

**BHARATHIAR UNIVERSITY: COIMBATORE-641046**  
**M.PHIL./Ph.D. - (FT/PT) – BIOCHEMISTRY**

**PART I: SYLLABUS**  
**(For the candidates admitted from the academic year 2018-19 onwards)**

**PAPER- I RESEARCH METHODOLOGY**

**UNIT I: Biostatistics**

Scientific writing - Writing methodology, results and discussion.

Correlation & regression.

Sampling distribution- students T test,  $\chi^2$  test.

Experimental design – CRD,RBD.

Analysis of experimental results- ANOVA, Duncan's Multiple range test.

**UNIT II: Immunotechnology**

Fluorescent antibody assay- Histochemical localization

RIA-principle and applications.

ELISA techniques - Principles and applications.

Immuno radiometric Assay - Principles and applications.

Stem cells- Embryonic stem cells, Adult cells, stem cell therapy. Gene therapy.

**UNIT III: DNA Technology**

Flow cytometry- Principle, abnormal chromosome analysis, karyotyping, COMET assay DNA fragmentation analysis.

PCR Methodology- designs of primers- RTPCR, Q-PCR, PCR in genomic analysis and diagnostic application.

PFGE- Principles, techniques & applications.

**UNIT IV: Protein Technology**

Natural Products- Detection of bioactive molecules by gas chromatography.

Mass spectrometry - Principles and application.

HPLC and HPTLC - Principle, instrumentation and application.

Capillary Electrophoresis- Principles, instrumentation and application.

X-ray diffraction, NMR & their uses in protein structure prediction

Principle and Instrumentation-UV Visible Spectroscopy, Fluorescent Microscopy.

**UNIT V: Bioinformatics**

Biological data bases – DNA sequence data bases & protein sequence data bases.

Genome database- Mouse genome database .

SRS- Similarity searching pairwise sequence alignment- BLAST, FASTA

Dynamic programming-local and global alignment, Needleman alignment.

Multiple sequence alignment- Phylogeny.

Structure database- Secondary structure prediction, Choufeat passman, Neural network methods.

Predicting 3 dimensional folds (Threading), Homology modelling, Molecular docking.

## REFERENCE:

1. S.P. Gupta(2004). Statistical methods.Sulthan chand & sons, Educational publishers, New Delhi.
2. Janis Kuby(2000) Immunology 3<sup>rd</sup> edition.
3. Richard A.Goldsby (2000) 5<sup>th</sup> edition. Immunology W.H.Freeman & Co. Newyork
4. D.A.Skoog & J.J. Leary, 1992. Principles of Instrument analysis 4<sup>th</sup> edition, Saunders college Pub.
5. Rastogi C.S.(2003)Bioinformatics- Concepts, skills and applications. CBS Publishers.
6. David Freifelder (2003). Molecular biology 2<sup>nd</sup> edition. Narosa publishing house, New Delhi.

## PAPER- II ADVANCED PAPER IN BIOCHEMISTRY

### UNIT I

#### Hormonal Regulation of Metabolism

Nucleic acid metabolism: Purine and Pyrimidine metabolism

protein metabolism urea cycle, aliphatic and aromatic amino acid metabolism

Inborn errors of metabolism- Albinism, Phenylketonuria, Maple syrup urine disease and Homocystinuria.

### UNIT II

#### Cell Biology and Molecular Biology

RNA editing, mi RNA and RNAi, and its applications.

Antisense RNA technology,signal sequence. hypothesis protein glycosylation, Protein degradation- ubiquitin pathway.

General principles of cell communication G- Protein-coupled receptors-structure and functions, cAMP and others second messengers –phosphotidyl inositol, diacyl glycerol,inositol1,4,5 triphosphate  $Ca^{2+}$ ; Receptor Tyrosine Kinases- structure and functions, importance of Ras, MAP Kinase cascade.

