

LABORATORY MANUAL IN MICROBIOLOGY



Pro. Dr. Balkrishna M. Sandikar

Mr. Shaileshkumar V. Mamdapure

Department of Microbiology,
Maharashtra Udayagiri College,
Udgir.

Kripa Drishti Publications, Pune.

LABORATORY MANUAL

IN MICROBIOLOGY

Prof. Dr. Balkrishna M. Sandikar
Mr. Shaileshkumar V. Mamdapure

Department of Microbiology
Maharashtra Udayagiri College,
Udgir.

Kripa-Drishti Publications, Pune.

Book Title: Laboratory Manual in Microbiology

**Authored By: Prof. Dr. Balkrishna M. Sandikar,
Mr. Shaileshkumar V. Mamdapure**

1st Edition

ISBN: 978-93-90847-47-1



Published: Oct 2021

Publisher:



Kripa-Drishti Publications

A/ 503, Poorva Height, SNO 148/1A/1/1A,
Sus Road, Pashan- 411021, Pune, Maharashtra, India.

Mob: +91-8007068686

Email: editor@kdpublications.in

Web: <https://www.kdpublications.in>

© Copyright Prof. Dr. Balkrishna M. Sandikar, Mr. Shaileshkumar V. Mamdapure

All Rights Reserved. No part of this publication can be stored in any retrieval system or reproduced in any form or by any means without the prior written permission of the publisher. Any person who does any unauthorized act in relation to this publication may be liable to criminal prosecution and civil claims for damages. [The responsibility for the facts stated, conclusions reached, etc., is entirely that of the author. The publisher is not responsible for them, whatsoever.]

Dedication

To Our Parents, Teachers and Students.

PREFACE

We feel blissful to make available our book 'Laboratory Manual in Microbiology' to the students, teachers and all other readers. This book is a sweet fruit to the honest and hard efforts for five years. Keeping in view the requirements of students and teachers, it is our humble attempt to present the contents in a lucid and appealing manner. The book constitutes '14' Sections including experiments conducted in the Microbiology laboratories with particular reference to the approach, requirements, procedure and interpretation of results and conclusion. The book constitutes '160' figures, Index in detail, Separate Appendices and List of References for further reading.

We have tried to cover maximum areas in Practical Microbiology along with the experiments in Biochemistry, Biotechnology, Environmental Science, Dairy Science and other courses related to these subjects. This book is very useful to conduct the experiments related to Agricultural Microbiology, Microbiology of Drinking Water and Sewage, Microbiology of Food and Milk, Air Microbiology, Microbial Metabolism (Enzymology), Genetics, Molecular Biology, Medical Microbiology, Pathology, Industrial Microbiology, etc. We hope that, this book would help to understand the basic concepts in Microbiology and make easy for the students and teachers to conduct experiments in microbiology and other subjects of life sciences. We heartily welcome the comments and suggestions from students and teachers which will be helpful for necessary improvements in subsequent edition.

On this occasion, it is our great pleasure and prime duty to express gratefulness to our family members, teachers, Honorable President and all other members of Maharashtra Education Society, Principal, Librarian and Other staff members of our college. We also express my sincere thanks to the Librarians of different Universities and Colleges where we visited for reference work regarding this book and Authors of many books those we referred.

We are thankful to Director Mrs. Rajani Adam, Kripa Drishti Publications, Pune to co-operate and publish this book. We are also thankful to all known and unknown personalities who have helped us to present this book.

Balkrishna M. Sandikar

Shaileshkumar V. Mamdapure

INDEX

General Rules/Instructions, Essential Things and Techniques Relates to Microbiology Experiments..... 1

I] Instructions Regarding Microbiology Experiments:.....	1
II] Some Basic Essential Things in Microbiology Laboratory:.....	2
III] Aseptic and Normal Techniques in Microbiology Laboratory:	11

