

## **SEMESTER - I**

### **B.C 101: CHEMISTRY OF BIOMOLECULES**

#### **UNIT - I**

##### **Amino acids and Peptides:**

Structural features - Stereoisomerism and optical activity, Nomenclature of chiral molecules - D, L - system and R-S system. Classification, Nomenclature and structure of standard amino acids. Structures and occurrence of nonstandard and nonprotein amino acids. Physical and chemical properties (due to carboxylic, amino and side chain groups). Acid - base titration of amino acids (Gly, His, Glu and its significance).

Peptide bond. Natural occurring peptides - Structures and functions. Physical and Chemical properties. Chemical synthesis of Peptides - liquid - phase and solid-phase techniques.

##### **Proteins:**

Classification and biological functions. Physico-Chemical properties. Forces stabilizing protein structure. Primary structure and its determination. Secondary structure - helical, Beta pleated structure. Ramachandran's plot. Fibrous proteins (Collagen, keratin, elastin, silk fibroin,) Super secondary structures.

#### **UNIT - II**

Tertiary and quaternary structures. Three - Dimensional structures of globular (Myoglobin, Haemoglobin, Sickle cell haemoglobin - structure and functional relationship), Determination of subunit composition. Denaturation - Melting temperature, effect of salts, Chaotropic agents. Protein folding – Anfinsen's studies on ribonuclease and Creighton's studies on BPTI; Chaperons, thermodynamics of protein folding. Prediction of protein structures - Choh and Fasman scheme. Chemical modification of proteins.

##### **Porphyryns and other Pigments:**

Classification nomenclature and properties of porphyryns. Structure and biological functions of metallo-porphyryns ( Hemes, chlorophylls, Cytochromes ), Xanthophylls, Carotenoids.

#### **UNIT - III**

##### **Carbohydrates:**

Classification - Review of isomerism. Cyclic structures and anomeric forms. Haworth projections, Conformational stability - boat and chair forms. Optical activity and Polarimetry. Structure and biological functions of mono-, di-, oligo-, and polysaccharides ( homo and hetero ). Sugar derivatives - alcohols, acids, amino sugars, deoxysugars, glycosides. Reaction of monosaccharide. Structural elucidation of oligosaccharides - Chemical and enzymatic (raffinose). Glycosoaminoglycans - classification, structure and biological functions. Glycoconjugates - structural features of proteoglycans, bacterial cell wall peptidoglycans,

teichoic acid. Glycoproteins - Classification, structure and biological functions. Isolation and analysis of carbohydrates - use of lectins.

### UNIT - IV

#### **Lipids:**

Classification and structure of fatty acids. Isomerism. Classification of lipids. Chemistry and biological function of eicosanoids, fats, waxes, phospholipids, non-phosphorylated lipids; terpenes, steroids. Physico-chemical properties and Characterization of fats and oils. Salient features of bacterial and plant lipids. Isolation and analysis of lipids.

#### **Nucleotides and Nucleic acids:**

Structure and properties of nitrogen bases, nucleosides, nucleotides. Conformation, Polynucleotide - Shorthand notation of structure. Nucleic acids - classification and their functions. Chemical synthesis of oligonucleotides. Determination of primary structure of DNA using Maxam-Gilbert method.

DNA - base composition, Chargoff's rules, Watson-Crick double helix, size ; different forms of DNA. Forces stabilizing structure of DNA. Physico-chemical properties. Denaturation - hypo and hyper chromic effect ; melting temperature. Renaturation kinetics - effect of salts, complexity. Hybridization and its significance.

Chemical differences between DNA and RNA and its significance. Different RNAs - mRNA, rRNA, tRNA and snRNA. Primary, secondary and tertiary structure of tRNA. Chemical and enzymatic hydrolysis of nucleic acids.

#### **Recommended Books:**

1. Biochemistry - Lubert Stryer ( Freeman Co ).
2. Text Book of Biochemistry with Clinical Correlations - Thomas M. Devlin (ed) Wiley-Liss ) - 4th Edition.
3. Biochemistry - Donald Voet and Judith Voet ( John Wiles and sons )
4. Lehninger Principles of Biochemistry - David L. Nelson and Michael M. Cox ( Macmillan - Worth ) - 3rd Edition.
5. Biochemistry - Garrett. R.H. and Grisham. C.M. ( Saundes College Publication ) - 2nd Edition.
6. Biochemistry - The Chemical Reactions of Living Cells Vol-I - David.E.Metzler. ( Harcourt - Acqoleric Press ) - 2nd Edition.
7. Text Book of Biochemistry - West and Todd (1998). ( Macrillian )
8. Biochemistry - Zuby
9. Nucleic acid Biochemistry and Molecular Biology - Mainwaring et al.( Blackwell )19.
10. Principles of Biochemistry - General Aspects - Smith etal ( Mc Grand Hill ) 1983.

## **BC 102 : ENZYMOLOGY**

### **Unit-I**

Historical aspects of enzymes. Remarkable properties of enzymes: catalytic power, specificity, regulation, transformation of different forms of energy. Holoenzyme, Apoenzyme, Coenzymes and cofactors. Nomenclature and classification of enzymes as per enzyme commission. Active site- Fisher and Koshland models. Formation of enzyme substrate complex – evidences.

Kinetic and chemical mechanisms of enzyme catalyzed reactions – Basic concepts of bioenergetics. The collision theory, activation energy and transition state theory. Basic concepts of kinetics of chemical reactions. Law of mass action and order of reactions. Initial velocity studies, progressive curves. Rapid reaction kinetics (Continuous flow and stopped flow techniques).

Assay of enzymes: By kinetics, determination of catalytic activity, coupled kinetic assays, RIA of enzymes. Immunoradiometric assay. Units of enzyme activity IU, Katal, turnover. Investigation of sub cellular compartmentation of enzymes. Enzyme histochemistry, the use of centrifugation.

### **Unit- II**

Kinetics of single substrate enzyme catalyzed reaction, equilibrium, steady state assumption - Michaelis-Menten (Briggs- Haldane) equation. Transformation of Michaelis- Menten equation. The Lineweaver Burk, Eadie-Hofstee and Hanes plots. Determination of  $V_{max}$ ,  $K_m$ ,  $K_{cat}$ , Specificity constant ( $K_{cat}/K_m$ ) and their significance. Effect of pH temperature, enzyme and substrate concentrations on enzyme activity, Kinetics of enzyme reactions having two or more substrates. Single displacement and double displacement reactions. Enzyme inhibition: Reversible inhibition-Competitive, Noncompetitive (pure, mixed) inhibition, partial inhibition. Substrate inhibition, allosteric and irreversible inhibition. Feedback inhibition.

### **Unit-III**

Mapping of active site: conformation and nature of active site. Models identification of functional groups essential for catalysis. Trapping the enzyme substrate complex use of substrate analogs, Enzyme modification by chemical procedures affecting amino acid side chain, treatment with proteases, applications of site directed mutagenesis for mapping of active site. The effect of changing pH. Factors contributing to the catalytic efficiency-proximity and orientation, covalent catalysis, acid-base catalysis, metal ion catalysis, factor of strain in enzyme catalysis. Mechanism of enzyme action- Lysozyme, Carboxy peptidase, Chymotrypsin and Ribonuclease.

### **Unit- IV**

Vitamin coenzymes, structure and functions. Enzyme regulation, allosteric enzymes, feed-back inhibition. Allosteric kinetics (ATcase), cooperativity, symmetry and sequential models. Hill equation-Plot. Covalent modification (Glycogen phosphorylase, Glutamine synthetase). Zymogen activation (Chymotrypsin). Isozymes (LDH). Multi-enzyme complexes (Pyruvate

dehydrogenase complex). Multifunctional enzymes. Modern concepts of evolution of catalysis – catalytic RNA (Ribozymes), abzymes (catalytic antibodies), Immobilized enzymes.

**Recommended books:**

1. Principles of Biochemistry, 1993. A.L.Lehninger, Nelson & Cox (CBS, India ) and new edition.
2. Principles of Biochemistry General Aspects 1983 – Smith et al., (McGraw Hill).
3. Biochemistry (2<sup>nd</sup> Edition) – Donald Voet and Judith Voet.
4. Biochemistry (4<sup>th</sup> Edition)- L.Stryer (Freeman).
5. Text Book of Biochemistry with clinical correlations (4<sup>th</sup> edition) – Thomas M. Devlin.
6. Text of Biochemistry (1908) – West & Todd (MacMillian).
7. Biochemistry – Zubay.
8. Understanding Enzymes (1985) – Trevor Palmer (John Wiley).
9. Fundamentals of Enzymology: The cell and molecular biology of catalytic proteins, 3<sup>rd</sup> edition Nicholas C.Price and Lewis Stevens (1999).
10. Biochemistry chemical reactions of living cells (Copy right 2001) David E.Matzler Vol.I
11. Biochemistry 2<sup>nd</sup> edition Christopher K.Mathews and K.E.Van Holdge (1995) (Benjamin and cumming).
12. Enzymes Biochemistry, Biotechnology, Clinical chemistry, Trevor Palmer.

## BC 103: CELL BIOLOGY AND MOLECULAR GENETICS

### Unit - I

Nucleus - Ultrastructure of nucleus and nuclear envelope. Nomenclature of chromosome, C-value paradox, dosage compensation. Organisation of eukaryotic chromosome - structure of nucleosome and extent of chromatin condensation in metaphase chromosome. Euchromatin and heterochromatin (constitutive and facultative). Nucleolus. Polytene and Lampbrush chromosomes.