### UNIT III

#### Genomics and Proteomics

Human Genome project - history, techniques and applications; NGS-principle and applications.

Anatomy of Prokaryotic and Human Genome; Genetic mapping and genetic markers- RFLP, Mini- and Micro satellite, STS and EST, SSCP,R APD, AFLP, SNPs. Analyzing Gene expression - DNA micro array. Epigenetics.

Proteome analysis- 2D gel electrophoresis; Protein-protein interactions- yeast two hybrid system and protein microarrays.

### UNIT IV

#### Biotechnology

Plant Biotechnology- Micro propagation, Organogenesis, somatic embryogenesis, artificial seeds, Haploidy: Protoplast fusion and Somatic hybridization. Transgenic plants.

Animal Biotechnology- Primary cell culture and cell line, tissue culture, organ culture; three Dimensional culture; Feeder layer; cell separation; cell synchronization; cryopreservation and revival, Transgenic animals.

## **UNIT V**

### **Intellectual Property Rights (IPR) and Bioethics**

Introduction to IPR and Patent.

World Trade Organization- Trademarks and copyrights.

Bioethics- Ethics in Bioscience, ethics in clinical trials and good clinical practice.

### **REFERENCE:**

1. Geoffrey Zubay (1993), Biochemistry, 3<sup>rd</sup> edition, Columbia University. Wm.C.Brown publishers., England.
2. Gerald Karp (2000). Cell and Molecular Biology, concepts and Experiments. 4<sup>th</sup> editions, John Wiley & sons, NewYork.
3. Lodish et. al (2000) Molecular Cell Biology, 5<sup>th</sup> edition, W.H. Freeman and company, NewYork.
4. D.Balasubramanian (1996). Concept in Biotechnology, Universal press India.
5. Freshney R.I(2000). animal Cell Culture, A practical Approach, John Wiley Publications, NewYork.
6. David W.Mount, (2001), Bioinformatics sequence and genome analysis, cold spring Harbor Laboratory press.
7. Pennigtons S.R and Dunn .M.J(2002), Proteomics, viva books pvt ltd.

## **PAPER III – 1. PLANT BIOCHEMISTRY AND PLANT THERAPEUTICS**

### **UNIT I**

Plant Therapeutics : Bioactive principles in herbs, plants with hepatoprotective , nephroprotective, hypoglycemic, anticancer, antibacterial, antiviral and antimalarial ,anti-inflammatory properties.

### **UNIT II**

Free radicals –types, sources, importance, producti on, free radicals induced damages, lipid peroxidation , measurement of free radicals, disease caused by radicals, reactive oxygen species, antioxidant defence system, enzymic and nom-enzymic antioxidants, role of antioxidants in prevention of diseases ,phytochemicals as antioxidants.

### **UNIT III**

Alkaloids, flavanoids, terpenoids, phenols-Occurrence ,distribution & functions ,Production of secondary metabolite in plants, stages of secondary metabolite production, uses of tissue culture techniques, elicitation, biotransformation- production of pharmaceutical compounds.

### **UNIT IV**

Principles-callus, meristem and organ culture, culture methods, culture media & preparations ,plant regeneration, protoplast technology, micropropogation in plants, somatic embryogenesis, somoclal selection.

### **UNIT V**

Carbon reactions in C<sub>3</sub>,C<sub>4</sub> & CAM cycle - calvin cycle, photorespiration, Nitrogen fixation-symbiotic & non symbiotic, role of nif and nod genes in nitrogen fixation, Techniques for plant transformation : Agrobacterium mediated gene transfer, process of T-DNA transfer, Direct gene transfer methods.

