Syllabus for Ph.D Entrance Test in Clinical Biochemistry, DMIMS University

Theme 1: Proteins & Enzymology

Unit A : Protein Biochemistry

Proteins and Mass Spectrometry: Overview of protein structure, protein folding, Ramchandran plot, domains and modules, binding sites within proteins, protein sequencing.

Basics of Mass Spectrometry, Ionization mechanisms- protonation, deprotonation, cationization, transfer of charged molecules to gas phase, electron ejection, electron capture, Mass analyzers-TOF, Ion trap, Quadrupole, Ionization methods-Electron Impact (EI), Chemical Ionization (CI), Fast Atom Bombardment (FAB), Field Description (FD), Electron Spray Ionization (ESI), Matrix Assisted Laser Desorption Ionization (MALDI), Protein Identification using MS.

Protein biosynthesis: Eukaryotic translation machinery, structure and assembly of the ribosome, initiation, elongation and termination of translation. Various factors involved in initiation, elongation and termination of translation. Formation of aminoacyltransfer RNA complex. Regulation of translation at genetic level.

Protein sorting and degradation: Intracellular protein sorting, movement of proteins between cellular compartments: gated, transmembrane and vesicular transport. Protein transport and translocation to nucleus, mitochondria, chloroplast, peroxisomes, endoplasmic reticular system. Protein degradation.

Protein Engineering : Design and construction of novel proteins and enzymes, Conformation of proteins in general and enzymes in particular, Effect of amino acids on structure of proteins, Energy status of a protein molecule, Structure function relations of enzymes, Physical methods such as x-ray crystallography for determination of protein structure, Site directed mutagenesis for specific protein function, Basic concepts for design of a new protein/enzyme molecule, Specific examples of enzyme engineering, -Dihydrofolate reductase and Subtilisin.

Unit B : Advanced Enzymology

Kinetics and Regulation of enzyme activity : Review of unisubstrate enzyme kinetics, multisubstrate enzyme kinetics, Co-operativity phenomenon, Hill and Scatchard plots, protein-ligand binding and its measurement, detailed mechanism of catalysis of serine protease, carbonic anhydrase, and PEP kinase, Metalloenzymes

Allosteric enzymes and multienzyme systems : Allosteric enzymes, sigmoidal kinetics and its physiological significance, symmetric and sequential modes of action and their significance, immobilized enzymes and their industrial applications, study of multienzyme complexes with respect to occurrence, isolation and their properties and polygenic nature eg. pyruvate dehydrogenase and fatty acid synthase.

Enzyme regulation : General mechanisms of enzyme regulation: Feedback inhibition and feed forward stimulation, repression and induction of enzymes, reversible and irreversible covalent modifications of enzymes, flexibility and conformational mobility of enzymes, convergent and divergent evolution of enzymes.

Bioenergetics and oxidative phosphorylation : Energy transformation, laws of thermodynamics, Gibbs energy, free energy change, redox potentials, phosphate potential, ion electrochemical potential, proton electrochemical potential, membrane potential, Chemi-osmotic theory, mitochondrial

respiratory chain, order and organization of carrier proton gradients, Characterization of Iron- Sulphur proteins and Cytochromes, Q cycle, Respiratory controls and oxidative phosphorylation, ATP synthase complex.

Unit C: Cell Biochemistry

Cell cycle and regulation : Review of cell cycle, divisional control, regulatory proteins, cyclin/cdk complexes, positive and negative regulation, inhibitory molecules, restriction points, regulation of DNA synthesis, regulation of degradation, check points, cell cycle arrest, role of cyclically activated protein kinases, transcriptional regulation.

Cell communication I: General principles of cell communication, extra cellular signals and their receptors, autocrine signaling and role of gap junctions, types of cell receptors, relay of signal and intracellular signal proteins, regulated proteolysis dependent signaling pathways (Notch, Wnt, hedgehog, NFKB)

Cell communication II: Informational transactions in eukaryotic cells- cyclic AMP facet, Study of G-proteins, signaling through G-protein linked cell surface receptors, signaling through enzyme linked cell surface receptors, Calcium messenger system, signaling via GMP.

