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SYLLABUS

For

Ph.D. Entrance Test

IN

BIOCHEMISTRY

BIOCHEMISTRY

Syllabus for Ph.D. Entrance Test

SECTION –A: RESEARCH METHODOLOGY (50% weightage)

UNIT I: Bio-analytical techniques

UNIT II: Research techniques in Molecular Biology

UNIT III: Immunological and microbial techniques

UNIT IV: Biostatistics, Computers and Bioinformatics

UNIT V: Scientific writing and Intellectual property right

SECTION –B: BIOCHEMISTRY (Subject Content) (50% weightage)

UNIT I: Biomolecules and Metabolism

UNIT II: Enzymology and Physiology

UNIT III: Cell biology, genetics and molecular biology

UNIT IV: Plant Biochemistry and Biotechnology

UNIT V: Nutritional and Clinical Biochemistry

SECTION –A: RESEARCH METHODOLOGY (50% weightage)

UNIT I: **Bio-analytical techniques**:-

Radioisotope techniques: Nature of radioactivity, properties of (α, β and λ-rays, measurement of radioactivity, use of radioisotopes in research. *In vivo and in vitro* labelling techniques, double labelling, quenching, internal standard, channel ratio, external standard ratio, emulsion counting, radioactive decay, autoradiography.

Electrophoretic techniques: Moving boundary and zonal electrophoresis, paper and gel electrophoresis, isoelectric focusing, 2Dimentional gel electrophoresis.

Centrifugation and ultra filtration techniques: Principle of sedimentation, cell fractionation, types of centrifugation, ultracentrifuge, filtration techniques.

Chromatography: Paper, TLC, Adsorption, partition, ion-exchange, reverse phase, gel filtration, affinity, gas chromatography, HPLC (High Pressure Liquid Chromatography).

Spectroscopy: Basic concepts and applications of X-ray diffraction, NMR, ESR, UV, IR, fluorescence, Raman, mass spectroscopy in structure determination of organic and biomolecules, CD and ORD.

Microscopy: Light, electron (scanning and transmission), phase contrast, fluorescence microscopy, freeze-fracture techniques, specific staining of organelles or marker enzymes.

UNIT II: **Techniques in Molecular Biology**:-

Recombinant DNA Technology

Methods of creating recombinant DNA molecule, splicing, properties of restriction endonucleases and their mode of action, selection/screening, construction of DNA library, genomic Vs cDNA library, chemical synthesis of gene, cloning vectors (X-phage, plasmid, M-13 phage, cosmid) shuttle vectors, yeast and viral vectors, expression vectors, uses of cloned gene, subcloning, sequencing by Sanger's method, proteins production in bacteria, site directed mutagenesis, RFLP, PCR, DNA finger printing, antisense-RNA technology, chromosomal walking.

Rapid DNA sequencing techniques and strategies details of a range of methodologies, e.g. plus and minus, dideoxynecleotide, partial ribosubstitution, Maxam and Gilbert. Use of thin gels, resolution etc. Interpretation of DNA sequences.

Rapid RNA sequencing techniques: plus and minus, dideoxy-nucleotide, Zimmern and Kaesberg, Peattie, Simoncsits et al., method etc. Interpretation of RNA sequence.

Cloning: Strategies for cloning in plasmid vectors, features of commonly used vectors, their purification and characterization. Identification of bacterial colonies that contain recombinant plasmids. Bacteriophage λ vectors, growth, purification. Cloning in Bacteriophage λ vectors.Cloning in cosmid vectors. Construction of Genomic DNA libraries in cosmid vectors. Enzymes used in molecular cloning, restriction enzymes, DNA-Polymerases, ligases, kinases, phosphatases, and nucleases. DNA binding proteins. Agarose gel and polyacrylamide gel electrophoresis, detection and extraction of DNA from gels. Construction and analysis of c-DNA; protocols and strategies for C-DNA cloning. Analysis of Genomic DNA by Southern Hybridization. Amplification of DNA by the polymerase chain reaction. Preparation of radiolabelled DNA and RNA probes. Synthetic oligonucleotides probes. Expression of cloned Genes in cultured cells. Screening expression with antibodies and oligonucleotides. Microarray chips and their applications.