### **REFERENCES:**

1. Khan,I.A and Khanum.A 2004. Role of Biotechnology in medicinal & aromatic plants,Vol 1 and Vol 10, Ukkaz Publications ,Hyderabad.
2. Purohit.S.S. 2005 Agricultural Biotechnology, Dr.Updesh Purohit Publishers, Jodhpur.
3. Slater.A. Scott.N.W and Fowler.M.R 2004.Plant Biotechnology-The genetic manipulation of plants,Oxford University Press, Oxford.
4. Singh.M.P and Panda .H 2005.Medicinal Herbs with their formulations, Daya Publishing House,Delhi

## **PAPER III – 2. CLINICAL BIOCHEMISTRY & CLINICAL TOXICOLOGY**

### **UNIT I**

Effects of physiochemical and biological factors on heavy metal toxicity, toxic mechanism- Carcinogenesis, teratogenesis & immunotoxicity. Bioassays for heavy metal toxicity, pathological and Histopathological examinations for heavy metal toxicity.

### **UNIT II**

Diagnostic enzymes in hepatobiliary disease, myocardial infraction, atherosclerosis, renal dysfunction. Cancer markers for oral, prostate, colorectal, breast and GI tract cancer, oncofetal cancer markers.

### **UNIT III**

Formation of free radicals, auto oxidation initiated by oxygen radicals, Influence of free radicals in metal toxicity. Free radical hepatotoxins- CCl<sub>4</sub> model .free radicals and cancer .Oxidative process in tissue injury. Detection of free radicals and radical ions. Role of free radicals in diseases.

### **UNIT IV**

Enzymic antioxidants- Chemistry, mechanism, antioxidant effect of SOD, catalase, Glulathione Peroxidase.

Non Enzymic antioxidants- source,chemistry,toxicity, biochemical functions,bioavailability,bioassays, Antioxidant effects of Vit A, Vit C, Vit E, glutathione and selenium.

### **UNIT V**

Medicinal plants-bioactive principles in medicinal plants. Methods of extraction, isolation, separation and screening of pharmacologically active plants-CNS,CVS, Hypoglycemic, Hepatoprotective, anti-allergic, anticancer, immunoactive plants. Plants protecting against oxidative stress, and chemotherapeutic products.

### **REFERENCES:**

1. Biochemistry oxygen toxicity, Annual review of Biochemistry Enrique cadinar, Vol 58,1989,pp 78-110
2. Free radicals in biology by William a.Pryor,Academic press 1980.,pp 96-150.
3. Heavy metal toxicity testing in environmetal samples, Reviews of environmental contamination and toxicology in chul, Kong ,Gabrial Bitton,Benkoopan,vol 142.1995.pp 130-136.
4. Methods of plant analysis,Phytochemical analysis by J.B.Harborne,chapman & Hall Ltd.1973 pp 1-26.
5. Pharmocology of medicinal plants and natural products by S.A.Dhanukar,R.A.Kulkarani, W.N.Rege, Indian Journal of Pharmacology,2000 S81-S118.
6. Selenium dependent enzymes-glutathione peroxidase. Annual review of Biochemistry by Thresser,stadman ,Vol 49.1980 pp 103-108.
7. Superoxide radicals & SOD by Irwin Fridowich Annual review of Biochemistry,Vol 64.1995 pp 97-106
8. Vitamins –Annual research review by Horrobin ,Eden Press Pub.,Vol.3. 1980.pp 59-82,91-105,218-291.

### **PAPER III – 3. ENZYMES AND ENZYME TECHNOLOGY**

#### **UNIT I**

Protein structure, functions, compositions and conformation of proteins. Enzyme catalysis (catalysis by serine proteases). Enzyme Kinetics. Proteins in solution and in membranes. Liposomes and their preparation.

#### **UNIT II**

Sources of enzymes for industry, extraction of enzymes for scientific and industrial purpose. Downstream processing of enzymes, Uses of soluble enzymes. Study of enzymes in aqueous biphasic systems. Effects of pH and temperature on enzyme activity.

