Semester: 4	
Course: Skill Enhancement Course-3	
Paper Title: Bioanalytical methods (Pr1) and Immunology (Pr2)	
Paper Code: S2BT230421P	**Credits: 3**
Hours/week: 3+4	
Category: Core/MDC/SEC/VAC: SEC	
Theory / Practical / Composite: Practical	
No of Modules : 2	

Course overview	This practical course provides hands-on training in essential bioanalytical and immunological techniques used in life science laboratories. The course focuses on developing experimental skills, understanding methodological principles, and interpreting laboratory data. In the bioanalytical section, students learn quantitative and qualitative analysis of proteins, amino acids, and lipids through spectrophotometric methods, colorimetric assays, chromatography, electrophoresis, protein precipitation, and chromatographic separation techniques. The immunology section introduces core antigen–antibody–based assays, including haemagglutination, immunodiffusion, ELISA, and Western blotting, enabling students to understand and perform widely used immunodiagnostic and research methods.

Course Outcome for Section 1 (Bioanalytical methods)

1. **Explain** the principles underlying spectrophotometric and colorimetric methods for protein estimation, including absorbance at 280 nm and the Modified Lowry method
2. **Perform** quantitative estimation of protein concentration using UV-spectrophotometric and biochemical assay techniques with appropriate standards and calculations.
3. **Apply** chromatographic techniques such as paper chromatography and thin-layer chromatography (TLC) to separate and identify amino acids and lipids.
4. **Analyze** the buffering behavior of amino acids by determining their buffering capacity across different pH ranges.
5. **Differentiate** and interpret protein separation patterns obtained from reducing and non-reducing SDS–PAGE, based on molecular weight and structural features.
6. **Apply** selective salting-out techniques using ammonium sulfate and analyze protein fractionation based on solubility differences.

Course Outcome for Section 2 (Immunology)

1. **Remember** Students will recall the reagents (like: antigens, antibodies, enzymes, substrates and others), biological components, and equipments used in Double Immunodiffusion, ELISA, and Western Blotting, and the key terminologies, assay formats, and detection systems used in such techniques.
2. **Understand** Students will develop an understanding of the immunochemical basis of agglutination reactions (including hemagglutination), precipitation reactions (including immunodiffusion), enzyme-linked detection (including ELISA), and immunoblotting (including Western Blotting), the rationale of each step of each assay, and how specificity, affinity, and avidity may influence the assay outcomes.
3. **Apply** Students will apply their knowledge to conduct Double Immunodiffusion tests using specific antigens and antibodies, and execute ELISA for qualitative and quantitative detection of antigens/antibodies, and Western Blotting technique for protein identification.

4. **Analyze** Students will analyze haemagglutination patterns and inhibition endpoints, differentiate between precipitin line patterns in Double Immunodiffusion, examine ELISA absorbance values using standard curves and controls, and Western blot band intensity, molecular weight and specificity, besides differentiating between true positives, false positives, and experimental artifacts.
5. **Evaluate** Students will critically evaluate the sensitivity, specificity, accuracy, and reproducibility of each immunological technique, the advantages and limitations of each, compare immunoassays for diagnostic, clinical, and research applications, and assess sources of experimental error and assay limitations.
6. **Create Students will be able to d**esign a custom immunoassay strategy for detecting a target antigen or antibody, propose modifications or improvements to existing protocols to enhance sensitivity, reduce background noise, or improve throughput, integrate multiple immunological techniques to solve complex experimental or diagnostic problems, and develop a mini experimental plan or workflow based on a given clinical/research scenario.

Prerequisite: **Basic knowledge about Immunology Theory**

SYLLABUS

Unit/Module	**Content**	**Hour of classes**
Section-1	• Estimation of protein concentration by taking absorbance at 280 nm • Estimation of protein concentration by Modified Lowry Method • Identification of amino acids and lipids by TLC and paper chromatography • Determination of buffering capacity of amino acids • SDS-polyacrylamide gel electrophoresis (reducing and non-reducing gel) • Selective salting out of proteins using ammonium sulfate precipitation • Size exclusion chromatography (demonstration)	2 classes/week
Section-2	• Haemagglutination assay and Haemagglutination inhibition assay – tutorial • Double immunodiffusion test using specific antibody and antigen • ELISA • Western Blotting	2 classes/week

Practical text/references

1. Owen JA, Punt J, Stranford SA. (2013). Kuby Immunology. 7th edition. W.H. Freeman and Company, New York.
2. Kit Manufacturers' Manuals
3. An introduction to practical biochemistry by David T Plummer

Evaluation		**Practical (25+25)**