Cell cycle - Overview of eukaryotic cell cycle, regulation of cell cycle by cell growth and extra cellular signals, Cell cycle check points, Regulators of cell cycle progression -MPF, cyclins and cyclin-dependent kinases. Cell differentiation

Cell death and proliferation – Apoptosis: definition, morphological and biochemical differences between apoptosis and necrosis, mechanism (internal and external signals) and significance. Brief account of biology of cancer.

### Unit - II

Cell Communication - General principles: Cell surface receptors (ion channel linked, G-protein linked and enzyme-linked receptors) and intracellular receptors, forms of intracellular signaling – Autocrine, paracrine, contact dependent, synaptic and endocrine signaling. Response of cell to signals. Intracellular signaling proteins: Different types and their role. Second messengers - cAMP pathway and role of calcium.

Cellular interactions - Microvilli, tight junctions, belt and spot desmosomes, gap junctions- Electrical coupling, The connexon, factor mediating cell-self recognition (aggregation factor).

Cytoskeleton - Structure and functions of actin, microfilaments and intermediary filaments.

### Unit - III

Modes of gene transfer in bacteria: Transformation – Discovery, and molecular mechanism of transformation. *In vitro* transformation. Transduction – Discovery. Development of generalized and specialized transducing particles. Conjugation – Discovery.  $F^+ \times F^-$ ,  $Hfr \times F^-$  and  $F' \times F^-$  (sexduction) crosses.

Benzer's studies on r-II locus of T4 bacteriophage – Establishment of Cistron. Recon and Muton. Complementation test. Modern concept of gene.

Mutations: Types, molecular mechanism of mutagenesis, analysis of mutants, site- directed mutagenesis, transposon mutagenesis.

Theory of probability: Chi-square test, Hardy and Weinberg equilibrium and its significance in understanding evolution.

## Unit - IV

Mendelian laws of inheritance – an overview. Genetic conventions, notations and terminology. Genetic recombination: Types (homologous (reciprocal and nonreciprocal), site specific and illegitimate). Different models of homologous recombination. Molecular mechanism of recombination.

Chromosome as a linkage unit. Coefficient of coincidence of double crosses, chiasma interference. Chromosomal mapping: Mapping by recombinational frequencies, transformation and interrupted mating. Chromosomal aberrations: Types (numerical and structural) and their significance in evolution.

Sex chromosomes; Determination of sex, genic balance theory, gynandromorphs. Sex- linked inheritance, Criss cross inheritance.

### Recommended Books:

1. Cooper Geoffrey, M. 2000. The Cell – a molecular approach. 2<sup>nd</sup> Edn. ASM Press. Washington.
2. Sharma AK & A Sharma. 1980. Chromosome techniques: Theory & Practice. Batterworth.
3. Alberts A et al. 1994. Molecular biology of the cell. Garland publ. New York
4. De Robertis EDP & EMF De Robertis. 2001. Cell and Molecular biology. Lippincott Williams & Wilkins. Bombay.
5. Freifelder D. 1990. Molecular biology. Narosa publ. house, New Delhi
6. Gardner E J & D P Snustad 1996. Principles of genetics. John Willey, New York.
7. Sambamurthy, AVSS. 1999. Genetics. Narosa publ. New Delhi.
8. Sinnot EW., LC Dunn & T Dobzhansky 1958. Principles of genetics. V edn McGraw Hill, New York.
9. Stansfield WD 1991. Theory & Problems in genetics III edn McGraw Hill, New York.
10. Strickberger MW 1996. Genetics III edn. McMillan, New York.
11. Winchester AM 1967. Genetics Oxford & IBH. New Delhi.

## **BC 104: TOOLS AND TECHNIQUES IN BIOLOGY**

### **Unit - I**

$P^H$  Determination – Measurement of  $P^H$ , biochemical buffers, selection of biochemical buffer, oxygen electrode and biosensors. Cell disruption methods: French press, sonication, freeze-thaw techniques, enzymatic method, use of liquid nitrogen in cell disruption. Centrifugation – Basic principles of sedimentation, types of centrifuges and rotors. Preparative ultracentrifugation - differential centrifugation, density-gradient, analytical ultracentrifugation and applications in determination of molecular weight, purity and detection of conformational changes in macromolecules.

### **Unit - II**

Separation methods - General principles and definitions. Methods based on polarity – Partition chromatography (Paper chromatography), adsorption chromatography (thin-layer chromatography), gas-liquid chromatography, reverse phase liquid chromatography. Methods based on size - Principle of Gel filtration, methodology and applications. Dialysis, ultra filtration, lyophilization. Methods based on affinity – Principle of Affinity chromatography, methodology and applications (purification of proteins, nucleic acids). High-performance liquid chromatography – Principle, instrumentation, practical procedure and applications. Ion-exchange chromatography – Principle, ion exchangers, methodology, pH and salt gradients for elution of proteins, amino acids and nucleotides. Amino acid analyzer.

### **Unit - III**

Electrophoresis – General principles and definitions. PAGE – Native-PAGE, SDS-PAGE, Isoelectric focussing, 2D electrophoresis, identification of novel proteins in 2D gels, capillary electrophoresis. Agarose gel electrophoresis - Preparation, separation and determination of molecular size of DNA, denaturing agarose gel electrophoresis and their applications, recovery of DNA from agarose gels. Pulse-field gel electrophoresis – principle, methodology and applications in separation of large DNA fragment.

### **Unit - IV**

Spectroscopy – Principles, laws of light absorption, Instrumentation and applications of UV-visible spectrophotometer, fluorescence spectroscopy, NMR, ESR, Mass spectroscopy – MALDI – TOF, ESI MS. Radioisotope Techniques – Types of isotopes, radioactive decay. Detection and measurement of radioactivity – GM counter, scintillation counter, autoradiography. Preparation of label compounds – pulse chase studies and tracer techniques, isotopes used in biology, safety methods in handling radioisotopes. Optical activity of biomolecules - CD and ORD – principles and applications. X-Ray diffraction – Basic principles, applications in the determination molecular structure of protein and nucleic acids.

**Recommended Books:**

1. Separation methods in biochemistry by S.J. Morris and P. Morris (Pitman)
2. The tools of Biochemistry by Terrance G. Cooper (Wiley)
3. Biochemical research technique (A practical introduction by Ed. John M. Wriggles worth
4. Analytical biochemistry by David J. Holmes and Hazel peck
5. A Biologist's guide to principles and techniques of practical biochemistry, 2<sup>nd</sup> edition Ed. by BL. Williams and K. Wilson (Edward Arnold)
6. Biophysical chemistry D. Freifelder, W.H. Freeman
- 7 Experimental techn. Ex ques in biochemistry by Drewer Pesec, AJ. And As worth, R.B.
8. Principles of Physical Biochemistry by K.E. Vanholdem W.C. Johnson, P.S. Ho, (Prentice Hall), 1998.
9. Manometer and biochemical techniques by W.W Umbrit and R.H. Burris (Burgens
10. The determination of Molecular Structure by P.J. Wheatly (Oxford Clarenders press) 1968
11. Molecular Biophysics – R.B. Setlow and E.C. Pollard (Addison Wiley publishing Co. USA)1962
12. Biophysics – Edited by W. Hoppe, W. Lehman Hi. Maskal and H. Ziegler (Springer verlag, Berlin) 1983
13. Practical biochemistry: Principles & Techniques – Edited by Wilson & Walker, Cambridge Univ. Press, New York) 1986.
14. Biological Spectroscopy by Iain D. Campbell and Raymond A. Dwek, the Benjamin/Cumming Pub. Co., California, London.
15. Biophysical Chemistry by C.R. Cantor and P.R. Schimmel, W.H. Freeman & Co., NY.
16. Introduction to Biophysical Methods for Protein and Nucleic Acid Research, Ed. J.A. Glasel and M.P. Deutscher, (Academics Press), 1995.

## **PRACTICALS**

### **BC P 101: BIOCHEMICAL PREPARATIONS AND QUANTITATIVE ANALYSIS**

Laboratory safety – Material Safety Data Sheets (MSDS), storage and handling of chemicals, chemical, biological and physical hazards, standard operating procedure (SOP) for laboratory safety, hazard management in the lab, hazard symbols, laboratory waste disposal.

1. General reaction of carbohydrates. Specific reactions of different sugars: arabinose, xylose, fructose, galactose, sucrose, maltose, lactose and Starch.
2. General reactions of proteins and amino acids. Precipitation reactions of albumins and globulins.
3. General reactions of lipids and cholesterol.
4. Isolation and estimation of cholesterol from brain.
5. Isolation and estimation of glycogen/starch from liver and potato.
6. Preparation of Casein from milk by Isoelectric precipitation.
7. Crystallization of albumin.
8. Estimation of proteins in biological samples :

- a. Biuret method.
- b. Folin-Lowry method.
- c. UV method.
- d. Bradfords dye binding method.

