

CONTENTS

I. GENERAL MICROBIOLOGY			
1. Introduction	001	25f) Catalase test	095
2. Laboratory rules and regulations.	002	25g) Oxidase test.	096
3. Basic requirements in the biology / biotechnology laboratory	006	25h) Urease test	098
4. Microscopy	018	25i) Nitrate test.	099
5. Cleaning and sterilization of glassware's.	025	25j) OF test.	100
6. Preparation of culture media	026	25k) Carbohydrate fermentation test	102
7. Sterilization	031	25l) Phenylalanine Deaminase test	103
8. Demonstration of ubiquitous nature of microbes.	036	25m) Decarboxylase test	104
9. Aseptic technique and culture inoculation	038	25n) ONPG test	106
10. Serial dilution technique	039	25o) Melonate utilization test	107
11. Pure culture technique.	042	25p) Esculin hydrolysis.	108
a) Pour plate technique	043	25q) Coagulase test	109
b) Spread plate technique.	044	25r) Optochin susceptibility test	111
c) Streak plate technique.	045	25s) Basitracin and SXT susceptibility test	112
12. Cultural variations of bacteria	047	25t) Novobiocin sensitivity test	113
13. Morphological variations of bacteria	050	25u) Bile solubility test	114
14. Staining techniques	051	25v) The CAMP test	115
14a) Smear preparation and fixation	051	25w) Litmus milk reaction	116
14b) Staining	053	25x) Starch hydrolysis	117
14ba) Simple staining	053	25y) Gelatin hydrolysis	118
14bb) Negative staining	054	25z) Casein hydrolysis	119
14bc) Gram staining	055	25aa) Lipid hydrolysis	120
14bd) Capsule staining	057	25ab) Salt tolerance test	121
14be) Spore staining	058		
14bf) Acid fast staining	060		
14bg) Albert's staining	062		
14bh) Silver staining for flagella	063		
14bi) Fontana staining	064		
14bj) Wayson staining	065		
15. Motility test	065		
16. Micrometry	067		
17. Cultivation of anaerobes.	068		
18. Preservation of microorganisms	074		
II. MICROBIAL PHYSIOLOGY		III. IMMUNOLOGY	
19. Measurement of growth and growth curve.	077	26. ABO blood grouping	123
20. Effect of pH on growth	081	27. Rh factor typing	125
21. Effect of temperature on growth	083	28. Demonstration of Lymphoid organs	126
22. Effect of salinity on growth	084	29. Antigen preparation	129
23. Effect of sunlight radiation on growth.	085	30. Polyclonal antibody Production	130
24. Effect of Disinfectant - Phenol Coefficient test	087	31. Blood collection and Serum preparation	131
25. Biochemical reactions of bacteria	088	32. Isolation and separation of PBMC	133
25a) Indole test	088	33. Antigen Antibody Reactions	135
25b) Methyl red test	089	33A. Agglutination reactions	135
25c) Voges proskauer test.	090	33Aa. Pregnancy test	136
25d) Citrate utilization test.	092	33Ab. Anti streptolysin O test	137
25e) Triple sugar iron test(H ₂ S Production)	093	33Ac. WIDAL test	139
		33Ad. RPR Test	140
		33Ae. CRP test	142
		33Af. Coombs Test	144
		33B. Precipitation	145
		33Ba. Radial immunodiffusion test	146
		33Bb. Double immunodiffusion test	147
		33Bc. Counter current Immunoelectrophoresis	148
		34. ELISA test	149
		35. Purification of IgG	151
		36. Ammonium sulphate precipitation of proteins	153
		37. Dialysis	155
		38. Separation of protein by SDS - PAGE	156
		39. Western blotting	160

