CONTENTS

	Contributors Preface Introduction	xi xiii xv
	 1. Obtaining and Maintaining Microbial Pathogens David G. Russell I. Introduction II. Biosafety Considerations III. Department of Health and Human Services Guidelines IV. Federal and Institutional Requirements V. Source of Pathogens VI. Culture and Maintenance of Pathogens VII. Conclusion 	1 1 2 2 3 3 4
PART I	Manipulation of Pathogens 2. Cultivation of Malaria Parasites William Trager	
	I. Introduction II. Culture Medium III. Serum IV. Erythrocytes V. Culture Systems VI. Synchronization VII. Gametocyte Production in Culture VIII. Cloning Methods IX. Cryopreservation X. Serum Replacement XI. Axenic (Extracellular) Development of Erythrocytic Stages of P. falciparum XII. Cultivation of Erythrocytic Stages of Other Species of Malaria Parasites XIII. In Vitro Development of Preerythrocytic and Sporogonic Stages of the Life Cycle References	7 8 8 10 10 16 18 20 20 21 21 21 22 23

3.	Molecular Tools for Genetic Dissection of the Protozoan Parasite Toxoplasma gondii		
	David S. Roos, Robert G. K. Donald, Naomi S. Morrissette, and A. Lindsay C. Moulton		
	 I. Introductory Overview II. In Vitro Culture of T. gondii Tachyzoites III. Molecular Transformation Systems for Toxoplasma IV. Summary and Outlook References 	28 32 44 59 61	
4.	Transfection Experiments with Leishmania		
	Jonathan H. LeBowitz		
	I. IntroductionII. Stable and Transient TransfectionIII. TransfectionReferences	65 66 70 76	
5.	Mutagenesis and Variant Selection in Salmonella Renée Tsolis and Fred Heffron		
	 I. Introduction II. Mutagenesis III. Screening of Variants IV. Genetic Analysis V. Perspectives References 	79 80 92 98 101 103	
6.	Mycobacterium: Isolation, Maintenance, Transformation, and Mutant Selection Nancy D. Connell		
	I. Introduction II. Biosafety Considerations III. Culture Media and Conditions IV. Isolation of Mycobacteria V. Maintenance of Stocks VI. Genetic Techniques VII. Mutagenesis VIII. Strain Construction IX. Mutant Selection and Isolation References	108 108 109 111 112 112 118 120 121 123	

PART II Microbial Adherence and Invasion Assays

7.	Modulation of Murine Macrophage Behavior in Vivo and in Vitro	
	Gregory J. Bancroft, Helen L. Collins, Lynette B. Sigola, and Caroline E. Cross	
	 I. Introduction II. Reagents and Solutions III. Animal Husbandry and Maintenance of Immunocompromised Mice IV. Eliciting and Harvesting Macrophages in Vivo 	130 131 134 136
	V. Adherence of Macrophages to Solid Substrates	139
	VI. Assays for Macrophage Phagocytic Activity	140
	VII. Analysis of MHC Class II Antigen Expression References	143 145
8.	In Vitro Assays of Phagocytic Function of Human Peripheral Blood Leukocytes: Receptor Modulation and Signal Transduction	
	Eric J. Brown	
	I. Introduction	147
	II. Phagocytosis Assays	148
	III. Phagocytic Receptors	156 159
	IV. Stimulation of Phagocytosis	160
	V. Phagosome Isolation References	162
9.	Bacterial Adhesion and Colonization Assays	
	Per Falk, Thomas Borén, David Haslam, and Michael Caparon	
	I. Introduction	165
	II. In Situ Screening of Host Receptor Distribution	166
	III. Bacterial Adherence to Cells in Culture	169
	IV. Biochemical Characterization of the Molecular Nature of Receptors	
	in Situ	172
	V. Bacterial Inhibition Experiments in Situ	174 175
	VI. In Vitro Assays for Bacterial Adhesion VII. Probing Eukaryotic Cell Glycoconjugates with Purified	173
	Bacterial Adhesins	185
	VIII. Concluding Remarks	187
	References	188
10.	Cytoadherence and the Plasmodium falciparum-Infected Erythrocyte	
	Ian Crandall and Irwin W. Sherman	
	I. Introduction	193
	II. Ligands for Adherence	194
	III. P. falciparum-Infected Red Cell Adhesions	196

