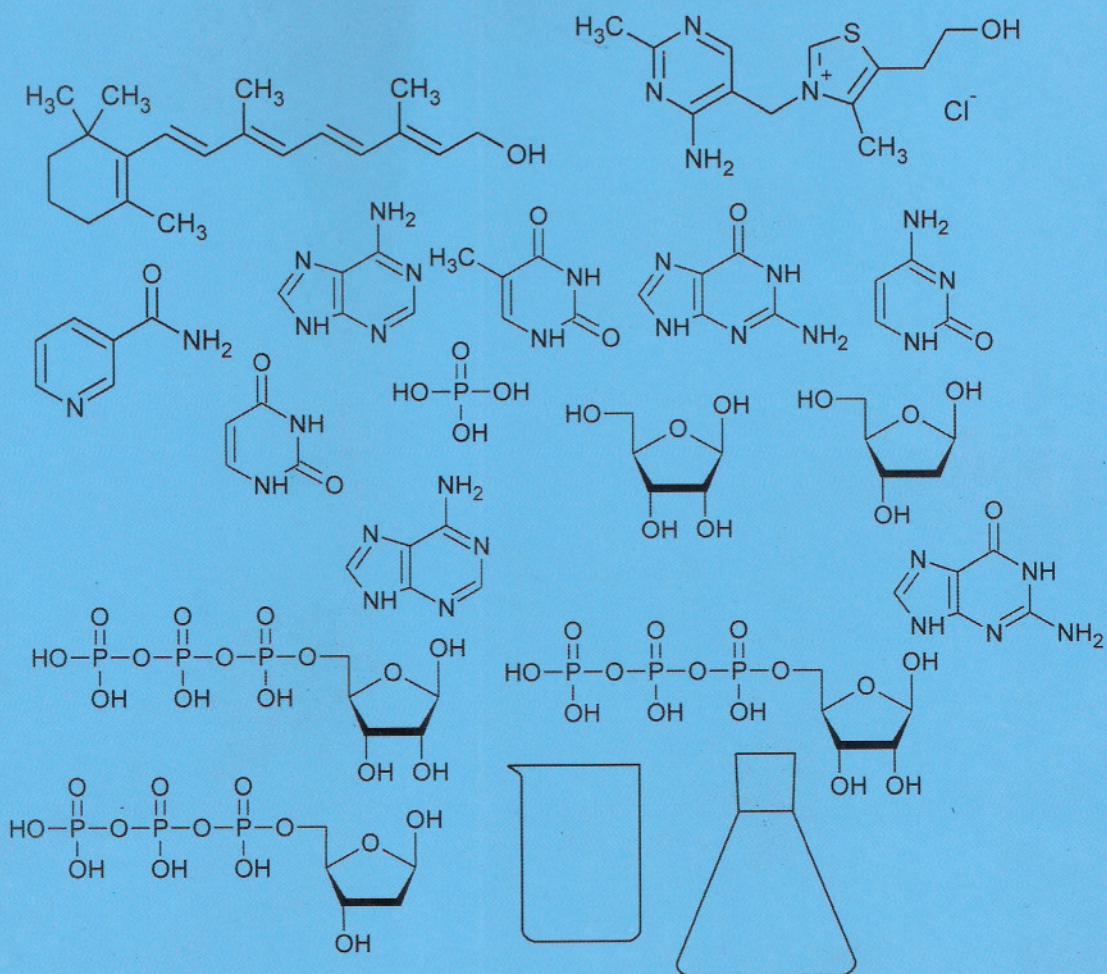


A PRACTICAL MANUAL

in Biochemistry & Clinical Biochemistry



Victor J. Temple,
Rachael Rowe, Nigani Willie
& Samson Grant

**A PRACTICAL MANUAL IN
BIOCHEMISTRY & CLINICAL
BIOCHEMISTRY**

**UNIVERSITY OF PAPUA NEW GUINEA
SCHOOL OF MEDICINE AND HEALTH SCIENCES**

DIVISION OF BASIC MEDICAL SCIENCES

**DISCIPLINE OF BIOCHEMISTRY &
MOLECULAR BIOLOGY**

**4th EDITION of
PRACTICAL MANUAL IN
BIOCHEMISTRY**

**Victor J. Temple,
RACHAEL ROWE, Nigani Willie
& Samson Grant**

Port Moresby
**University of Papua New Guinea Press
2013**

This Practical Manual in Biochemistry & Clinical Biochemistry is a modified and updated version of the “Practical Manual in Biochemistry” by Dr. G.R.E. Swaniker, Dr. A. C. Banerjee and Mrs. S. Smillie.

