Methods in Molecular Biology series

Title: Serum / Plasma Proteomics

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Preface

Since its early beginnings for religious and 'medicinal' purposes, blood science has become a cornerstone of medicine – especially in the areas of clinical chemistry, disease diagnosis and therapeutic monitoring. Because blood constituents, primarily proteins, reflect diverse physiological, pathological and pharmacological states they are of great clinical significance –i.e., blood can be considered the 'window of disease'. The ease with which blood (especially, its plasma / serum components) can be sampled in a non-invasive manner makes it a logical choice for diagnostic screening applications.

Over the past decade we have witnessed the advent of more powerful proteomics technologies that allow deeper drilling of the blood proteome. These technological improvements have, in part, fuelled the quest for discovery of novel blood-based biomarkers of disease.

This volume describes recent developments in the relatively new area of blood proteomics. One significant technical challenge of any blood proteome analysis is the issue of sample complexity – for example, the dynamic range of protein concentrations in blood is in the order of 10^9-10^{10}, which is beyond any single protein fractionation method. Thus, in order to examine low-abundance proteins in blood it is necessary to perform serial fractionation. Section A of this volume comprises 7 chapters devoted to fractionation strategies for in-depth blood proteome analysis. This Section also includes a chapter devoted to enriching for a sub class of proteins, many of which are of very low-abundance not typically seen in whole blood proteome analysis – the glycoproteins.

Another major challenge facing serum-based biomarker discovery, and blood proteomics in general, relates to blood collection and storage. Not to put too fine point on it, if screening blood samples for the purpose of discovering novel biomarkers, one needs to be sure that 'normal' and 'disease' blood samples are in fact being compared, and not variances in blood collection/ storage protocols. These vexed issues are covered in 3 chapters in Section B. To aid blood proteome researchers, we also include current standard operating procedures (SOPs) for plasma collection for the purpose of clinical research, the measured concentrations of many plasma proteins from quantitative assays, and reference ranges for blood tests. We also include a summary of the major findings of an international collaborative effort, involving 38 laboratories, that was conducted by the Human Proteome Organization (HUPO) in 2005 to address the effects of pre-analytical variance and different proteomic protocols on acquiring a comprehensive blood proteome. These aids are appended at the end of the Volume.

Needless to say, quantitative assaying of blood-based biomarkers is an important aspect of clinical chemistry. Section C of this Volume includes 4 chapters with detailed protocols for performing both antibody-based (e.g., multiplex fluorescent microsphere-based assay using the BioPlex system) and non-antibody based (e.g., mass spectrometry-based multiple reaction monitoring, MRM) quantitative assays. Additionally, an updated protocol for analyzing glycated proteins in human plasma of patients presenting with glucotoxicity is presented.

Section D of this volume includes 5 chapters focusing on proteome analysis of blood cell compartments (e.g., platelet concentrates and phosphoproteome analysis of platelet plasma membrane), circulating nanomembraneous vesicles (exosomes) and blood-related fluids such as tissue interstitial fluid. Any text covering blood proteome analysis would be found wanting if data management, statistical design, and bioinformatic challenges were not covered. This topic is detailed through 4 chapters in Section E, along with a featured protocol for using the PeptideAtlas, an essential adjunct to any MRM assay design.

Serum/Plasma Proteomics is a comprehensive resource of protocols for areas, pre-analytical through to analytical, of plasma and serum proteomics. This book, contributed by leading experts

in the field, provides a valuable foundation for the development and application of blood-based proteomics.

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Recommended reading

Starr, D., (2002). *Blood: An Epic History of Medicine and Commerce*. 2nd ed. New York: HarperCollins Publishers. p464. ISBN 0-688-17649-6.