RESEARCH METHODOLOGY

Research methods:

Definition of problem, necessity of defining problem, technique involved in defining a problem. Surveying the available literature. Research design, objective, methods of collecting data, sampling methods, information required for solving a problem.

Philosophy and Ethics:

Introduction to philosophy: definition, nature and scope, concept, branches.

Ethics: definition, moral philosophy, nature of moral judgements and reactions.

Scientific Conduct:

Ethics with respect to science and research; Intellectual honesty and research integrity.

Scientific misconducts: Falsification, Fabrication, and Plagiarism (FFP).

Redundant publications: duplicate and overlapping publications, salami slicing. Selective reporting and misrepresentation of data.

Publication Ethics

Publication ethics: definition, introduction and importance.

Best practices/standards setting initiatives and guidelines: COPE, WAME, etc; Conflicts of interest.

Publication misconduct: definition concept, problems that lead to unethical behavior and vice versa, types.

Open Access Publications

Open access publications and initiatives. SHERPA/RoMEO online resource to check publisher copyright & self-archiving policies. Software tool to identify predatory publications developed by SPPU. Journal finder / journal suggestion tools viz. JANE, Elsevier Journal Finder, Springer Journal Suggester, etc.

Publication Misconduct

Group Discussions

Subject specific ethical issues, FFP, authorship; Conflict of interest

Complaints and appeals: examples and fraud from India and abroad.

Software tools

Use of plagiarism software like Turnitin, Urkund and other open source software tools.

Databases and Research Metrics

Databases

Indexing databases;

Citation databases: Web of Science, Scopus, etc.

Statistics:

Statistics: introduction, definition, population and universe, sample and population. Measure of central tendency and dispersion. Probability distribution – normal, binomial and Poisson distribution. Mean, mode, standard deviation, standard error, t-test and ANOVA.

ANALYTICAL BIOCHEMISTRY

Preliminary techniques in Biochemistry: Animal models, types of studies, mutant organisms (auxotroph), cultured animal and plant cells and plant as models.

Cell fractionation techniques: Cell lysis, homogenization, extraction, salting in and salting out. Dialysis and Ultrafiltration-Artificial membranes, semipermeable membranes, Donnan membrane equilibrium and biological significance of osmosis.

Centrifugation: Svedberg's constant, sedimentation velocity and sedimentation equilibrium.

Ultra centrifugation: Differential and density gradient centrifugation, construction of preparative and analytical ultracentrifuge.

Chromatographic techniques: Principles, procedure and applications of paper, TLC, adsorption, ion exchange, gel filtration, affinity, GLC, chromatofocusing, HPLC and FPLC.

Electrophoretic techniques: Polyacrylamide gel electrophoresis, SDS-PAGE, 2D-electrophoresis, agarose gel electrophoresis, isoelectric focusing, pulsed field electrophoresis, high voltage electrophoresis,

capillary electrophoresis, isotachophoresis. Separation of proteins, lipoproteins and nucleic acids. Visualizing separated components; staining, fluorescence, PAS staining, zymogram and reverse zymogram.

Spectroscopic techniques: Principles of colorimeter, spectrophotometer, fluorimeter. Beer- Lambert's Law and its limitations. Extinction coefficient, fluorescent probes and their applications. Principle and applications of NMR, IR, CD/ORD.
Radioisotope techniques: Radioactivity, stable and radioactive isotopes. Methods of detection of isotopes. GM counters, liquid scintillation counters and autoradiography. Units of radioactivity, half-life of radioisotopes. Radiation monitoring and its hazards. Application of radioactive tracer in biology.

Radioisotopes in Biology: 3H, 14C, 32P, 131I, 35S, concept of half-life, decay constant, detection and quantitation- GM counter and solid and liquid scintillation counter. Specific activity, autoradiography and their applications.
Mass spectroscopy: Theory and construction of mass spectrometer. Ionization, fragmentation, m/e, time of flight, MALDI and ESI.
Labelling: Using plant system (monosaccharides and polysaccharides), animal system, chemical (Glucose1-14C) and enzymatic methods (disaccharides). Labelling of ATP (α-P32 and γ-P32), proteins and nucleic acids.