9 Titration curve of amino acid and calculation of pK and pI value.

10 Estimation of amino acids by formal titration.

11. Estimation of amino acid by Ninhydrin method.

12. Estimation of tyrosine by Millon's-reaction.

13 Identification of N-terminal group of proteins by Sanger's method.

14. Estimation of fructose in Fruit-juice.

15. Estimation of Carbohydrates by Anthrone Method.

16. Estimation of Carbohydrates by Nelson Somogyi Method.

17. Preparation of Lecithin from egg yolk and estimation of lipid phosphorus.

18. Preparation and properties of cytochrome C from pig heart.

19. Preparation of ATP from Rabbit Muscle.

20. Isolation of chloroplast and assay of Hill reaction by spectrophotometry.

21. Isolation of chitin from shells.

**BC P 102: ANALYTICAL METHODS**

1. Preparation of buffers and measurement of pH
2. Verification of Beer's law.
3. Determination of molar extinction coefficient of p-nitrophenol.
4. Isolation and spectrophotometric characterization of plant pigments
5. Isolation of Mitochondria from rat liver by density gradient centrifugation
6. Detection of changes in the conformation of BSA by Viscosity Measurement.
7. Effect of pH on the conformation of BSA.
8. Separation of amino acids by Paper Chromatography.
9. Separation of amino acids by TLC.
10. Separation of sugars by TLC.
11. Effect of solvent system on the R<sub>f</sub> value of two solutes using TLC.
12. Isolation & separation of Brain Lipids solvent extraction and TLC.
13. Separation of amino acids by Paper Electrophoresis.
14. Separation of amino acids by Ion-exchange Chromatography.
15. Separation of Proteins by PAGE.
16. Determination of subunit composition and molecular weight of Proteins by SDS-PAGE.
17. Desalting of proteins by Dialysis.
18. Separation of Blue dextran and cobalt chloride on Sephadex G-25.
19. Separation of biomolecules by gel filtration.
20. Absorption spectra of phenol red, amino acids and nucleic acid.
21. Measurement of inversion of sucrose by Polarimetry.
22. Two dimensional separation of amino acids

## SEMESTER II

### **BC 201: MICROBIOLOGY , BIOENERGITICS AND HUMAN NUTRITION**

#### **UNIT - I**

##### Microbiology

Historical development of microbiology - Scope of microbiology. – Spontaneous generation theory, germ theory of disease. Isolation of microorganisms- Direct and indirect, methods of maintenance of cultures.

Microscopy: Bright-field microscopy, Dark-field microscopy, fluorescence microscopy, phase contrast microscopy, transmission electron microscopy, scanning electron microscopy. Applications of Microscopy in Biology.

General characteristics of bacteria, protozoa, fungi, algae, cyanobacteria, rickettsias. Spirochetes and archaeobacteria.

Outline classification of bacteria as per Bergey's Manual of Systematic Bacteriology. Ecological grouping of bacteria.

Ultra structure of bacterium. An account of variant ( Capsule, flagellum, pilus, endospore ) and invariant ( cell wall, cell Membrane, Mesosomes, Nucleoid, ribosomes ) components of bacterial cell.

Nature and properties of Viruses. Viruses of Plants, vertebrates and microorganisms. Morphology, size, ultra structure and life cycles of some representative viruses - TMV, T4, Lambda and SV40, Prions, Viroids.

#### **Unit II**

Cell growth and kinetics of bacterial growth, Normal and biphasic growth curve, batch and continuous cultures, chemostats.

Nutritional requirement of bacteria - phototroph, chemotrophs, organotrophs, methylotrophs mixotrophs. Saprophytes; symbiotic and parasitic modes of nutrition. Fermentative and sulphur reducing bacteria.

Anaerobic and aerobic Growth. A brief note on animal, plant and human diseases caused by microorganisms. Introduction to Dairy microorganisms: Fundamentals of control- control by physical agents, chemical agents, antibiotics and other chemotherapeutic agents. Assay of antimicrobial action.

#### **UNIT-III**

Bioenergetics :Thermodynamic principles – Chemical equilibria; free energy, enthalpy ( $H_0$  entropy (S). Free energy changes in biological transformations in living systems; High energy compounds. Energy change, oxidation-reduction reactions. Organization of electron carriers

and enzymes in mitochondria. Classes of electron-transferring enzymes, inhibitors of electron transport. Oxidative phosphorylation. Mitochondrial transport system. Microsomal electron transport; photorespiration, cyclic and non-cyclic reactions; photo-chemical events associated with pigment system – I and II. Utilization of oxygen by oxygenases, superoxide dismutase and catalase.

#### Unit - IV

##### Human nutrition

Composition of food, criteria of energy value, measurement of energy value of food – direct and indirect methods. RQ of foods and its significance. Basal metabolism and measurement of BMR. Factors affecting BMR. Specific dynamic action of foods, sources and physiological functions of carbohydrates, proteins, fats. Dietary fiber and its role. Complete and incomplete proteins. Essential fatty acids. Nitrogen balance, methods employed to evaluate nutritive value of dietary proteins.

Vitamins - fat and water soluble vitamins – structure, sources, daily allowances, physiological role, deficiency and toxicity symptoms, balanced diets and caloric requirements for people of various ages, sex and physiological state.

Minerals and essential ultra trace elements – calcium, copper, iron, iodine, zinc, cobalt, selenium, fluorine, manganese and molybdenum.

##### Reference Books:

1. Text book of Microbiology – William Burrows.
2. The Microbial World – R.Y.Steiner et al.
3. Microbiology – Peleczar, Reid and Chan.
4. Biology of Microorganisms – Sandes T. Lyles.
5. Fundamentals of Microbiology – M.Frebisher.
6. Diseases of Crop plants- G.Rangaswamy.
7. Microbiology – Pyalkin.
8. Plant Pathology – J.C.Walker.
9. Principles of Microorganisms – Brooks.
10. Molecular Genetics of Bacteria, 2<sup>nd</sup> edition 1995, Jeremy W.Dale John Wiley And Sons.
11. General Microbiology 5<sup>th</sup> edition. R.Y.Stainer et al.
12. Microbiology 4<sup>th</sup> edition, Prescott, Harley, Klein (McGraw Hill).
13. Biochemistry . Berg, Tymoczko and Stryer, Freeman Publications.
14. Principles of Biochemistry – A.L.Lehninger(CBS Publishers).
15. Biochemistry – Lubert Stryer (5<sup>th</sup> Edition).
16. Principles of Biochemistry – General aspects – Smith et al., (8<sup>th</sup> edition).
17. Harper's Review of Biochemistry – Martin et al., ( Lange).
18. Text Book of Biochemistry with clinical correlation – Thomas M.Devlin (John Wiley) 2<sup>nd</sup> & 4<sup>th</sup> Edition.
19. Text Book of Biochemistry – West et al., 1966 (MacMillan).
20. Biochemistry 2<sup>nd</sup> ed. C.K.Mathews and K.E.Van Holde (1995) (Benjamins/Cummings).
21. Biochemistry 2<sup>nd</sup> ed Donald Voet and J.G.Voet (1994)(John Wiley).

## **BC 202: METABOLISM OF CARBOHYDRATES AND LIPIDS.**

### **Unit-I**

Catabolism, Anabolism - Glucose as fuel-Major and Minor metabolic pathways-Glycolysis-Importance of phosphorylated intermediates, Fates of pyruvate. Preparatory phase and payoff phase. Regulation of glycolysis. Fermentation. Metabolism of maltose, lactose, sucrose, fructose, mannose and galactose. Pentose phosphate pathway and its significance. Glucuronic acid pathway and ascorbic acid pathway.

### **Unit-II**

TCA cycle: pyruvate dehydrogenase complex, Reactions of the TCA cycle and regulation. Amphibolic pathway. Anapleortic reaction. The glyoxylate cycle. Control of the concentration of a given enzyme.

Gluconeogenesis and regulation. Futile cycles in carbohydrate metabolism. Glycogen metabolism and regulation. Biosynthesis of Starch, Biosynthesis of glycoproteins. CO<sub>2</sub> Fixation, C<sub>3</sub> and C<sub>4</sub> pathways (Hatch- Slack pathway). Disorders of carbohydrate metabolism- glycogen, Lactose, Galactose and fructose.

### **Unit -III**

Lipid digestion, absorption and transport. Fatty acid oxidation - Fatty acid activation, Transport across the mitochondrial membrane. Oxidation: oxidation of unsaturated, odd-chain fatty acid, peroxisomal  $\beta$ -oxidation. Regulation of fatty acid oxidation.  $\alpha$ -oxidation and  $\omega$  -oxidation. Degradation of triacyl glycerol and phospholipids. Ketone bodies - Formation and utilization. Biosynthesis of Fatty acids- Transfer of Mitochondrial acetyl Co-A to cytosol, formation of malonyl Co-A, Fatty acid synthase complex, biosynthesis and regulation of fatty acid synthesis.(Long chain fatty acids and unsaturated fatty acids) Biosynthesis of prostaglandins, Thromboxanes and Leucotrienes. Biosynthesis of Triacyl glycerol's and its regulation. Biosynthesis of glycerophospholipids, sphingophospholipids and sphingoglycolipids. Sphingolipid storage diseases. Biosynthesis of cholesterol and its regulation, entry of cholesterol esters into cells. Fates of cholesterol.

### **Unit - IV**

Lipoprotein metabolism. Chylomicrons, VLDL, LDL, HDL. Integration of carbohydrate and lipid metabolism. Role of liver and adipose tissue in lipid metabolism- fed & fast conditions. Metabolic integration, Compartmentation, Disorders related to carbohydrate and lipid metabolism.

**Recommended Books:**

1. Principles of Biochemistry – A.L.Lehninger(CBS Publishers).
2. Biochemistry – Lubert Stryer (5<sup>th</sup> Edition).
3. Principles of Biochemistry – General aspects – Smith et al., (8<sup>th</sup> edition).
4. Harper's Review of Biochemistry – Martin et al., ( Lange).
5. Text Book of Biochemistry with clinical correlation – Thomas M.Devlin (John Wiley) 2<sup>nd</sup> & 4<sup>th</sup> Edition.
6. Text Book of Biochemistry – West et al., 1966 (MacMillian).
7. Biochemistry 2<sup>nd</sup> ed. C.K.Mathews and K.E.Van Holde (1995) (Benjamins/Cummings).
8. Biochemistry 2<sup>nd</sup> ed Donald Voet and J.G.Voet (1994)(John Wiley).

