

LS406A**Life Sciences Practicals I****8 Credits**

Coordinator: Prof. Sneh Lata Panwar

*Participating Faculty (Module-wise):**Biochemistry: Prof. S.L. Panwar*, Dr. V.Yadav**Cell Biology: Dr. N. Mondal*, Dr. A. Bansal**Genetics: Prof. N. Ramchiary*, Dr. B.Chaudhary, Dr. Ekta Rai**Microbiology: Prof. A.S. Kharat**

	S.No.	Topic	Faculty Name/ Contact Hours
Biochemistry (2 credits)	1.	<i>Basic Concepts of Preparing Solutions:</i> Molarity; Percent solutions; Calculating Molarity of Percent solutions; Preparation of Tris and Phosphate Buffers; Preparation of stock solutions of Acids and Bases	SLP and VY
	2.	Recipes and preparation of reagents for experiments	
	3.	Assay to determine the activity and specific activity of an enzyme	VY
	4.	Partial purification of an enzyme and its qualitative analysis on SDS-PAGE	VY
	5.	Quantitative analysis of protein samples using the Lowry and Bradford methods	VY
	7.	<i>Characterization of Enzymes:</i> Kinetics of the enzyme; Effect of pH and temperature on enzyme activity	VY
	8.	<i>Analysis of Lipids:</i> Extraction of Phospholipids from egg yolk	SLP
	9.	<i>Analysis of Lipids:</i> Estimation of lipids by the Fiske & Subbarow method	SLP
	10.	<i>Analysis of Lipids:</i> Separation of various phospholipids by thin-layer chromatography	SLP
	11.	<i>Analysis of Lipids:</i> Estimation of Cholesterol	SLP
	12.	<i>Analysis of Lipids:</i> Theory and demonstration of lipid analysis by GC-MS	SLP

Cell Biology (2 credits)	1.	<i>Cell culture techniques:</i> Handling of mammalian cell culture of epithelial cell lines L-132 and HeLa- revival, plating, and preparation of freeze-downs.	NM/AB
	2.	<i>Microscopy- Parts of microscopes, Care & handling:</i> (i) Bright field microscope and Phase contrast microscope (ii) Fluorescence Microscopes: Inverted microscope; upright microscope (iii) Stereo microscope (iv) Applications for studying cell development and cell cycle progression. (v) Confocal microscopy demonstration; Applications for nuclear staining, calculating nuclear to cytoplasmic ratio of data already collected, cytoskeleton labelling for target protein localization within a cell at different stages of eukaryotic microorganism development.	AB/NM
	3.	<i>Visualization of DNA damage by Microscopy:</i> (i) Treating HeLa cells with Doxorubicin for different time points (ii) Acridine Orange/Propidium Iodide staining of cells followed by Fluorescence microscopy (iii) Trypan Blue staining of cells (iv) Counting live and dead cells by hemocytometer and quantifying the effect of the drug	NM/AB
	4.	<i>Visualization of Cell Cycle stages by Microscopy:</i> Processing of cells and analysis to study different phases of cell cycle; Demonstration of stages of plasmodium by microscopy Demonstration of Flow Cytometry Flow cytometry data analysis	AB/NM
Genetics (2 credits)	Animal Genetics using <i>Drosophila melanogaster</i> model		
	1.	Handling and maintaining cultures, and studying the life cycle of <i>Drosophila melanogaster</i> .	ER
	2.	Identification and analysis of different mutant lines/phenotypes of <i>Drosophila melanogaster</i> .	
	3.	Performing genetic crosses with <i>Drosophila melanogaster</i> to understand Mendelian inheritance.	
	4.	Calculating genetic distances by analyzing recombination frequencies from a trihybrid cross.	
	5.	Studying polytene chromosomes through salivary gland isolation in <i>Drosophila melanogaster</i> .	
	Plant Genetics: Construction of Genetic Map in Biparental Mapping Population		
	1.	DNA extraction of mapping population (approx. 96 individuals) derived from two contrasting parents	NR; BC

