

Contents

Preface	vi
Basic Microbiology Laboratory Safety	xii
Biosafety Levels for Selected Infectious Agents	xv
Microorganisms Used or Isolated in the Lab	
Exercises in This Manual	xvi

PART 1 Microscopy 1

L 1 Brightfield Microscopy	3
2 Darkfield Microscopy	13
3 Phase-Contrast Microscopy	17
4 Fluorescence Microscopy	25
5 Microscopic Measurements	33

PART 2 Survey of Microorganisms 39

L 6 Microbiology of Pond Water—Protists, Algae, and Cyanobacteria	41
L 7 Ubiquity of Bacteria	55
L 8 The Fungi: Molds and Yeasts	59

PART 3 Manipulation of Microorganisms 69

L 9 Aseptic Technique	71
L 10 Pure Culture Techniques	81

PART 4 Staining and Observation of Microorganisms 93

L 11 Smear Preparation	95
L 12 Simple Staining	101
13 Negative Staining	105
L 14 Capsular Staining	109
L 15 Gram Staining	113
L 16 Spore Staining: Two Methods	119
L 17 Acid-Fast Staining: Kinyoun Method	125
18 Motility Determination	129

PART 5 Culture Methods 135

19 Culture Media Preparation	137
20 Preparation of Stock Cultures	147
L 21 Enumeration of Bacteria: The Standard Plate Count	151
22 Slide Culture: Fungi	163

PART 6 Bacterial Viruses 167

23 Determination of a Bacteriophage Titer	171
--	-----

24 A One-Step Bacteriophage Growth Curve	177
25 Isolation of Phage from Flies	183
26 Phage Typing	189

PART 7 Environmental Influences and Control of Microbial Growth 193

L 27 Effects of Oxygen on Growth	195
L 28 Temperature: Effects on Growth	203
L 29 pH and Microbial Growth	209
L 30 Water Activity and Osmotic Pressure	213
L 31 Ultraviolet Light: Lethal Effects	217
32 The Effects of Lysozyme on Bacterial Cells	221
33 Evaluation of Alcohol: Its Effectiveness as an Antiseptic	227
L 34 Antimicrobial Sensitivity Testing: The Kirby-Bauer Method	231
L 35 Evaluation of Antiseptics: The Filter Paper Disk Method	243
36 Effectiveness of Hand Scrubbing	249

PART 8 Identification of Unknown Bacteria 257

L 37 Morphological Study of an Unknown Bacterium	259
38 Cultural Characteristics	265
L 39 Physiological Characteristics: Oxidation and Fermentation Tests	269
L 40 Physiological Characteristics: Hydrolytic and Degradative Reactions	281
L 41 Physiological Characteristics: Multiple Test Media	287
L 42 Use of Bergey's Manual	295

PART 9 Miniaturized Multitest Systems 303

43 Enterobacteriaceae Identification: The API 20E System	305
44 Enterobacteriaceae Identification: The Enterotube II System	311
45 O/F Gram-Negative Rods Identification: The Oxi/Ferm Tube II System	321
46 Staphylococcus Identification: The API Staph System	329

PART 10 Diversity and Environmental Microbiology 335

- 47 Isolation of an Antibiotic Producer: The *Streptomyces* 337
- 48 Nitrogen Cycle: Ammonification 343
- 49 Symbiotic Nitrogen Fixation: *Rhizobium* 349
- 50 Free-Living Nitrogen Fixation: *Azotobacter* 353
- 51 Denitrification: *Paracoccus denitrificans* 359
- 52 The Winogradsky Column 365
- 53 Purple Nonsulfur Photosynthetic Bacteria 371
- 54 Sulfate-Reducing Bacteria: *Desulfovibrio* 375
- 55 Bacterial Commensalism 379
- 56 Bacterial Synergism 381
- 57 Microbial Antagonism 383

PART 11 Applied Microbiology 387

- 58 Bacterial Counts of Food 389
- 59 Bacteriological Examination of Water: Most Probable Number Determination 393
- 60 Bacteriological Examination of Water: The Membrane Filter Method 403
- 61 Reductase Test 407
- 62 Temperature: Lethal Effects 411
- 63 Microbial Spoilage of Canned Food 417
- 64 Microbiology of Alcohol Fermentation 423

PART 12 Bacterial Genetics and Biotechnology 427

- 65 Mutant Isolation by Replica Plating 429

- L** 66 Bacterial Transformation 433
- L** 67 Polymerase Chain Reaction for Amplifying DNA 443
- L** 68 Plasmid Isolation 447

PART 13 Medical Microbiology 455

- L** 69 The Staphylococci: Isolation and Identification 457
- L** 70 The Streptococci and Enterococci: Isolation and Identification 469
- L** 71 Gram-Negative Intestinal Pathogens 483
- 72 A Synthetic Epidemic 491

PART 14 Immunology and Serology 499

- 73 Slide Agglutination Test: Serological Typing 503
- 74 Slide Agglutination Test for *S. aureus* 505
- 75 Slide Agglutination Test for *Streptococcus* 511
- 76 The Heterophile Antibody Test 517
- 77 Blood Grouping 523

Appendix A Tables A-1

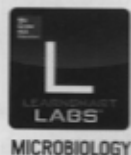
Appendix B Indicators, Stains, Reagents A-6

Appendix C Media A-9

Appendix D Identification Charts A-14

Reading References R-1

Index I-1



Indicates a LearnSmart Lab™ activity is available for all or part of this exercise. For more information, visit mhhe.com/slabsmicro.