## **BC 203: MOLECULAR BIOLOGY**

### **Unit - I**

#### **DNA Replication and repair:**

Modes of replication. Experimental evidences for semi-conservative mode of replication - Meselson–Stahl, and Cairns experiments. Replication fork, continuous and discontinuous DNA synthesis. Enzymes and proteins in replication - Single strand DNA binding proteins (SSB), Helicases, Topoisomerases, DNA ligases. Priming by RNA polymerase and primase. DNA polymerases - *E.coli* DNA polymerase I, II and III, and Eukaryotic DNA polymerases. Replication of *E. coli* chromosome and M13 genome. Rolling circle replication in  $\lambda$  bacteriophage. Eukaryotic DNA replication. Autonomous replication sequences (ARS). Regulation of ColE1 plasmid DNA replication. Termination and fidelity of DNA replication. Nearest neighbour base pair analysis. Inhibitors of DNA replication. Reverse transcriptase. DNA damage and repair: Photoreactivation, direct reversal of damage, excision repair, recombination repair. SOS response.

### **Unit - II**

#### **Transcription (RNA Biosynthesis):**

Polynucleotide phosphorylase. RNA polymerases - structure of *E. coli* RNA polymerase, and nature of eukaryotic RNA polymerases. Promoters and their characterization. Enhancer sequences. Initiation, elongation and termination of RNA synthesis. Monocistronic and polycistronic RNAs. Post-transcriptional modifications of eukaryotic hnRNA – capping, methylation and polyadenylation. RNA splicing and splicing mechanisms. Splicing of nuclear pre-tRNA, group I and group II introns, and pre-mRNA splicing. Excision of multiple introns. Role of catalytic RNA.

### **Unit - III**

#### **Translation (Protein synthesis):**

Elucidation of the genetic code - experimental studies of Nirenburg and Khorona. General features of genetic code, codon degeneracy and universality. Mitochondrial genetic code, tRNA role in protein synthesis. Amino acyl-tRNA synthetases, wobble hypothesis. Mechanism of initiation, elongation and termination of protein synthesis. Translational factors. Inhibitors of protein synthesis - antibiotics and other inhibitors. Post-translational modifications. Protein sorting and targeting. Signal hypothesis - signal sequences, signal recognition particle, and molecular chaperones, protein degradation, Lysosomal degradation. The ubiquitin pathway - protein stability and N-end rule.

### **Unit - IV**

#### **Regulation of gene expression :**

House-keeping genes, constitutive genes, and regulatory genes. Induction and repression. Regulatory proteins – DNA-binding motif of regulatory proteins. Role of zinc fingers, leucine zippers, helix-turn-helix. Regulation of gene expression in prokaryotic operons. Negative regulation and positive regulation. Fine structure of *lac* operon. Repressor and the catabolite

activator proteins in gene regulation of *lac* operon. Dual functions of the repressor in *ara* operon. Transcriptional control by attenuation in *trp* operon. Regulation of gene expression in  $\lambda$  and *nif* operon. Regulation of gene expression in eukaryotes – *gal* operon in yeast. Eukaryotic translation control - translation control by heme, interferon, mRNA masking, antisense RNA. Hormones and environmental factors affecting gene expression. Homeotic genes and their regulation.

### Recommended Books:

1. Molecular Biology of the Cell – B. Alberts, D. Bray, J. Lewis, M. Raff, K. Roberts and J.D.Watson (Garland Publishing , New York and London)
2. Molecular Biology – A Comprehensive Introduction to Prokaryotes and Eukaryotes – D. Freifelder (Jones and Bartlett, USA)
3. Recombinant DNA : A Short Course – J.D. Watson, J. Tooze and D.T.Kurtz (Scientific American Book, W.A.Premon).
4. Molecular cloning : Laboratory Manual – Maniatis, E.F. Fritsch and J. Sambrook (Cold Spring Harbor Laboratory, New York).
5. Modern Genetics (2<sup>nd</sup> Edition, 1984 ) – A.J. Ayala and W. Castra (Goom Helns, London).
6. Techniques in Molecular Biology (1992) – Ed., J. Walker and W. Castra (Geom Helns, London).
7. Practical Methods in Molecular Biology (1991) – R.F. Schecleif and P.C. Wensik (Springer Verlag).
8. Genes V. (1994) – Benjamin Lewin (Oxford University Press ).
9. Molecular biology of the Gene 4<sup>th</sup> Edition by – Watson, Hopkins, Roberts, Steitz and Weiner
10. Biochemistry - Donald Voet and Judith Voet ( John Wiles and sons )

## **BC-204: BIOSTATISTICS AND BIOINFORMATICS**

### **Unit - I**

Data - Data types, collection of data, classification and tabulation. Measures of central tendencies – Mean, median and mode. Measures of variation - Range, quartile deviation, mean deviation and standard deviation. Coefficient of variation. Probability – Addition and multiplication theories, conditional probability and probability distributors – Binomial, poisson and normal distribution.

### **Unit - II**

Correlation and linear regression – Scatter diagram method – Karl Pearson's Rank correlation methods. Regression lines – fitting of regression lines by least squares method – Regression coefficients and properties. Small sample tests – t, F and chi square tests. ANOVA – one way and two way classifications. Duncan's multiple range (DMR) test.

### **Unit - III**

Introduction - Origin of bioinformatics biological data (genome projects), computer and information technology contributions. Disciplines of bioinformatics – Genomics, transcriptomics, proteomics, functional genomics, structural genomics, metabolomics, pharmacogenomics, structure prediction and drug design.

Genome projects - General introduction to genome projects (rice genome project and Mycobacterium tuberculosis genome project). Special emphasis on Human Genome Project (HGP) – Science behind HGP, benefits of HGP, ELSI of HGP in use of genetic information, genetic testing standard, quality and commercialization.

Biological database - Introduction of database (DB), need, organization, search of DB. An overview of biological databases - NCBI, EMBL, DDBJ, SWISS-PROT, PDB, KEGG.

### **Unit - IV**

Database querying with NCBI using key words, sequences (proteins and genes), finding similarities, identifying genes and proteins from different organisms.

Sequence alignment - Introduction, significance of sequence alignments and use of dot matrices.

Pairwise and multiple sequence alignment (MSA) using Clustal programs.

Sequence analysis - concepts of sequence analysis and their importance. BLAST – blastn, blastp, blastx, tblastx, output analysis matrix BLOSSUM, PAM, e-value.

Proteomics - Introduction, principle, technique, 2-D database. Gel analysis software, post gel analysis, MALDI-TOF. Significance and applications of proteomics in modern biology.

**Recommended Books:**

1. Statistical methods. S.P. Gupta
2. Fundamentals of mathematical statistics. S.C Gupta & Kapoor
3. Statistical methods in biological and Health Science. J. S. Milton & J.O. Tsokan.
4. Primrose SB. Principles of Genome Analysis: a guide to mapping and sequencing DNA from different organisms. 2nd Ed. 1998. Blackwell Science: Oxford. ISBN 0-632-04983-9.
5. Genome Mapping: A practical approach. Dear P (Editor). 1st Ed. 2000. Oxford University Press: Oxford, New York.
6. Developing Bioinformatics Skills. Alfonso Valencia and Blaschke. L (2005) Oreille's Publication.
7. Bioinformatics sequence, structure and data banks ed. By Des Higgins Willie Taylor.(2006)
8. "Bioinformatics: A Practical Guide to the Analysis of Genes and Proteins" (Andreas D. Baxevanis, B. F. Ouellette), Paperback, 2nd ed., 470 pp., ISBN: 0471383910, Publisher: Wiley, John & Sons, Inc., Pub.
9. David W. Mount, Bioinformatics: Sequence and Genome Analysis, 2nd edition, Cold Spring Harbor Laboratory, 2004, ISBN 0-87969-687-7.

## PRACTICALS

### **BC P 201: ENZYMOLOGY**

#### I. Assay the following enzymes

1. Amylase from Saliva
2. Urease from Horse-grass
3. Acid phosphatase from potato
4. Alkaline phosphatase from Serum
5. Cholinesterase from Blood
6. SDH from Liver
7. Invertase
8. Trypsin
9. LDH from Serum ( Isoenzymes )
10. Thenberg method – xanthine oxidase.
11. Purification and study of enzyme kinetics with respect to substrate and enzyme concentrations, pH, Temperature, Activators and inhibitors and Immobilization using some of the above enzymes (Papain from latex of papaya).

### **BC P 202 : MICROBIOLOGY & CELL BIOLOGY**

1. Sterilization Techniques :  
Autoclaving, hot-air oven sterilization. Sieve filtration, membrane filtration.
2. Preparation of routine laboratory media – Nutrient broth, LB broth ,agar media.
3. Isolation of bacteria, *Actinomyces* and fungi from soil/water.
4. Development of a single colony from a bacterial culture.
5. Techniques of maintenance of stock cultures.
6. Staining techniques. Simple, differential, acid-fast and spore staining.
7. Measurement of microbial cells.
8. Motility (Hanging drop) of microbial cells.
9. Microscopic observation of microbial culture.
10. Study of growth of bacteria, cyanobacteria and fungi.
11. Effect of pH, temperature and osmotic concentrations on bacterial growth.
12. Identification of bacteria based on fermentation, starch hydrolysis, gelatin liquifraction, urease activity, catalase activity and nitrate reductase activity.
13. Oligodynamic action of metals on bacteria.
14. Microbiological assay of antibiotics.
15. Preparation of cytological slides for mitosis using root tips.
16. Preparation of cytological slides for meiosis-I using flower buds; chiasma frequency
17. Identification of different stages of mitosis and meiosis.

