>Supplemental Testing</i>		7
<i>Non-Reactive Biopattern</i>		8
Applications		8
<i>GN Card</i>		8
<i>GP Card</i>		9
<i>YST Card</i>		19
<i>BCL Card</i>		23

Validation Procedure	27
Potential New Applications	27
Conclusion	27
References	27
About the Author	32
<b>2. Detection of Microorganisms Using Impedance Microbiology and the bioMerieux Bactometer® System</b>	<b>33</b>
<i>Patricia Rule</i>	
History	33
Instrument	34
<i>Equation</i>	36
Theory	36
Applications	39
CFU Determination	40
Bioburden and Microbial Limits	42
<i>Sterility Testing</i>	43
<i>Antimicrobial Effectiveness/Preservative Efficacy Testing</i>	44
<i>Disinfectant/Sanitizer Testing</i>	45
Impedance as a Predictive Indicator	48
Conclusion	49
References	49
About the Author	53
<b>3. Biolog: Modern Phenotypic Microbial Identification</b>	<b>55</b>
<i>Barry R. Bochner</i>	
The Biolog System and Modernization of Phenotypic Testing – An Overview	55
A Brief History of Microbial Identification	57
The Biolog System	59

Problems with Species Not in the Database	62
Retrospective Trending	63
Validation of Identification Systems	63
Perspectives on Phenotypic ID Versus DNA-based ID	64
The Basic Steps of Phenotypic Testing	65
The Importance of Standardization and Precision in the Testing Regime	66
<i>Isolation of a Pure Culture</i>	66
<i>Cultivation on Appropriate Agar Media</i>	67
<i>Performance of the Gram Stain</i>	68
<i>Selection of the Appropriate Test Panel</i>	69
<i>Preparation of the Inoculum</i>	69
<i>Inoculation of the Panel</i>	70
<i>Incubation of the Panel</i>	70
<i>Reading and Interpretation of the Results</i>	70
Conclusions and the Future of Phenotypic Testing	71
References	72
About the Author	73
<b>4. Under the Microscope: Microbial Identifications in Pharmaceutical and Biopharmaceutical Quality Control Laboratories, “An Era of Coarse to Fine Adjustment”</b>	<b>75</b>
<i>Mary Griffin and Dona Reber</i>	
Regulatory Considerations	75
Methods	78
Validation	84
Looking Ahead	85
Case Study	85
<i>Met Assay Acceptance Criteria</i>	87
<i>Accepted Identification</i>	87
Results and Discussion	88
Current Example of a Process Flow Approach for Identification	92

<i>Situation</i>	92
<i>Solution</i>	92
Trending	93
QC Pharmaceutical and Biopharmaceutical Microbiology Laboratories Meet the Business Need	96
Summary	97
Conclusion	98
References	98
About the Authors	100
<b>5. The Growth Direct Test: A Rapid, Non-destructive, Automated System for Microbial Enumeration</b>	<b>103</b>
<i>Roanna London, Julie Schwedock, Andrew Sage, Michael Michalek, Heather Valley, Joe Lacirignola, Paula Welter, Luis Jimenez, Steven Buhl, and Don Straus</i>	
Moving Towards Rapid Microbial Enumeration in Pharmaceutical Microbiology	103
How the Growth Direct Test Works	105
<i>Demonstrating Equivalence to Visual Plate Counting</i>	109
<i>Imaging Growing Microcolonies Over Time for Improving Accuracy</i>	111
<i>Assessing Accuracy at Lowest Levels of Contamination</i>	112
<i>Dynamic Range</i>	114
<i>Detecting a Broad Range of Microbes</i>	115
<i>Time to Detection for Model Organisms</i>	116
<i>Important Parameters Underlying Time to Detection</i>	117
<i>The Range of Microbes That Can Be Detected When the Duration of Culturing is Defined</i>	119
Applying the Growth Direct System to Key Pharmaceutical Microbiology QC Applications	120
<i>Rapid Detection of Microbes in Water Samples</i>	120
<i>Detecting Stressed Microbes</i>	122