Section-1: Laboratory Instruments..... 15

1.1 Microscope	15
1.2 Autoclave:.....	22
1.3 Hot Air Oven:	24
1.4 Incubator:.....	25
1.5 Refrigerator:.....	27
1.6 pH Meter:.....	28
1.7 Agarose Gel Electrophoresis Unit	29
1.8 Laminar Air Flow System:	30
1.9 Anaerobic Jar:.....	32
1.10 Distillation Apparatus:.....	32
1.11 Bioreactor or Fermenter:.....	33
1.12 Rotary Shaker:	35
1.13 Centrifuge and Ultracentrifuge:	35
1.14 Colorimeter and Spectrophotometer:.....	39
1.14.1 Colorimeter:	39
1.14.2 Spectrophotometer:	42
1.15 Chromatography Apparatus:.....	43
1.16 Magnetic Stirrer:	45
1.17 Polymerase Chain Reaction (PCR) Instrument (Thermocycler):	46
1.18 Membrane Filter Assembly:	47
1.19 Colony Counter:	48
1.20 Balance:	49
1.21 Homogenizer:	50

Section-2: Stain and Staining Techniques 51

Experiment- 2.1	53
Simple Staining / Monochrome Staining	53
Experiment 2.2.....	54
Negative Staining / Background Staining / Relief Staining	54
Experiment 2.3.....	55
Gram Staining:	55
Experiment 2.4.....	57
Acid-fast Staining.....	57

Experiment 2.5	59
Capsule Staining by Maneval's Method	59
Experiment 2.6	61
Capsule Staining by Anthony Method	61
Experiment 2.7	62
Metachromatic Granules Staining by Albert's Method	62
Experiment 2.8	64
Cell Wall Staining by Chances Method	64
Experiment 2.9	65
Lipid Staining or PHB Granule Staining by Burdon's Method	65
Experiment 2.10	66
Endospore Staining by Schaeffer-Fulton Method	66
Experiment 2.11	68
Flagella Staining	68
Experiment 2.12	70
Blood Staining by Leishman's Method	70
Experiment 2.13	72
Staining of Fungi using Lactophenol Cotton Blue	72
Experiment 2.14	75
Staining of VAM Fungi	75
Experiment 2.15	76
Staining of Yeast Cells Using Basic Stains	76
Experiment 2.16	77
Staining of Actinomycetes	77
Experiment 2.17	78
Microscopic Observation of Protozoa	78
Section-3: Pure Culture Techniques	80
I] Types of Nutrient Media:	80
A] Types of Laboratory Media Based on Its State:	80
B] Types of Laboratory Media Based on Components Used:	81
C] Types of Laboratory Media Based on Type of Microorganism to be Grown	82
II] General Steps Involved in Preparation of Nutrient Medium:	82
III] Adjustment of pH: An Essential Step	83
Experiment 3.1	83
Preparation of Nutrient Broth and Nutrient Agar	83
Experiment 3.2	85
Preparation of MacConkey's Broth and MacConkey's Agar	85
Experiment 3.3	86
Preparation of Potato Dextrose Broth and Potato Dextrose Agar	86
Experiment 3.4	87
Isolation and Identification of Bacteria from Mixed Culture	87
Experiment 3.5	94
Study of Motility of Bacteria by Hanging Drop Technique	94
Experiment 3.6	96
Measurement of Size of Bacterial Cell by Micrometry Technique	96

Experiment 3.7.....	98
Study of Bacterial Growth Curve.....	98
Experiment 3.8.....	100
Cultivation of Anaerobic Bacteria	100
Experiment 3.9.....	107
Study of Biochemical Characters of Bacterial Pure Cultures	107
Experiment 3.10.....	118
Isolation of <i>Saccharomyces</i> species from Fruits	118
Experiment 3.11.....	119
Isolation and Identification of Fungi.....	119
Experiment 3.12.....	121
Preservation of Pure Cultures (Stock Culture Maintenance)	121
Section-4: Effect of Different Factors on Growth of Microorganisms	124
Experiment 4.1.....	124
Effect of Temperature on Growth of Bacteria	124
Experiment 4.2.....	126
Effect of pH on Growth of Bacteria.....	126
Experiment 4.3.....	127
Effect of Salt Concentration on Growth of Bacteria	127
Experiment 4.4.....	128
Effect of Molecular Oxygen (Air) on Bacterial Growth.....	128
Experiment 4.5.....	129
Effect of Copper Ions on Bacterial Growth	129
Experiment 4.6.....	131
Effect of Dyes on Bacterial Growth.....	131
Experiments 4.7	132
Effect of U. V. Light on Growth of Bacteria on Bacterial Growth.....	132
Section-5: Agricultural Microbiology	133
Experiment 5.1.....	133
Isolation and Identification of Bacteria from Soil.....	133
Experiment 5.2.....	135
Isolation and Identification of Fungi from Soil.....	135
Experiment 5.3.....	136
Isolation and Identification of Actinomycetes from Soil	136
Experiment 5.4.....	138
Isolation and Identification of <i>Azotobacter</i> species from Soil	138
Experiment 5.5.....	140
Isolation and Identification of <i>Rhizobium</i> Species from Root Nodules	140
Experiment 5.6.....	143
Isolation of Phosphate Solubilizing Bacteria from Soil	143
Experiment 5.7.....	144
Determination of R: S Ratio of Soil	144
Experiment 5.8.....	146
Quantitative Analysis of Bacteria in Soil by Standard Plate Count.....	146