## SEMESTER –III

### BC 301: GENETIC ENGINEERING

#### Unit I

Genetic engineering - Introduction and outlines of gene cloning. DNA cutting and joining - Enzymatic cleavage of DNA. Restriction and modification enzymes – Classification, nomenclature and importance of restriction endonucleases. Restriction mapping, DNA ligases, polynucleotide kinase, alkaline phosphatases, S1 nuclease, terminal transferase, Bal 31 nuclease. Polymerase chain reaction - principle, types (RT-PCR, nested and inverse PCR), primer design, and applications of PCR. Sequencing methods – Sanger's, and Maxam-Gilbert's method. Automated sequencing.

#### Unit II

Cloning vectors – Characteristics of a vector. Natural plasmids used as vectors – advantages and disadvantages. Artificial plasmids and their importance as cloning vectors. Vectors used for cloning in *E.coli*. (plasmids, bacteriophage derivatives, cosmids, BACs), yeast (YACs, shuttle vectors), higher plants (Ti plasmid derivatives, caulimovirus) and animal cells (constructs of SV 40 and retroviruses). Characteristics of expression vectors. Generation of DNA fragments containing a gene (shot-gun method, southern analysis, and cDNA synthesis). Joining of DNA fragments to vector molecules, cohesive termini ligation and blunt end ligation – linkers, adaptors and homopolymer tails. Preparation of DNA probes, Construction of DNA libraries – genomic and cDNA libraries. Screening of recombinants for a positive clone – Genetic, biochemical and hybridization methods. Microarrays and macroarrays.

#### Unit III

Introduction of Recombinant DNA molecules into appropriate hosts – Competent cells preparation, electroporation, microinjection and particle bombardment method, and selection of transformants. *Agrobacterium* - mediated transformation of plant cells – Identification of transformed cells and micropropagation of transformed cell into callus, and regeneration of transgenic plants. Transgenic plants and transgenic animals.

Transfection - Salient features and its significance in transforming animal cells. *In vitro* packaging of recombinant cosmids. Introduction of recombinant cosmids into *E.coli* cells.

#### Unit IV

Expression of cloned genes - Construction of expression vectors: Vectors having inducible *lac*, *taq* promoters. Use of IPTG and its role in induction of a cloned gene. Expression of proteins with His tag and its significance in simultaneous expression and purification of recombinant proteins. Cloning and over expression. Somatostatin, insulin, interferon, and vaccines. Production technologies – synthesis of plantibodies. Problems associated with expression of cloned genes – inclusion bodies, solubilisation and reconstruction of expressed proteins.

DNA Finger Printing - RAPD, RFLP and AFLP analysis. Markers linked to drug and disease resistant genes. Application of RFLP in forensic science, pedigree analysis, biodiversity, genetic

counseling and germ plasm maintenance. Antisense technology and its application. Use of transposon tagging.

### **Recommended Books:**

1. Molecular Cloning: A Laboratory manual, J. Sambrook, E.Ffrisch and T. Maniatis, Old Spring Harbor Laboratory Press New York, 2000
2. DNA Cloning : a Practical Approach, DM Glover and BD Hames, IRL Press
3. Molecular and Cellular methods in Biology and Medicine. PB Kaufman,W.Wu.D Kim and LJ Cseke, CRC
4. Methods in EnzymologyVol. 152, Guide to Molecular Cloning Techniques, SL Berger and AP Kimmel, Academic Press, Inc San Diego, 1998
5. Methods in Enzymology Vol. 185, Gene Expression Technology, D V Goeddel, Academic Press, Inc. San Diego, 1990
6. DNA Science. A first Course in Recombinant Technology, DA Mickloss and GA Freyer, Cold Spring Harbor Laboratory Press, New York 1990
7. Molecular Biotechnology (2<sup>nd</sup> Edn) SB Primrose, Blackwell Scietific Pub. Oxford, 1994
8. Milestones in Biotechnology. Classic papers on Genetic Engineering. JA Davies and WS Reznikoff, Butterworth-Heinemann, Boston, 1992
9. Route Maps in Gene Technology, MR Walker and R Rapley, Biackwell Science Ltd, Oxford 1997
10. Molecular Biotechnology – Glick
11. Concepts of Biotechnology D. Balasubramanian
12. Principles of Gene Manipulation by Old and Primrose, Blackwell

## **BC 302 : Metabolism of Nitrogen compounds**

### **Unit-I**

Nitrogen cycle. Non-biological and biological nitrogen fixation. Nitrogenase system. Utilization of nitrate ion, ammonia incorporation into organic compounds. Synthesis of glutamine and regulatory mechanism of glutamine synthetase.

General metabolic reactions of amino acids. Amino acid transamination, deamination; oxidative deamination, non oxidative deamination ( amino acid oxidases and deaminases), amino acid decarboxylation, role of folic acid, vitamin B1, B6, and B12 in amino acid metabolism. Krebs urea cycle , regulation of urea cycle.

### **Unit-II**

Metabolic breakdown of individual amino acids. Ketogenic and glucogenic amino acids; Biosynthesis and regulation of aspartate family amino acids (Aspartate kinase), branched chain amino acids, histidine, tryptophan, phenylalanine. Metabolic defects of amino acid metabolism.

### **Unit - III**

Amino acids as biosynthetic precursors – formation of Creatine, serotonin, melatonin, histamine, anserine, carnosine. Polyamines:GABA,melanin, catecholamine. Biosynthesis and degradation of porphyrin (Heme). Porphyrins (Fixation of sulphur and sulphur cycle). Non-ribosomal peptide synthesis-glutathione, cyclic antibiotics (Gramicidin).Biosynthesis of secondary metabolites -

Biosynthesis of nucleotide coenzymes – nicotinamide coenzymes, Flavin coenzymes and coenzyme– A.

Biosynthesis of alkaloids – Morphine, Ajmalicine. Plant growth regulators – Auxin (IAA), Ethylene.

### **Unit- IV**

Biosynthesis degradation and regulation of purine and pyrimidine nucleotides, chemical inhibition of biosynthesis of nucleotides.

Biosynthesis of deoxyribonucleotides, regulation of ribonucleotide reductase. Salvage pathway. Disorders of purine and pyrimidines nucleotide metabolism.

**Recommended books:**

1. Principles of Biochemistry – A.L.Lehninger(CBS Publishers).
2. Biochemistry – Lubert Stryer (5<sup>th</sup> & 6<sup>TH</sup> Edition ).
3. Principles of Biochemistry – General aspects – Smith et al., (8<sup>th</sup> edition).
4. Harper's Review of Biochemistry – Martin et al., ( Lange).
5. Text Book of Biocehmistry with clinical correlation – Thomas M.Devlin (John Wiley) 2ne & 4<sup>th</sup> Edition.
6. Text Book of Biochemistry – West and Todd, 1966 (MacMillian).
7. Biochemistry 2<sup>nd</sup> ed. C.K.Mathews and K.E.Van Holde (1995) (Benjamins/Cummings).
8. Biochemistry 2<sup>nd</sup> ed Donald Voet and J.G.Voet (1994)(John Wiley).
9. Biocemistry – The Chemical Reactions of Living Cells Vol-II, David E. Metzler.
10. Biochemistry – J.L. Zubay
11. Biochemistry – Garrett and Grishame C.M. (Saundes College publication )- 2<sup>nd</sup> Edition.
12. Biochemistry and Molecular biology of plants by Buchanan, Gruissem, and Jones. American Society of Plant Physiologists. Rockville, Maryland.
13. Introduction to plant Biochemistry. IInd Ed. Goodwin and Mercer, CBS Publication and distributors, New Delhi
14. Plant Biochemistry by Dey, PM and Harborne JB, Academic Press, New York.

## BC 303 : IMMUNOLOGY

### UNIT - I

#### **Introduction:**

Science of Immunology. Historical background of Immunology, Biological aspects of Immunity; Self and non-self recognition, specificity, memory of immune system. Cells of immune system, stem cell, megakaryocyte, lymphoid cell, myeloid cell, erythroid cell, granulocytes, agranulocytes, T-cell and B-cells.

#### **Antigens:**

Essential features of Ag, Haptens, Carrier molecule, Cross reactivity. Immunization protocols – Selection of experimental animals, route of immunization, test bleeding, preparation of antiserum and its storage. Role of adjuvant in immunizations, Freund's complete and incomplete adjuvants.

**Antibodies:** Nature, primary structure of immunoglobulin, light chain, heavy chain, variable region, constant region, Hinge region, enzymatic fragmentation of Ig. Domain structure of Ig and significance. Classification of Ig: Types - IgG, IgM, IgA, IgD and IgE (origin, structure and functions).

#### **Theories of Ab formation:**

Instructive, selective, clonal selection; theories and evidences; Immunological memory. Antibody diversity theories - Introduction, assembly and rearrangement of polypeptide chains in Ig. Light chain formation, V-J joining, Heavy chain formation, V-D-J- joining. Diversity theories: Mini gene theory, Mutation theory, Germ line theory.