UNIT III: **Immunological and microbial techniques:-**

Antigens, haptens and antibodies. Fine structure and subclasses of antibodies. Clonal selection theory and genetic basis of antibody diversity, immunoglobulin class switching. Antigen-antibody interactions. Immunodiffusion, immunoelectrophoresis, RIA, EIA, ELISA, fluroscent labelling and fluroscent cell sorter. Monospecific and bispecific antibodies. Hybridoma technology and monoclonal antibodies, catalytic antibodies. Western blotting. Purification and characterization of antibodies/proteins.

Basic microbial methods, Nutrition, physiology and growth of microbial cells. Microbial growth: The definition of growth, mathematical expression of growth, growth curve, measurement of growth and growth yield, synchronous growth, continuous culture

Gram positive and gram-negative organisms. Structure and functions of peptidoglycan in gram-positive and gram-negative organisms. Functions of polymeric components in outer membrane and acidic polymers in gram-negative organisms.

UNIT IV: **Biostatistics, Computers and Bioinformatics:-**Population, Sample, variable, parameter, primary and secondary data, screening and representation of data. Frequency distribution, tabulation, bar diagram, histograms, pie diagram, cumulative frequency curves. Mean median, mode, quartiles and percentiles, measures of dispersion: range, variance, standard deviation, coefficient of variation, symmetry: measures of skewness and kurtosis

Sample space, events, equally likely events. Definition of probability (frequency approach), independent events. Addition and multiplication rules, conditional probability, Examples Bernoulli, Binomial, Poisson and Normal distributions. Mean and variance of these distributions (without proof). Sketching of p.m.f. and p.d.f, Use of these distributions to describe in biological models. Model sampling and Simulation study. Scatter plot, correlation coefficient (r), properties (without proof), Interpretation of r, linear regression. Fitting of lines of regression, regression coefficient, coefficient of determination.

Use of random numbers to generate simple random samples with replacement and without replacement. Sampling distribution and standard deviation of sample mean. Stratified sampling and its advantages.

Hypothesis, critical region, and error probabilities. Tests for proportion, equality of proportions, equality of means of normal populations when variance known and when variances are unknown. Chi-square test for independence. P-value of the statistic. Confidence limits, Introduction to one way and two-way analysis of variance.

History of development of computers, Basic components of computers, Hardware; CPU,input, output, storage devices. Software; operating systems, Programming languages (Machine, Assembly and Higher level)

Introduction to MSEXCEL-Use of worksheet to enter data, edit data, copy data, move data. Use of in-built statistical functions for computations of Mean, S.D., Correlation, regression coefficients etc. Use of bar diagram, histogram, scatter plots, etc. graphical tools in EXCEL for presentation of data. Introduction to MSWORD word processor editing, copying, moving, formatting, Table insertion, drawing flow charts etc.

Introduction to Internet and use of the same for communication, searching of database, literature, references etc. Introduction to Bioinformatics, Databank search- Data mining,

Data management and interpretation, BLAST, Multiple sequence alignment, Protein Modeling, Protein structure Analysis, Docking, Ligplot interactions, Genes, Primer designing, Phylogenetic Analysis, Genomics and Proteomics.

UNIT V: **Scientific writing and Intellectual property right:-**

Objectives of research:

Motivation and objectives – Research methods and Methodology. Types of research– Descriptive and Analytical, Applied and Fundamental, Quantitative and Qualitative, Conceptual and Empirical.

Defining and formulating the research problem - Selecting the problem - Necessity of defining the problem - Importance of literature review in defining a problem – Literature review– Primary and secondary sources – reviews, treatise, monographs-patents – web as a source – searching the web - Critical literature review – Identifying gap areas from literature review - Development of working hypothesis.

