SARDAR PATEL UNIVERSITY
VALLABH VIDYANAGAR

Programme: MSC (CBCS)
Syllabus with effective from: 2018-19

Industrial Biotechnology
Semester III

PS03CIBT21: Immunotechnology

Unit I

Introduction to immune system: mechanisms of barrier to entry of microbes / pathogens;
Cells and organs of the immune system involved in innate and adaptive immunity: cells of the immune system, primary and secondary lymphoid organs, Hematopoiesis and its regulation.
Innate immunity: receptors of the innate immunity (TLR and sensing of PAMPs, CLR,RLR and CLR); Inflammatory responses.
Antigens: antigenicity, and immunogenicity. B and T cell epitopes.

Unit II

Antibody: Structure of immunoglobulin; classes of immunoglobulins, Signal transduction pathways emanating from the BCR.
The Organization and Expression of Lymphocyte Receptor Genes: Hozumi and Tonegawa's Experiment, Multigene organization of Ig Gene, Mechanism of VDJ recombination, B cell receptor expression, allelic exclusion,B cell isotype switching and somatic hypermutation; expression of membrane bound and soluble immunoglobulin; T cell receptor genes and expression
Complement system: Overview of classical, alternative and lectin complement pathways, functions of complement, regulation of complement, complement deficiencies, microbial complement evasion strategies

Unit III

The Major Histocompatibility Complex and Antigen Presentation: The structure and function of MHC molecules, general organization and inheritance of MHC, self – MHC restriction, endogenous and exogenous pathway of antigen processing and presentation; cross presentation of exogenous antigen, presentation of non peptide antigens.
Cytokines: properties, receptors, associated diseases, therapeutic applications, cytokine signaling pathways: JAK-STAT and FAS-FASL signaling pathways.

Unit IV

Basics of Antigen-antibody interactions: Agglutination, precipitation, RIA and ELISA.
Cell and antibody mediated effector response: Antibody mediated effector response (Neutralization, opsonization/ phagocytosis, complement fixation, ADCC); Cell mediated effector response (Generation of effector CTL's, Granzyme and Perforin Mediated Cytolysis, Fas-FasL Mediated Cytolysis, NK cell mediated cytolysis
Immunity to infection: Immunity to viruses, Immunity to bacteria and fungi, Immunity to parasites (protozoa and worms).

References:

1. Owen, J. A., Punt, J., & Stranford, S. A. (2013). ***Kuby immunology*** (7^{th} Edn). New York: WH Freeman.
2. Murphy, K., & Weaver, C. (2016). ***Janeway's immunobiology*** (9^{th} Edn) Garland Science.
3. Male, D., Brostoff, J., Roth, D., & Roitt, I. (2012). ***Immunology*** (8^{th} Edn) *With STUDENT CONSULT Online Access*. Elsevier Health Sciences.
4. Abbas, A. K., Lichtman, A. H., & Pillai, S. (2014). ***Cellular and molecular immunology*** (6^{th} Edn) Elsevier Health Sciences.
5. Relevant review articles / research papers / handouts of latest development in the subject.

PS03CIBT22: Downstream processing

UNIT-1

Introduction to downstream processing principles, characteristics of biomolecules and bioprocesses. Cell disruption for product release – mechanical, enzymatic and chemical methods. Pretreatment and stabilisation of bioproducts.

UNIT-2

Physical methods of separation: centrifugation and filtration.
Adsorption, liquid-liquid extraction, aqueous two-phase extraction, membrane separation – ultrafiltration and reverse osmosis, dialysis, precipitation of proteins by different methods.

UNIT-3

Purification methods: Chromatography – principles, instruments and practice, adsorption, reverse phase, ion-exchange, size exclusion, hydrophobic interaction, bioaffinity and pseudo affinity chromatographic techniques.

UNIT-4

Purification strategies: Case studies of animal based products -Tissue Plasminogen Activator, erythropoietin; plant based products- shikonin and seed proteins; bacterial products- lipases, amylase, subtilisin, ethanol and citric acid.

References:

1. Gary Walsh Proteins: Biochemistry and Biotechnology. 2nd edition, Wiley Blackwell. 2002
2. J.C. Janson and L. Ryden, (Ed.) – Protein Purification – Principles, High Resolution Methods and Applications, VCH Pub. 1989.
3. R.K. Scopes – Protein Purification – Principles and Practice, Narosa Pub., 1994.
4. B. Sivasanker –Bioseperations –Principles and Techniques, Prentice –Hall of India, 2005.
5. Roger G.Harrison, Paul Todd- Bioseparations Science and Engineering, Oxford University Press, 2006.
6. D. G. Rao, Introduction to Biochemical Engineering, Tata McGraw-Hill Education, 2005.

PS03CIBT23: Enzyme Technology and Enzyme Engineering

Unit I

Introduction to enzymology and historical developments in enzymology
Enzyme Structure and classification.
Practical Enzymology: Enzyme Activity, assay, factors affecting enzyme activity, progress curve, rate enhancement, enzyme activators, coenzyme and cofactors,
Enzyme specificity
Enzyme purification: Objectives and strategy, separation techniques, test of purity, case study.

