

## Contents

Foreword by Dr Anil K Jain	υ
Foreword by Dr NP Singh	vii
Preface to the Third Edition	ix
Preface to the First Edition	x
Index of Competencies	xv

#### Section 1: General Microbiology Practicals

- 1. Rules and Safety Precautions to be Observed in the Microbiology Laboratory 3
- 2. GLP, Biohazard, Biosafety Levels and Biosafety Cabinets 5
- 3. Universal Presence of Microbes 11
- 4. Microscope: General Instructions about Use and Care 13
- 5. Microscopy and Micrometry 15
- 6. Methods of Sterilization and Disinfection 19
- 7. Biomedical Waste Disposal 23
- 8. Table of Commonly Used Culture Media in Microbiology and their Uses 25
- 9. Demonstration of Common Culture Media and Biochemical Reactions 29
- 10. Culture Methods 41
- 11. Description of the Appearance of Growth on Solid or in Liquid Media 45
- 12. Demonstration of Methods of Antimicrobial Susceptibility Testing 47
- 13. Miscellaneous Arthropods of Medical Importance (Including Tables) 53
- 14. Demonstration of Methods for Diagnosis of Viral Infections 59
- 15. Antigen–Antibody Reactions 63

#### Section 2: Perform and Identify the Different Causative Agents of Infectious Diseases by Microscopy

- 16. Demonstration of Formol-ether Concentration Method for Stool Examination 75
- 17. Demonstration of Normal Stool Findings 77
- 18. Practical: Preparation of Saline and Iodine Wet Mount of Stool Sample for Diagnosis of Ova/Cysts 81
- 19. Practical: To Stain Smear by Ziehl-Neelsen Technique for Diagnosis of Acid-fast Bacilli 83
- 20. Practical: To Stain a Smear by Albert Staining for Diagnosis of C. diphtheriae 87
- 21. Practical: To Stain a Smear by Gram Stain for the Diagnosis of Bacterial Infections 89
- 22. Practical: To Perform Motility Preparation of Bacteria for the Diagnosis of Bacterial Infections 95
- 23. Demonstrations of other Staining Methods for Microscopy 97
  - I. Demonstration of Spore Staining and Anaerobic Methods for Diagnosis of Anaerobic Bacteria 97
  - II. Demonstration of Silver Impregnation Staining Method for Diagnosis of Spirochetes 103
  - III. Demonstration of LPCB Stain and Characteristic Features of the Common Fungi and their Diagnostic Methods 107

#### Section 3: Identify the Causative Agent of Malaria and Filaria

- 24. Demonstration of Preparation of Blood Film 113
- 25. Practical: To Study Peripheral Blood Smear Examination for the Characteristic Features for Malarial Parasites 119
- 26. Practical: To Study Peripheral Blood Smear Examination for the Characteristic Features for Microfilaria for Diagnosis of Filariasis 125
- 27. Demonstration of Morphological Forms of Leishmania donovani 127

#### Section 4: Identify the Common Etiologic Agents of Diarrhea and Dysentery

- 28. Table of Common Etiologic Agents Causing Diarrhea/Dysentery and their Method of Detection 133
- 29. Practical: To Study the Characteristic Features of the Cysts of Entamoeba histolytica, Entamoeba coli and Giardia lamblia 135
- 30. Practical: To Study the Characteristic Features of the Eggs of Helminths—Cestodes 139
- 31. Practical: To Study the Characteristic Features of the Eggs of Helminths—Nematodes 143
- 32. Practical: To Study the Characteristic Features of the Oocysts of Coccidian Parasites 149
  - Table of Common Ova/Cysts Found in Wet Mount Stool Microscopy 150
- 33. Practical: To Study the Characteristic Features of Escherichia coli 155
- 34. Practical: To Study the Characteristic Features of Shigella spp. 159
- 35. Demonstration of the Characteristic Features of Vibrio cholerae 163

Section 5: Identify the Different Modalities for Diagnosis of Enteric Fever

36. Practical: To Study the Characteristic Features of Salmonella typhi 169

#### Section 6: Identify the Microbial Agents Causing Rheumatic Heart Disease and Infective Endocarditis

- 37. Agents Causing Infective Endocarditis 177
- 38. Practical: To Study the Characteristic Features of Streptococcus pyogenes 179

