

METHODS IN MOLECULAR BIOLOGY

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Serum/Plasma Proteomics

Methods and Protocols

Second Edition

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Preface

Human blood is one of the most informative and important proteomes from a clinical perspective. Blood, plasma, and serum are the predominant samples used for diagnostic analyses in clinical practice and are available in biobanks from thousands of clinical studies. The fact that blood constituents, primarily proteins, reflect diverse physiological, pathological, and pharmacological states makes them of great clinical significance—i.e., blood can be considered the “window of physiology and disease”. The ease with which blood (especially its plasma/serum components) can be sampled in a noninvasive manner makes it a logical choice for diagnostic screening applications. Characterization of plasma and serum proteins (both in qualitative and quantitative terms) should provide a foundation for the discovery of candidate markers for disease diagnosis and development of new therapeutics. Mass spectrometry (MS)-based proteomics is a technology capable of discovering biomarkers in blood. However, MS-based blood proteomics is extremely challenging for a number of reasons, most prominently the significant large dynamic range of protein abundances. Over the past several years, we have witnessed the advent of more powerful proteomics technologies and fractionation strategies that allow identification and accurate, multiplexed, quantitative monitoring of a diverse range of components from blood proteome. Improvements in discovery-based proteomics are focused toward increased multiplex quantitation and overcoming sample complexity through fractionation, increased mass ranges through improvements in labeling efficiency, and reduced user costs. Advances in targeted proteomics are being directed toward biomedical research and clinical applications, such as large-scale quantification, improvements in method development, throughput, data processing and analysis, and the utilization of fast-scanning high-resolution accurate-mass instruments to analyze low-abundance proteins in complex biological matrices. Further developments in informatics analyses, software developments, and computational tools are providing insights into large data sets and open-source data along with large-scale application of bioinformatics. Collectively, these improvements have, in part, fueled the quest for the discovery and monitoring of novel blood-based biomarkers and their modifications during disease.

This updated volume describes recent developments in blood proteomics—providing key insights and recommendations into processing and handling strategies, fractionation, posttranslational modification analyses, antibody-based approaches, and key developments in discovery and targeted proteomics toward clinical assay development. Part I of this volume comprises three chapters devoted to blood collection, handling and processing, and storage. Part II relates to fractionation strategies for in-depth blood proteome analysis and posttranslational modifications and includes seven chapters. Part III relates to proteome analyses of blood cell components, including platelets and red blood cells, circulating extracellular vesicles/exosomes, and related biofluids, while Part IV relates to detailed protocols for performing discovery and targeted antibody-based quantitative assays. Part V provides key insights into mass spectrometry-based discovery (global) and targeted (multiple/selected reaction monitoring) approaches. Part VI relates to studies focusing on key proteomics-based developments in biomarker discovery utilizing blood products.

To aid blood proteome researchers, we also include current standard operating procedures (SOPs) for plasma and serum collection for the purpose of clinical research, measured concentrations of many plasma proteins from quantitative assays, and reference ranges for blood tests. We also include a detailed overview of reference ranges for current blood tests. Such reference ranges for blood tests are studied within the field of clinical chemistry. These aids are appended at the end of the volume.

An Updated Serum/Plasma Proteomics is a comprehensive resource of 35 chapters and protocols for areas—pre-analytical through to analytical—of plasma and serum proteomics to assess human health and disease. This updated volume, contributed by leading experts in the field, complements the initial volume *Serum/Plasma Proteomics* and provides a valuable foundation for the development and application of blood-based proteomics.

David W. Greening

Richard J. Simpson

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