Cancer: Causes and types of cancer, viral carcinogenesis, tumor suppressors, oncogenes and signal transduction, growth and spread of cancer, molecular basis of cancer therapy, molecular markers. Programmed cell death and its regulation.

Theme 2: Immunology
Unit A : Immunology

The Immune system, Immunoglobulins and TCR : History of Immunology, Innate Immunity: effector mechanisms involved; PAMPs, PRRs, Phagocytosis, Lysis, blocking, extra cellular killing etc . Biochemistry and biology of TLRs, Inflamatory processes, inflamamasomes interrelationship between innate and adaptive immunity.

Immunoglobulins and TCR : Immunochemistry: Antigen antibody reaction, its kinetics and thermodynamics; Structure, functions of immunoglobulins; Ig genes and their expression, Generation of Ab diversity. BCR, TCR, Organization and re-arrangement of TCR genes, TCR diversity.

Anatomy of Immune system : Immunological cells, tissues and organs. Maturation, activation and differentiation of B and T cells. MHC genes and their polymorphism, Structure and function of MHC molecules. Clonal selection theory Cell surface molecules : Ig super family, integrins , selectins , chemokine receptors and other accessory molecules, Cytokines and chemokines

Immune response by T and B lymphocytes : Cellular and molecular mechanisms of Ab production, humoral and cell mediated immunity, Antigen processing and presentation, T and B cell interaction. Super antigens.

Immunological Techniques : Immunochemical techniques including immunodiffusion, RIA, EIA, agglutination, immunoflorescence, immunoelectron microscopy, immunoelectrophoresis. HLA typing, leukocyte migration inhibition technique, delayed hypersensitivity technique, cytotoxicity assay. Monoclonal Ab's, hybridoma and other technologies, Abzymes.

Unit B : Advanced Immunology

Complement system: Alternative and Classical pathway of complement activation

Immune networks: Homeostasis in the immune system-termination of normal immune responses, network hypothesisIn vivo immunity to viruses, bacteria, fungi, protozoa, worms etc

Immunological tolerance and Autoimmunity : Immunologic tolerance, T lymphocyte tolerance-central and peripheral, Apoptosis in Lymphocytes-pathways and biochemical mechanisms, effector mechanisms, Tolerance induced regulatory T cells, B lymphocyte tolerance- Central and Peripheral, Homeostasis in the immune, pathogenesis and therapeutic approaches to autoimmunity.

Tumor and Transplantation Immunology, Hypersensitivity : General features of tumor immunity, tumor antigens, Immune response to tumor and evasion, Immunotherapy, Types of hypersensitivity, Effector mechanisms of immunologic tissue injury and disease.

Immunodeficiency and Vaccinology : MHC and disease susceptibility, immune deficiency disorders, Active immunization (immune prophylaxis), passive immunization, adjuvants, modern approaches to vaccine development, role of vaccines in the prevention of disease.

Theme 3: Clinical Biochemistry

Unit A : Clinical Biochemistry

Automation in clinical biochemistry, gastric and blood disorders : Automation in Clinical Biochemistry- Instrumental concept, Selection of Instrument, Quality assurance, Control of pre-analytical and analytical variables, External and internal quality control measurements.

Gastric disorders: Disorders of gastric function, method of evaluation, pancreatic diseases, Steatorrhoea, Malabsorption syndrome test for their evaluation.

Blood Disorder: Review of mechanism of coagulation and fibrinolysis, abnormalities in blood coagulation, variation of plasma proteins, abnormalities of blood formation, anemia, haemoglobinopathies, clinical significance of fecal and urine analysis.