<i>Rapid Detection of Microbes in Environmental Air Samples</i>	123
<i>Testing Surfaces Using Contact Plates</i>	127
<i>Rapid Microbial Limit Testing</i>	129
<i>Rapid In-process Bioburden Testing</i>	130
<i>Testing the Feasibility of a Rapid Sterility Test Using the Growth Direct System</i>	131
Conclusion	133
References	134
About the Authors	135
<b>6. ATP Bioluminescence Using Millipore's Milliflex® Rapid System</b>	<b>137</b>
<i>Serge Ohresser</i>	
Introduction	137
<i>Rapid Detection of Microorganisms Using ATP Bioluminescence</i>	137
Milliflex Rapid System: Basics and Principles	138
<i>Bioluminescent Reaction</i>	138
<i>Sample Prep</i>	141
<i>Reagent Spraying</i>	142
<i>Signal Detection and Image Processing</i>	143
<i>How to Validate for Successful Regulatory Acceptance</i>	149
<i>Robustness</i>	149
<i>Ruggedness</i>	157
<i>Accuracy</i>	159
<i>Range and Linearity</i>	161
<i>Limit of Detection</i>	163
<i>Precision</i>	164
<i>Specificity</i>	165
<i>Food and Beverage Industry: Iced Tea</i>	167
Conclusions	172
References	173
About the Author	174

<b>7. ATP Bioluminescence Using the Celsis System</b>	<b>175</b>
<i>Lori Daane</i>	
Introduction	175
RapiScreen™ and ATP Bioluminescence	176
RapiScreen for Products Low in Background ATP	177
Sample Compatibility	177
Organism Specificity	178
Sensitivity	178
RapiScreen for Products Low in Background ATP	179
RapiScreen for Products High in Background ATP	181
Overview of the Celsis Advance™ Luminometer	183
Celsis Advance.im Information Management Software	185
The Evolution of ATP Bioluminescence: AKuScreen™	188
References	191
About the Author	191
<b>8. Selection and Validation of the Celsis Advance ATP Analysis System for Product Release Testing for Non-Sterile Pharmaceuticals</b>	<b>193</b>
<i>Vian Lach</i>	
Current Status	193
Changes Required	194
Potential Rapid Microbiology Technologies	194
<i>ATP Analysis Principle</i>	<b>195</b>
Celsis Advance Luminometer	197
ATP Analysis Application	198
Analysis of Conventional Microbiology	198
Factors Affecting Sensitivity of ATP Analysis	199
<i>Instrument Sensitivity</i>	199
<i>Reagent Background</i>	199
<i>Medium ATP Background</i>	200
<i>Microbial ATP Content</i>	200
Practical Application of ATP Analysis	200

ATP Analysis for Non-sterile Pharmaceuticals	201
Feasibility Studies	201
<i>Examining the Microbial Status of Products to be Tested</i>	203
<i>Examining the Product Formulations for Microbial Contamination Sources</i>	204
<i>Examining the Product Formulations for Sources of ATP</i>	204
<i>Examining the Products for Materials that Interfere with ATP Analysis</i>	204
<i>Examining the Products for Materials that Quench the Light Emitted During Analysis</i>	204
Practical Application of ATP Analysis	205
Estimating Microbial Numbers by ATP Analysis	206
Result Interpretation from ATP Analysis	207
<i>All Samples are Negative</i>	207
<i>The Largest Sample Aliquot is Positive and All Other Aliquots are Negative</i>	207
<i>More Than One Sample Aliquot is Positive</i>	208
Choice of Culture Media	208
Media Quality Control	209
Selection of Test Cultures	210
Preparation of Cultures for Validation Testing	210
Validation of Testing Methods	212
Sample Testing Strategies Using ATP Analysis	213
Sample Testing Scheme EP	214
Sample Testing Scheme USP	214
Sample Testing Scheme Harmonized	214
Choosing a Testing Scheme for ATP Analysis Validation	215
Validation Principles for Compendial Tests	216
ATP Analysis Methods	217
Incubation Requirements for Samples	218
Validation Testing Schemes	224
Validation Result Interpretation	228
Practical Application of ATP Analysis	229
Conclusions	231
References	232
About the Author	232