#### **UNIT III**

Major types of enzyme immobilization. Techniques employed for immobilizing enzymes, kinetics of immobilized enzymes. Advantages and disadvantages in the utilization of soluble enzymes immobilized enzymes and immobilized cells. Different types of reactors of immobilized enzymes and their applications.

#### **UNIT IV**

Application of ELISA and EMIT in Clinical analysis. Different types of Biosensors-potentiometric, amperometric, piezo electric and immuno biosensors. Electro analytic applications of enzymes, Methods of coenzyme regeneration .Biochips and Biocomputers.

#### **UNIT V**

Restriction endonucleases and their Uses, DNA ligase, DNA polymerase and their uses in Biotechnology, site directed mutagenesis, enzyme catalysis in organic solvents, artificial enzymes, ribozymes and Abzymes.

#### **REFERENCES:**

1. Robert.K.Scopes-Protein purification practice, Sprincer verlag,1982
2. C.Branden and J.Tooze-Introduction to protein structure, Garland pub, New York.
3. Alanwisheman-Handbook of Enzyme Biotechnology II, John Wiley & Sons,1985
4. J. Tampion and M.D. Tampion-Immobilized cells, Principles and applications. Cambridge University Press, New York, 1987.
5. M.F. Chaplin & C. Bucke, Enzyme Technology, Cambridge University press, New York, 1990.
6. M.J.C. Crabbe, Enzyme Biotechnology, Ellis Horwood, New York
7. Review of Enzyme function in organic solvents.L.J.Biochem, 203, 25-32.1992.FEBS.1992

## **PAPER III – 4. DNA TECHNIQUES & CLINICAL APPLICATIONS**

### **UNIT I**

Constructing restriction maps, Reporter Genes, Assay of Chloramphenicol, acetyl transferase and  $\beta$ -galactosidase activity.

PCR, Primer design, Cloning of PCR Products, Types of PCR, Anchored, Alu and RT-PCR. RAPD, Microsatellite and Quantitative PCR. Multimutation screening using PCR and ligation – Principles and Applications. Molecular diagnosis of viral and bacterial pathogens using PCR.

### **UNIT II**

Prokaryotic Expression system, pUR, pT 7-7, pET, pQE, expression vectors, pRIT, pGEX, pMAL vectors.

Expression Strategies, Preparation of expression vector, Foreign DNA ligation, transformation and characterization of protein by N-terminal sequencing and by LCMS

### **UNIT III**

Eukaryotic vectors, vectors for propagation of cloned DNA in yeast –episomal vectors, integrating vectors, engineering a foreign gene for intracellular expression, yeast (*S.cerevisiae*) promoter systems & terminators, yeast signal sequences, culture and transformations methods of commonly used *S.cerevisiae* strain, Induction and analysis of intracellular expressions.

### **UNIT IV**

Purification of expressed proteins from E.coli, purification of soluble recombinant proteins, Preparation of cell lysate, ion exchange chromatography, gel filtration, metal chelate affinity chromatography (MCAC) and immunoaffinity column.

Purification of inclusion bodies, *In vitro* refolding of proteins, verifying protein integrity. Techniques for measuring protein stability and processing of secreted protein.

### **UNIT V**

Probes-non radioactive labeling of nucleic acids-systems, substrated, labeling methods-Nick translation, oligo or random primer and PCR labeling. Efficiency of the methods. Detection of probes- Autoradiography- chemiluminogenic and different chromogenic substrates. Molecular diagnosis of genetic disease-cystic fibrosis, muscular dystrophy, Huntingtons disease, Fragile-X-syndrome, Retinoblastoma, Alzheimers disease, Amyotrophic lateral sclerosis.