Patent and Intellectual Property, Intellectual Property Areas: Patents, Trademarks and Copyrights, Parts of a Patent; The process of obtaining a patent; who can obtain a why obtain a patent?; Recent changes in IPR and patent policies.

Methodology of scientific research

The nature of scientific methods; Design of experiment, Review writing, Abstract, Discussion, References, Bibliography, Hypothesis, Data presentation in research paper, synopsis, concept note, Significance of statistical methods.

SECTION –B: BIOCHEMISTRY (Subject Content) **(50%** weightage)

UNIT I: Biomolecules and Metabolism

Biomolecules:-

Carbohydrates and their derivatives

Monosaccharides and related compounds, glycosidic bond, disaccharides, polysaccharides, heteropolysaccharides.

Lipids

Fatty acids, Phospholipids, Cholesterol and related steroids, other biological lipids. Glycerolipids, sphingolipids, the eicosanoids.

Nucleotides and nucleic acids

Genetic significance of nucleic acids, structural properties of DNA, chemical synthesis of DNA, conformational behavior of RNA, nucleoproteins.

Analysis of nucleic acids

Isolation of nucleic acids, radioactive labeling of nucleic acids, restriction endonucleases, plasmids, purification of complementary DNA strands, hybridization by blotting, determining base sequence of DNA, preparation of DNA complementary to RNA.

Amino acids, peptides and polypeptides

Amino acids, peptides and polypeptides, determination of amino acid composition of proteins, determination of amino acid sequence of proteins, chemical synthesis of peptides and polypeptides.

3-D structure of proteins

Information for folding, forces that determine folding, hierarchy of structural organization, functional diversification of proteins.

Characterization and purification of proteins

Methods of protein characterization, methods of protein purification.

Centrifugation, Dialysis, Lyophilization, Ultrafilteration, Chromatography, Electrophoresis,

Metabolism:-

Carbohydrates metabolism:

Glycolysis, citric acid cycle its function in energy generation and biosynthesis of energy rich bonds, pentose phosphate pathway and its regulation. Alternate pathways of carbohydrate metabolism.Gluconeogensis, interconversions of sugars.Biosynthesis of glycogen, starch and oligosaccharides. Regulation of blood glucose homeostasis. Hormonal regulation of carbohydrate metabolism.

Lipids metabolism

Fatty acid biosynthesis: Acetyl CoA carboxylase, Fatty acid synthase, desaturase and elongase. Fatty acid oxidation: (x, 0, w oxidation and lipoxidation. Lipid Biosynthesis: Biosynthesis of triacyglycerols, phosphoglycerides and sphingolipids, Biosynthetic pathways for terpenes, steroids and Prostaglandins. Ketone bodies: Formation and utilisation. Metabolism of Circulating lipids: chylomicrons, LDL, HDL and VLDL. Free fatty acids. Lipid levels in pathological conditions.

Amino Acids metabolism

Biosynthesis and degradation of amino acids and their regulation. Specific aspects of amino acid metabolism. Urea cycle and its regulation, In-born errors of amino acid metabolism.

Nucleic Acids

Biosynthesis or purines and pyrimidines, Degradation if purines and pyrimidines

Regulation of purine and pyrimidine biosynthesis, Structure and regulation of ribonucleotide reductase.

Biosynthesis of ribonucleotides, deoxyribonucleotides and polynucleotides

Inhibitors of nucleic acid biosynthesis

UNIT II: Enzymology and Physiology:-

Enzymology:

Review of unisubstrate enzyme kinetics and factors affecting the rates of enzyme catalyzed reactions. Michaelis pH functions and their significance.

Classification of multisubstrate reactions with examples of each class. Kinetics of multisubstrate reactions. Derivation of the rate of expression for Ping Pong and ordered Bi Bi reaction mechanism. Use of initial velocity, inhibition and exchange studies to differentiate between multisubstrate reaction mechanisms.

Concept of Convergent and Divergent evolution of enzymes.

Methods of examining enzyme-substrate complexes.