<b>9. Using ATP Bioluminescence for Microbiological Measurements in Pharmaceutical Manufacturing</b>	<b>233</b>
<i>Lucia Ceresa and Peter Ball</i>	
Introduction	233
Guidance Documents on Adopting Rapid Microbiology Methods	234
Detection of Microbial Contamination Using ATP Bioluminescence	234
The Pallchek™ Rapid Microbiology System	237
Controlling Background	239
Practical Aspects of Routine Usage	240
Applications	242
<i>Early Release of Pharmaceutical Products</i>	242
<i>Monitoring Water for Injection</i>	245
<i>Environmental Monitoring</i>	245
<i>Other Key Applications</i>	245
Validation and Regulatory Approval	246
Acknowledgements	248
References	248
About the Authors	249
<b>10. Rapid Steam Sterilization Biovalidation Using Biological Indicators and the Pallchek™ Luminometer</b>	<b>251</b>
<i>Gilberto Dalmaso</i>	
Abstract	251
Introduction	252
Background	253
<i>Process Validation</i>	254
<i>Developing a Sterilization Process</i>	254
<i>Validation of RMMS for BIs</i>	257
<i>Reagent ATP Dilutions</i>	261
<i>Microbial Dilutions – Geobacillus stearothermophilus</i>	263

<i>Test Protocol for BIs Biovalidation and Preliminary Study</i>	266
<i>Validation of Short Incubation Period BIs Validation</i>	268
Discussion	269
Conclusion	269
<i>Moving from Data to Knowledge</i>	269
<i>Future Directions</i>	270
References	272
About the Author	272

**11. Detection of Microbial Contamination for Cell Therapy Products: Validation of an Automated Microbial Detection System **273****

*Gary C. du Moulin, Grace Kielpinski,  
Sam Prinzi, John Duguid, and Ann Price*

Introduction	273
The Need for Rapid Microbial Detection Methods for Cell Therapy	274
<i>Carticel: Autologous Cultured Chondrocytes</i>	275
Detection Platforms	276
Validation Acceptance Criteria	277
Selection of Microbial Challenge Strains and Preparation of Inocula	278
Phase 1: Early Stage Validation Studies	279
<i>Materials and Methods</i>	280
<i>Results</i>	281
Phase 2: Test Condition Optimization Studies	282
Phase 3: Late Stage Validation Studies	284
<i>Results</i>	285
<i>Conversion of Classic Model to 3D Model Study</i>	286
<i>Conversion of Glass Bottles to Plastic Bottles Study</i>	287
Conclusion	288
Acknowledgements	288
References	289
About the Author	290

<b>12. Viability-based Technologies: Solid-phase Cytometry Using Chemunex ScanRDI®</b>	<b>291</b>
<i>Pascal Yvon</i>	
Part 1: The ScanRDI System	291
<i>Direct Labeling, Direct Detection and Enumeration</i>	292
<i>A Simple, Three-step Protocol</i>	294
<i>Sample Analysis Using ScanRDI is a Simple Three-step Process</i>	294
<i>Discrimination/Data Processing</i>	298
<i>Results in 90 Minutes with the Sensitivity of One Cell</i>	300
PART 2: ScanRDI APPLICATIONS	302
<i>Process Water Testing</i>	302
<i>Cell Culture Process Monitoring</i>	304
<i>Environmental Monitoring (Air, Surfaces, Personnel)</i>	308
<i>Antimicrobial Effectiveness Testing</i>	310
<i>Final Product Release</i>	311
<i>Drinking and Raw Water Testing</i>	311
Part 3: Return On Investment and Regulatory Compliance	312
<i>An Investment with Immediate Return</i>	312
<i>Complies with Worldwide Regulatory Standards</i>	312
Part 4: Summary	313
Acknowledgements	313
References	313
About the Author	315
<b>13. Validation of the ScanRDI® for Purified Water Testing</b>	<b>317</b>
<i>Patrick J. McCormick, Susan E. Norton, and Stephen P. Costanzo</i>	
Introduction	317
Grades of Process Water	318
<i>Purified Water</i>	318
<i>Water for Injection</i>	319
Monitoring Water System Quality	319
The ScanRDI System	320