Endocrinology I: Insulin and glucagon: Various types of hyperglycemia, Diabetes mellitus Ketonemia ,ketonuria , Experimental diabetes , Hypoglycemia, Polyurea, Glucose tolerance test.Thyroid: Iodine metabolism, Hypo and Hyper thyroidism, B.M.R.and other test for evaluation of thyroid function.Parathyroid: Calcium and phosphorus metabolism. Abnormalities of Parathyroid function and methods of evaluation.

Endocrinology II : Adrenal: Addison's disease and pheochromocytoma, Disorders of steroid metabolism, Test for evaluation of adrenal functions.

Pituitary: Pituitary hormones, Clinical syndromes and their evaluation.

Liver disorders:

Liver disorders: Jaundice, fatty liver and liver function tests. Renal function test.
Cerebrospinal fluid: Composition in health and disease. Lipid profile in health and disease.
Elements of Clinical Enzymology: Isoenzymes in health and disease.
Clinical significance of GOT, GPT, Creatine kinase, LDH etc.
Biochemical diagnosis of disease by enzymatic evaluation.

Unit B : Advanced Clinical Biochemistry

Aging and Neurological Disorders : Current view and theories of aging, auto immune connection and HLA association, processes of aging and biochemical alteration, DNA damage, protein oxidation and axonal transport in aging, nutritional intervention as anti-aging therapy.Alzheimer's disease: Causes, symptoms, diagnosis, pathogenesis, genetics, APP, ApoE, PS2, tau protein, risk factors and therapeutic approaches.Progeria.Parkinson's disease: Causes, symptoms, diagnosis, pathogenesis, genetics and therapeutic approaches

Obesity: Theories, lipid metabolism, adipose tissue anomalies. Genetic basis of familial obesity, effects of neuropeptides and leptin in nutrient partitioning. Obesity related derangements in metabolic regulation. Therapeutic approaches

Molecular and Metabolic Diseases: Human gene map, genetic diversity, polymorphism, genetic linkage, chromosomal disorder. Monogenetic Disorders: Autosomal dominant, autosomal recessive, X-linked, Multifactorial disorders, Genetic heterogeneity. Allelic heterogeneity, Pathogenesis of genetic disease, Galactosemia, Hemophilia, Sickle cell anemia, Muscular dystrophy, Hypercholesterolemia, Gout, Turner's syndrome

Reproductive Biochemistry: Overview of reproductive system and reproduction, biochemistry of reproductive disorders (male & female), Influence of various factors in reproduction with special reference to role of prostaglandins and gonadotrophins. Mechanism and methods of birth control and possible biochemical consequences thereof. Biochemical marker's in infertility disorders. Techniques involved in assisted reproductive technology (ART). Culture media and cell culture techniques in ART programme.

Unit C : Applied Nutritional Biochemistry

Clinical nutrition: Role of a dietician: role and responsibilities of a dietician, nutrition counseling, professional ethics and obligations. Diet therapy-Rationale for diet therapy (normal diet, modifications of the diet to the light diet, soft diet, full liquid diet, clear liquid, tube feeding); Routes for diet therapy-enteral and parental; use of biochemical parameters in the planning of diets, use of computers in the planning of diets.

Nutritional Counselling and Dietetics : Nutritive value of different food groups and changes due to cooking in various food groups. Storage of foods, food quality and factors affecting food quality. Control of food quality. Use of additives, Classification and applications.

Community Nutrition : Opportunities in community Nutrition, Assessing Community Resources, assessing target population, program planning, Assessing community's nutritional resources, Food insecurity, food assistance programs, world hunger and food insecurity. Principles of nutrition education and policy making, national nutrition agenda

Applied Nutrition and Public Health : Concept of Health, Nutrition and Public Health Nutrition, Demographic trends in India and the significance of certain indices of Health and Nutrition situation of a community IMR, MMR, TFR, Birth rate, Death rate, Life expectancy etc.) Major nutritional problems in developing countries. Dietary surveys-methods, ways of interpretations and analysis, recommendations based on survey findings. Assessment of nutritional status: biochemical and anthropometry.

