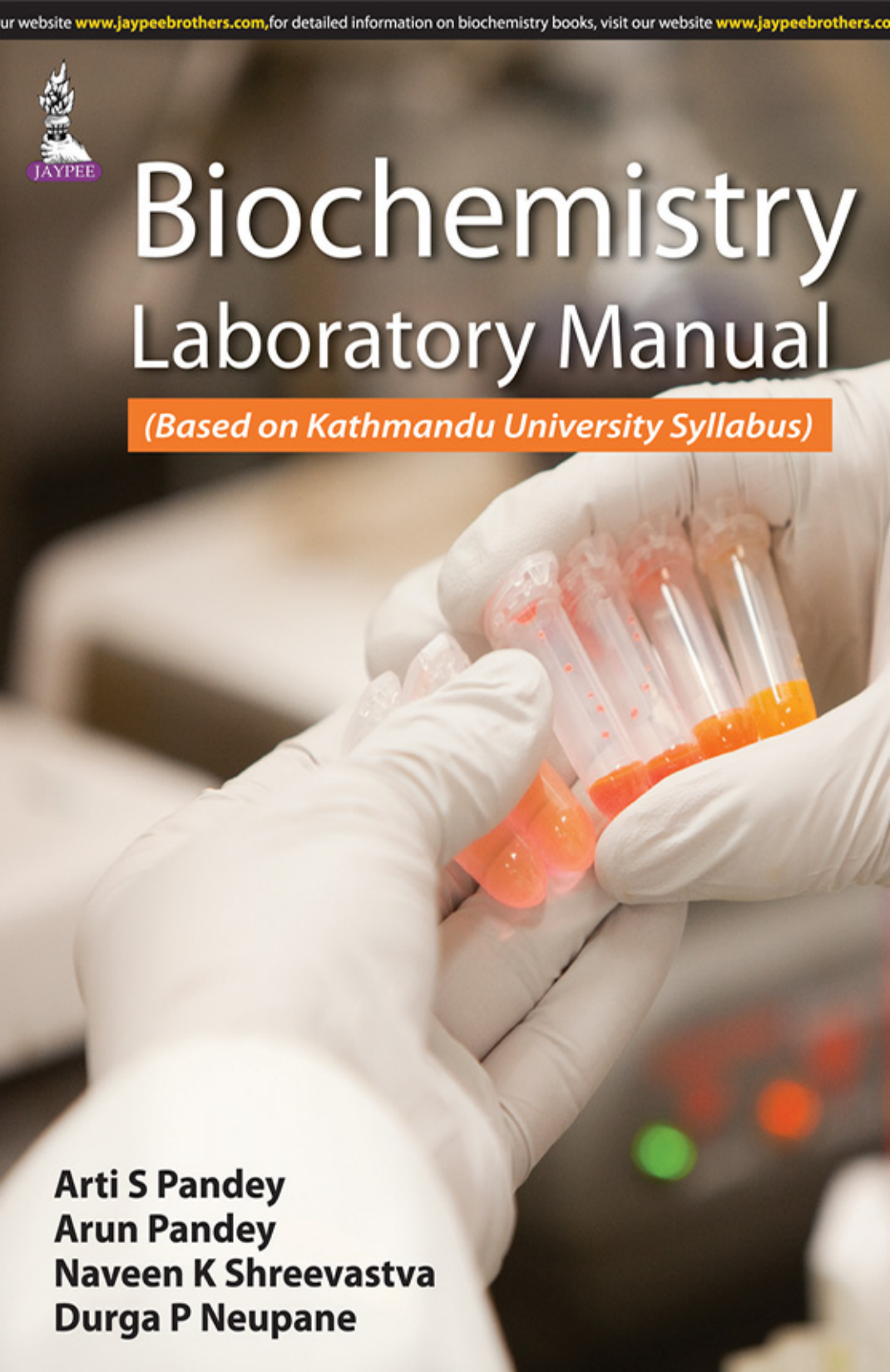




Biochemistry Laboratory Manual

(Based on Kathmandu University Syllabus)



**Arti S Pandey
Arun Pandey
Naveen K Shreevastva
Durga P Neupane**

Contents

MBBS I

Experiment 1: Use of Micropipettes	3
• Introduction	3
• Use	3
• Procedure for micropipetting	4
• Checking your pipetting skill	5
Experiment 2: Buffers and pH	7
• Introduction	7
• pH meter	8
Experiment 3: Qualitative tests for Carbohydrates	11
• Introduction	11
• Reactions of monosaccharides, disaccharides and polysaccharides	11
Experiment 4: Qualitative Tests for Proteins	20
• Introduction	20
• Biuret reaction (general test for protein)	20
• Heat coagulation test	21
• Precipitation by salt solution (ammonium sulfate)	21
Experiment 5: Separation of Serum Proteins by Gel Electrophoresis	25
• Introduction	25
• Principle	26
• Quantitation	27
• Hyperproteinemias	28
• Hypoproteinemias	28
Experiment 6: Principles of Colorimetry	32
• Introduction	32
• The photoelectric colorimeter	33