BIOMOLECULES

Carbohydrates: Classification of carbohydrates. Chemistry of monosaccharides: pentoses. Hexoses, deoxyglucose, amino sugars muramic acid, Linkages in sucrose, lactose and maltose, trehalose and glycosides. Isolation of polysaccharides: Homopolysaccharides and heteropolysaccharides, starch, cellulose, glycogen, hyaluronic acid, chondroitin sulphate, chitin, xylans, bacterial cell wall polysaccharides, blood group polysaccharides.
Structure elucidation: degradation, graded acid hydrolysis, periodate oxidation, degradation of oxopolysaccharides, methylation, and acetylation. Glycoproteins: N- and O-glycosylation, lectins, carbohydrates in tissue engineering. Proteoglycans: Agreecan, Syndecan, and Decorin. Pectin and pectic polysaccharides.
Amino acids, Peptides and Proteins: Features of the peptide bond, naturally occurring peptides: glutathione enkaphalins and endorphins. Chemical synthesis of peptides; Khorana's solution phase synthesis, Merrifield's solid phase synthesis.
Determination of amino acid compositions: Acid and base catalyzed hydrolysis, separation, quantification, determination of N and C terminal residues, determination of site of glycosylation and type of linkage (o-glycosyl and n-glycosyl).
Elucidation of structure of proteins: Isolation of proteins; overview of purification and criteria of purity. Determination of primary structure: Sequencing strategies; N-terminal and C-terminal, sequencing methods. Automated sequanators. Determination of s-s-bond position.
Secondary structure of proteins: α, β sheet, β bend, β turn and super secondary structures. Secondary structure prediction methods: Ramachandran plot, Chou and Fasman algorithm. Tertiary and quaternary structures.
Factors responsible for protein folding: Anfinsen’s experiment. Weak forces of interaction; hydrogen bonding, Vander Waal’s forces, London forces, ionic interactions, hydrophobic interactions, S-S bridges, peptide bond, glycosidic bond, phospodiester bond, and allolysine. Denaturation and renaturation of proteins, molten globule. 3D Structure of myoglobin hemoglobin, immunoglobulin, collagen, chymotrypsin and keratin. Molecular Chaperons.
Lipids: Classification of lipids; oils, fats, and waxes. Occurrence and properties of fatty acids, esters of fatty acids, cholesterol, phosopholipids, glycolipids, sphingolipids, cerebrosides and gangliosides.

Lipid mediators: Eicosanoids, prostaglandins, leukotrienes, prostacyclins, thrombaxanes, DAG, ceramide and PAF.

Nucleic acids: Isolation of RNA and DNA from biological samples. Physico-chemical properties of nucleic acids- melting of DNA, Tm; factors affecting Tm, Cot curve, classification of DNA based on cot curve. Chemical reactions of DNA and RNA.

Structure of nucleic acids: Primary, secondary and tertiary structure of DNA; Watson and Crick model; B and Z DNA, other models of DNA structure. palindromic sequences, cruciforms. DNA protein interaction; zinc finger,leucinc zipper, helix-turn-helix, other motifs, DNA bending and kinks. Secondary structure of tRNA and clover leaf model. Nucleic acid sequencing- Maxam- Gilbert method, dideoxy method. Chargaff's rule.

PHYSIOLOGY AND NUTRITION

Physiology: Basic body plan in humans, location of organs and their basic functions.

Circulatory system: Blood-Composition, cells, plasma proteins and lipoproteins. Erythrocytes; structure and function. WBC; types, differential count, functions. Platelets and their functions. Buffer systems, hemostasis, Mechanism of blood clotting, role of vit K, digestion of clot, anticoagulants, blood volume, blood pressure and their regulations. Hematopoiesis. Plasma lipoproteins and their functions, HDL, LDL, VLDL, chylomicrons. CSF; composition and function.

Respiratory System: Lungs, structure and functions, gas exchange, oxygen binding by hemoglobin, factors affecting oxygenation and acid-base balance.

Digestive secretions - Composition, functions and regulation of saliva, gastric, pancreatic, intestinal and bile secretions. Mechanism of HCl production in the stomach and Gastrointestinal hormones. Digestion and absorption of carbohydrates, lipids and proteins.

Hepatobiliary System: Anatomy of the liver, blood supply, cells; hepatocytes, endothelial cells and Kupffer cells, secretory and excretory function and formation of bile.

Excretory system- Structure of nephron, formation of urine- glomerular filtration, tubular reabsorption of glucose, water and electrolytes, tubular secretion. Kidney hormones. Regulation of acid base, electrolyte and water balance. Respiratory and metabolic acidosis and alkalosis.

Muscular system - Smooth, skeletal and cardiac muscles. Contractile and other proteins of muscle. Fine structure of muscle fibre, neuromuscular junctions. Mechanism of muscle contraction.

Concepts of Nutrition: Introduction, essential nutrients and their classification. Proximate analysis of foods.

Macronutrients: Carbohydrates- Digestible and non-digestible, dietary fibre. Proteins- Essential amino acids, malnutrition, Kwashiorkor and Marasmus. Lipids-triglycerols, phospholipids, cholesterol and essential fatty acids.

Micronutrients: Pro-vitamins, anti-vitamins, sources, requirements, functions and deficiency symptoms of vitamin-C , thiamine, riboflavin, niacin, pyridoxine, folic acid, vitamin B12, pantothenic acid, biotin & Vitamin- A,D,E and K. Absorption, requirements and functions of Zn, Ca, P, Mg, Cl, Fe.