Experiment 5.9	148
Demonstration of Nitrification.....	148
Experiment 5.10	149
Demonstration of Denitrification.....	149
Experiment 5.11	150
Demonstration of Ammonification	150

Section-6: Microbiology of Drinking Water and Sewage152

Experiment 6.1	154
Quantitative Analysis of Water by Most Probable Number (MPN).....	154
Experiment 6.2	157
Qualitative Analysis of Water.....	157
Experiment 6.3	162
Differentiation of <i>Escherichia Coli</i> from other Coliforms by Eijkman Test.	162
Experiment 6.4	162
Qualitative Analysis of Water by Membrane Filter Technique	162
Experiment 6.5	164
Study of Biological Oxygen Demand (BOD).....	164
Experiment 6.6	166
Isolation of Coliphages from Sewage	166

Section-7: Microbiology of Food and Milk168

Experiment 7.1	168
Bacteriological Examination of Food by Standard Plate Count (SPC) Method	168
Experiment 7.2	170
Bacteriological Examination of Food by Direct Microscopic Count (DMC)	170
Experiment 7.3	173
Determination of Microbiological Quality of Milk by Methylene Blue Reduction Time Test (MBRT).....	173
Experiment 7.4	175
Microbiological Analysis of Milk by Resazurin Test.....	175
Experiment 7.5	177
Determination of Pasteurization Efficiency of Milk by Phosphatase Test	177
Experiment 7.6	179
Isolation and Enumeration of Bacteria from Food.....	179
Experiment 7.7	180
Isolation of Fungi from Spoiled Food.....	180

Section-8: Microbiology of Air181

Microorganisms in Air and Their Analysis	181
Experiment 8.1	181
Microbiological Analysis of Air by Settling Plate Technique	181
Experiment 8.2	183
Bacteriological Analysis of Air by Solid Impaction	183

Experiment 8.3.....	185
Bacteriological Analysis of Air by Liquid Impingement.....	185
Experiment 8.4.....	186
Isolation and Identification of Air-Borne Phytopathogenic Fungi.....	186
Section-9: Study of Enzymes.....	187
Experiment 9.1.....	188
Demonstration of Gelatinase Activity.....	188
Experiment 9.2.....	189
Demonstration of Urease Activity	189
Experiment 9.3.....	191
Demonstration of Caseinase Activity.....	191
Experiment 9.4.....	193
Demonstration of Lecithinase Activity	193
Experiment 9.5.....	194
Demonstration of Catalase Activity	194
Experiment 9.6.....	196
Demonstration of Desulfurase Activity.....	196
Experiment 9.7.....	198
Demonstration of Amylase Activity	198
Experiment 9.8.....	199
Estimation of α Amylase Activity.....	199
Experiment 9.9.....	202
Study of Hemolysin.....	202
Experiment 9.10.....	203
Study of Coagulase Activity	203
Section-10: Biochemistry and Microbial Metabolism	204
Experiment 10.1.....	204
Detection of Carbohydrates by using Benedict's reagent	204
Experiment 10.2.....	205
Estimation of Carbohydrate by Anthrone Method	205
Experiment 10.3.....	207
Detection of Proteins using Biuret Reagent	207
Experiment 10.4.....	208
Estimation of Protein by Folin-Lowery Method	208
Experiment 10.5.....	210
Estimation of Amino Acid by Rosen's Method	210
Experiment 10.6.....	213
Estimation of Reducing Sugar by Sumner's Method.....	213
Experiment 10.7.....	215
Detection of Biomolecules by Paper Chromatography	215
Experiment 10.8.....	219
Detection of DNA by Agarose Gel Electrophoresis	219