Unit II

Enzyme Kinetics:
Chemical reaction kinetics and catalysis
Single substrate kinetics: Equilibrium and Steady state kinetics, significance of Km, Vmax & Kcat, enzyme efficiency
Multisubstrate kinetics: General rate equation, compulsory order, random order and ping-pong mechanisms and their primary and secondary plots.
Enzyme inhibition and its kinetics: Reversible and irreversible inhibition, competitive, non-competitive and uncompetitive, mixed, partial, substrate and allosteric inhibition.
Thermal kinetics: Effect of temperature on reaction rate, enzyme stability, Arrhenius equation and activation energy.

Unit III
Mechanism of Enzyme Action:
Enzyme mechanisms: Factors affecting catalytic efficiency, Mechanism of Lysozyme, Chymotrypsin, Carboxypeptidase, Restriction endonuclease, Aspartate transcarbomylase.
Allosteric enzymes and sigmoidal kinetics: Protein ligand binding, Co-operativity, MWC & KNF models,
Multienzyme enzyme complexes.

Unit IV
Methods to study enzymes and its mechanisms
Enzyme engineering: Chemical modification of enzymes: methods of modification of primary structure, catalytic and allosteric properties, use of group specific reagents.
Enzymes in non conventional media, Enzymes as analytical reagents.
Isoenzymes and its physiological significance, Ribozymes and Abzymes.

Reference Books:

1. Fundamentals of Enzymologist : Nicholes C. Price and Lewis Stevens, Oxford Univ. Press.
2. Enzyme Structure and mechanism: Alan Fersht, Reading, USA.
3. Understanding Enzymes: Trevor Palmer
4. The chemical kinetics of enzyme action: K. J. Laider and P. S. Bunting, Oxford University Press, London.
5. Enzymes: M. Dixon, E. C. Webb, CJR Thorne and K. F. Tipton, Longmans, London.
6. Proteins: Thomas Creighton

PS03CIBT24: Lab I (Practicals based on PS03CIBT21 and PS03CIBT22)

PS03CIBT25: Lab II (Practicals based on PS03CIBT23 and PS03EIBT2X)

PS03EIBT21- Bio manufacturing Principles and Practice

Unit I

Overview and design of biomanufacturing, quality by design approach, technical considerations, phases and scale up: life cycle of manufacturing, raw material considerations, compliance and quality in biomanufacturing, lean biomanufacturing;

Standard manufacturing operating procedures of biotechnology, including upstream and downstream processing of proteins, and quality control of protein production, and final fill and finish of product; Case studies to be included at least: therapeutic proteins, monoclonal antibodies, human vaccines.

Unit II

Introduction to quality system, main elements of a quality system; Essential of quality system; Practical implementation of a quality system; Structure of quality manual, correlation between GMP requirements (WHO) and ISO 9001:2000.

Unit III

Personnel: Principles of human resource management, duties of senior management, organizational structures, qualification and profiles requirement.

Premises: Official requirements, material & personnel flow and layout, air cleanliness classes and grades, construction elements, barrier systems, isolators and safety cabinets, building services, heating ventilation air conditioning (HVAC), process gases, qualification of premises and HVAC systems, pharma monitoring of HVAC systems, particle monitoring.;

Process Validation: Official requirements, Validation - a key element of quality management, validation planning and procedure, validation documentation, process validation and product lifecycle ; Cleaning Validation: Official requirements, how to validate cleaning procedures.

UNIT-IV

Production: Sanitation, GMP in production process, sterilisation processes, aseptic processing, freeze-drying, testing for sterility, testing for endotoxins, testing for leakage and for particles, microbiological monitoring, packaging materials, packaging process. Information, national bodies and pharmaceutical associations; Pharmacopeia; EU directives and guidelines, USA: CFR and FDA guidelines, ICH-guidelines, PIC/S guidelines, GMP of other regions, WHO guidelines.

References:

1. Introduction to Biomanufacturing, by Northeast Biomanufacturing Center and collaboration, 2012.
2. Introduction to Biomanufacturing, by Mark Witcher. In Encyclopedia of Industrial Biotechnology.
3. Good Manufacturing Practices for Pharmaceuticals (e-resource): A Plan for Total Quality Control. Sidney Willig and James Stoker
4. Biotechnology Operations: Principles and Practices, by John M. Centanni, Michael J. Roy; CRC press
5. Learn Biomanufacturing, 1st Ed.; by Nigel Smart; Woodhead Publishing
6. GMP Manual; Publisher Maas & Peither America, Inc. GMP Publishing.

PS03EIBT22: Metabolic Engineering

UNIT-I

Stoichiometry, kinetics and thermodynamics of cellular reactions. Material balances on pathways and whole cell balances; Over and under-determined systems; Data consistency for over-determined systems. Regulation of metabolic pathways; role of enzymes, substrate, product and regulatory molecules; Hierarchical control in cellular systems.