#### Section 7: Identify the Common Etiological Agents of Upper Respiratory Tract Infections (Gram Stain)

- 39. Table of Agents Causing Upper and Lower Respiratory Tract Infections 185
- 40. Practical: To Study the Characteristic Features of Corynebacterium diphtheriae 189
- 41. Practical: To Study the Characteristic Features of *Staphylococcus aureus* 193
- 42. Other Agents Causing Upper Respiratory Tract Infections, e.g. *Streptococcus pyogenes, Staphylococcus aureus* (Revision Practical) 199

#### Section 8: Identify the Common Etiological Agents of Lower Respiratory Tract Infections (Gram Stain and Acid-fast Stain)

- 43. To Study the Characteristic Features of Klebsiella spp. 203
- 44. To Study the Characteristic Features of Mycobacterium tuberculosis 205
- 45. To Study the Characteristic Features of Pseudomonas spp. 209
- 46. Other Agents Causing Lower Respiratory Tract Infections, e.g. *Staphylococcus aureus*, *Pneumococcus, Aspergillus* spp. (Revision Practical) 213

#### Section 9: Identify the Microbial Agents Causing Meningitis

- 47. Table of Agents Causing Meningitis 219
- 48. Practical: To Study the Characteristic Features of Gram-negative cocci Neissseria meningitidis 225
- 49. Other Agents Causing Meningitis, e.g. *Staphylococcus aureus*, *Streptococcus* spp, *E. coli*, *Klebsiella* spp., *M. tuberculosis* (Revision Practical) 227

#### Section 10: Demonstrate Infection Control Practices and Use of Personal Protective Equipment (PPE)

- 50. Practical: Demonstrate Hand Hygiene as an Infection Control Practices 235
- 51. Practical: Demonstrate Types and Use of PPE 239

#### Section 11: Demonstrate the Appropriate Method of Collection of Samples in the Performance of Laboratory Tests in the Detection of Microbial Agents Causing Infectious Diseases

52. Specimens and Collection 245

- Demonstrate Method of Collection of Blood Samples from Children and Adults 245
- Demonstrate Method of Inoculation of Blood for Blood Culture in the Detection of Microbial Agents Causing Septicemia 245
- Amount, Number and Type of Blood Cultures 245
- Transport and Incubation of Blood Culture Bottles 245
- How to Minimize Contamination in Blood Culture 245
- Other Types of Microbiological Specimens Used for Detection of Microorganisms Causing Infectious
   Diseases 245
- Criteria for Rejection of a Microbiological Sample 245
- Transport and Storage of Microbiological Samples when Delay in Transport and Testing 245
- Various Microbiology Specimens with Respect to Type of Infection 246

#### Section 12: Demonstrate Respect for Patient Samples Sent to the Laboratory for Performance of Laboratory Tests in the Detection of Microbial Agents Causing Infectious Diseases

53. Practical: To be able to Demonstrate Need for Confidentiality Pertaining to Patient Identity with Regards to Laboratory Results 261



## Rules and Safety Precautions to be Observed in the Microbiology Laboratory

- 1. Wash your hands with soap and water after handling the infectious material.
- 2. Wear laboratory coats in the laboratory.
- 3. Keep the nails clean and short.
- 4. Tie up long hair, while working in the laboratory.
- 5. Avoid eating, drinking and smoking in the laboratory.
- 6. Decontaminate the working area with appropriate disinfectant after spillage of potentially infected material.
- 7. Carry-out the laboratory procedure following standard precautions.
- 8. Avoid mouth pipetting as far as possible.
- 9. Perform all the laboratory procedures in a way that minimizes the aerosol formation.
- 10. Bring practical manual in all the practical classes.
- 11. Draw properly labeled diagrams neatly, get it corrected from your teachers.
- 12. Leave your microscope and work seat clean before going out of practical lab.
- 13. Keep your bag away from working tables.
- 14. Come exactly at 2.00 pm for practical and tutorials.