Theme 4: Molecular Biology

Unit A: Basic Molecular Biology

Eukaryotic and Prokaryotic chromosomes

Chromatin structure: Histones, DNA, nucleosome morphology and higher level organization; Functional states of chromatin and alterations in chromatin organization. **Chromosome organization:** Metaphase chromosomes: centromere and kinetochore, telomere and its maintenance; Holocentric chromosomes; Heterochromatin and euchromatin, position effect variegation; Chromosomal domains (matrix, loop domains) and their functional significance.

Organization of nuclear and organellar genomes; C-value paradox, Repetitive DNA-satellite DNAs and interspersed repeated DNAs, Transposable elements, LINES, SINES, Alu family and their application in genome mapping

Concept of a gene: Conventional and modern views. Fine structure of gene, split genes, pseudogenes, non- coding genes, overlapping genes and multi-gene families.

Replication and Recombination

Replication: Review of replication in bacteria, plasmid and viruses, Models of DNA replication. DNA replication in eukaryotes. Eukaryotic DNA polymerases and their roles, origin of replication, Autonomously Replicating Segments (ARS) in yeast, elongation, lagging strand synthesis, and termination.

Recombination: DNA recombination: Homologous, site specific and transposition, Homologous recombination: Holliday Model, Messelsson -Radding Model, Rec BCD pathway. Site specific recombination: Lambda phage ingration, and excision earrengement,of immunoglobulin genes. Transposition: Prokaryotic transposition, Insertion sequence,s, and mere complex transposons (eg Tn3), conservative and replicative transposition. Eukaryotic transposable elements: yeast and Drosophila transposons, retroviruses, and retrovirus like elements.

Transcription

Review of prokaryotic transcription, transcription in eukaryotes: Eukaryotic RNA polymerases and their subunit structure, Class I, II and III promoters, Upstream elements, enhancers and silencers, General transcription factors, Class I, II, III genes and their functions elongation factors, TBP structure and its role in transcription, mediators. Structure of transcription activators, zinc fingers, homeodomains, helix loop helix, bZIP, beta barrels.

Unit B : Advanced Molecular Biology

Regulation of eukaryotic gene expression at transcriptional level Overview of transcription by RNA Polymerases I, II, and III Anatomy of a protein-coding gene. Basal transcription by RNA polymerase II: Subunits of Pol II; general transcription factors; Activators, How the initiation complex is assembled, How initiation occurs. Speeding up the process: Enhancers, TAF's and how they work

Regulated transcription: transcription factors: Zinc-fingers (Sp1; the first such factor identified) Leucine zippers, Basic helix loop helix, Homeodomains, DNA binding domains, Activating domains RNA Elongation: HIV TAT/TAR. RNA polymerase III and regulation of 5S rRNA

Regulation of eukaryotic gene expression at post translational, translational and post-translational levels : Regulation at tpst-transcriptional level: Effect of cap and polyadenylation on splicing, trans and alternative splicing, RNA editing, mRNA stability and transport. Regulation at Translational level: Global regulation through eIF2 and eIF4E/eIF4E-BP. Specific regulation through 5' UTRs using RNA structure e.g. ODC. Specific regulation through 5' UTR/protein interactions e.g. ferritin in eukaryotes and ribosomal proteins in prokaryotes. Specific regulation through 3' UTRs e.g. 15-LOX. Regulation at post-translational level: Control of the level of active proteins, regulation of proteolysis

Regulatory RNAs: Historical background, RNA interference as regulatory mechanism in eukaryotes Slicer and dicer, synthesis and function of RNAi molecules in plants, chromatin remodeling in human disease and diagnosis.

Epigenetics : Background, chromosomal inheritance taking fission yeast as an example, DNA methyltransferases, DNA methylation maintenance, histone modification and regulation of chromatin structure, bivalent histones, DNA demethylation, histone demethylation.