## REFERENCES:

1. Recombinant DNA Technology-Raymond .L.Rodringuez & Robert C. Tait-Benjamin Cummings pub.1983.
2. Recombinant DNA Mrthodology-WU,Grossman,Moldave-Academic Press1989
3. DNA Cloning 1, 2, 3& 4 –D.M. Glover & B.D. Hames-IR L Press.
4. Molecular Cloning Vol.1, 2, 3-Sambrook, Fritsch & T. Maniatis-Cold spring Harbor Laboratory press, 1989.
5. Gene and Biology of Cancer-Harold Varmus, Robert A. Wrinbert-Scientific American library, 1993.
6. PCR-C.R. Newton & A. Graham. Bios Scientific Publishers .1995
7. PCR 1& 2 – A Practical Approach-M.J. Mc Pherson, B. D. Hames and G.R. Taylor-Oxford University Press,1995
8. Molecular Biology- A Project approach – Susan J. Ka rcher. Academic Press, 1995.
9. Essential Molecular Biology-Vol.I and II. T. A. Brown.
10. PCR-based Diagnostics in infectious Disease. Garth .D. Ehrilich, Steven .T. Greenberg. Blackwell Scientific Publication.
11. Non-isotope probing, Blotting and Sequencing, 2<sup>nd</sup> edition, Edited by Larry.J.Kricka, Academic Press, 1995.



## **PAPER III – 5. ENVIRONMENTAL BIOCHEMISTRY**

### **UNIT I**

Concept of an ecosystem. Structure and functions of ecosystem. Producers, consumers and decomposers. Energy flow in an ecosystem. Ecological succession. Food chains, food webs and ecological pyramids.

Introduction, types, characteristic features, structure and function of the following ecosystem. Forest ecosystem, Grassland ecosystem, Desert ecosystem, Aquatic ecosystems (Pond, streams, lakes, rivers, oceans, estuaries.)

### **UNIT II**

Causes, effects and control measure of, Air pollution, Water pollution, Soil pollution, Noise pollution, Thermal Pollution, Bioleaching,

### **UNIT III**

Introduction – Definition, genetic, species and eco system diversity. Value of Biodiversity : Consumptive use, Productive uses social, ethical, aesthetic and option values. Biodiversity at global, National and local levels. Hot spots of biodiversity, Threats to biodiversity  
Endangered and endemic species of India  
Conservation of biodiversity: In-situ and Ex-situ conservation of biodiversity-Conservation of Germplasm.

### **UNIT IV**

Enzymes: Immobilization of enzymes, enzymes engineering, isolation and culturing of microorganisms from contaminated area. Production of enzymes, fermentation, antibiotics, use of microbes to treat sewage water and industrial effluents and mining.

### **UNIT V**

Pollution Control: Cleaner technologies, reducing environment impact of industrial effluents, chemical pesticides, herbicides and fertilizers. Renewable source of energy through waste materials; biogas, energy crops, cellulose current levels of biodiversity.

### **REFERENCES:**

1. Elements of biotechnology, P.K.Gupta, Rastogi and Company, Meerut, India.
2. An Introduction to Genetic Engineering, Desmond S.T.Nicoll-Cambridge University.
3. Biotechnology, Kesar, Trehar Wiley, Eastern India.
4. Microbiology, Michael Pelczar, Tata-Mc Graw Hill Publishing Company, New Delhi.
5. Recombinant DNA-a short course, J.D.Watson, Scientific American Bank.

## PAPER III – 6. CANCER BIOLOGY AND IMMUNOLOGY

### UNIT I

Biology of cancer-Phenotype of a cancer cell. Causes of cancer-DNA tumor viruses, RNA tumor viruses, cell cycle and its control. Role of protein kinases, checkpoints, kinase inhibitors and cellular response.

### UNIT II

Programmed cell death (Apoptosis)-Intracellular proteolytic cascade, cascade of caspase proteins, adapter proteins, Bcl-2, IAP family proteins, extra cellular control of cell division. TNF family of death receptor and its signaling.

### UNIT III

Genetic basis of cancer-oncogenes, tumor suppressor genes, aberrations in signaling pathways. oncogenic mutations in growth promoting proteins, Mutations causing loss of growth –inhibition and cell cycle control, role of carcinogens and DNA repair in cancer.

