

Laboratory Manual of Microbiology, Biochemistry and Molecular Biology



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Laboratory Manual of Microbiology, Biochemistry and Molecular Biology

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*To
Our Reverend
DADA*



A Scholar, Philosopher and Guide

FOREWORD

Science at times is defined as study of ideas verifiable through experiment. It is well known the world over that quality of science learning and teaching is directly proportional to experiences in the laboratory. In fact, this is one of the weaknesses in the science education in our country. The way we conduct our laboratories actually makes a mockery of the subject. As a result, the theory and the practices become virtually disjoint elements of the same subject with either aspects having no bearing on the other.

I myself do not have any readymade recipe through which both theory and practicals could be integrated so that science learning becomes a holistic experience. My wonderful and learned friends are bringing out a laboratory manual of Microbiology, Biochemistry and Molecular Biology. By a cursory look through the table of contents, I am getting a feeling that this book may provide a partial solution to the problems which I have indicated above.

I am told that this book is designed to meet the requirements of undergraduate and post graduate students appearing for examination in Bioscience, Biotechnology, Microbiology, Biochemistry and Biochemical Engineering. I am further informed that it is a much needed effort since most of the practical books available in the market focus only on one or two specializations; in contrast this laboratory manual provides a snapshot to most of the common experiments prescribed by any University.

I must say that authors have well utilized their experience and expertise to bring this book to the current form. There is no doubt in my mind that this book will be received well by the students and research labs.

Last, but not the least, I am glad that the three jewels identified and polished by Dada are adding yet another feather in the cap of Banasthali.

Congratulations and all the best!

May 9, 2011

Aditya Shastri
Vice Chancellor
Banasthali University
Banasthali

PREFACE

The applications of Microbiology, Biochemistry and Molecular Biology have led to the accumulation of a huge body of knowledge and phenomenal growth in procedures and methodologies. In recent years many novel methods have been developed and the old methods have been improved. Nevertheless the time tested classical techniques have been retained. The comprehensive practical training for any student studying biological sciences is must. Though many practical books are available in the market but this is an unique combination of protocols that covers maximum (about 80%) of the practicals of various Indian universities for UG and PG courses in Bioscience, Biotechnology, Microbiology, Biochemistry and Biochemical Engineering.

While preparing this laboratory manual the efforts have been made into various aspects of laboratory practices for the beginners like; the do's and don'ts of working in any laboratory, concepts and terminologies used, and how to prepare the solutions/reagents. The protocols given here have been tested by authors during their long teaching experience. The book has been divided into four sections, the first one is **Introduction** which is subdivided into laboratory etiquette and safety, molecular, empirical and formula weight, planning a solution of a particular molarity, accuracy and calibration, buffers etc. Second part is about **Instruments: Principle and Precautions** which elaborates various commonly used equipments needed to perform different experiments. The third part of the book is about **Experiments** which has all the traditional to latest experiments with principle, requirements, procedure, results and observation and precautions in three major areas of Bioscience and Biotechnology i.e. **Microbiology, Biochemistry and Molecular Biology**. At the end a rather comprehensive **Appendix** is given as section four.

The microbiology section contains all the basic techniques used in laboratories and industries. It also consists of some advanced microbiological experiments related to industrial microbiology. The second section deals with the basic practicals associated with carbohydrates, lipids, amino acid analysis, and chromatographic techniques. Besides, other advanced techniques such as isoelectric focusing, SDS-PAGE etc. have also been included. As molecular biology has become an integral part of almost all biological courses, the third section of this book is dedicated to all the techniques related to and used in molecular biology. Some experiments related to mitochondrial and chloroplast DNA

isolation, recombinant DNA transformation and selection, blotting and hybridization techniques which are generally not found in most practical books, have also been included here. All the three sections of the book i.e. microbiology, biochemistry and molecular biology have been written by authors who have long experience and are well versed with conducting practicals related to their expertise.

One person who has been instrumental for the initiation of this project and who has rendered his whole hearted support at all times for the successful completion of this book is none other than Dr. Ravi Israni. We owe him our heartfelt gratitude. The authors also wish to acknowledge the support and guidance provided by Prof. Aditya Shastri, Vice Chancellor and Prof. Vinay Sharma, Head, Dept. of Bioscience and Biotechnology of Banasthali University. For the preparation of this book we have consulted many practical books and we would like to extend our thanks to all the authors of these books namely K.R. Aneja, R.C. Dubey, D.K. Maheshwary, R.N. Bhattacharya, S. Sadasivam, A. Manickam etc.

Last but not least, we are greatly indebted to the almighty and our family members for their blessings and making our path smooth.

Jyoti Saxena
Mamta Baunthiyal
Indu Ravi

ABBREVIATIONS

A	- Absorbance
APS	- Ammonium persulphate
BPB	- Bromophenol blue
BSA	- Bovine Serum Albumin
BTH	- Benzo (1,2,3) thiadiazole-7-carbothionic acid S-methyl ester
bp	- Base pairs
CTAB	- Cetyltrimethylammonium bromide
CAT	- Chloramphenicol acetyl transferase
Da	- Daltons
DAB	- 3, 3'-Diamino benzidine
DEPC	- Diethyl pyrocarbonate
DMAB	- <i>p</i> - Dimethyl amino benzaldehyde
DMF	- N, N-dimethyl formamide
DMSO	- Dimethyl sulfoxide
DTT	- Dithiothreitol
DW	- Distilled water
EDTA	- Ethylenediamine tetra acetic acid
EtBr	- Ethidium bromide
EMB	- Eosine-methylene blue
FW	- Formula weight
g	- gram
GAA	- Glacial acetic acid
GFP	- Green fluorescent protein
GUS	- β -Glucuronidase
HEPA	- High Efficiency Particulate Air
HEPES	- N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid
H ₂ O ₂	- Hydrogen peroxide
HCl	- Hydrochloric acid
HPLC	- High performance liquid chromatography
h	- Hours
IR	- Infra red
IPTG	- Isopropyl-thiogalactoside
kDa	- kilodaltons
kbp	- kilobase pairs
Km	- Michaelis-Menton constant
L	- litre

LB media	- Luria Bertani media
LMW	- Low molecular weight marker
M	- Molar
MW	- Molecular weight
Mr	- Relative molecular weight
mg	- milligram
min	- minutes
ml	- milliliter
µg	- microgram
µl	- microlitre
µkat	- microkatal
µm	- micromoles
nm	- nano meters
N	- Normal
OD	- Optical Density
pfu	- Plaque forming units
psi	- Pounds per square inch
ppm	- parts per million
Pi	- inorganic phosphate
PBS	- Phosphate buffered saline
PCR	- Polymerase chain reaction
PDA	- Potato Dextrose Agar
PVDF	- Polyvinylidene difluoride
Rf	- Relative front
RCF	- Relative centrifugal force
RPM	- Revolutions per minute
S	- Svedberg unit
Sec	- seconds
SDS-PAGE	- Sodium dodecyl sulphate polyacrylamide gel electrophoresis
TE	- Tris EDTA
TBS	- Tris buffered saline
Tm	- melting temperature
TEMED	- N, N, N', N'-Tetra methyl ethylene diamine (hydroxy methyl) amino methane
TLC	- Thin layer chromatography
Tris	- Tris (hydroxymethyl) aminomethane
UV	- Ultra violet
v	- velocity
Vmax	- maximum velocity
v/v	- volume by volume
w/v	- weight by volume
X-gal	- 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside

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