Theme 5: Biotechnology and Bioresearch

Unit A : Biotechnology

rDNA technology : Genomic and cDNA libraries, DNA manipulation enzymes, isolation of specific genes.Gene cloning: REs, vectors-plasmids, cosmids phage vectors, M13 phage vectors, phagemids expression vectors with strong promoters, inducible, vectors produce fusion proteins and their isolation, Eucaryotic expression system, shuttle vectors, YAC, BAC insertion of DNA and its ligation to carrier DNA, introduction of DNA in cells, gene synthesis, gene libraries. Application of recombinant DNA technology in medicine, agriculture industry and environmental sciences.

Gene control systems in bacteria and bacteriophage lambda: Mechanism of induction and repression, constitutive expression various control mechanisms, positive regulation, negative regulation, attenuation, operon hypothesis with special reference to mal/gal, ara and histidine operons, Regulatory mechanisms in bacteriophage lambda.

Regulation of gene expression at various levels (transcription, post transcriptional and translational) DNA-protein interactions: Lambda family of repressor, trp repressor.

Biochemical engineering: Biochemical Engineering: Bioreactors and related equipment and instrumentation, types of bioreactor (Batch, semi batch, CSTF, recycle etc), reactor analysis, reactor design, reactor for recombination proteins.

Fermentation technology: Fermentation technology, microbial culture reaction, genetic modification, use of mutants, recombinant DNA technology and application in fermentation technology, microbial growth kinetics, sterilization, fermentation process kinetics, analysis of rate pattern and kinetic groups, fermentation process types, control of environmental variables, recovery of fermentation products, isolation and purification and use of immobilization techniques.

Bioinformatics and Drug Designing: Introduction to Bioinformatics: Applications of Bioinformatics, Bioinformatics resources. Biological Databases: Overview to Biological Databases, Nucleotide Databases (GenBank, DDBJ, ENA), Protein sequence databases (Uniprot, Swiss prot, Prosite , Pfam, Prodom), Protein structure databases (PDB, SCOP, CATH). Sequence analysis: Sequence similarity search, BLAST, FASTA, CLUSTAL.

Genomics: Introduction to Genomics, Comparative Genomic Databases, Objective of Genome Comparisons, Genome Alignments

Proteomics: Overview of Proteomics, Experimental Techniques, Bioinformatics Approaches, Protein-Protein

Interaction, Databases and software. Software's for Drug Designing. Structure Based Drug Designing, Ligand Based Drug Designing.. Virtual Screening. Homology Modeling and Chimera Generation

Unit B : Biochemical Research Techniques

Research Methodology:

1. Introduction to research methodology: History and definition of research, role of theory, hypothesis, sampling, variables, randomness, selection of problem, purpose of research and research reporting
2. Experimental research: Early experimentation, experimental groups, control groups, variables, method of controlling variables, designing and validation of experiments
3. Methods and tools in research: Research tools and its reliability and validity, quantitative and qualitative studies, observation, inquiry forms, Q methodology, data collection, limitations and sources of error
4. Types of data Analysis: Descriptive data analysis, Inferential data analysis, Computer data analysis

Biostatistics : Principles and practice of statistical methods in biological research, samples and populations, Basic statistics-average, statistics of dispersion, coefficient of variation, confidence limits, Probability distribution, normal, binomial and Poisson distribution. Mean variants, standard deviations and standard error, correlation and regression, test of statistical significance, and analysis of variance and covariance.

Data Retrieval, Scientific Writing and Presentation

1. Information Search & Data Retrieval, Tools for Web Search , Data Retrieval Tools, Data Mining of Biological Databases.
2. Report Writing, Significance of report writing, different steps in report writing, types of report, layout of research paper.
3. Mechanics and precautions of writing research reports for scientific journals, popular magazines, seminars/symposia/ conferences/workshops, poster session.
4. Presentation– Oral & Written Presentations in classrooms, scientific meets & public audience. Defence of research thesis.

Computers: Introduction of computer networks- Topologies and designs; Basics of computer operating systems-windows and Linux; Introduction to Markup language-Hyper Text Markup Language (HTML) and Extensive Markup Language (XML); Spreadsheets and Presentation software.

