Abstract of the Thesis entitled **"Paper-Based Microfluidic Platform for estimation of Hematocrit, Creatinine and Total Protein from Whole Blood"** submitted by **Smriti Sinha** (Enrollment No: 18MM92R02)

Despite significant progress in global healthcare, low and middle-income countries continue to face a disproportionately high burden of morbidity and mortality from preventable diseases. Early diagnosis and timely treatment are essential to reducing this burden; however, the reliance of conventional centralized diagnostic systems on complex infrastructure and logistics renders them largely inaccessible in resource-limited settings. To bridge this gap, extensive research has focused on developing Point of Care (POC) diagnostic technologies capable of performing tests at or near the patient site. While POC technologies have advanced considerably, devices allowing direct biomarker detection from blood, the most informative biological matrix, remain limited. To address this limitation, this dissertation presents the development of a paper-based diagnostic platform engineered for direct biomarker detection from whole blood in a simple, rapid, and cost-effective manner. Initially, blood hematocrit estimation was achieved through automated analysis of blood spreading patterns on paper using a custom-developed smartphone application. The integrated design enabled both quantitative and qualitative assessments simultaneously. Validation against a standard hematology analyzer demonstrated clinically acceptable performance, highlighting the potential of the device for routine health screening, critical care monitoring, and large-scale anemia detection in resource-constrained environments. Furthermore, salt-functionalized paper was employed for plasma separation from whole blood to facilitate subsequent biosensing applications. Systematic optimization of paper properties, NaCl functionalization, and device geometry resulted in a plasma separation efficiency of up to 74% without requiring blood dilution. The separated plasma was then utilized for Bromocresol Green-based colorimetric sensing of human serum albumin, which showed strong correlation with gold-standard results, confirming its diagnostic utility. Considering the limitations of colorimetric biosensing, particularly its susceptibility to subjective interpretation and relatively low sensitivity and specificity, this study further advanced toward developing the electrochemical paper-based biosensor to achieve higher analytical performance. A graphite-based conductive ink was synthesized using graphite powder, ethanol–acetone as solvents, and cellulose acetate as a binder. Electrodes fabricated via stencil printing of the developed ink exhibited favourable morphological, rheological, and electrochemical properties. The fabricated paper sensor was subsequently employed for the non-enzymatic electrochemical detection of creatinine and total protein, two critical biomarkers for kidney and liver function assessment. Creatinine detection relied on its coordination with ferric ions in a NaCl electrolyte, while total protein detection was based on an electrochemical adaptation of the biuret reaction, wherein peptide bonds bind with cupric ions to form stable complexes. The sensor demonstrated clinically relevant detection ranges with excellent sensitivity, specificity, and stability. Furthermore, the developed sensor was integrated with on-chip plasma separation for individual detection of creatinine and total protein during clinical validation, which demonstrated high accuracy and reproducibility. Overall, this work demonstrates a proof-of-concept for an efficient, reliable, user-friendly, and cost-effective paper-based biosensor for direct on-card estimation of haematocrit, total protein, and creatinine from whole blood, representing a promising step toward decentralized and patient-centric healthcare delivery.

Keywords: Biosensors, Blood, Plasma, μPAD, Colorimetric sensors, Hematocrit, Smartphone app, Electrochemical sensors, Conductive ink, Iron, Biuret reagent, Creatinine, Total protein