Basal metabolism: Determination of Basal Metabolic Rate (BMR) - Experimentally and by calculation average BMR for Indians. Factors affecting BMR. Energy requirements for different physical activities, Standard Dynamic Action (SDA) of food. Nutritive value of proteins.

Recommended Daily Allowance (RDA): Calories from carbohydrates, lipids, proteins.

Special aspects: Nutrition during pregnancy, lactation and old age. Nutrition for infants and children. Factors affecting the nutritional status.

ENZYMOLOGY

Introduction to Enzymes: Nature of enzymes, localization, isolation, purification and characterization of enzymes. Criteria of purity of enzymes, fold purity. Nomenclature and IUB classification of enzymes.

Enzyme specificity, specific activity, assay methods; coupled enzyme assays, continuous, end point and kinetic assay. Units of enzyme activity, IU and Katal. Industrial and Biomedical applications of enzymes.

Enzyme kinetics: Michaelis-Menten equation, initial velocity approach, steady state approach. Vmax, Km and their significance. Linear transformation of Michaelis-Menten equation; Lineweaver-Burk plot, Eadie-Hofstee, Wolf and Cornish-Bowden. Scatchard plot. Rate of a reaction, order and molecularity. Ist order reaction kinetics. Rectangular hyperbola, Michaelis- Menten equation as rectangular hyperbola, asymptote, linear transformation, calculation of slope, intercept. Effect of pH, temperature and substrate concentration.

Enzyme Inhibition: Types of reversible inhibitors - competitive, non-competitive, un-competitive and mixed inhibitors. Partial inhibition, substrate inhibition and allosteric inhibition. Irreversible inhibition.

Kinetics of bi-substrate reactions: Sequential mechanism, compulsory order and random order mechanism, non-sequential mechanism, ping pong mechanism, distinction between different kinetic pathways using primary and secondary plots. Inhibition studies in the characterisation of bi-substrate reactions.

Mechanisms of enzyme catalysis: Active site structure; methods of determining active site structure, isolation of ES complex, affinity labelling, chemical modification studies and active site structure investigation.

Nature of enzyme catalysis: Transition state theory, proximity and orientation, orbital steering, acid base catalysis, covalent catalysis, metal ion catalysis, nucleophilic and electrophoilic catalysis, intramolecular catalyses, entropy effects. Effect of temperature and pH on enzyme catalysed reaction.

Mechanisms of action of specific enzyme: Chymotrypsin; zymogen activation, acid-base catalysis, charge relay network. Lysozyme, alcohol dehydrogenase, ribonuclease, carboxypeptidase A, RNA as an enzyme.

Coenzymes: The mechanistic role of the following coenzymes in enzyme catalyzed reactions-nicotinamide nucleotides, flavin nucleotides, pyridoxal phosphate, coenzyme A, thiamine pyrophosphate and biotin, Folate coenzymes.

Monomeric and oligomeric enzymes: Monomeric enzymes-the serine proteases, zymogen activation. Sulphahydryl enzymes-papain. Oligomeric enzymes-isoenzymes (LDH) and multi-enzyme complexes-(Pyruvate dehydrogenase complex).

Allosteric enzymes: Binding of ligands to proteins - Co-operativity, the Hill equation, equilibrium dialysis technique. Sigmoidal kinetics: The MWC and KNF models. Significance of sigmoidal behaviour. Allosteric enzymes and metabolic regulation. Study of ATCase- as typical allosteric enzyme.

METABOLISM OF FUEL MOLECULES

Introduction: Basic concepts in metabolism: catabolism, anabolism, catabolic, anabolic and amphibolic pathways.

Carbohydrate metabolism*:* Introduction, glycolytic pathway, energetics and regulation of glycolysis, fate of pyruvate, oxidation of pyruvate. Citric acid cycle and its regulation, energetics, anaplerosis. Gluconeogenesis and its regulation, Cori cycle, glyoxylate cycle. glucose paradox. Futile cycles and their applications. Entry of other carbohydrates into glycolysis-fructose and galactose.

Glycogen and starch metabolism: Biosynthesis and degradation of starch and glycogen and its regulation. Glycogen storage disorders. Lactose intolerance, fructosuria, galactosemia. HMP pathway and its regulation.

Hormonal regulation of glucose metabolism: Effect of insulin and glucogon, catecholamines, growth hormones and carticosteroids on carbohydrate and lipid metabolism in different tissues. Action of thyroid hormones and their mechanisms.