- Verification of Beer-Lambert's law using bromophenol blue 34

Experiment 7: Estimation of Serum Total Protein and Determination of Albumin to Globulin Ratio 38

- Introduction 38
- Principle 38
- Reagents 38
- Procedure 39
- Observation 39
- Calculation 39
- Normal range (adults) 40

Experiment 8: Genomic DNA Isolation, Amplification of the D1S80 Locus and DNA-Gel Electrophoresis 42

- Introduction 42
- Materials and methods 43
- The D1S80 locus 44
- Gel electrophoresis and DNA fragment analysis 46

Experiment 9: Estimation of Blood Glucose by GOD-POD Method 49

- Introduction 49
- Principle 49
- Specimen collection 49
- Reagents 50
- Sample collection 50
- Procedure 50
- Observation 50
- Normal range (serum/plasma glucose) 51
- Comment on the result 51

Experiment 10: Estimation of Serum Total Cholesterol by Modified Zak's Method 53

- Introduction 53
- Principle 53
- Reagents 54

		• Procedure	54
		• Observation	55
		• Calculation	55
		• Normal range for serum cholesterol (recommended by NCEP)	55
		• Comment on the result	55
Experiment 11: Estimation of Serum Triglycerides			57
		• Introduction	57
		• Principle	57
		• Procedure	57
		• Observations	58
		• Calculation	58
		• Normal values: (A/C to NCEP-ATP II)	58
		• Comment on the result	59
Experiment 12: Estimation of Serum Uric Acid by Caraway's Method			61
		• Introduction	61
		• Principle	61
		• Reagents	62
		• Procedure	62
		• Observations	62
		• Calculation	63
		• Normal range	63
		• Comment on the result	63
MBBS II			
Experiment 13: Determination of Serum Amylase Activity by Amylolytic Method			67
		• Introduction	67
		• Principle	67
		• Reagents	68
		• Procedure	68
		• Normal range	69
		• Comment on the result	69

Experiment 14: Estimation of Serum Aspartate Transaminase (AST) Activity 71

- Introduction 71
- Principle 71
- Reagents 72
- Procedure 72
- Observations 72
- Normal range 73
- Comment on the result 73

Experiment 15: Estimation of Serum Alanine Transaminase (ALT) Activity 75

- Introduction 75
- Principle 76
- Reagents 76
- Procedure 76
- Observations 77
- Calculation 77
- Normal range 77
- Comment on the result 78

Experiment 16: Estimation of Serum Bilirubin by Modified Jendrassik/Grof Method 80

- Introduction 80
- Principle 81
- Reagents 81
- Procedure 81
- Observations 82
- Calculation 82
- Normal range 83
- Comment on the result 83

Experiment 17: Estimation of Serum Urea by Diacetyl Monoxime (DAM) Method 87

- Introduction 87
- Principle 88
- Reagents 88

• Procedure	88
• Observation	89
• Calculation	89
• Normal range	89
• Comment on the result	90
Experiment 18: Estimation of Serum Creatinine by Jaffe's Alkaline Picrate Method	91
• Introduction	91
• Principle	91
• Reagents	92
• Procedure	92
• Observation	92
• Calculation	93
• Normal range	93
• Comment on the result	93
• True creatinine	93
Experiment 19: Urinary Creatinine (Jaffe's Method) and Creatinine Clearance	95
• Introduction	95
• Principle	95
• Procedure	95
• Observation	96
• Calculation	96
• Normal range	97
• Comment on the result	97
• Normal range	98
• Comment on the result	98
Experiment 20: Urinalysis	101
• Introduction	101
• Physical examination	101
• Chemical examination	102
• Abnormal chemical constituents	103

Experiment 21: Urinalysis (Abnormal Constituents)	108
• Physical examination of urine	108
• Chemical examination	108
• Result	109
• Interpretation	109
Experiment 22: Oral Glucose Tolerance Test (OGTT): Principle and Interpretation	112
• Introduction	112
• Preparatory phase	112
• Procedure	113
Experiment 23: Molecular Diagnosis: Example Sickie Cell Anemia using ASO and RFLP	116
• Introduction	116
• Sickie cell anemia	116
Reference Biochemical Values	121
Reagents	122

Experiment

4

Qualitative Tests for Proteins

INTRODUCTION

Normal adults excrete a small amount (upto 160 mg/day) of proteins in urine. This consists of albumin, Tamm-Horsfall protein, some other plasma proteins, glycoproteins, etc. This small amount of protein is not detectable by routine methods. Increased amount of proteins in urine, i.e. proteinuria can be caused by increased glomerular permeability (e.g. albuminuria), reduced tubular reabsorption (e.g. β_2/α_1 microglobulinuria), increased secretion of protein from the renal tract (e.g. Tamm-Horsfall proteinuria) or increased concentration of low molecular weight proteins in the plasma (e.g. Bence Jones proteinuria). Proteinuria may be physiological or pathological. Physiological proteinuria may be seen in last weeks of pregnancy, in severe stress like excessive physical activity, etc. Pathological proteinuria may be seen in conditions such as nephrotic syndrome, pyelonephritis, glomerulonephritis, severe urinary tract infection, etc.