### UNIT - II

#### **Immunity:**

Types: Active and passive immunity. Cell mediated immunity, humoral immunity, Factors involved in T-cell and B-Cell activation. Immune response-Primary immune response and secondary immune response; immunological memory and principle of vaccination.

#### **Natural resistance and nonspecific defense mechanism:**

Natural resistance-Species, racial, individual resistance, Non-specific defense mechanisms - Compliment activation, phagocytosis and mechanism of phagocytosis, Phagolysosome and respiratory burst. Natural killer cell and Interferon. Antiviral mechanism of gamma interferon

#### **Compliment pathway:**

Nature, cascade pathway, components of compliment pathway. Types: Classical pathway and alternative pathway. Classical pathway, intermediate steps, anaphylotoxin, immune adherence and immuno conglutinins. Regulation of C3b, formation of MAC, functions of compliment pathway.

### UNIT - III

**Hypersensitivity (HS):** General introduction, normal immune reactions, tissue protection and tissue damage, hyper immune reactions. Hypersensitivity state and reactions. Classification of hypersensitivity reactions.

**Type - I:** General historical background of allergies. Prophylaxis reaction. Allergies and anaphylaxis - IgE, Mast cell degranulation, biologically active agents released in Type I reactions, wheel and erythematous reaction. Clinical manifestations and treatment of allergic reactions.

**Type - II:** Antibody mediated HS reactions; Mechanism, pathogenicity and cases of type II reactions; Hemolytic disease of new born (HDN).

**Type - III:** Immune complex mediated HS reactions: Mechanism & pathogenicity of type III reactions. Soluble immune complexes and insoluble immune complex mediated reactions. Arthus reaction. Farmer's lung, Librarian's lung, fox furriers disease, Serum sickness, nephritis.

**Type - IV:** Delayed type (or) cell-mediated HS reactions; Mechanism and pathogenicity, Tuberculin reaction, acute late graft rejection and Contact dermatitis.

**Type - V:** Stimulatory HS reactions. Mechanism and pathogenicity, T-cell stimulation, Grave's disease, Neonatal hypothyroidism.

**Blood Groups:**

A,B,AB,O and Rh system, significance, practical applications of immuno Hematology in blood transfusions, maternal fatal incompatibility - Erythroblastosis foetalis.

**Auto immunity and Immune suppression:**

Introduction, Auto recognition, classification of auto immune diseases, Hashimoto disease, thyrotoxicosis, myasthenia gravis, good pasture's syndrome, Systemic lupus erythematosus, Auto immune hemolytic anemia, Rheumatoid arthritis, male infertility, Ulcerative colitis, Thrombocytopenic purpura, Pernicious anemia.

**Immune suppression:** Types, General mechanism of immune suppression, lymphoid cell abolition, and immune suppressive drugs (azothioprine, methatrexate, cyclophosphamide, Cyclosporin-A, Steroids, lymphomodulins).

**Transplantation:**

Terminology, Auto graft, Isograft, Allograft, Xenograft. Immunological basis of transplantation reactions, genetics of transplantation antigens, mechanism and types of rejections, GvH reaction. Prevention of graft rejection- cell ablation and ISD therapy.

## UNIT - IV

**Immune Deficiencies:**

Introduction, primary and secondary deficiencies. T-cell, B-cell and combined immuno deficiencies, Complement system deficiency. Acquired immuno deficiency syndrome. SCID.

**Major Histocompatibility Complex (MHC):**

MHC in mice and HLA in man-fine structure and functions only.

**Immunological Techniques:**

Ag-Ab interactions, Precipitations and agglutinations. Lattice hypothesis. Precipitation reactions - Ring test, single diffusion test, two dimensional diffusion, single radial immuno diffusion

(SRID). Immune Electrophoresis – counter current IE, cross over IE, rocket IE, 2 D- immune electrophoresis.

Agglutination tests:- Haemagglutination reactions (ABO and Rh), Widal test, VDRL test, Agglutination inhibition test. Complement fixation test – Principle technique and applications.

Enzyme Immuno Assay: ELISA – Overview of enzyme immuno assays, principle and methodology of ELISA – Sandwich ELISA and Indirect ELISA. Applications of ELISA in biology.

Radio immuno assay – Radio labeled and unlabeled antigen. Principle and methodology of RIA. Applications of RIA. Doping test. IRMA (Immuno Radiometric Assay).

### **Monoclonal antibody and Hybridoma technology:**

General Introduction, Polyclonal antibodies, significance of monoclonal antibodies. Hybridoma technology, General Immunization of animals, isolation of lymphocytes, Myeloma cell lines, fusion of myeloma cell with antibody producing B-cell. Fusion methods, selection and screening methods for positive hybrids with HAT selection, cloning methods. Production, purification and characterization of monoclonal antibodies. Engineering of monoclonal antibodies- chimeric mouse-human Mab, grafted CDRs, chimeric immunotoxin, hetero conjugate. Application of monoclonal antibodies in biochemical research, in clinical diagnosis and treatment. Production of human monoclonal antibodies and their applications.

### **Recommended Books:**

1. Essentials of Immunology - Ian & Roit - Blackwell Scientific Publications.
2. Fundamentals of immunology - William C. Boyed (Wiley Toppan).
3. Introduction to Immunology - John W. Kinball.
4. Fundamentals of Immunology - Otto S. View and others.
5. Immunology - D.M. Wier.
6. Immunology - Jains Kubay, (2001) Second Edition, W H Frecman & Company New York.
7. Cellular and Molecular Immunology 3<sup>rd</sup> ed. Abul K. Abbas Andrew, K. Lichtman Jordan S. Pober.



## **BC 304 : MOLECULAR PHYSIOLOGY**

### **UNIT - I**

Composition of Blood, Plasma proteins, Blood Cells - RBC - Structure, composition, functions, WBC, platelets. Blood coagulation and fibrinolysis, Haemoglobin- Transport of gases ( O<sub>2</sub> and CO<sub>2</sub> transport, oxygen dissociation curve, Bohr's effect, Hamberger's process ) . Events in haematopoiesis. Other body fluids, CSF and Lymph .

Structure and function of kidney, urine formation, composition of urine. Outlines of functional anatomy of gastrointestinal tract, secretions of stomach, liver, pancreas, duodenum & small Intestine and regulation. Physiology and biochemistry of digestion and absorption of food stuffs.

### **UNIT - II**

Structure and organization of Muscle cells, types of muscles -striated muscle, cardiac & smooth muscle. Molecular organization of contractile systems. Molecular mechanism of contraction and relaxation of muscle - actin, myosin, tropomyosin, titin, nebulin. Role of calcium, troponin C, calsequestrin, ryanodine. Differences and comparison among skeletal, cardiac & smooth muscles. Role of nitric oxide as vasodilator in smooth muscle.

Structure of Nerve cell, origin of membrane potential, mechanism of propagation of nerve impulse in unmyelinated and myelinated nerve fibers. Refractory period, characteristics of stimulus, strength - duration relationship. Hodgkin - Huxley formulation. Membrane properties from current voltage relations. Patch-Clamp technique. Models of squid axon, Electrical properties of receptors. All or none principle, Synapse - types of synapses, transmission at adrenergic and cholinergic nerve endings. Neurotransmission, chemical and electrical theories - EPSP and IPSP. Blood brain barrier. Neurotransmitters, biogenic amines, amino acids, peptides, use of agonists and antagonists.

### **UNIT - III**

Introduction to Biological Membranes - Functions and importance of Bio membranes. The diversity of Bio-membranes, Development of membrane models. Fluid mosaic model, biochemical composition, Molecular and Macromolecular membrane structure, Membrane components lipids - proteins - carbohydrates, membrane asymmetry, lipids -Proteins. Protein mobility, Factors influencing membrane Fluidity. Artificial membranes and liposomes-Preparations and applications.

Studies using Fluorescence anisotropy. The diversity of membrane proteins. Erythrocyte membrane, LDL receptor, Bactereorhodopsin, carbohydrates.

## UNIT - IV

### **Membrane Transport:**

Diffusion across cellular Membranes, Active, passive and mediated transport mechanisms. Energetics of transport system, passive transport anion exchange proteins, active transport. Active transport of  $\text{Na}^+.\text{K}^+$  ( $\text{Na}^+.\text{K}^+.$  ATPase)  $\text{Ca}^{2+}$  ( $\text{Ca}^{2+}$  ATPase ),  $\text{Na}^+$  dependent symport system ( Sugars coupled to phosphorylation, group translocation. ( gama-glutamyl cycle ). Transport process in bacterial ( systems) Membranes – proton motive force in bacterial transport processes. Ionophores, Gap junctions. Endocytosis-clathrin, Exocytosis. Pinocytosis. Receptor mediated endocytosis. Membrane biogenesis.

Receptors- Nature of receptors, Classification of receptors \_ Nuclear receptor family, Cell membrane receptors – Ion channel linked, G- Protein linked, Enzyme linked receptors. Signalling proteins –Thyrosine kinases, Serine/Threonine kinases.

### **Recommended Books:**

1. Dynamics of biological membranes – M.D.Henslay and K.K.Stainless.
2. Biochemistry – Voet and Voet.
3. Cell and molecular biology – Shieler and Bianchi.
4. Principles of Biochemsitry – Lehninger, Nelson and Cox, CBS publishers.
5. Biochemistry – West et al.
6. Biochemistry – L.Stryer.
7. Physiology – Vidyaratan.
8. Medical Biochemsitry – M.N.Chatarjea & Rana Shinde.
9. Harper's review of biochemistry – 25<sup>th</sup> edition by R.K.Murray et al., (Appleton and Lange).