Lipid Metabolism: Degradation of triacylglycerols, phospholipids and sphingolipids and regulations; lipase, hormone sensitive lipase, phospholipases and sphingomyelinase. Fatty acid degradation; α and β and ω-oxidation. Knoop's experiment, saturated and unsaturated fatty acids. Formation of ketone bodies and their oxidation. Energetics and biosynthesis of fatty acids; fatty acid synthetase complex, chain elongation and desaturation. Pathways in plants and animals, conversion of linoleate to arachiodnante (scheme only).

Cholesterol synthesis and degradation and regulations: Metabolism of circulating lipids; chylomicrons, HDL, LDL and VLDL. Reverse cholesterol transport by HDL. Oxidized lipids and their metabolism, Foam cell formation. Regulation of blood cholesterol, triglycerides, LDL and HDL. Obesity, and mechanisms, exercise and regulation of energy metabolism.

Phospholipid biosynthesis and regulations: Denovo pathway and inter conversion, biosynthesis of phospholipids, sphingolipids, ether lipids and glycolipids. Degradation and biosynthesis of gangliosides and cerebrosides. Biosynthesis of prostaglandins, thromboxanes and leukotrienes.

Integration of metabolic pathways: Integration of carbohydrate and lipid metabolism, and their regulation and manipulation.

Thermodynamics: I, II and III laws of thermodynamics. Enthalpy, entropy, free energy and chemical equilibrium. High energy compounds- Energy currency, ATP, ADP, creatine phosphate, phosphoenol pyruvate as energy rich compound.

Mitochondrial electron transport: Entry of reducing equivalents for oxidation; malate-aspartate shuttle, glycerol phosphate shuttle. Organization of respiratory chain complexes, structure and function of the components; Fe-S proteins, cytochromes, Q cycle, proton transfer, P/O ratio, respiratory control, oxidative phosphorylation, uncouplers and inhibitors, sequence of electron carriers based on redox potentials. ATP synthesis, ATP synthase complex, binding change mechanism, proton motive force, Mitchell's hypothesis.

CELL BIOLOGY AND ENDOCRINOLOGY

Cell Biology: Types of cells, Extracellular matrix, Cytoskeletal elements and cell-cell Interactions-Adhesion. Cell division and Cell cycle-Mitosis and meiosis, Cell cycle phases and Programmed cell death. Biomembranes-Composition of plasma and organelle membranes, Singer and Nicholson's model and its salient features. Membrane domains- Caveolae and Rafts. Technique used to study the membranes structure-FRAP and single particle tracking. Preparation and usage of liposomes and erythrocytes ghosts. Membrane asymmetry. Protein-protein and protein-lipid interactions in membranes. Protein and lipid trafficking in membranes. Membrane Transport: Passive, facilitated and exchange diffusion, Fick's law of diffusion and active transport. Structure and function of Na-K ATPase and $Ca2^{+}$ATPase. Ion channels, ionophores and aquaporins. Receptor mediated endocytosis and exocytosis.

Disorders associated with membrane transport systems-Cystic fibrosis. Bacterial transport system.

Nervous system: Division of nervous system-neutron structure and types. Role of NGF, N-CUM and other specialized proteins. Resting membrane potential of excitable cells. Mechanism of initiation and propagation of action potential. Voltage gated ion channels (sodium, potassium and calcium). Design and use of patch clamp in measuring membrane potential. Depolarization and hyperpolarization in post-synaptic cells. Synaptic transmission, neurotransmitters, biogenic amines, amino acids and neuropeptides. Storage and exocytosis of neurotransmitters. Termination of neurotransmitters action. Acetylcholine receptors, nicotinic and muscarinic adrenergic receptors, other neurotransmitters receptors. Mechanism of synaptic transmission, receptor integrated ion channels and G-protein mediated ion channels. Use of agonists and antagonists of neurotransmitters in Biochemistry and medicine.

Endocrine System: Endocrine organs in man. Location and inter relationship of endocrine glands in man; chemistry of hormones produced by hypothalamus, pituitary, thyroid, parathyroid, pancreas, adrenals, gonads and intestine. Functions and abnormalities- hypo and hyper production of hormones secreted by;

pituitary, thyroid, pancreas, adrenals and gonads. Structure and control of hypothalamus: Hormones produced; GRH, somatostatin, TRH, CRH, GnRH.

Pituitary-anatomy and structure- Hormones of anterior, posterior and median lobes. Proopiomelanocortin. Testes and ovaries- hormones produced by testes and ovaries, menstrual cycle. Regulation of hormone production and release: hypothalamus-pituitary-target organ axis and regulation by feedback mechanism. Conversion of cholesterol to steroid hormone.

Mechanism of action of Hormone: Peptide hormones- General mechanisms of cell signalling by hydrophilic factors, transmembrane receptors, G protein coupled receptors, receptor tyrosine kinase, eicosanoid receptors. **Second messengers:** 1P3, DAG, cAMP, protein kinases. Nitric oxide signalling; generation and action. **Growth factors:** Structure, mechanism of action and receptors of EGF, PDGF, NGF and IGF. Isolation and characterization of insulin receptor. **Steroid hormones-** Steroid receptors, isolation and characterization of steroid receptors. Receptor down regulation, desensitization and up regulation. Pineal gland, Melotonin and circadian rhythm.