Flexibility and conformational mobility of enzymes.

Methods for measuring kinetic and rate constants of enzymic reactions and their magnitudes.

Enzymes Turnover and methods employed to measure Turnover of enzymes. Significance of enzymes Turnover.

Protein - Ligand binding, including measurement, analysis of binding isotherms. Cooperativity phenomenon. Hill and Scatchard Plots.

Allosteric enzymes, Sigmoidal kinetics and their physiological significance. Symmetric and sequential modes for action of allosteric enzymes and their significance.

Immobilized enzymes and their industrial applications. Effect of partition on kinetics and performance with particular emphasis on changes in pH and hydrophobicity.

Multienzyme system : Occurrence, isolation and their properties. Polygenic nature of multienzyme systems. Mechanism of action and regulation of pyruvate dehydrogenase and fatty acid synthetase complexes. Immobilized Multienzyme Systems and their applications.

Structure and function of coenzymes

Thiamine pyrophosphate, Pyridoxal phosphate, Nicotinamide, flavins, phosphopentetheine, alfa-lipoic acid, biotin, folate, vitamin B12, Iron containing coenzymes, coenzymes in methanogenesis.

Porphyrines

Porphyrin nucleus and classification, important metalloporphyrins occuring in nature, detection of porphyrins Spectrophotometry, bile pigments, chemical and physiological significance by and fluoroscence.

Mechanisms of enzyme catalysis

Enzyme catalyst and other chemical catalyst, unique features of enzyme catalysts, trypsin family of enzymes, chymotrypsin catalytic mechanism, carboxipeptidase A, pancreatic RNAase A, lysozyme, lactate dehydrogenase.

Physiology:

Blood: Composition and functions of plasma, erythrocytes including Hb, leukocytes and thrombocytes plasma proteins in health and diseases.

Blood coagulation - mechanism and regulation. Fibrinolysis, transfers of blood gases oxygen and carbon dioxide. Role of 2,3 DPG, Bohr effect and chloride shift.

Hydrogen ion homeostasis- Factors regulating blood pH - buffers, respiratory and renal regulation. Acid-base balance - metabolic and respiratory acidosis and alkalosis.

Digestive system: Composition, functions and regulation of saliva, Gastric, pancreatic, intestinal and bile secretions - digestion and absorption of carbohydrates, lipids, proteins, nucleic acids, minerals and vitamins.

Excretory system: Structure of nephron, formation of urine, glomerular filtration, tubular re-absorption of glucose, water and electrolytes - tubular secretion.

Regulation of water and electrolyte balance, role of kidneys and hormones in their maintenance.

The endocrine glands: secretion and function - reproduction, pregnancy and lactation. Biochemistry of vision.

UNIT III: Cell biology, genetics and molecular biology:-

Cell Biology:

Cell classification: Cell variability (size, shape, complexity, functions).

Structural Organisation of prokaryotic and eukaryotic cells. The ultra structure of nucleus, mitochondria, endoplasmic reticulum, rough and smooth, Golgi apparatus, lysosomes and peroxisomes and their functions. Plant and animal cells: variation in structure and function.

The cytoskelton - microtubules and microfilaments.