## **PRACTICALS**

### **BC P 301: IMMUNOLOGY**

1. RBC count.
2. Total WBC count
3. WBC Differential count.
4. Erythrocyte Sedimentation Rate (ESR)
5. Packed Cell Volume (PCV)
6. Estimation of Haemoglobin (Hb)
7. Mean Cell Haemoglobin and Mean Cell RBC volume
8. Colour Index and Volume Index of RBC
9. Osmotic fragility of RBC
10. Raising of antibodies in rabbits/chicks/rats.
11. Ouchterloney double Immuno diffusion.
12. Single Radial Immuno diffusion
13. Rocket immunoelectrophoresis
14. Cross over Immunoelectrophoresis
15. Detection of auto immune disease (auto immune haemolytic anaemia)
16. Detection and semi-quantitative estimation of HCG by ELISA
17. Detection of antigen by latex agglutination inhibition test.
18. Detection of rheumatoid arthritis (Auto immune disease) by slide agglutination test.
19. Haemeagglutination tests for identification of human blood groups.
20. Detection by viral fever by slide agglutination tests.

### **BC P 302: MOLECULAR BIOLOGY**

1. Isolation of DNA from bacterial, plant, animal cells
2. Estimation of DNA by diphenylamine method.
3. Isolation of RNA from yeast cells
4. Estimation of RNA by orcinol method
5. Estimation of DNA and purity determination by UV absorption method
6. Determination of melting temperature  $T_m$
7. Isolation of plasmid PJA from *E.coli* HB
8. Detection and differentiation of open circular, Linear and closed covalent circular (CCC) plasmid DNA by submarine gel electrophoresis
9. Transformation of *E. coli* HB 101/JM 103. with ampicillin resistant plasmid PJA
10. Transfection of M13 DNA into *E.coli* JM 103
11. Isolation of Phage  $\lambda$
12. Isolation of single and double stranded M13 DNA
13. Conjugation: Use of broad host range plasmid RP4 in demonstrating conjugal transfer of plasmid in Bacteria.
14. Catabolite repression: Evidence of  $\beta$ -Galactosidase induction in presence of lactose in *E.coli* strains.

## **SEMESTER – IV**

### **BC 401: CLINICAL BIOCHEMISTRY**

#### **UNIT - I**

Introduction and maintenance of Clinical Biochemistry laboratory - hazards; Units of measurement; reference values - factors affecting reference values. Quality control in laboratory - use of external and internal standards with WHO standards. Selection and evaluation of methods. Automation in clinical laboratory ; sources of biological variation. Collection and preservation of samples. Sampling methods.

Acid - base balance, Buffer systems, Role of lungs, red cells, kidney in regulating the pH of body fluids. Metabolic and respiratory acidosis and alkalosis ; mixed acidosis. Compensation mechanism. Tests used to evaluate acid-base status of blood and their significance. Glomerular and tubular functions. Test for evaluation. Measurement and significance of nonprotein nitrogen compounds. Estimation of glomerular filtration rate ; Assessment of renal concentrating and diluting ability. Measurement of renal blood and plasma flow. Assessment of glomerular permeability. Renal tubular acidosis. Nephritis and nephrotic syndrome.

#### **UNIT -II**

Functions of liver Metabolism of bilirubin - formation, transport, hepatic uptake and transport, conjugation, excretion, bacterial degradation. Bile acid metabolism and formation and secretion of bile; enterohepatic circulation.

Liver function tests related to bilirubin, protein, carbohydrates, lipid metabolism ; detoxification, synthetic, excretory. Serum enzymes in liver disease. Jaundice - Classification, types, diagnosis and differential diagnosis.

Factors affecting enzyme levels in plasma or serum. Selection of enzyme tests. Techniques of enzyme assay. Measurement of isoenzymes and isoforms. Plasma enzymes in diagnosis and prognosis - aminotransferases, Creatine Kinase, LDH, alpha-amylase, phosphatases, GGT, G6PDH, Cholinesterase ; Isoenzymes of LDH and alkaline phosphatase. Clinical application of plasma enzymes in liver and muscle disease.

Cardiac markers - Isoenzymes of CK and LDH, Myoglobin, Cardiac troponin I and T. Clinical utility of cardiac markers in detection of acute myocardial infarction, minor myocardial injury, and in monitoring reperfusion following thrombolytic therapy.

Gastric function. Regulation of gastric secretion. Composition of gastric juice. Tests for gastric function - Fractional test meal, Histamine test meal, pentagastrin test, Insulin stimulation test. Gastric analysis. Significance of serum pepsinogen and gastrin.

Pancreatic exocrine secretions - Composition Tests in pancreatic disease - Directly following duodenal stimulation and by indirect procedures. malabsorption syndrome due to pancreatic and intestinal diseases and their investigation - Faecal fat, butter fat test meal, D-Xylose excretion test. Disaccharidase deficiency and their investigation.

Plasma proteins- Their variation in disease. Paraproteinemia, Proteinuria - Glomerular and tubular.

#### UNIT - IV

##### **Disorders of Carbohydrate Metabolism:**

Importance and regulation of blood glucose level, Hypo and Hyperglycemia. Diabetes mellitus - Classification, etiology pathogenesis, clinical and laboratory features. Diagnosis of diabetes mellitus - GTT ( oral and I.V. ), random, fasting and postprandial blood glucose level, glycosuria, ketone bodies, glycolated Hb, plasma insulin, C-Peptide. Metabolic complication of Diabetes mellitus - hyperglycemia and nonketotic coma, hypoglycemia, Lactic acidosis, ketoacidosis, atherosclerosis, nephropathy.

Hypoglycemia - fasting and provoked. Stimulation test ( I.V. glycogen and leucine test ), extending GTT ; Hypoglycemia in children - neonatal and early infancy.

##### **Lipid metabolism:**

Plasma lipids and lipoproteins and their functions and metabolism. Disorders of lipoprotein metabolisms. Diagnosis of lipoprotein disorders and management.

##### **Porphyryne metabolism:**

Porphyrinuria, porphyrias - Classification, types. Test based in the investigation of porphyrias.

##### **Recommended Books:**

1. Tietz Textbook of Clinical chemistry - Carl.A.Burtis, Edward R.Arnold (W.B. Saundes Co) 3rd Edition.
2. Varley's Practical Clinical Biochemistry - (Ed) Alan H. Gowenlock, J.R.McMurray, .M.McLauchlan (Heinemann Medical Books ). 6th Edition.
3. Clinical Biochemistry - Metabolic and Clinical Aspects - (Ed) W.J.Marshall & S.K. Bargert (Churchill Linigstorl )
4. Practical Clinical Biochemistry - Vol I and II - Harold Varleg. A.H. Gowenlock, M.Beh - ( CBS Publication ) 5th Edition.
5. Clinical Chemistry in Diagnosis and Treatment - Joan F. Zilva dn P.R.Pannali ( Lloyd - Luke ) 2nd Edition.
6. Harper's Review of Biochemistry - Martin et al ( Longman ) 25th Edition.
7. Clinical Diagnosis and Management of Laboratory methods. J.B.Henr ( Saunders ) 23rd Edition.
8. Lecture notes on clinical biochemistry by Whitvy-Percyrob et al
9. Illustrated Biochemistry by Lippin Cots

## **BC 402: APPLIED BIOCHEMISTRY**

### **Unit- I**

Biopesticides - Insecticidal Toxins of *Bacillus thuringiensis* – Mode of action – structure of toxin gene – Bio-control through Genetic Engineering.

Bioreactors – Large-Scale fermentation – types of reactors – continuous fermentation – Batch fermentation – fed-batch fermentation – Solid-state fermentation. Downstream processing – production of Ethanol, Butanol, Organic Acids – Citric acid, Ascorbic acid, Amino acids – Lysine and Glutamic acid. Antibiotics (Penicillin, Tetracyclin and Streptomycin). Production of single cell proteins.

### **Unit-II**

Creation of Transgenic animals – Retroviral vector method – DNA microinjection – cloning by nuclear transfer – YAC Transgenesis - Transgenic Sheep, Goats, Pigs, Birds.

Gene Therapy – Ex-vivo gene therapy – In-vitro gene therapy – viral and non-viral gene therapy. Generation of anti-sense RNA – Anti-sense RNA technology and its significance in agriculture and medicine. Immobilization of whole cells – Enzymes and their applications.

### **Unit-III**

Animal Cell Culture : physical, chemical and metabolic functions of constituents of culture media and reagents –Adaptation of mammalian cells to growth in serum free and protein free media.

Techniques of cell culture, primary and established cell culture, biology of cultured cells, measuring parameters of growth and cytotoxicity, Immortalization strategies of mammalian cells.

Stem cells and embryonic cell culture, organ and Histotypic cultures and their applications. Regeneration of dentin skin and wound repair, three dimensional culture and biology of tissue engineering.

Scaling up of animal cell culture – Micro carrier culture, cell synchronization, culturing of animal cells in Fluidized bed reactors.

### **Unit-IV**

Outlines of Plant Tissue Culture – plant transformation Ti-plasmids mechanism of T-DNA transformation – Engineering herbicide resistant- insect resistance – disease resistance in plants – production of golden rice.

Molecular Markers: marker aided breeding – RFLP markers, Linkage analysis – AFLP – QTL map based cloning and molecular marker assisted selection.