The 2nd Edition of this manual was edited in 1992 by Dr. J. G. O. Harding and Mrs. B. R. Alfred. The 2nd Edition was also reprinted in 1998. The 3rd Edition was a modified and updated version of the 2nd Edition by Assoc Prof. Victor J. Temple, Mr. Nigani Willie and Mr. Samson Grant. This 4th Edition is an updated version of the 3rd Edition.

This 4th Edition is an updated and greatly modified version of the 3rd Edition.

4th Edition: A Practical Manual in Biochemistry & Clinical Biochemistry

ISBN: 978 – 9980 – 84 – 919 – 9

Edited by: Assoc. Prof. Victor J. Temple, Ms Rachael Rowe, Mr. Nigani Willie & Mr. Samson Grant

Published by UPNG Press and Uni-Bookshop

P.O. Box 413, University PO, UPNG Campus, Waigani, N.C.D, Port Moresby PNG

Email: upngbooks@gmail.com

ACKNOWLEDGEMENTS

The editors would like to acknowledge the undergraduate students in the School of Medicine and Health Sciences (SMHS) University of Papua New Guinea (UPNG) for their enthusiasm and commitment to the study of Biochemistry and Molecular Biology. We acknowledge Ms Jennie Bautau-Grant in the Biochemistry laboratory, for her dedicated service and hard work in keeping the laboratory fully functional at all times. Many thanks also to the technical officers in BMS. We thank all our colleagues in the Division of Basic Medical Sciences (BMS) for their support. We are extremely grateful to Ms Theresa Dunamb, the secretary in BMS, for providing the electronic copy of the 2nd edition of this manual. Constructive comments and recommendations for improvement in the next edition of this manual are most welcome.

#	CONTENT	Page #
1	Table of content-----	3
2	Chapter 1: -----	5
3	Introduction: Safety in the Biochemistry Laboratory: Guidelines and Precautions:-----	5
4	Practical Reports & Assignments-----	7
5	Chapter 2-----	8
6	Structure of water; Water as a major solvent -----	8
7	Section one: Solutions: Molarity (Molar concentration)-----	8
8	Normality-----	10
9	Percent-of-solute; Percent based on weight-----	10
10	Osmolarity-----	10
11	SECTION TWO: Dilutions; Simple and Serial dilutions:-----	11
12	Practical Session One: Preparation and Dilution of Solutions -----	13
13	Chapter 3 -----	16
14	SECTION ONE: The pH scale; Ionic Product of Water (K _w): Significance of the Ion Product of Water: -----	16
15	Relationship between the Ionic Product (K _w) of water and the pH scale -----	17
16	SECTION TWO: Acids, Bases and Buffers -----	18
17	Strength and Concentration of acidic and basic solutions; -----	18
18	Bronsted and Lowry concepts of acids & bases (Conjugate Acid Base Pair)	18
19	Buffers, Range of Buffer and Buffer Capacity: -----	19
20	Handerson-Hasselbalch equation; How does a buffer work? -----	19
21	The pH range of a buffer solution; Buffer Capacity; The pH METER -----	20
22	Practical Session Two: Measurement of pH; using pH Meter -----	22
23	Practical Session Three: Section A: Buffer Solutions -----	24
24	Practical Session Three: Section B: Saliva -----	27
25	Chapter 4 -----	30
26	Colorimetry and Spectrophotometry -----	30
27	Transmittance; Absorbance; Lambert's Law; Beer's Law; Beer-Lambert's equation -----	31
28	Molar Extension Coefficient; Limitations of Beer-Lambert's Equation -----	31
29	Colorimeter and Spectrophotometer -----	32
30	Practical Session Four: Colorimetry and Spectrophotometry -----	34
31	Chapter 5 -----	38
32	Estimation and Separation of Proteins in Blood -----	38
33	Section One: Estimation of Serum / Plasma Proteins -----	38
34	Section Two: Separation of Serum Proteins -----	40
35	Practical Session Five: Estimation of Proteins in Blood -----	43
36	Practical Session Six – A: Separation of Proteins using cellulose strips -----	47
37	Practical Session Six – B: Separation of serum proteins in 1.0% Agarose Gel	52
38	Chapter Six -----	55
39	Chromatography: -----	55
40	Absorption, Affinity, Gas-liquid, Gel-filtration or molecular sieving, Partition chromatography -----	55
41	Practical Section Seven: Paper Chromatography of Amino Acids in Urine ----	58
42	Practical Session Eight: Semi-quantitative assessment of Aspirin by Thin Layer Chromatography (TLC) -----	60
43	Chapter Seven -----	63
44	Estimation of Amylase Activity in Saliva and Serum -----	63
45	Practical Session Nine: Estimation of Salivary Amylase Activity -----	63
46	Chapter Eight -----	67
47	Blood Glucose -----	67
48	Practical Session Ten: Determination of Glucose in Serum -----	68
49	Chapter Nine -----	71
50	Estimation of Serum Total Cholesterol -----	72
51	Practical Session Eleven: Estimation of Cholesterol in Serum -----	72
52	Chapter Ten -----	75