Section-11: Genetics and Molecular Biology224

Experiment 11.1224
 Extraction of DNA from Bacterial Cells224
Experiment 11.2226
 Estimation of DNA by Diphenylamine Test.....226
Experiment 11.3228
 Extraction of RNA from Yeast Cells228
Experiment 11.4230
 Estimation of RNA230
Experiment 11.5231
 Induction of Lactose Operon231
Experiment 11.6233
 Effect of U.V. Radiations.....233
Experiment 11.7235
 Study of Photoreactivation.....235
Experiment 11.8237
 Isolation of Antibiotic Resistant Mutant by Induced Mutation (Gradient Plate Technique)237
Experiment 11.9239
 Study of Transformation239
Experiment 11.10242
 Study of Conjugation242
Experiment 11.11245
 Isolation of Auxotrophic Mutants of *Escherichia coli* by Replica Plate Technique.....245

Section-12: Industrial Microbiology248

Experiment 12.1248
 Primary Screening of Antibiotic Producers using Crowded Plate Technique248
Experiment 12.2250
 Primary Screening of Amylase Producing Bacteria from Soil250
Experiment 12.3251
 Primary Screening of Organic acid Producer251
Experiment 12.4253
 Production of Bioinoculants using *Azotobacter* species253
Experiment 12.5255
 Production and Estimation of Citric Acid by Titration.....255
Experiment 12.6258
 Penicillin Bioassay258
Experiment 12.7262
 Ethanol production using *Saccharomyces* species on Laboratory scale262
Experiment 12.8263
 Estimation of Ethanol by Specific Gravity Method.....263
Experiment 12.9264
 Detection of Ethanol by Iodoform Test264

Experiment 12.10.....	265
Estimation of IAA by using Salkowski's Reagent.....	265
Experiment 12.11.....	267
Production of Wine using Grapes	267
Section-13: Medical Microbiology and Pathology	269
Experiment 13.1.....	269
Isolation and Identification of <i>Salmonella</i> Species	269
Experiment 13.2.....	273
Isolation and Identification of <i>Shigella</i> Species	273
Experiment 13.3.....	274
Isolation and Identification of <i>Staphylococcus</i> species.....	274
Experiment 13.4.....	278
Isolation and Identification of <i>Vibrio cholerae</i>	278
Experiment 13.4.....	279
Isolation of Normal Flora of Human Skin	279
Experiment 13.6.....	281
Total Leucocytes (WBC) Count in Blood.....	281
Experiment 13.7.....	283
Total Erythrocyte (RBC) Count in Blood	283
Experiment 13.8.....	284
Diagnosis of Typhoid by Widal test (Slide test)	284
Experiment 13.9.....	286
Diagnosis of Typhoid by Widal test (Tube test)	286
Experiment 13.10.....	286
Venereal Disease Research Laboratory (VDRL) Test	286
Experiment 13.11.....	288
Separation of Serum and Plasma.....	288
Experiment 13.12.....	289
Staining of Blood for Malaria Parasite.....	289
Experiment 13.13.....	291
Detection of Blood Group.....	291
Experiment 13.14.....	293
Physical Examination of Urine	293
Experiment 13.15.....	296
Detection of Carbohydrates in Urine Using Benedict's reagent	296
Experiment 13.16.....	297
Detection of Glucose in Urine by using Strip Method.....	297
Experiment 13.17.....	298
Detection of Bile Pigment (Bilirubin) in Urine Sample by Faucet Test	298
Experiment 13.18.....	299
Detection of Bile Salt in Urine Sample by Hay's Sulphur Powder Test.....	299
Experiment 13.19.....	299
Detection of Blood in Urine by Benzidine Test.....	299
Experiment 13.20.....	300
Detection of Acetone or Ketone Bodies in Urine by Rothra's Nitropruside Test	300