40. Immunofluorescence test	162	D. CLINICAL PARASITOLOGY	
IV. MEDICAL MICROBIOLOGY			
A. Clinical Biochemistry and Haematology			
41. Total erythrocyte count	164	73. Identification of intestinal parasites	236
42. Total leukocyte count	166	73a. Microscopic methods	236
43. Total platelet count	168	73aa. Saline wet mount	236
44. Differential count	170	73ab. Iodine wet mount	237
45. Haemoglobin estimation	173	73b. Concentration of stool parasites	238
46. Erythrocyte sedimentation rate.	174	73ba. Sedimentation method	238
47. SGOT	175	73bb. Floatation method	239
48. SGPT	177	74. Identification of blood and tissue parasites	243
49. Serum Cholesterol analysis	179	74a. Thin Blood film preparation	243
50. Serum sugar analysis	181	74b. Thick blood film preparation	243
51. Estimation of Urine albumin	182	74c. Giemsa staining	244
52. Estimation of Urine bile salt	183	74d. Wrights staining	244
53. Estimation of Urine sugar.	184	74e. Leishmans staining	246
B. CLINICAL BACTERIOLOGY			
54. Isolation of normal flora from mouth / oral cavity	185	75. Isolation of bacteria from soil	248
55. Isolation of bacteria from sputum	186	76. Isolation of fungi from soil	250
56. Isolation of bacteria from upper respiratory track	189	77. Isolation of actinomycetes from soil	252
57. Isolation of bacteria from stool	191	78. Isolation of Cyanobacteria from soil	254
58. Isolation of bacteria from wound (Pus)	193	79. Isolation of phosphate solubilizers from soil	255
59. Isolation of bacteria from blood	195	80. Assessment of VAM colonization	257
60. Isolation of bacteria from urine	197	81. Isolation and cultivation of Rhizobium from legumes	258
61. Identification of <i>Escherichia coli</i>	200	82. Testing antagonistic activity of soil microbes.	260
62. Identification of <i>Salmonella sp.</i>	201	83. Isolation of microbes from rhizosphere, non rhizosphere and rhizoplane.	261
63. Identification of <i>Shigella sp.</i>	203	84. Analysis of Sodium and potassium by flame photometer	263
64. Identification of <i>Staphylococcus aureus</i>	205	85. Trace element analysis by AAS	264
65. Identification of <i>Streptococcus pyogenes</i>	207	86. Estimation of soil mineral contents	265
66. Identification of <i>Corynebacterium diphtheriae</i>	209	86a) pH	265
67. Identification of <i>Pseudomonas aeruginosa</i>	210	86b) Nitrate	265
68. Antibiotic sensitivity assay	212	86c) Nitrite	267
69. Assessment of Minimal inhibitory concentration	213	86d) Sulphate	268
C. CLINICAL MYCOLOGY			
70. Isolation of Fungi from clinical samples	216	86e) Phosphate	269
70a. KOH mount	218	86f) Calcium	270
70b. KOH-DMSO-INK mont / stain	219	86g) Magnesium	271
70c. KOH- Calcoflour fluorescent-stain	220	86h) Chloride	272
70d. Indian ink Preparations	221	86i) Fluoride	273
70e. Giemsa Stain for Histoplasma capsulatum	222	86j) Silica	274
71. Identification of mould on primary culture	222	86k) Ammonia	275
71a. Block inoculation	224	VI. ENVIRONMENTAL MICROBIOLOGY AND BIOTECHNOLOGY	
71b. Scarification	225	87. Isolation of Air Borne Bioparticles	277
71c. Slide culture technique	225	88. Effect of high salt concentration on microbial growth	279
71d. Cellophane tape mount	226	89. Oligodynamic action of heavy metals on microbes	280
71e. Tease preparations	227	90. Isolation of Coliforms from sewage	282
71f. Lactophenol cotton blue staining	227	91. Estimation of total solids in effluent sample	283
71g. Hair Perforation Test	228	92. Analysis of TDS of effluent	284
72. Identification of yeast	229		
72a. Germ tube test	232		

93. Estimation of total suspended solids of effluent	285	126. Ligation of insert DNA to vector DNA	350
94. Determination of Biological oxygen Demand	285	127. Polymerase Chain reactions	351
95. Determination of Chemical Oxygen Demand	287	128. Southern Blotting	352
96. Microbial degradation of Cellulose	289	129. Northern Blotting	355
VII. FOOD AND DAIRY MICROBIOLOGY		130. Mutagenesis	357
97. Bread preparation	291	130a. Spontaneous mutation	357
98. Enumeration of microorganism from bread	293	130b. Isolation of UV induced mutants of <i>E. coli</i>	358
99. Microbial examination of curd	294	130c. Induced mutation – EMS mutagenesis	358
100. Yoghurt preparation	295	131. Isolation of protoplast and spheroplast	360
101. Production of Sauerkraut	296	131a. Protoplast isolation	360
102. Isolation and identification of microbes from fruits and vegetables	297	131b. Spheroplast isolation	361
103. Isolation of Salmonella from poultry products	300	132. Transformation	362
104. Isolation of microorganism form grains	301	133. Transduction	364
105. Determination of Thermal Death Point (TDP) and Thermal Death Time(TDT)	302	134. Conjugation-interupted	365
106. Analysis of aflotoxin by Thin Layer Chromatography	304	135. Ampicillin selection of auxotrophs	366
107. Qualitative analysis of milk	306	X. VIROLOGY	
107a. Direct Microscopic examination of milk	306	136. Isolation of coliphage from sewage	368
107b. Standard plate count	307	137. Determining Bacteriophage Titors	369
108. Quantitative examination of milk	308	138. Cultivation of viruses in embryonated egg	371
108a. Methylene blue reductase test	308	139. Chick Embryo Fibroblast technique for virus cultivation	372
108b. Resazurin test	310	XI. BIOCHEMISTRY	
109. Portability test of water	311	140. Preparation buffers.	374
109a. Most Probable number test	311	141. pKa Value determination	375
109b. Presence Absence coliform Test	315	142. Beer lamberts Law verification	377
109c. Membrane filter technique for the quality analysis of water	316	143. Quantitative estimation of carbohydrates	379
110. Mushroom cultivation	318	144. Quantitative estimation of Proteins	383
111. Alcohol fermentation from fruit juice (wine)	321	145. Quantitative estimation of aminoacids	385
112. Preparation of Beer	323	146. Quantitative estimation of lipids	388
VIII. INDUSTRIAL MICROBIOLOGY AND BIOTECHNOLOGY		147. Estimation of Carbohydrates by Anthrone method	390
113. Bio ethanol production	324	148. Estimation of Reducing sugar by DNS method	391
114. Immobilization of yeast cell using sodium alginate	325	149. Estimation of Reducing sugar by Benedict's method	392
115. Amylase production	327	150. Estimation of Reducing sugar by Nelson – Somogyi methods	394
116. Protease production	329	151. Estimation of sugar by Folin wu method	395
IX. MICROBIAL GENETICS, MOLECULAR BIOLOGY AND MICROBIAL BIOTECHNOLOGY		152. Estimation of proteins by Lowry method	396
117. Isolation of chromosomal DNA from bacteria	332	153. Estimation of proteins by Biuret test	398
118. Isolation of chromosomal DNA from Goat Liver	336	154. Estimation of proteins by Bradford methods.	398
119. Isolation of chromosomal DNA from human peripheral Blood	336	155. Determination of acid number of an edible oil	399
120. Isolation of DNA from Plants	338	156. Determination of saponification number of edible oil.	401
121. Isolation of RNA from human blood	340	157. Determination of iodine number of an edible oil	403
122. Isolation of plasmid DNA	342	158. Estimation of DNA by Diphenylamine method.	405
123. Restriction digestion of DNA	343	159. Estimation of RNA by Orcinol method.	406
124. Principles and application of agarose gel electrophoresis	344	160. Estimation of alcohol	408
125. Elution of DNA	346	161. Separation of amino acids by paper chromatography.	409
		162. Estimation of free aminoacids by Ninhydrin method	410