Insect hormones: Structure and function of moulting hormone, ecdysone, juvenile hormones, Biochemistry of Plant hormones.

Pheromones: Mechanism of perception and action. Use of pheromones in control of agricultural pests.

METABOLISM OF NITROGEN COMPOUNDS

Nitrogen Cycle: Introduction to biological and non-biological nitrogen fixation, brief introduction to *nif* genes, utilization of nitrate and nitrites, regulation of nitrate reductase.

Catabolism of amino acids: Study of degradation pathways of the individual amino acids in animal, plant and microbial systems-Glucogenic and ketogenic amino acids and their significance. Degradation of amino acids forming pyruvate (alanine, glycine, threonine, serine, cysteine, cysteine and tryptophan) oxaloacetate (aspartic acid and asparagine), α- ketoglutarate (glutamic acid, glutamine, arginine, histidine and proline), succinyl CoA (valine, isoleucine, threonine and methionine), Fumarate (phenylalanine, tyrosine) acetoacetate and/or acetyl CoA (leucine and lysine), pyruvate, formaldehyde, acetoacetate and/or acetyl CoA (tryptophan), and fumarate, acetoaetate and/or acetyl CoA (phenylalanine and tyrosine). Inherited disorders associated with glycine, aromatic, branched- chain, basic and sulfur containing amino acid metabolism.

Biosynthesis of amino acids: in animal, plant and microbial systems-Biosynthesis of non -essential amino acids from pyruvate (alanine), intermediates of glycolysis (serine, tyrosine), biotransformation of serine to glycine and cysteine, and TCA cycle (aspartic acid, asparagine, glutamic acid and glutamine), non- essential amino acid s(glycine, proline and arginine), and essential & non – essential amino acid (cysteine). Biosynthesis of essential amino acids from aspartate family of amino acids (threonine, lysine and methionine), pyruvate family of amino acids (valine and leucine), pyruvate and α-ketobutyrate family of amino acid (isoleucine) aromatic family of amino acids from glycolysis intermediates (phenylalanine, and tryptophan) and histidine. Regulation of amino acid biosynthesis by sequential and concerted feedback inhibition.

Amino acid Metabolism: General metabolic reaction of amino acids– transamination, pseudotransamnation, glucose–alanine cycle, oxidative deamination (glutamate dehydrogenase), minor pathways of amino acid degradation – transdeamination, amino acid oxidase, and non – oxidative deamination (α– deaminase, dehydrase, asparaginase and glutaminase). Assimilation of ammonia, formation of amino acid amides by glutamine synthetase and its regulation. Urea cycle– regulation and metabolic disorders. Biosynthesis of creatine and creatine phosphate, polyamines– putrescine, spermidine and spermine, glutathione (γ-glutamyl cycle), physiologically active amines (serotonin. γ-amino butyric acid, histamine, and catecholamines – dopamine, epinephrine and epinephrine).

Heme Metabolism: Biosynthesis and degradation of porphyrin, porphyrias, jaundice and Hemoglobinopathies.
Nucleotide Metabolism: Biosynthesis of purine and pyrimidine nucleotides and their inter conversion, regulation of biosynthesis. Other pathways of purine nucleotide formation. Biosynthesis of deoxyribonucleotides and coenzymes nucleotides. Chemical inhibition of the biosynthesis of nucleic acid precursors. Degradation of purine and pyrimidines, and disorders associated with their metabolism; gout, Lesch-Nyhan syndrome, oroticaciduria, and xanthinuria.

IMMUNOLOGY

History and scope of immunology: Types of immunity- innate and adaptive. Immune reactive cells. Humoral and cell mediated immunity. Anatomy of lymphoid organs- primary lymphoid organs, secondary lymphoid organs and lymphtic system. Antigens-chemical nature, types, antigenicity, haptens, epitopes, antigenic determinants, adjuvants and super antigens. Valency of antigen, epitope analysis.

Immunoglobulins: Basic structure, functions, theories of antibody formation, classes and immunoglobulin super family. Antigenic determinants on immunoglobulins. Methods of raising polyclonal antibodies. Monoclonal antibodies – production and application. Antibody diversity- mechanism contributing to diversity, somatic recombination, rearrangement and generation of antibody diversity. Class switching.