53	Renal (Kidney) Function I: -----	75
54	Practical Session Twelve: Urinalysis Part I: -----	76
55	Section I: Using Multistick -----	79
56	Section II: Physical Examination of Urine -----	80
57	Determination of Specific Gravity of Urine -----	80
58	Section III: Chemical Analysis of Urine -----	80
59	Tests for Proteins in Urine -----	82
60	Tests for Glucose in Urine: -----	82
61	Test for Ketone Bodies: Rothera's Test: -----	83
62	Test for Bilirubin & Urobilinogen in Urine -----	83
63	Practical Session Thirteen: Urinalysis Part II -----	88
64	Microscopic Examination of Urine -----	88
65	Chapter Eleven -----	93
66	Renal (Kidney) Function II: -----	93
67	Determination of Serum Urea: -----	93
68	Practical Session Fourteen: Estimation of Urea in Blood (Serum) -----	95
69	Chapter Twelve -----	99
70	Renal (Kidney) Function III: -----	99
71	Creatinine Clearance -----	99
72	Practical Session Fifteen: Creatinine Clearance -----	101
73	Chapter Thirteen -----	106
74	Renal (Kidney) Function IV: Urinary Stones (Nephrolithoisis or Renal Calculi) -----	106
75	Practical Session Sixteen: Estimation of Uric Acid in Urine and Serum: -----	108
76	Section 1: Procedure for Determination of Uric Acid in Urine: -----	110
77	Section II: Determination Of Uric Acid In Serum: -----	111
78	Chapter Fourteen -----	113
79	Liver Function Tests: Functions of the Liver: -----	113
80	Biochemical Parameters in LFT -----	113
81	Diagnostic significance of AST, ALT, ALP, GGTP -----	114
82	Total Protein (Albumin and Globulins) -----	115
83	Practical Session Seventeen: Estimation of Serum Bilirubin -----	117
84	Types of jaundice: Haemolytic or Pre-hepatic; Hepatic; Post-hepatic -----	117
85	Estimation of Bilirubin in Serum -----	118
86	Practical Session Eighteen: Serum Alkaline Phosphatase -----	121
87	Practical Session Nineteen: Estimation of serum ALT and AST -----	126
88	Chapter Fifteen -----	132
89	Diabetes Mellitus (DM): -----	132
90	Practical Session Twenty: Oral Glucose Tolerance Test and Urinalysis -----	133
91	Chapter Sixteen: Molecular Biology -----	141
92	Practical Session Twenty One: Extraction of DNA -----	142
93	Chapter Seventeen: Assignments -----	147
94	References -----	147
95	Appendix -----	148