### UNIT IV

Immunity- Active, passive, humoral and cell mediated immunity. Therapeutic uses of cytokines and cytokine receptors. Test for lymphocyte function. B cell and T cell immuno deficiency diseases. Clinical laboratory methods for the detection of antigens and antibodies. Test for histocompatibility antigens.  
Autoimmune disease- RA, SLE.

### UNIT V

Techniques-FISH techniques, Real time PCR, Western blotting, ELISA.  
Immunocytochemistry, immunohistochemistry, flow cytometry, cell sorting, fluroscent microscopy and confocal microscopy, Cancer immunotherapy- Monoclonal Ab therapy.

### REFERENCES:

1. Gerald Karp (2004) .Cell and Molecular Biology, Concepts and Experiments .4<sup>th</sup> edition, John Wiley & Sons, New York.
2. Lodish et.al. (2004) Molecular Cell Biology,5<sup>th</sup> edition, W.H.Freeman and Company, New York.
3. Bruse Albert's et.al. (2002) Molecular biology of the cell, 4<sup>th</sup> edition, Garland Science, New York.
4. Benjamin Lewin (2004) Genes VIII, Prentice Hall.
5. Sam brook, J., Fritish, E. F., and Maniatis, T., (1989). Molecular cloning: A Laboratory Manual.Vol. 1, 2, 3. Cold Spring Harbor Laboratory Press, New York.
6. Beatty, B et al., (2002) FISH, A Practical Approach, Oxford University Press , Oxford.
7. Ormerod, M. (2000) Flow Cytometry: A Practical Approach, 3<sup>rd</sup> ed. Oxford University Press, Oxford.
8. Paddock, S.W. (1998) Confocal Microscopy : Methods and Protocols, Humana Press , US.

## **PAPER III – 7. FREE RADICAL BIOLOGY AND ENDOCRINOLOGY**

### **UNIT I**

Basic Concepts .Definition of the analytical processes, integrated automation for clinical laboratory, development of standards for laboratory automation, Blood analyzer.

### **UNIT II**

Formation of free radicals, auto oxidation initiated by oxygen radicals, influence of free radicals in metal toxicity, free radicals hepatoxins, CCl<sub>4</sub> model. Free radicals & cancer. Oxidative process in tissue injury. Detection of free radicals by ESR Spectroscopy.

### **UNIT III**

Enzymic Antioxidants-Chemistry, mechanism, antioxidant effects of superoxide dismutase, catalase Glutathione peroxidase.

### **UNIT IV**

Sources , chemistry , toxicity, biochemical functions, bioassay, antioxidant effects of Vitamin A, Vitamin C, Vitamin E, Glutathione and Selenium.  
Trace elements- Introduction, sources, biochemical functions of zinc, copper, magnesium & iron.

### **UNIT V**

Definition, general characteristics, biosynthesis, bioassay of steroid hormones, peptide hormones, adrenal hormones, thyroid hormones.  
Vasoactive peptide hormones and pheromones (social hormones).

### **REFERENCES:**

1. Tietz N.W. (1994), Fundamentals of Clinical chemistry. W.B.Saunders's company.
  2. Harper's Biochemistry (1993) 23<sup>rd</sup> edition, Prentice Hall International Inc, USA.
  3. Free Radicals in Biology by William A. Pryor, academic Press, 1980. pp 96-150.
  4. Selenium dependent enzymes-glutathione peroxidase. Annual review of Biochemistry by Thresser, C.Stadman, Vol . 49.1980. pp 103-108.
  5. Superoxide radicals and SOD by Irwin Fridowich, Annual review of Biochemistry, Vol. 64.1995.pp97-106.
  6. Vitamins-annual research review by Horrobin, Eden Press Pub., and Vol.3.1980.pp 59-82, 91-105,218-291.
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