Cellular Basis of Immunity: Primary and secondary immune response. Reticuloendothelial system, T, B and accessory cells. Development of T and B cells. Sub sets of T and B cells. T-helper cells, T-killer cells, T-suppressor cells. T and B cell receptors, antigen processing and presentation. T and B interaction. Cytokines and co-stimulatory molecules; lymphokines, interleukins, structure and function of IL-lβ, IL-2, TNF-α. Suppression of immune response, immunoglobulin genes, generation of immunoglobulin diversity, gene rearrangement and other mechanisms, clonal selection theory of Burnet.

MHC: MHC gene and its polymorphism, role of MHC in immune response and transplantation. **T and B cell lymphocytes**: origin, differentiation, characterization and functions. T cell and B cell receptor complexes. Antigen processing and presentation. Cytokines and co-stimulatory molecules. Role in immune response. T and B cell interactions.
Complement system- components, receptors, activation of complement pathways and its biological consequences. Major histocompatibility complex (MHC) genes and products. Role of MHC antigens in immune response, MHC antigens in transplantation.
Non-specific defences in man: Barriers to infection; skin, mucous membrane, inflammation,
hyper sensitivity reactions (Type I, II, III and IV).
Transplantation: Autograft, isograft, allograft and xenograft. Graft rejection, graft vs. host reaction.

Tumour immunology: Tumour associated antigens, factors favouring tumour growth, immune surveillance. Tumour necrosis factor-α and β.
Disorders of immunity: Immunological tolerance, auto immune disorders, AIDS, SCID.
Vaccines: Adjuvants, vaccines and their preparations. Polyclonal and monoclonal antibodies; hybridoma technique.
In vitro antigen-antibody reaction: Precipitation, agglutination, complement fixation, immuno diffusion, immunoelectrophoresis, immunofluorescence, RIA, ELISA.
Defence system in plants: Host parasite interaction and defence system in plants.

CLINICAL BIOCHEMISTRY AND DIETETICS

Blood: Blood Haemostasis, Composition, blood count, total, differential and platelet count. Blood group studies, Rhesus factor, ESR- its determination and importance in disease. Blood coagulation factors,

mechanism and its regulation. Plasma proteins, profile in health and diseases. Abnormal haemoglobins, Disorders of haemoglobins- thalassemmia, sickle cell anaemia. Anaemias Microcytic, macrocytic and normocytic, CSF analysis.

Diagnostic Enzymology: Clinical significance of enzymes like SGOT, SGPT, LDH, CPK, Alkaline and acid phosphatase, amylase.

Kidney profile: Assessment of renal function-clearance tests and their importance in assessment of kidney functions. Laboratory investigations of kidney disorders- UTI, kidney stones, Nephritis, Urolithiasis, Dialysis, Uremia, Hyporuricemia.

Liver profile: Biochemical indices of hepatobiliary diseases, Bile pigments- Formation of bilirubin, urobilinogen, bile acids, Jaundice- pre-hepatic, hepatic, post hepatic. Diagnosis Liver function tests, Diseases of liver- Hepatitis, Cholestasis Cirrhosis, Gall stone.

Disorders of carbohydrate metabolism: Diabetes- aetiology, classification, management, laboratory investigations. GTT, GlycatedHb, Diabetic complications, inborn errors of carbohydrate metabolism- Glycogen storage diseases, Galactosemia, Lactose intolerance, Pentosuria. Disorders of Lipid metabolism- Plasma lipoproteins and their functions, Hyperlipoproteinaemia- classification, Primary and secondary, Hypercholesterolemia, Ketosis and its significance. Disorders of amino acid and protein metabolism- Inborn errors of amino acid metabolism- PKU, Alkaptonuria. Disorders of purine and pyrimidine metabolism- Gout, Lesch-Nyhan syndrome, Xanthuria, Oroticaciduria. Cardiovascular disorders- Major cardiovascular system- Atherosclerosis- risk factors, pathogenesis, diagnosis and prognosis. Gastrointestinal disorders: Fractional gastric analysis, Hypo and hyperacidity, Gastric ulcers, Malabsorption syndrome.

Dietetics: Introduction to nutrition. Food pyramid. Diet planning and introduction to diet therapy. Nutritional requirements for different age groups, anaemic child, expectant women, and lactating women. Diet planning for prevention and cure of nutritional anaemia.

Diet therapy: Functional foods, dietary considerations during fever, and typhoid, malaria, influenza and tuberculosis patients. Prevention, and correction of obesity, underweight, and metabolic diseases by diet therapy. Dietary interventions to correct and or manage gastrointestinal diseases (indigestion, peptic ulcer, stomach carcinoma, constipation, diarrhoea, steatorrhoea, irritable bowel syndrome.

Diets in liver diseases - Hepatitis, cirrhosis, cholecysthetis and cholelithiasis. Functional foods based diet therapy for diabetes, cardiovascular disease, nephritis, and genetic disorders (PKU, galactosemia, lactose-intolerance, fructosurea) and cancers.