Types of tissues, epithelium - types, epithelial apices - glycocalyx, microvilli. The basement membrane - structural features and characteristics. The extracellular matrix-collagen, elastin, fibrillin, fibronectin, laminin and protroglycans.
Culture techniques to study cell division - cell division by mitosis and meiosis. Cell cycle.
Cell differentiation - organogenesis, morphological, functional and biochemical maturation of tissues.Biochemistry of cancer - carcinogenesis, characteristics of cancer cell, agents promoting carcinogenesis.
Genetics: Mendelian laws and basic genetics
Molecular Biology:
DNA replication
Semiconservative replication of DNA, DNA synthesis in prokaryotes, replication of a viral chromosome, replication of the E. coli chromosome, DNA synthesis and chromosomal replication in eukaryotes, control of DNA replication, specific inhibitors of DNA replication, DNA degradation, DNA repair, DNA recombination.
RNA synthesis
Different classes of RNA, DNA dependent synthesis of RNA, eukaryotic transcription, other RNA synthesis, posttranscriptional modification and processing of RNA, Degradation of RNA by ribonucleases, inhibitors of RNA metabolism.
Protein synthesis
The cellular machinery of protein synthesis, steps in translation, deciphering the genetic code, code word assignments, inhibitors of protein synthesis, posttranslational modifications of proteins, intracellular protein degradation, lysosomes and protein degradation.
Regulation of gene expression in prokaryotes
Regulation of gene expression in *E. coli*, regulation of gene expression in bacterial viruses.
Regulation of gene expression in eukaryotes
Gene regulation in unicellular eukaryotes, gene regulation in multicellular eukaryotes, regulatory phenomena associated with development.

UNIT IV: Plant Biochemistry and Biotechnology
Structure and functions of plant cell (including cell wall, plasmodesmata, meristematic cells, vacuoles, secretary systems and root quiescent zone), Isolation of cell organelles, absorption, adsorption and transport of water and ions in plants. Evapotranspiration.
Photosynthesis - structure of organelles involved in photosynthesis in plants and bacteria. Proton gradients and electron transfer in chloroplasts of plants and in purple bacteria - differences from mitochondria. Light receptors - chlorophyll, light harvesting complexes, bacteriorhodopsin, rhodopsin as ion pump.
Photosystems I and II, their location, mechanism of quantum capture and energy transfer between photosystems - ferridoxin, plastocyanin, plastoquinone, carotenoids.
The Hill reaction, photophosphorylation and reduction of CO2.
C_3 C^4 and CAM metabolism, light and dark reactions. Light activation of enzymes, regulation of photosynthesis. Photorespiration.
Biological nitrogen fixation and ammonia assimilation.
Nitrate and sulphate reduction and their incorporation into amino acids.
Translocation of inorganic and organic substances.
Secondary plant metabolism

Special features of secondary plant metabolism, formation of phenolic acids, tannins, lignins, lignans, pigments, terpenes, terpenoids, plant phenolics, alkaloids and surface waxes - their biosynthesis and functions, cell wall components.

Plant hormones - Growth regulating substances and their mode of action. Molecular effects of auxin in regulation of cell extension and of gibberellic, abscisic acids and cytokinins in the regulation of seed dormancy, germination, growth and development, and embryogenesis.

Biochemistry of seed development and fruit ripening.

Defence system in plants.

Tissue culture and transgeneic plants.

Plant pathogens and integrated defense response to infection

Gene silencing as an adaptive defense

Natural products and plant disease resistance

Programmed cell death

Hypersensitive response of plants and mitochondrial function

Biochemistry of airborne signals during plant defense

Co-evolution and plant resistance to natural enemies

Production of transgenic organisms: microbes

Producing proteins in bacteria and fungi

Methods to produce transgenic plants

Herbicides, manipulating herbicide tolerance in plants

benefits and problems of herbicide tolerance in plants

Plants with increased resistance to insects

Biotechnology and tomato, genetic modifications of foods, oils and starches

Improving plant tolerance to environmental stress

Production of transgenic animals

Release of transgenic organisms in the environment

Safety and regulation of genetically engineered food

UNIT V: Nutritional and Clinical Biochemistry

Nutrition:

Basic Concepts: Composition of human body. Energy content of foods. Measurement of energy expenditure: Direct & indirect calorimetry. Definition of BMR and SDA and factors affecting these. Thermogenic effects of foods. Energy requirements of man and woman and factors affecting energy requirements.

Carbohydrates: Dietary requirements and sources of available and unavailable carbohydrates. Physico-chemical properties and physiological actions of unavailable carbohydrates (dietary fibre).

Proteins: Protein reserves of human body. Nitrogen balance studies and factors influencing nitrogen balance. Essential amino acids for man and concept of protein quality Cereal proteins and their limiting amino acids. Protein requirement at different stages of development.