UNIT-II

Pathway manipulation strategies for overproduction of various metabolites, examples of ethanol overproduction, overproduction of intermediates in main glycolytic pathway and TCA cycle like pyruvate, succinate *etc.*; Need for multiple genomic modifications; Modulating fluxes in desired pathways; Tools for multiple genomic modifications examples- TALENS CRISPR-Cas systems as well as traditional systems of gene knock ins and knock outs and promoter engineering.

UNIT-III

Metabolic pathway synthesis; Relation with bioprocess design; BIOBRICKS approaches; Introduction to tools of synthetic biology. Metabolic flux analysis; Building stoichiometric matrix; Steady state and pseudo steady state assumptions; Using different optimizing functions to solve linear programming problem.

UNIT-IV

Flux Balance Analysis: understanding flux cone and constraints; Introducing additional constraints from thermodynamics; Brief introduction to developments in this area; MOMA (Minimization of Metabolic Adjustment), iFBA (Integrated Flux Balance Analysis) *etc.* Experimental determination of metabolic fluxes; C13 labeling, NMR and GC-MS based methods for flux determination.

References:

1. Stephanopoulos, G.N., Aristidou, A.A., Nielsen, J. (1998) *Metabolic Engineering: Principles and Methodologies*. 1st ed. San Diego: Academic Press.

2. Smolke, C.S. (2010) *Metabolic Pathway Engineering Handbook: Fundamentals*. 1st ed. New York: CRC Press.

3. Smolke, C.S. (2010) *Metabolic Pathway Engineering Handbook: Tools and Applications*. 1st ed. New York: CRC Press.

PS03EIBT23: Omics and Computational Biology

Unit I Genomics and methods in genomics

Introduction to the proteome and the genome, codon bias, gene expression, Genome size-C value paradox, DNA sequencing: Maxam- Gilbert, Sanger, Pyrosequencing, automated DNA sequencing. Other features of nucleic acid sequencing. Analysis and Annotation-ORF Exon-intron boundaries, DNA Microarray technology:The generation of cDNA expression libraries, their robotic arraying, Complex hybridization on DNA chips.
Transcriptomics: Comparative transcriptomics, Differential gene expression; Genotyping/SNP detection; Detection technology; Computational analysis of microarray data.

Unit II Proteomics and methods in proteomics

Relationship between protein structure and function, Identification and analysis of proteins by 2D analysis; Spot visualization and picking; Tryptic digestion of protein and peptide fingerprinting; Common ionization methods for peptide/protein analysis; Introduction to Mass spectrometers; MALDI-TOF and LCMS analyses
Protein-protein interactions: Solid phase ELISA, pull-down assay (using GST-tagged protein), far western analysis, surface plasmon resonance technique, Yeast two hybrid system, Phage display; Protein interaction maps.
Protein arrays-definition, applications- diagnostics, expression profiling. Uses of automated technologies to generate protein arrays and chips.

Unit III Introduction to computational biology basics and biological databases

Computers in biology, Overview of biological databases, nucleic acid & protein databases, primary, secondary, functional, composite, structural classification database, Sequence formats & storage **Pairwise and multiple sequence alignments:** Local alignment, Global alignment, Scoring matrices - PAM, BLOSUM, Gaps and penalties, Dot plots. Dynamic programming approach: Needleman and Wunsch Algorithm, Smith and Waterman Algorithm, Hidden Markov Model: Viterbi Algorithm. Heuristic approach: BLAST, FASTA. Building Profiles, Profile based functional identification.

Unit IV Genome analysis

Polymorphisms in DNA sequence, Introduction to Next Generation Sequencing technologies, Whole Genome Assembly and challenges, Sequencing and analysis of large genomes, Gene prediction, Functional annotation, Comparative genomics, Probabilistic functional gene networks, Human genome project.**Structure visualization:** Retrieving and drawing structures, Macromolecule viewing platforms, Structure validation and correction, Structure optimization, Analysis of ligand-protein interactions; Tools such as PyMol or VMD.

References:

- Discovering Genomics, Proteomics and Bioinformatics, A,M, Campbell, C,S,H, Press,
- (2003).
- Essential of Genomics and Bioinformatics C,W, Sensen, Wiley (2003).
- Hand book of Comparative Genomics: Principle and Methodology by Cecilia Saccone,
- GrazianoPesole, Wiley-LISS publication (2003).
- Proteomics: From protein sequencing to function by S.R. Pennington and M.J. Dunn,
- Private Ltd (2001).
- Introduction to Proteomics by Daniel C, Liebler, Humana Press.
- Mount, D. W. (2001). Bioinformatics: Sequence and Genome Analysis. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
- Bourne, P. E., & Gu, J. (2009). Structural Bioinformatics. Hoboken, NJ: Wiley-Liss.
- Lesk, A. M. (2004). Introduction to Protein Science: Architecture, Function, and Genomics. Oxford: Oxford University Press.
- Campbell, M &Heyer, L. J. (2006), Discovering Genomics, Proteomics and Bioinformatics, Pearson Education.