BIURET REACTION (GENERAL TEST FOR PROTEIN)

This test is positive for all compounds containing more than one peptide linkage (tripeptide onwards). All proteins give a positive biuret test.

Principle

Protein reacts with Cu^{2+} ions in alkaline medium to form a blue to violet/purple-colored complex; which is due to formation of co-

ordination complex between Cu^{++} (cupric ions) and peptide bonds of proteins.

Procedure	Observation	Inference
1 ml of protein solution in a test tube +1 ml 5% NaOH + 2 drops of 0.5% copper sulfate solution. Mix well		

HEAT COAGULATION TEST

Principle

Proteins lose their solubility in water as the temperature is raised. This is due to a loss of its tertiary structure or “denaturation”. When heated directly, a protein solution will show precipitation or solidifying of protein, a process called coagulation. An example is, heating of egg albumin, which coagulates into a solid, opaque mass.

Procedure	Observation	Inference
Fill 2/3rd of test tube with the protein solution. Heat upper-half of the tube while holding it in a tilted position. Cool and add 2–3 drops of 1% acetic acid		

PRECIPITATION BY SALT SOLUTION (AMMONIUM SULFATE)

The process of protein precipitation by the addition of a neutral salt such as ammonium sulfate is known as salting out. The phenomenon is explained on the basis of dehydration of protein molecules by salts. The cations and anions of salts neutralize the ionic groups on proteins, discouraging interactions with water. This causes increased protein-protein interaction, resulting in molecular aggregation and precipitation. The amount of salt required for protein precipitation depends on the size (molecular

weight) of the protein molecule. In general, the higher the molecular weight of protein, lower is the salt required for precipitation. Larger molecules (e.g. globulins) require half saturation with ammonium sulfate and smaller molecules (e.g. albumin) require full saturation for precipitation.

Procedure	Observation	Inference
A. Half saturation test: Take 3 ml protein solution in a test tube and add 3 ml saturated solution of ammonium sulfate. Mix well and keep for 5 min. Filter Perform the biuret test with the filtrate: 1 ml of the filtrate + 1 ml of 40% NaOH + 2 drops of 1% CuSO_4 solution. Mix well		
B. Full saturation test with ammonium sulfate and perform biuret test with filtrate: 3 ml specimen + add solid ammonium sulfate till saturated, filter and perform biuret test Biuret test: 1 ml of filtrate + 1 ml of 40% NaOH + 2 drops of CuSO_4 solution. Mix it well		

QUESTIONS

1. What is denaturation of protein?

- 2. Why does albumin require full saturation with ammonium sulfate for precipitation, whereas globulin requires half saturation?**

- 3. Is the presence of albumin in urine normal? Comment.**

- 4. When do different types of protein appear in urine?**

5. When are the conditions of hypoalbuminemia and hyperalbuminemia seen? Explain.

JAYPEE BROTHERS

Biochemistry

Laboratory Manual

This *Biochemistry Laboratory Manual* is meant for students of the MBBS program that is being carried out in the Medical Colleges under Kathmandu University in Nepal. The manual is based upon the Kathmandu University School of Medical Sciences curriculum for MBBS, and complements what is being taught in theory classes. Clinically relevant questions about the experiments that stress application of a biochemical test in diagnosis are included at the end of each chapter. The manual tries to minimize writing on the part of the students, so that they focus on the results of their experiments, with comments on the results themselves as well as their clinical relevance. The book includes exercises on protein and DNA electrophoresis, which can be taught as a demonstration, or carried out by each student on his own blood samples.

All the authors are faculty members at Kathmandu Medical College (Basic Sciences) in Duwakot, Bhaktapur, Nepal, and teach Medical Biochemistry to MBBS, BDS and Nursing students.

Arti S Pandey PhD in Biochemistry from Montana State University, USA, is an Associate Professor and Head of the Department with 11 years of teaching experience at Kathmandu Medical College, Kathmandu, Nepal.



Arun Pandey MSc (Medical Biochemistry) is an Assistant Professor with 6 years of teaching experience at Nepal Medical College, as well as Kathmandu Medical College, Kathmandu, Nepal.



Naveen K Shreevastva MSc (Medical Biochemistry) is a Lecturer and has 7 years of teaching experience at Kathmandu Medical College, Kathmandu, Nepal.



Durga P Neupane MSc (Medical Biochemistry) is a Lecturer with 7 years of teaching experience at Kathmandu Medical College, Kathmandu, Nepal.



Available at all medical bookstores
or buy online at www.jaypeebrothers.com



JAYPEE BROTHERS
Medical Publishers (P) Ltd.
www.jaypeebrothers.com

Join us on [facebook.com/JaypeeMedicalPublishers](https://www.facebook.com/JaypeeMedicalPublishers)

Shelving
Recommendation
BIOCHEMISTRY

ISBN 978-93-5152-651-3



9 789351 526513