# Index of Competencies

Code	Competency	Chapter	Page no
MI1.2	Microscope: General instructions about use and care	4	13
MI1.2	Microscopy and micrometry	5	15
MI1.5, 8.5, 8.6 8.7, 8.8	Methods of sterilization and disinfection	6	19
MI8.5, 8.6, 8.7, 8.8	Biomedical waste disposal	7	23
MI1.1	Demonstration of common culture media and biochemical reactions	9	29
MI1.1	Culture methods	10	41
MI1.1	Description of the appearance of growth on solid or in liquid media	11	45
MI1.6	Demonstration of methods of antimicrobial susceptibility testing	12	47
MI1.1, 1.7	Demonstration of methods for diagnosis of viral infections	14	59
MI1.8, 8.15	Antigen-antibody reactions	15	63
MI1.2	Demonstration of formol-ether concentration method for stool examination	16	75
MI1.2	Demonstration of normal stool findings	17	77
MI1.2	Preparation of saline and iodine wet mount of stool sample	18	81
MI1.2	Ziehl-Neelsen (ZN) staining	19	83
MI1.2, 6.2	Albert staining	20	87
MI1.2	To stain a smear by Gram stain for the diagnosis of bacterial infections	21	89
MI1.2	To perform motility preparation of bacteria for the diagnosis of bacterial	22	95
	infections		
MI4.1	Demonstrations of other staining methods for microscopy	23	97
MI7.1	<ul> <li>Demonstration of silver impregnation staining method for diagnosis of spirochetes</li> </ul>	23	103
MI1.2, 8.1	• Demonstration of LPCB stain and characteristic features of the common fungi and their diagnostic methods	23	107
MI2.5, 2.6	Preparation of blood film	24	113
MI2.5, 2.6	Peripheral blood smear examination for malarial parasites	25	119
MI2.5, 2.6	Peripheral blood smear examination for microfilaria	26	125
MI2.5, 2.6	Morphological forms of Leishmania donovani	27	127
MI1.2, 3.2, 8.15	Entamoeba histolytica, Entamoeba coli and Giardia Iamblia	29	135
MI1.2, 3.2, 8.15	Eggs of helminths (cestodes)	30	139
MI1.2, 3.2, 8.15	Eggs of helminths (nematodes)	31	143
MI1.2, 3.1, 8.15	To study the characteristic features of the oocysts of coccidian parasites	32	149
MI1.2, 3.2, 8.15	Escherichia coli	33	155
MI1.2, 3.2, 8.15	Shigella	34	159
MI1.2, 3.2, 8.15	To study the characteristic features of Vibrio cholerae	35	163
MI3.3, 3.4	Salmonella typhi	36	169
MI2.3, 8.15	Agents causing infective endocarditis	37	177
MI1.2, 2.3, 4.1, 4.3, 8.15	Streptococcus pyogenes as a causative agent of rheumatic fever	38	179
MI6.2	To study the characteristic features of Corynebacterium diphtheriae	40	189

### xvi Microbiology Practical Manual

Code	Competency	Chapter	Page no
MI1.2, 4.3, 6.2, 8.15	Staphylococcus aureus	41	193
MI1.2, 2.3, 4.1, 4.3, 6.2, 8.15	Other agents causing upper respiratory tract infections, e.g. <i>Streptococcus pyogenes, Staphylococcus aureus</i> (revision practical)	42	199
MI1.2, 6.2, 6.3	To study the characteristic features of Klebsiella spp.	43	203
MI1.2, 6.3, 8.15	To study the characteristic features of Mycobacterium tuberculosis	44	205
Ml1.2, 4.1, 4.3, 6.3, 8.15	To study the characteristic features of <i>Pseudomonas</i> spp.	45	209
MI1.2, 6.2, 6.3	Other agents causing lower respiratory tract infections, e.g. <i>Staphylococcus aureus, Pneumococcus, Aspergillus</i> spp. (revision practical)	46	213
MI1.2, 5.3, 8.15	To study the characteristic features of gram-negative cocci Neisseria meningitidis	48	225
MI1.2, 5.3, 8.15	Other agents causing meningitis, e.g. <i>Staphylococcus aureus, Streptococcus</i> spp., <i>E.coli, Klebsiella</i> spp., <i>M. tuberculosis</i> (revision practical)	49	227
MI8.7	Demonstration of infection control practices	50	235
MI8.7	Personal protective equipment (PPE)	51	239
MI1.1, 8.9, 8.10	Specimens and collection	52	245
MI8.11	Demonstrate confidentiality pertaining to patient identity in laboratory results	53	261