163. Separation of lipids by thin-layer chromatography.	411	189. Mounting of genitalia in <i>Drosophila melanogaster</i>	450
164. Separation of plant pigments by column chromatography.	412	190. Mounting of the sex comb in <i>Drosophila melanogaster</i>	450
165. Determination of the effect of pH on the Activity of human salivary α -amylase	415	191. Monohybrid experiments in <i>Drosophila melanogaster</i>	451
166. Determining the effect of temperature on the activity of human salivary α -amylase	416	192. Dihybrid experiments in <i>Drosophila melanogaster</i>	452
167. Demonstrating the Presence of Catalase	416	193. Demonstration of the law of independent assortment	453
XII. PLANT TISSUE CULTURE		194. Demonstration of law of segregation	455
168. Preparation of media for plant tissue culture	418	195. Determination of Phenomenon of Segregation – Artificial	456
169. Sterilization, inoculation and incubation of explants	421	196. Determination of Phenomenon of independent assortment – Artificial	457
170. Isolation of protoplasts	422		
171. Protoplast Fusion (Somatic Hybridization)	424	XV. CELL BIOLOGY	
172. Suspension Culture	425	197. Mitosis in onion root	459
173. Regeneration of plants using callus culture	428	198. Meiosis in flower buds of <i>Allium cepa</i> (Onion)	461
174. Anther/Ovule Culture Protocol	430	199. Meiosis in Grasshopper Testis (<i>Poecilocerus pictus</i>)	464
XIII. ANIMAL CELL CULTURE		200. Barr Body staining from buccal epithelial cells	465
175. Washing and sterilization of glasswares for animal cell culture	431	201. Normal Human Karyotyping	465
176. Preparation of media for animal cell culture	433	202. Preparation of Polythene Chromosomes	468
177. Preparation of TVG Buffer	435	203. Isolation of Chloroplasts from Spinach Leaves	469
178. Aseptic Technique and Good Cell Culture Practice	436		
179. Primary cell Culture and maintenance of cell lines	437	XVI APPENDIX	
180. Trypsinizing and subculturing cells from a monolayer	438	Appendix A – Culture Media	472
181. Subculture of Adherent Cell Lines	439	Appendix B – Staining Reagents	479
182. Subculture of Semi-Adherent Cell Lines	440	Appendix C – Biochemical test Media and Reagents	481
183. Subculture of Suspension Cell Lines	441	Appendix D – Spotters	484
184. Cell Viability Test by Trypan Blue Exclusion	442	Appendix E – Sources of Microorganisms	511
185. Assay of cell viability by dye uptake	443	Appendix F – Sources of media, chemicals and Glassware's	512
186. Cell Fusion/Hybridoma Production	443	Appendix G – Calculations	513
XIV. GENETICS		Appendix H – pH and pH indicators	518
187. Lifecycle of <i>Drosophila melanogaster</i>	447	Appendix I – Glossary	519
188. Culturing techniques and handling of flies	448	Appendix J – Periodic table	544
		Appendix K – Bibliography	545
		Appendix L – Index	546