	IV. Cytoadherence, an <i>in Vitro</i> Model of Sequestration: Practical Considerations of Cytoadherence Assays References	198 207
PART III	The Study of Intracellular Pathogenesis	
	11. Purification of <i>Plasmodium falciparum</i> Merozoites for Analysis of the Processing of Merozoite Surface Protein-1 Michael J. Blackman	
	 I. Introduction II. Parasite Culture and Synchronization III. Merozoite Isolation IV. Assay for Secondary Processing of the Merozoite Surface Protein-1 (MSP-1) 	213 214 216 217
	References	220
	12. In Vitro Secretory Assays with Erythrocyte-Free Malaria Parasites Kasturi Haldar, Heidi G. Elmendorf, Arpita Das, Wen Lu Li, David J. P. Ferguson, and Barry C. Elford	
	I. Introduction II. Release and Separation of Late Ring and Trophozoite Stage Parasites from the Erythrocyte Membrane (EM) and Tubovesicular	222
	Membrane (TVM) Network III. Synthesis and Secretion of Proteins by Intact, Ring/Trophozoite Parasites	230
	 IV. Organization of Secretory Activities at Different Stages of the Asexual Life Cycle V. Release of Pigmented Trophozoits and Schizonts from Infected Erythrocytes by Osmotic Shock in Isoosmolar 	234
	Dipeptide-Based Media References	240 245
	13. Intracellular Survival by Legionella	
	Karen H. Berger and Ralph R. Isberg	
	I. Introduction	247
	II. Laboratory Cultivation of L. pneumophilaIII. Tissue Culture of U937 Cell-Derived Macrophages	248 248
	IV. Intracellular Thymineless Death EnrichmentV. Identification of Intracellular Growth Mutants from Enriched	249
	Bacterial Pools Using "Poke Plaque" Assays	256
	VI. Additional Remarks References	257 258

14.	Isolation and Characterization of	
	Pathogen-Containing Phagosomes	
	Prasanta Chakraborty, Sheila Sturgill-Koszycki, and David G. Russell	
	I. Introduction II. Choice of Pathogens and Particles III. Choice of Macrophage IV. Particle Adherence and Internalization Conditions V. Cell Lysis Conditions VI. Isolation of Phagosomes VII. Analysis of Phagosomal Constituents VIII. Storage and Handling of Two-Dimensional SDS-PAGE Data IX. Shortcomings References	261 263 264 265 266 269 271 273 273 275
15.	Immunoelectron Microscopy of Endosomal Trafficking in Macrophages Infected with Microbial Pathogens	
	David G. Russell	
	I. Introduction	277
	II. The Host-Pathogen Interplay	278
	III. Intersection with the Endosomal Pathway IV. Processing of Infected Macrophages for	279
	Immunoelectron Microscopy	280
	V. Blocking Cryosections and Incubation with Primary Antiserum	281
	VI. Gold-Conjugated Second Antibodies.	282
	VII. Controls	283
	VIII. Final Preparation of the Grids	284
	IX. Routine Protocol for Analysis of Fluid-Phase Trafficking References	286 287
	References	207
16.	Measuring the pH of Pathogen-Containing Phagosomes	
	Paul H. Schlesinger	
	I. Introduction	289
	II. Materials	299
	III. Procedures	302
	IV. When Things Are Not Perfect, or Even Very Close	307
	V. Conclusion References	309 309
17.	Techniques for Studying Phagocytic Processing of Bacteria for Class I or II MHC-Restricted Antigen Recognition by T Lymphocytes Clifford V. Harding	
	I. Introduction	313
	II. Generating MHC-I- and MHC-II- Restricted T Cells to Detect Antigen Processing	315

III. Antigen Presentation AssaysIV. Observations and Implications References	319 324 324
Index	327
Volumes in Series	335