**Recommended books:**

1. Culture of Animal Cells, (3<sup>rd</sup> Edition), R.Lan Freshney, Wiley – Liss.
2. Animal Cell Culture – Practical Approach, Ed. John R.W.Masters,
3. Animal Biotechnology – Ranga.
4. Concepts of Biotechnology, D.Balasubramanian, K. dharmalingam, Kuntala, Jayaraman, 1999.
5. Molecular Biotechnology – Glick and Pasteurnak.
6. Plant Biotechnology. J.Hammond, P.McGarvey and V.Yusibov (Eds.):Springer Verlag, 2000.
7. Biotechnology in Crop Improvement H.S.Chawla International Book Distributing Company.1998.
8. Plant Biotechnology, Purohit 2000.

## **BC-403: ENDOCRINOLOGY**

### **UNIT - I**

An account of cell signaling process ( Autocrine, paracrine, Telecrine actions ). Historical aspects of endocrinology, general characteristics and classification of hormones. Assay of hormones (bioassay, chemical assay, RIA, ELISA ). Mechanism of action of steroid and peptide hormones Molecular mechanism of signal transduction - nature and types of receptors, ligand-receptor interactions, Scatchard plot, up and down regulation of receptors, concept of second Messengers with focus on cAMP, cGMP, Calcium, I P3 Nitric oxide. Mechanism of generation of second messengers and their action. Biological rhythms & biological clocks.

### **UNIT - II**

#### **Hormones of Hypothalamus and Pituitary:**

Synthesis, storage, secretion, transport, mechanism of action and biological actions hypothalamic, adenohipophysial and neurohypophysial hormones. Hypothalamic and pituitary disorders.

A brief account of structure and function of pheromones and insect hormones.

### **UNIT - III**

A detailed account of thyroid, parathyroid, adrenal medullary and cortical, pancreas (insulin, glucagon, and pancreatic polypeptide) and gut hormones, ANF (atrial natri uretic factor). Renin-angiotensin system and renal hormones. Disorders of thyroid, adrenal glands, prostaglandins. Hormones of thymus pineal glands and other peptide growth factors.

### **UNIT - IV**

Hormones of Gonads : Chemistry, Synthesis, storage, secretion and regulation, physiological and biochemical aspects of hormones of Gonads, puberty and hormonal control of puberty, hormonal regulation of menstrual cycle. Oral contraceptives. Disorders associated with Gonadal hormones.

#### **Recommended Books:**

1. Harper's review of biochemistry – 25<sup>th</sup> edition by R.K.Murray et al., (Appleton and Lange).
2. Cell and Molecular Biology – Shieler and Bianchi.
3. Text book of Biochemistry with clinical correlations (eds.) T.M.Devlin (John Wiley).
4. Endocrinology by Mac.E.Hadley (Prentice Hall).
5. Tietz text book of clinical chemistry by Carl. A.Burtis and Edward R.Ashwood.
6. Essential endocrinology J.E.Wise (Oxford Press).

## BC 404 : BIOCHEMICAL TOXICOLOGY, CANCER, PHYTOCHEMISTRY AND GENOMICS

### UNIT I

Toxicology: Scope: Fundamentals of toxicology, types (natural, chemical ) and sources of toxicants, absorption, mechanism of toxicity, evaluation of toxicity, Metabolism of xenobiotics, detoxification: hydroxylation monooxygenase reactions, isoforms of cytochrome P-450. Conjugation reactions. Toxicokinetics and risk assessment markers of toxicity, diagnosis, treatment and prevention, Alcohol and drug addiction and drug metabolism, food adulterants, additives and colorants.

The biochemical basis of neuro - psychiatric disorders- Myasthanea gravis, Huntington disease strokes, Parkinson's disease, Alzheimer's disease, Schizophrenia. Trace elements toxicology.

### UNIT II

Cancer, types of cancer, Differences between normal and cancer cells- Warburg effect, contact inhibition of movement, loss of cellular affinity, alterations in cytoskeleton, cell surface, decreased serum requirements and secretion of growth factors, differential gene expression. Factors causing cancer-physical, chemical and biological agents. Oncogenes – Oncogene families :Protein kinases (*Src, abl, erbB1*), GTP binding proteins (H-ras, K-ras), Growth factors (*sis*), nuclear proteins (*myc, myb, fos*), Hormone receptors (*erbA*), unclassified. Retroviral oncogenes, protooncogenes, oncogenes in human cancer, functions of oncogene products. Tumor suppressor genes – Identification, role in cell cycle control, functions of tumor suppressor gene products (RB, P<sup>53</sup>, P<sup>16</sup>, P<sup>21</sup>, P<sup>TEN</sup>). Role of oncogenes and tumor suppressor genes in tumor development. Mitogenic polypeptide growth factors. Metastasis

Stem cells, Embryonic and adult stem cells, Unique properties and markers. Maintenance and the potential applications in molecular biotechnology.

### UNIT III

Secondary metabolites of plants – structure, distribution and functions of Phenolic compounds – simple phenols, phenol carboxylic acids, phenol propanes, lignins. Structure and functions of flavonoids. Terpenoids : structure, functions and Biosynthesis. Alkaloids and their pharmaceutical uses. Structure, properties and applications of leptins.

Structure, physiological functions and mode of action of phytohormones – auxins, gibberellins, cytokines, ethylene and abscisic acid.

### UNIT IV

Phylogenetic analysis: Introduction to Phylogenetic tree building methods (Neighbor Joining and UPGMA methods). Differences between Parsimony and Maximum likelihood approaches. Consensus trees and Bootstrap support.

Functional Genomics: Introduction to Microarray Technologies, Gene chips, Time course experiments, Types of Microarrays and their applications.

Proteomics: Introduction to Proteomics technology and Protein chips. Applications of proteomics.

Transcriptomics: Introduction to miRNA, siRNA, RNAi, Antisense RNA and their prediction methods. RNA interference technology, EST sequencing, EST databases.

Structural Genomics: Goals and Applications of Structural genomics initiatives.

Comparative Genomics and Phylogenomics: Introduction to Comparative Genomics and resources for Comparative genomics and phylogenomics.

Pharmacogenomics: Introduction to Drug Discovery and Pharmacogenomics

## RECOMMENDED BOOKS

1. Cooper Geoffrey, M. 2000. The Cell – a molecular approach. 2<sup>nd</sup> Edn. ASM Press. Washington
2. Alberts A et al. 1994. Molecular biology of the cell. Garland publ. New York
3. De Robertis EDP & EMF De Robertis. 2001. Cell and Molecular biology. Lippincott Williams & Wilkins. Bombay.
4. Genes VII– Benjamin Lewin (Oxford University Press ).
5. Molecular biology of the Gene 4<sup>th</sup> Edition by – Watson, Hopkins, Roberts, Steitz and Weiner
6. Biological concepts and techniques in toxicology- an integrated approach by – Jim E Riviere.
7. Toxicologic biomarkers by- AP. Decaprio.
8. Toxicokinetics and Risk assessment by – J.C. Lipscomb, EV. Ohanian.
9. Introduction to biochemical toxicology by- Ernest Hodgeson and Frank.E. Guthrie (Elsevier press)
10. Biochemistry and Molecular Biology of plants – Bob B. Buchanan, Grussem W and Russell L. Jones.
11. Plant Biochemistry-3<sup>rd</sup> Edition – Heldt – Elsevier publications
12. Introduction to plant Biochemistry – Second edition by T.W. Goodwin and Mercer E.I
13. Molecular modeling
14. Bioinformatics: A Practical Guide to the Analysis of Genes and Proteins" (Andreas D. Baxevanis, B. F. Ouellette), Paperback, 2nd ed., 470 pp., ISBN: 0471383910, Publisher: Wiley, John & Sons, Inc., Pub.
15. David W. Mount, Bioinformatics: Sequence and Genome Analysis, 2nd edition, Cold Spring Harbor Laboratory, 2004, ISBN 0-87969-687-7.

## **PRACTICALS**

### **BC P 401: FOOD ANALYSIS**

1. Estimation of moisture, fiber content of foods.
2. Preparation of ash
3. Estimation of reduced ascorbic acid by DCPIP method.
4. Estimation of total ascorbic acid by DNPH method.
5. Estimation of Thiamine by colorimetry/fluorimetry.
6. Estimation of Riboflavin by Fluorimetry.
7. Estimation of Niacin by Cyanogen bromide method.
8. Estimation of available Lysine in food.
9. Estimation of copper in food.
10. Estimation of magnesium in food.
11. Estimation of iron in food.
12. Estimation of calcium in food.
13. Estimation of nitrogen in food by Kjeldahl method.
14. Estimation of fat in food (Gravimetry).
15. Determination of Saponification value of oil.
16. Determination of Iodine value in oil.
17. Determination of Acid value of an oil.

### **BC P 402: CLINICAL BIOCHEMISTRY**

1. Estimation of blood glucose
2. Estimation of blood urea.
3. Determination of creatinine clearance.
4. Estimation of creatine in serum.
5. Estimation of uric acid in serum.
6. Estimation of serum total protein.
7. Estimation of Serum albumin.
8. Agar gel electrophoresis of serum proteins.
9. Agar gel electrophoresis of serum lipoproteins.
10. Estimation of Serum cholesterol (HDL+LDL)
11. Determination of SGOT
12. Determination of SGPT
13. Estimation of serum calcium
14. Estimation of serum phosphate
15. Gastric analysis
16. Estimation of serum bilirubin
17. Determination of thymol turbidity
18. Determination of zinc turbidity
19. Determination of urine ascorbic acid
20. Tests for abnormal constituents in urine
21. Use of diagnostic kits.