Lipids: Major classes of dietary lipids. Properties and composition of plasma lipo- proteins. Dietary needs of lipids. Essential fatty acids and their physiological functions.

Electrolytes and water balance: Electrolyte concentrations of body fluids. Acid base regulation by the human body. Concept of metabolic and respiratory acidosis and alkalosis.

Minerals: Nutritional significance of dietary calcium, phosphorus, magnesium, iron, iodine, zinc and copper.
Vitamins: Dietary sources, biochemical functions and specific deficiency diseases associated with fat and water-soluble vitamins. Hypervitaminosis symptoms of fat- soluble vitamins.
Nutritional requirements during pregnancy, lactation and of infants and children.
Processed Food: Food processing and loss of nutrients during processing and cooking
Anti-nutrients: Naturally occurring food born toxicants: Protease inhibitors, Hemagglutins, Hepatotoxins, Allergens, Oxalates, Toxins from Mushrooms, Animal food stuffs and sea foods.
Protein energy malnutrition (PEM): Aetiology, clinical features, metabolic disorders and management of Marsmus and Kwashiorkar diseases.
Starvation: Techniques for the study of starvation. Protein metabolism in prolonged fasting. Protein sparing treatments during fasting. Basic concept of High protein, low caloric weight reduction diets.
Obesity: Definition and classification. Genetic and environmental factors leading to obesity Obesity related diseases and management of obesity. Role of leptin in regulation of body mass.
Clinical nutrition: Role of diet & nutrition in the prevention and treatment of diseases: Dental caries, Fluorosis, Renal failure, Hyperlipidemia, Atherosclerosis & Rheumatic disorders, Inherited metabolic disorders: Phenyl ketonuria, Maple syrup disease. Hemocystinuria, Galactosemia, Gout, Diabetes Insipidus and Diabetes Mellitus.
Food allergy: Definition, Role of antigen, host and environment. Types of Hypersensitivities. Diagnosis and management of allergy.

Clinical Biochemistry:
Introduction to laboratory principles and instrumentation in Clinical Biochemistry.
Automation in the Clinical Biochemistry
Instrumental concepts
Chemical reaction phase
Measurement approaches
Selection of instruments.
Quality Assurance
Control of Pre-analytical variables
Control of analytical variables
External and internal quality control measurements.
Disorders of Carbohydrate Metabolism
Diabetes mellitus
Glycohemoglobins.
Hypoglycemia's.
Ketone bodies
Various types of glucose tolerance tests. Glycogen storage diseases
Galactosemia
Lipids, Lipoproteins and Apolipoproteins
Physiology of lipids/lipoproteins, lipidosis
Clinical inter-relationships of lipids (sphingolipidosis, multipisclerosis), Lipoproteins and apolipoproteins.
Diagnostic tests for apolipoproteins, HDL-cholesterol, LDL-cholesterol and triglycerides disorders.

Disorders of Amino Acid Metabolism

Phenylalaninemia, homocystineuria, tyrosinemia and related disorders, aminoacidurias.

Evaluation of Organ Function Tests

Assessment and clinical manifestations of renal, hepatic, pancreatic, gastric and intestinal functions, bilirubin metabolism.

Clinical presentation and diagnosis of various organ diseases.

Diagnostic Enzymes: Aspartate aminotransferase, Alanine aminotransferase, Creatine kinase Aidolase, Lactate dehydrogenase

Enzyme tests in determination of myocardial infarction, pancreatitis, biliary diseases

Hormonal Disturbances

Protein hormones, anterior pituitary hormones, posterior pituitary hormones, steroid hormones, adrenocortical steroids, reproductive endocrinology, thyroid function.

Disorders of Mineral Metabolism

Hypercalcemia, hypocalcemia, normocalcemia, hypophosphatemia, hyperphosphatemia.

Detoxification Mechanism in the Body

Enzymes of detoxification - polymorphism in drug metabolizing enzymes.

Detection of toxic substances by specific procedures.