Systems Biology-An introduction. Introduction to Metagenomics.

Unit C : Bioresearch Techniques

Flow cytometry : Principles of flow cytometry, Instrument overview, principle of fluorescence, sample preparation, data analysis and applications of flow cytometry. Overview, Fludics, Generation of Scatter and fluorescence (Optical bench, optical filters, signal detection, Treshold), Data Analysis, (Data Collection and Display, gating, data analysis for subsetting applications, Data analysis for their applications) Sorting, Lasers, and Alignment (Working of lasers and laser alignment)

Animal cell culture techniques : Animal cell Culture: Cell culture (adherent and suspension), basic equipment, cell culture media-Components, sterility, buffering capacity, growth requirements, supplementation of serum antibiotic and antimycotic agents, preparation of medium, advantages and limitations of Primary cell culture clonal cell lines, basic technique a of animal cell, subcultuing disaggregation, method for quantitation of cells in culture, counting chamber, counters, cell viability determination, cytotoxicity assay and its applications, cell apoptosis assay and its applications, 3 D cultures.

DNA techniques: Isolation, Sequencing, Restriction Nucleases, Gel Electrophoresis, DNA probes Nucleic acid hybridization: Southern blotting, DNA fingerprinting and DNA typing, DNA Library, DNA sequencing: Sanger and Maxam Gilbert, Restriction Mapping, DNase foot printing, DMS foot printing, knockouts PCR: RFLP, RAPD, AFLP, SNP

RNA techniques: Isolation, Hybridization, Northern Blotting, in vitro labelling with radioisotopes and chemical markers, Mapping and quantifying transcripts: S1assay, primer extension, run off transcription. Transcription rate measurement in vivo: Nuclear run on transcription, reporter gene N transcription. si RNA technology/ gene silencing techniques, its applications, microarrays, ribozyme technology.

Biochemical methods : Co-immunoprecipitation, BiFC bimolecular fluorescence complementation, affinity electrophoresis, Pull down assays, label transfer, yeast two hybrid screens, phage display

Biophysical methods : Fluorescence polarization/anisotropy; Fluorescence correlation spectroscopy; FRET, BRET; 2D-FT NMR spectroscopy; Protein–protein docking , Isothermal Titration Calorimetry (ITC); Isothermal Titration Calorimetry (ITC)

Unit D : Clinical Research

Pre-Clinical Research: Animal studies, acute & chronic toxicity of drugs; regulations for number & types of animals, protocols for animal experimentation. Biochemical & histopathological studies of animals after drug administration. Mechanism & cause of death. Routine toxicity studies & special toxicity studies; carcinogenicity, mutagenicity & teratogenicity to be given special emphasis.

Phases of Clinical Research : Phases of clinical research, number of volunteers in phase I & types of patients in phase II to IV. Importance of these investigations. Post marketing surveillance (PMS) & pharmacovigilence in case of clinical investigations after marketing authorization. Importance of ethical committee, protocol design, documentation in clinical trials. Bio Availability and Bio Equivalence studies Pharmacokinetics, Pharmacodynamics, Genomic studies

Good lab practices : Good Clinical Practices, Good Manufacturing Practices and Good Laboratory Practices Principles of ICH-GCP, History GCP, declaration of Helsinki, Belmont report, Nuremberg code, Tuskgee trial. Schedules Y and its amendments, ICMR Guidelines Composition, functions & operations of IRB/IEC ethics of clinical trials Health Authorities- CDSCO, US-FDA, EMEA and other

Roles and Responsibilities of different stake holders in Clinical Research-Sponsor, CRO, SMO, Ethics Committee, Investigator, CRA, CRC, Patients and other, Departments in Clinical Research-Operations, Business Development, Regulatory, Pharmacovigilance, Data Management, Centralized Monitoring, Quality, Finance. Important documents in Clinical Research and importance of documentation in Clinical Research. Stages in clinical trial-Feasibilities, PSSV, SIV, SMV and Close-out visits, Audits and Inspections