Experiment 13.21	301
Estimation of Blood Urea	301
Experiment 13.22	302
Detection of Protein in Urine by using Sulphosalicylic Acid	302
Experiment 13.23	303
Detection of Protein in Urine by Strip Method	303
Experiment 13.24	304
Microbiological Analysis of Urine	304
Section- 14: Preparation for Microbiology Experiments.....	305
14.1 Preparation of Laboratory Media:	305
14.2. Preparation of Biochemical Test Reagents.....	312
14.3 Preparation of Stains and Staining Reagents	314
14.4 Preparation of Indicator Dyes:.....	318
14.5 Preparation of Buffers	320
14.6. Preparation of Other Miscellaneous Solutions	323
Appendix-I: Commonly Used pH Indicator Dyes	325
Appendix-II: McCrady's Table.....	326
Appendix-III: Important Elements, Their Molecular Weights and Atomic Weights	328
References	330

About the Book

The book 'Laboratory Manual in Microbiology' covers maximum experiments conducted in Microbiology Laboratories of all the Degree Colleges affiliated to different Universities in India. It also includes some experiments in other subjects related to Life-sciences like Biotechnology, Biochemistry, Environmental Science, Dairy Science, Food Technology, Agrotechnology, etc. The book starts with the General Rules/Instructions, Essential things and Techniques relates to Microbiology experiments. It constitutes 13 Sections including 150 experiments those routinely conducted in the Microbiology laboratories and Section: 14 regarding- Preparation of reagents, indicators, stains and buffers. The book constitutes 160 figures those facilitates the understanding of experiments, Book contents in detail, and separate Appendices regarding- Commonly used pH indicator dyes, McCrady's table, International atomic weights of elements, Units of measurements and their interconversions. List of references at the end of book helps to get additional information about specific experiment. We hope that, this book will be very useful for all the Teachers, Students and Laboratory Assistants in Departments of Life-sciences, especially the Microbiology.

About the Authors



Dr. Balkrishna M. Sandikar

He is working as a Professor in Microbiology, at Maharashtra Udayagiri College, UDGIR Dist. Latur affiliated to Swami Ramanand Teerth Marathwada University, Nanded- Maharashtra.

He has 33 years teaching experience to students of Microbiology, i.e. since the date of his appointment 2nd July 1988. He is conducting research work especially in the field 'Biological control and plant growth promotion'. He has been awarded M. Phil. degree in microbiology for his research work on Role of fluorescent pseudomonads in growth promotion of Cassia uniflora in May 2000, by University of Pune. He has been completed minor research project sanctioned and financially supported by University Grants Commission Pune, on Biological control of damping-off diseases during 2002-2004. He has been awarded Ph.D. degree in Microbiology by Swami Ramanand Teerth Marathwada University, Nanded, for his research work on Studies on biological control agents against soil-borne fungal pathogens of crop plants in March 2009.

He has been published research papers in national and international journals regarding his research. He has been selected on advisory board of International Journal of Plant Protection, published by Hind Agricultural Research and Training Institute, Muzaffarnagar, U.P. He has worked as In-charge Principal of Maharashtra Udayagiri College, UDGIR Dist. Latur.

His first book Applied Microbiology has been published by Himalaya Publishing House, Mumbai in 2013, second book Fundamental Microbiology by Books and Allied, Publications Kolkata in 2021 and Basic Biochemistry and Microbial Metabolism in 2021 by Himalaya Publishing House, Mumbai.



Mr. Shaileshkumar V. Mamdapure

He has completed his B.Sc. in 2012 from Maharashtra Udayagiri College, UDGIR and M. Sc. Microbiology in 2014 from School of Life sciences, Swami Ramanand Teerth Marathwada University, Nanded- Maharashtra. Presently he is working as an Assistant Professor (CHB) in Microbiology, at Maharashtra Udayagiri College, UDGIR Dist. Latur affiliated to Swami Ramanand Teerth Marathwada University, Nanded- Maharashtra. He is actively involved in teaching and research since last 6 years regarding different fields of Microbiology. He has published research papers in National and International peer reviewed journals.



Kripa-Drishti Publications

A-503 Poorva Heights, Pashan-Sus Road, Near Sai Chowk,
Pune – 411021, Maharashtra, India.

Mob: +91 8007068686

Email: editor@kdpublications.in

Web: <https://www.kdpublications.in>

Price: ₹ 500

ISBN: 978-93-90847-47-1

