

Name of the Programme: Ph.D. Microbiology

Course Code: MIC-700

Title of the course: Research Methodology

Number of Credits: 04

Effective from AY: 2022-23

<b>Pre-requisites for the Course:</b>	Provisional registration for Ph.D. in Microbiology	
<b>Course Objectives:</b>	<ul style="list-style-type: none"><li>• To introduce aspects of research methodology pertaining to microbiology</li><li>• To sensitize about the hazards and norms of safety in microbiology laboratory</li><li>• To expose the students to biological data handling and statistical tools used for analysis</li><li>• To familiarize the students with various tools and techniques required in conduct of microbiological research –for isolation, characterization, purification, etc of microorganisms and biomolecules</li></ul>	
<b>Content:</b>	<p><b>1. Safety in Microbiology laboratory</b> Introduction and importance of safety in laboratory, Classification and types of hazards and safety measures, fire hazard and safety procedures, handling of hazardous chemicals, and solvents and hazards associated with instruments. First-Aid and its role in life saving during accident. Classification of biohazard, biosafety levels and procedures of handling biohazardous materials, Roles and types of personal safety equipment.</p> <p><b>2. Data handling and statistical analysis</b> Statistical analysis of any replicative measurements, Accuracy, precision, population and sample, true value, mean, standard deviation, standard error, Gaussian distribution, confidence limits and its estimates, Hypothesis testing (Z-test, t-test), Experimental designing (Factorial Design), Chi-square, F-test, ANOVA analysis. Use of computation software for statistical analysis. Mathematical modelling, Response surface methodology for 2 and 3 factors, and other advance statistics.</p> <p><b>3. Methods for handling microorganisms</b> Culture media and Growth system, Assay/monitoring methods for growth (Absorbance/packed volume/dry and wet weight/protein/ pigment content); colony forming units (cfu), plaque forming unit (pfu). Maintenance of cultures and taxonomic placement including keys of classification. Assay for Toxicity: LD50, MIC; Assay of Lethality: TDT, TDP, D<sub>10</sub>; Assay for enzymes and other biochemical reactions, including kinetics, Methods and evaluation of cell disruptions, Common reference biomolecules (proteins, lipids, nucleic acids, carbohydrates), SI units, Molarity, Molar, Moles, Buffers, Buffering capacity, Molecular weights, Nomograms, Common detergents used in microbiology</p> <p><b>4. Microscopic techniques</b> Microscopy and Micrometry, Stains and staining procedures, Photomicrometry, optical systems resolution, Phase Contrast microscopy, fluorescence microscopy, SEM</p>	<p>4 Hours</p> <p>8 Hours</p> <p>6 Hours</p> <p>4 Hours</p>

	<p><b>5. Molecular Techniques</b> Isolation, detection, characterisation of genomic and plasmid DNA. Endonucleases and restriction mapping. Common vectors, protocols for scoring recombinants, transformants and transconjugants. Isolation of RNA and their types. Thermal denaturation curve and calculation of G+C%. Use of X-gal, IPTG, PCR amplification of DNA, Nucleic acid hybridization (Southern and Northern blot techniques).</p> <p><b>6. Microbial cells and physiology</b> Fungi, Yeast, Algae, Cyanobacteria, Viruses, Eubacteria and Archaea. Biochemical activities of microorganisms-photosynthesis, respiration, fermentation. Energy acquisition pathways: EMP, HMP, ED, TCA Physiological and Nutritional characteristics of Microbial types. Genome organisation in microorganisms.</p> <p><b>7. Extraction, Separation and purification of biomolecules</b> Electrolyte / Solvent separation / Extractions, Centrifugation, Chromatography: adsorption, Ion exchange, affinity and Size exclusion, HPLC; Electrophoresis, ion-selective electrode, pH, pKa, pl, ampholyte, LC-Mass.</p> <p><b>8. Characterization of biomolecules</b> GC-Mass, AAS, Flame Photometer, NMR &amp; ESR, CD, IR, UV-Visible, Fluorimetry, Luminometry, Radioisotopy (Counter decay, safety of isotopes and usage)</p>	<p>6 Hours</p> <p>8 Hours</p> <p>10 Hours</p> <p>14 Hours</p>
<b>Pedagogy:</b>	Lectures/tutorials/assignments	
<b>References/ Readings:</b>	<ol style="list-style-type: none"> <li>1. Arora, PN and Malhan, PK, Biostatistics, Himalaya Publishing House. (2020)</li> <li>2. Davis, BD., Dulbecco, R, Eisen, HN &amp; Ginsberg, HS, Microbiology, Harper and Row Publishers. (1980)</li> <li>3. Haaland, PD, Experimental design in biotechnology. CRC press. (2020)</li> <li>4. Moat, AG, Foster, JW and Spector, MP eds., Microbial Physiology. John Wiley &amp; Sons. (2003)</li> <li>5. Plummer, DT, An introduction to practical biochemistry, Tata McGraw Hill (2001)</li> <li>6. Prudent practices in the laboratory: handling and management of chemical hazards, The National Academies Press, USA. (2011)</li> <li>7. Rao, KS, Biostatistics for Health and Life Sciences. Himalaya Publishing House, India (2017)</li> <li>8. Sadasivam, S, Manickam, A, Biochemical methods. New Age (P) International. (2007)</li> <li>9. Sambrook, J, Fritsch, EF, and Maniatis, T, Molecular cloning: a laboratory manual, Cold Spring Harbor Laboratory Press, New York (1989)</li> <li>10. Singh, Y.K., Fundamentals of Research Methodology and Statistics, New Age International Pvt. Ltd., India (2006)</li> <li>11. Silverstein, RM, Bassler, GC &amp; Morrill, TC, Spectrometric Identification of Organic Compounds, John Wiley, Singapore. (1991)</li> <li>12. Skoog, DA, Holler, FJ, &amp; Crouch, SR, Principles of Instrumental Analysis, Cengage Learning. (2017)</li> <li>13. Voet, D, Voet, JG and Pratt, CW, Fundamentals of biochemistry: life at the molecular level. John Wiley &amp; Sons. (2018)</li> </ol>	

	14. Wilson, K, & Walker J., Principles and Techniques of Practical Biochemistry, Cambridge University Press (2002)
<b>Course Outcomes:</b>	<ol style="list-style-type: none"><li>1. Execute safe laboratory practices in research.</li><li>2. Apply the knowledge of research methodology to plan and execute the experiments independently.</li><li>3. Analyse the microbiological data and perform statistical analysis.</li><li>4. Demonstrate the use of various equipment and techniques for microbiological research pertaining to isolation, characterization and identification of microorganisms and their biomolecules.</li></ol>