MOLECULAR BIOLOGY

DNA Replication: Central dogma of Molecular biology, Structure of DNA and forces stabilizing DNA structure. Genome organization in prokaryotes and eukaryotes. Experimental evidences-DNA as the genetic material and semiconservative mode of DNA replication. Models of DNA replication. Characterization, composition and mechanism of action and role of *E. coli* DNA Polymerase I, and III, E. coli and Phage DNA Ligase, topoisomerases, primosome complex, helicase, primase, ssDNA stabilizing proteins. Brief study of E. coli DNA polymerase II. Mechanism of E. coli DNA replication (trombone model). Origin of replication and events occurring on replication fork- topological problems, initiation, elongation, and termination of E. coli DNA replication. Leading strand, lagging strand, Okazaki fragments. Proof of discontinuous synthesis of DNA. Fidelity of replication-proofreading and nick translation, nearest neighbour base frequency analysis. Eukaryotic DNA polymerases, Mechanism of replication of Eukaryotic DNA, mitochondrial and chloroplast DNA. Regulation of eukaryotic DNA replication and inhibitors of DNA replication. DNA replication in adenovirus, polyoma and SV 40. Rolling circle mode of DNA replication. Replication of ss +RNA viruses, ss–RNA viruses, dsRNA- reovirus, and retroviruses.

DNA repair; Existence of repair systems, direct repair systems, excision repair- base excision and nucleotide excision repair, photo reactivation. Post replication repair; mismatch repair, SOS repair (by passing damaged DNA during replication) and recombination repair.
Transcription: Structure of gene. Characterization and mechanism of action of prokaryotic RNA polymerase. Significance of sigma factor. Mechanism of transcription in E. coli. Initiation of prokaryotic transcription; bacterial promoters, Closed and open initiation complexes, promoter clearances. Sigma factors, concept of mRNA, elongation of RNA synthesis, termination; rho-dependent and independent termination. Processing of RNA in prokaryotes. Inhibitors of RNA synthesis.

Eukaryotic RNA polymerases: Classification and transcription units. Initiation at RNA pol I, II, and III promoters. Elongation and termination of eukaryotic transcription process. Post transcriptional modification of eukaryotic tRNA, and rRNAs, role of RNPs, RNase-P, polynuceotide kinase in modification. Post transcriptional modification of eukaryotic mRNAs; capping, and tailing. Intron splicing; Properties and role of snRNPs in splicing, mechanism of splicing by class-I (GU-AG), and class-II (GU-AC) introns, spliceosome, alternative splicing.
Genetic code: Genetic code and its significance. Deciphering of the genetic code; Nierenberg and Khorana's work. General features of genetic code. Mitochondrial genetic code. Co-linearity of genes and proteins. Coding properties of tRNA; wobble hypothesis. **Ribosomes:** Prokaryotic ribosomes; molecular components, *in vivo* assembly, dissociation of subunits, and polysomes. Eukaryotic components and their assembly. Organelle ribosomes.
Translation: Initiation factors, elongation factors and termination factors of translation in prokaryotes and eukaryotes. Mechanism and process of protein synthesis in prokaryotes and eukaryotes; steps involved in protein synthesis, amino acid activation, exchange of ribosomal subunits, binding of mRNA to ribosomes, direction of protein synthesis and reading of mRNA. Protein chain initiation, elongation and termination. Comparative account of eukaryotic and prokaryotic translation. Inhibitors of prokaryotic and eukaryotic translation. Post-translational modifications of proteins. Synthesis of secretory and membrane proteins; signal sequence hypothesis. Mechanism of translational control.

BIOCHEMICAL GENETICS AND GENE REGULATION

Genetics: Introduction, Nature of genetic material; Prion chromosomes and genes. Mutation, types of mutation, mutagens, mechanism of mutation, induction and isolation of mutants and their role in genetic studies.
Classical genetics: Review of classical genetics; work on *Pisumsativum*, *Drosophila melanogaster*, *Neurosporacrassa*etc. Inheritance (sex – linked and others), population genetics, extranuclear inheritance. Sex determination, Morgan's discovery of sex linked inheritance, pattern of inheritance of sex linked genes, X-linked traits in humans. Identification of sex chromosomes, XX, XY, mechanism of sex determination.