	2.	PCR analysis of mapping population DNAs with molecular marker(s)	NR; BC
	3.	Marker band scoring after resolving in agarose gel	NR; BC
	4.	Construction of genetic map using MapMaker/Joinmap or any other mapping programmes publicly available	NR; BC
	5.	Presentation of genetic maps and interpretation	NR; BC
	Plant Genetics: Quantitative Trait Loci Mapping in Biparental Cross Derived Mapping Population		
	6.	Scoring of quantitative traits in a segregating mapping population	NR; BC
	7.	Mapping of Quantitative Trait Loci using different programs	NR; BC
	8.	Presentation of QTLs in linkage maps and interpretation	NR; BC
Microbiology (2 credits)	1.	Basic concepts in microbial media: Minimal, Complete/Complex, Differential, Enrichment & Selective and Special media; Test of Sterility	ASK
	2.	Principles of pure culture techniques: Streak plate, Spread plate, and Pour plate technique; Preservation of Microbial stocks -80 deep freezer	ASK
	3.	Microscopic visualization of microorganisms: Negative staining, Monochrome staining, Differential staining - Gram staining, Special staining; Capsule staining-Maneval's method (<i>Acinetobacter baumannii</i> and <i>Klebsiella pneumonia</i>), Endospore staining using - Negative endospore (<i>Bacillus spp.</i>)	ASK
	4.	Measurement of bacterial motility using (<i>Escherichia coli</i> OR <i>Pseudomonas aeruginosa</i>): Hanging drop preparation, swarming growth, and Craigie's tube method	ASK
	5.	Bacterial culture characteristics: Sugar fermentations; Biochemical tests: Urease, Triple Sugar Iron Agar slant, Amino acid Decarboxylase	ASK
	6.	Water Microbiology: Preliminary, Presumptive, and Confirmatory tests: MacConkey agar, IMViC, MPN, Endo agar, and EMB agar test	ASK
	7.	Growth Curve to study the effects of Temp, pH, and oxygen concentration on the growth of microorganisms (<i>E. coli</i> , <i>K. pneumonia</i> , <i>A. baumannii</i> , <i>S. sonnei</i> , <i>E. coli</i>)	ASK
	8.	Effect of antibiotics on bacteria: Agar disc and agar well diffusion assay - Kirby-Bauer disc diffusion test to determine antibiotic sensitivity on Gram-negative ESKAPE bacteria	ASK
	9.	Effect of antibiotics on bacteria: Determination of Minimum Inhibitory Concentration of antibiotics on Gram-negative ESKAPE bacteria	ASK
	10.	Effect of antibiotics on bacteria: Biofilm formation on <i>A. baumannii</i> at sub-MIC of cephalosporins and polymyxins	ASK

	11.	In vitro infection: Adherence and Invasion of respiratory bacterial pathogen <i>K. pneumonia</i> , <i>A. baumannii</i> , <i>P. aeruginosa</i> to human lung cancer cells A549	ASK
	12.	To study the effect of <i>Triadax procumbens</i> plant extract/s and silver nanoparticles as antimicrobial and anticancer potential	ASK

Further Reading:

Biochemistry

1. Practical Biochemistry: Principles and techniques by Keith Wilson and John Walker
2. Lehninger- Principles of Biochemistry by David L Nelson, Michael M Cox
3. Biochemistry: Voet & Voet

Cell Biology

Handout/references will be provided by the concerned teacher in the practical class

Genetics

1. Methods in Yeast Genetics and Genomics, 2015 Edition: A CSHL Course Manual
2. Guide to Yeast Genetics and Molecular Biology, Guthrie, C. and Fink, G.R. Meth. Enzymol. Volume 194

Microbiology

1. Microbiology, J.G. Cappuccino, N. Sherman, Pearson Education Publications
2. Essential Microbiology, Stuart Hogg, John Wiley and Sons Limited
3. Microbiology: A Human Perspective, E.W. Nester, D.G. Anderson, C.E. Roberts, N.N. Pearsall, M. T. Nester McGraw Hill Higher Education
4. Microbiology by M. J. Pelczar Jr, E.C.S. Chan and N. R. Kreig
5. General Microbiology, 5th Edition, R. Y. Stanier
6. Fundamental Principles of Bacteriology, A. J. Salle
7. Fundamentals of Microbiology, 9th Edition, by M. Frobisher
8. Manual of Environmental Microbiology, C. J. Hurst, R. L. Crawford, G. R. Knudsen, M. J. McInerney, L. D. Stetzenbach. ASM Press
9. Microbiology, L.M. Prescott, J. P. Harley, D.A., Klein, McGraw Hill International Edition
10. General Microbiology. H.G. Schlegel, Cambridge University Press