Validation of Analytical Parameters	321
<i>Accuracy</i>	323
<i>Linearity</i>	324
<i>Precision</i>	325
<i>Range</i>	327
<i>Limit of Detection and Quantitation</i>	328
<i>Specificity</i>	331
<i>Ruggedness and Robustness</i>	331
<i>Equivalency - Purified Water Analysis</i>	331
Summary and Conclusion	334
References	335
About the Authors	338
<b>14. Validation of the ScanRDI® for Microbial Detection in Mammalian Cell Culture Systems</b>	<b>339</b>
<i>Amy McDaniel</i>	
Introduction and Purpose	339
Principle of the Method	340
Master Plan Overview	342
<i>Instrument Qualification Summary</i>	343
<i>Method Validation Summary</i>	343
<i>Method Evaluation</i>	344
<i>Concurrent Testing</i>	346
Example of ScanRDI® Usefulness in Contamination Detection	349
<i>Method Validation</i>	350
<i>Limit of Detection</i>	351
<i>Specificity</i>	352
<i>Robustness</i>	352
<i>Ruggedness (Intermediate Precision)</i>	353
<i>Crossover Studies</i>	354
Conclusion	355
Acknowledgements	355
References	356
About the Author	356

<b>15. Viability-based Technologies: Digital Flow Cytometry Using Chemunex D-Count® and BactiFlow®</b>	<b>357</b>
<i>Pascal Yvon</i>	
Part 1: The D-Count and BactiFlow Systems	358
<i>Direct Labeling, Direct Detection, and Enumeration</i>	359
<i>A Simple Protocol</i>	361
<i>BactiFlow Protocol</i>	363
<i>Discrimination/Data Processing</i>	364
<i>Fully Traceable Results in Hours, Not Days</i>	366
Part 2: D-Count and BactiFlow Applications	368
<i>Pharmaceutical Products</i>	369
<i>Real-time Process Water Testing</i>	370
<i>Cosmetics and Personal Care Products</i>	372
<i>Food Products</i>	373
<i>Specific Detection and Enumeration</i>	375
<i>Complies with Worldwide Regulatory Standards</i>	377
Summary	377
Acknowledgements	378
References	378
About the Author	378
<b>16. Rapid Enumeration of Microorganisms Using Advanced Analytical's RBD 3000</b>	<b>379</b>
<i>Ann M. Steger</i>	
Introduction	379
Basics of the RBD 3000	380
Operating Modes	381
<i>Total Viable Organism Detection</i>	381
<i>Antibody-Specific Microbial Detection</i>	382
Fluidic System	383
Optical System	384
Electronic System	384
RBD 3000 Hardware	385

RBD 3000 Software	388
Defining Analysis Box Parameters	388
Sample Enumeration on the RBD 3000	390
Applications for the RBD 3000	391
<i>Purified Water Analysis Using the RBD 3000</i>	391
<i>Specific Microbial Detection Using the RBD 3000</i>	392
Validation	395
Conclusion	395
References	396
About the Author	396
<b>17. Rapid Microbial Counting by Flow Cytometry: Validation and Implementation for Research and Development (R&amp;D) Applications</b>	<b>397</b>
<i>Kimberly Conner Kozak and     Donald E. Langworthy</i>	
Objectives	397
Technology Theory	398
Validation and Applications of Flow Cytometry	398
<i>Validation for Bacteria and Protozoa</i>	399
<i>Bacterial Enumeration with a Loose Adsorbent</i>	405
<i>Bacterial Membrane Integrity</i>	409
Conclusions	413
References	415
About the Authors	415
<b>18. The RBD 3000 Rapid Bacterial Enumeration System as an Alternative to Traditional Pour Plate Enumeration</b>	<b>417</b>
<i>Pastora Hasher Homesley</i>	
Faster, Better, Cheaper	418
Assessment of Available Technologies	419