Bacterial genetics: Bacterial chromosome, plasmids; fertility, resistance, colicinogenic and others. Recombination in bacteria. Mechanism of recombination, transposable genetic elements, transformation and conjugation in bacteria. Linkage map of bacterial chromosomes.
Human Genetics: Biochemical events occurring during mitosis and meiosis. Structure of chromatin; nucleosomes and higher orders of organization. Chromosome banding, Chromosome mapping based on recombination frequency data. Gene structure in eukaryotic organisms, introns, exons, pseudogenes, gene clusters, spacers, repetitive sequences and transposons. Overview of human genome project, mapping of human genes; techniques used, assignment of important genes. Transposition in human chromosomes. Chromosomal abnormalities.
Regulation of gene expression in prokaryotes: Principles of regulation of gene expression. Outline of transcriptional regulation, Induction, repression, constitutive/basal level expression. Genes involved in

regulation; regulator, promoter, operator and structural genes- activators and repressors. Identification of control regions by DNase-foot printing, gel mobility assay methods.
The operon model; Regulation of gene expression at transcriptional level. Concept of positive regulation and negative regulation. Operon concept- study of structure and regulation of Lac operon, Jacob and Monod hypothesis- Catabolite repression; role of cAMP and cAMP-receptor protein (CRP/ CAP) in the expression of glucose-sensitive operons, structure and functions of CAP. Structure function and regulation of tryptophan operon in *E.coli*, Concept and process of negative regulation, repression and attenuation in tryptophan operon. Structure and regulation of arabinose operon, and histidine operon. Structure and functions of λ repressor, Cro, and λcII. Anti-termination as a mechanism of regulation.

Eukaryotic gene expression: Levels of control of gene expression in eukaryotes. Regulation of gene expression in yeast. Control of galactose genes in yeast. Regulation of gene expression- β-globin gene, DHFR gene. Histone modification. Brief study of regulation of developmental genes in Drosophila.

DNA binding protein motifs: Zinc finger, leucine zipper, helix-turn-helix and other motifs. **Regulation at the level of post translational modification:** proteins stability, N-end rule, PEST and other sequences, ubiquitin mediated degradation.

GENETIC ENGINEERING AND BIOTECHNOLOGY

Genetic Engineering: Extraction and purification of nucleic acids (DNA and RNA) from biological sources. Definition, aims and objectives of recombinant DNA technology, restriction-modification systems, restriction enzymes; type I, II and III, specificity, sticky ends and blunt ends, isoschizomers.

Gene Cloning: Basic principles and tools and techniques of gene cloning: Characteristics and applications of restriction endonucleases and modifying enzymes. Methods of Isolation of gene/ DNA fragment for cloning. Methods for gene cloning: *in vivo*- cloning in *E. coli*. *In vitro*- polymerase chain reaction. Characteristics and applications of Plasmid, Cosmid, Phagemid, M13phage vector, λvector, BAC, PAC, and YAC. Selection of suitable vectors for cloning, expression and sequencing of DNA fragments.
Ligation: Blunt end and sticky end ligation, use of linkers and adopters, homo polymer tailing, colony hybridization, plaque hybridization.
Transformation: Micro injection, electroporation, lipofection, calcium phosphate method, protoplast fusion/somatic cell hybridization and biolistic methods. Transgenic plants and animals, gene knock out.

Identifying the right clones: Direct screening; insertional inactivation of marker gene, visual screening, and plaque phenotype. Indirect screening; immunological techniques, hybrid arrest translation, hybrid select translation. Screening using probes; construction of gene probes, hybridization and labelling.

Techniques: DNA sequencing, shot gun and orderly sequencing, chromosome walking, PCR; analysis of products, nested PCR, applications of PCR in cloning, agriculture and medicine. RT-PCR technique and applications. Real time PCR for quantification.
Blotting techniques: Dot blot, Southern, Northern, Western blot, DNA finger print assay, gel retardation assay.
Applications: Gene therapy, applications in agriculture medicine, industry. GM foods, terminator gene, negative impact of genetic engineering.
Biotechnology: Industrial microorganisms and their characteristics, Primary and secondary metabolites. Fermenter: Design of batch fermenter, CSTR, semicontinuous and continuous feed-batch fermenters. Fermentation types. Bioprocess development.
Organism and strain improvement: origin of industrial strain, Isolation, and strain improvement.
Medium and growth conditions: Raw materials and fermentation media, optimization of growth and culture conditions, growth Kinetics and product formation kinetics, Rheological parameters to be

considered for scale-up of bioprocess from lab to industrial scale. Methods of cell Immobilization, Fed-batch and continuous fermentations by immobilized systems. Downstream process, Recovery and purification of products.

Production of amino acids- glutamic acid, and lysine, organic acids- acetic acid, citric acid, Itaconic acid. Health care products- vitamins, antibiotics. Alcohols- bioethanol, butanol. Acrylonitrile, biogas and Biopolymers. **Production of enzymes** (amylase, proteases, cellulases, xylanases,) from bacterial and fungal strains by solid-substrate and submerged fermentation.

Environmental and agriculture Biotechnology: Natural control of insect pests, Production of biopesticides. Development of specialized microorganisms for bioremediation of toxic environmental pollutants (PAHs, pesticides, industrial effluents). Bioremediation of toxic industrial pollutants and pesticide contaminated sites