

# Contents

|                     |     |
|---------------------|-----|
| Preface             | v   |
| Laboratory Protocol | vii |

## PART 1

### Microscopy

|                              |    |
|------------------------------|----|
| 1. Brightfield Microscopy    | 2  |
| 2. Darkfield Microscopy      | 9  |
| 3. Phase-Contrast Microscopy | 11 |
| 4. Fluorescence Microscopy   | 17 |
| 5. Microscopic Measurements  | 22 |

## PART 2

### Survey of Microorganisms

|                                       |    |
|---------------------------------------|----|
| 6. Protozoa, Algae, and Cyanobacteria | 26 |
| 7. Microscopic Invertebrates          | 35 |
| 8. Aseptic Technique                  | 39 |
| 9. The Bacteria                       | 46 |
| 10. The Fungi: Yeasts and Molds       | 48 |

## PART 3

### Microscope Slide Techniques

|                                                 |    |
|-------------------------------------------------|----|
| 11. Negative Staining                           | 56 |
| 12. Smear Preparation                           | 58 |
| 13. Simple Staining                             | 62 |
| 14. Capsular Staining                           | 63 |
| 15. Gram Staining                               | 64 |
| 16. Spore Staining: Two Methods                 | 67 |
| 17. Acid-Fast Staining:<br>Ziehl-Neelsen Method | 69 |
| 18. Acid-Fast Staining:<br>Fluorescence Method  | 70 |
| 19. Motility Determination                      | 72 |

## PART 4

### Culture Methods

|                                                                                   |     |
|-----------------------------------------------------------------------------------|-----|
| 20. Culture Media Preparation                                                     | 76  |
| 21. Pure Culture Techniques                                                       | 82  |
| 22. Cultivation of Anaerobes                                                      | 89  |
| 23. Bacterial Population Counts                                                   | 93  |
| 24. Slide Culture: Autotrophs                                                     | 99  |
| 25. Slime Mold Culture                                                            | 100 |
| 26. Slide Culture: Molds                                                          | 103 |
| 27. Isolation of Anaerobic Phototrophic Bacteria:<br>Using the Winogradsky Column | 106 |

## PART 5

### Bacterial Viruses

|                                                          |     |
|----------------------------------------------------------|-----|
| 28. Isolation of Phage from Sewage                       | 113 |
| 29. Isolation of Phage from Flies                        | 116 |
| 30. Burst Size Determination:<br>A One-Step Growth Curve | 120 |

## PART 6

### Microbial Interrelationships

|                            |     |
|----------------------------|-----|
| 31. Bacterial Commensalism | 126 |
| 32. Bacterial Synergism    | 127 |
| 33. Microbial Antagonism   | 128 |

## PART 7

### Environmental Influences and Control of Microbial Growth

|                                                                           |     |
|---------------------------------------------------------------------------|-----|
| 34. Temperature: Effects on Growth                                        | 130 |
| 35. Temperature: Lethal Effects                                           | 132 |
| 36. pH and Microbial Growth                                               | 134 |
| 37. Osmotic Pressure and Bacterial Growth                                 | 135 |
| 38. Oligodynamic Action                                                   | 136 |
| 39. Ultraviolet Light: Lethal Effects                                     | 137 |
| 40. Evaluation of Disinfectants:<br>The Use-Dilution Method               | 139 |
| 41. Evaluation of Alcohol:<br>Its Effectiveness as a Skin Degerming Agent | 141 |
| 42. Evaluation of Antiseptics:<br>The Filter Paper Disk Method            | 145 |
| 43. Antimicrobial Sensitivity Testing:<br>The Kirby-Bauer Method          | 145 |
| 44. Effectiveness of Hand Scrubbing                                       | 148 |

## PART 8

### Identification of Unknown Bacteria

|                                                                         |     |
|-------------------------------------------------------------------------|-----|
| 45. Preparation and Care of Stock Cultures                              | 152 |
| 46. Morphological Study of Unknown                                      | 154 |
| 47. Cultural Characteristics                                            | 157 |
| 48. Physiological Characteristics:<br>Oxidation and Fermentation Tests  | 161 |
| 49. Physiological Characteristics:<br>Hydrolytic Reactions              | 170 |
| 50. Physiological Characteristics:<br>Miscellaneous Tests               | 174 |
| 51. Use of <i>Bergey's Manual</i> and<br><i>Identibacter Interactus</i> | 177 |

## Contents

### PART 9

#### Miniaturized Multitest Systems

- 52. Enterobacteriaceae Identification:  
The API 20E System 185
- 53. Enterobacteriaceae Identification:  
The Enterotube II System 189
- 54. O/F Gram-Negative Rods Identification:  
The Oxi/Ferm Tube II System 194
- 55. Staphylococcus Identification:  
The API Staph-Ident System 198

### PART 10

#### Microbiology of Soil

- 56. Microbial Population Counts of Soil 202
- 57. Isolation of an Antibiotic Producer  
from Soil 203
- 58. The Nitrogen Cycle 206
- 59. Nitrogen-Fixing Bacteria 208
- 60. Ammonification in Soil 212
- 61. Isolation of a Denitrifier from Soil:  
Using Nitrate Agar 215
- 62. Isolation of a Denitrifier from Soil:  
Using an Enrichment Medium 217

### PART 11

#### Microbiology of Water

- 63. Bacteriological Examination of Water:  
Qualitative Tests 222
- 64. The Membrane Filter Method 226
- 65. Standard Plate Count:  
A Quantitative Test 228

### PART 12

#### Microbiology of Milk and Food Products

- 66. Standard Plate Count of Milk 230
- 67. Direct Microscopic Count of Organisms in Milk:  
The Breed Count 231
- 68. The Reductase Test 234
- 69. Bacterial Counts of Foods 236
- 70. Microbial Spoilage of Canned Foods 237
- 71. Microbial Spoilage of Refrigerated Meat 239
- 72. Microbiology of Alcohol Fermentation 241
- 73. Microbiology of Yogurt Production 243

### PART 13

#### Bacterial Genetic Variations

- 74. Mutant Isolation by  
Gradient Plate Method 246
- 75. Mutant Isolation by Replica Plating 248
- 76. Bacterial Mutagenicity and Carcinogenesis:  
The Ames Test 250

### PART 14

#### Medical Microbiology and Immunology

- 77. A Synthetic Epidemic 254
- 78. The Staphylococci:  
Isolation and Identification 257
- 79. The Streptococci:  
Isolation and Identification 262
- 80. Gram-Negative Intestinal Pathogens 270
- 81. Urinary Tract Pathogens 274
- 82. Slide Agglutination Test:  
Serological Typing 279
- 83. Slide Agglutination (Latex) Test:  
For *S. aureus* Identification 281
- 84. Tube Agglutination Test:  
The Heterophile Antibody Test 283
- 85. Tube Agglutination Test: The Widal Test 285
- 86. Phage Typing 287
- 87. White Blood Cell Study:  
The Differential WBC Count 288
- 88. Total WBC Count 292
- 89. Blood Grouping 296
- 90. The Snyder Caries Susceptibility Test 299

- Laboratory Reports 301
- Descriptive Charts 417
- Appendix A Tables 425
- Appendix B Indicators, Stains, Reagents 435
- Appendix C Media 439
- Appendix D Identification Charts 445
- Appendix E The Streptococci 455
- Appendix F *Identibacter interactus* 459
- Reading References 473
- Index 475