

---

# Contents

<i>Preface</i> .....	<i>v</i>
<i>Contributors</i> .....	<i>xi</i>

## PART I BLOOD PROCESSING AND HANDLING STRATEGIES

1	Protocols for the Isolation of Platelets for Research and Contrast to Production of Platelet Concentrates for Transfusion .....	3
	<i>Rosemary L. Sparrow, Richard J. Simpson, and David W. Greening</i>	
2	Collection of Plasma Samples in Areas with Limited Healthcare Access .....	19
	<i>Alicia Johnson, Camila Braga, Pedro de Magalhães Padilha, and Jiri Adamec</i>	
3	Blood Collection Processing and Handling for Plasma and Serum Proteomics .....	33
	<i>Conor McCafferty, Natasha Letunica, Ella Swaney, Cai Tengyi, Paul Monagle, Vera Ignjatovic, and Chantal Attard</i>	
4	Preparation of Cryoprecipitate and Cryo-depleted Plasma for Proteomic Research Analysis .....	41
	<i>Rosemary L. Sparrow, Richard J. Simpson, and David W. Greening</i>	

## PART II DISCOVERY-BASED MASS SPECTROMETRY

5	High-Throughput Proteome Profiling of Plasma and Native Plasma Complexes Using Native Chromatography .....	53
	<i>Aleksandr Gaun, Niclas Olsson, John C. K. Wang, Dan L. Eaton, and Fiona E. McAllister</i>	
6	High-Throughput and In-Depth Proteomic Profiling of 5 $\mu$ L Plasma and Serum Using TMTpro 16-Plex .....	81
	<i>Yan Zhou, Rui Sun, Sainan Li, Xiao Liang, Liujia Qian, Liang Yue, and Tiannan Guo</i>	
7	An Optimized Data-Independent Acquisition Strategy for Comprehensive Analysis of Human Plasma Proteome .....	93
	<i>Haoyun Fang and David W. Greening</i>	
8	In-Depth Blood Proteome Profiling by Extensive Fractionation and Multiplexed Quantitative Mass Spectrometry .....	109
	<i>Xue Zhang, Huan Sun, Zhen Wang, Suiping Zhou, Yingxue Fu, High A. Anthony, and Junmin Peng</i>	
9	Early Cancer Biomarker Discovery Using DIA-MS Proteomic Analysis of EVs from Peripheral Blood .....	127
	<i>Camila Espejo, Bruce Lyons, Gregory M. Woods, and Richard Wilson</i>	

## Contents

### PART III DEVELOPMENTS IN TECHNOLOGIES FOR PLASMA PROTEOMICS

- 10 Strategies to Enrich, Identify, and Characterize Glycoproteome in Blood Plasma Using Liquid Chromatography High-Resolution Mass Spectrometry..... 155  
*Saravanan Kumar*
- 11 Proteome Analysis of Whole Blood Collected by Volumetric Absorptive Microsampling ..... 173  
*Mark P. Molloy, Cameron Hill, Matthew J. McKay, and Ben R. Herbert*
- 12 Proteomic Applications and Considerations: From Research to Patient Care ..... 181  
*Natasha Letunica, Conor McCafferty, Ella Swaney, Tengyi Cai, Paul Monagle, Vera Ignjatovic, and Chantal Attard*

### PART IV ENRICHMENT & DETECTION STRATEGIES

- 13 Immunoaffinity Mass Spectrometry Diagnostic Tests for Multi-Biomarker Assays..... 195  
*Scott Bringans, Tammy Casey, Jun Ito, Tasha Lumbantobing, Ronan O'Neill, and Richard Lipscombe*
- 14 Secretome Profile of Leukocyte-Platelet-Rich Fibrin (L-PRF) Membranes ..... 207  
*Lidia Hermida-Nogueira, Juan Blanco, and Ángel García*
- 15 Quantification of Proteins in Blood by Absorptive Microtiter Plate-Based Affinity Purification Coupled to Liquid Chromatography-Mass Spectrometry..... 221  
*Frank Klont, Oladapo Olaleye, and Rainer Bischoff*
- 16 Glycomics-Assisted Glycoproteomics Enables Deep and Unbiased N-Glycoproteome Profiling of Complex Biological Specimens ..... 235  
*The Huong Chau, Anastasia Chernykh, Julian Ugonotti, Benjamin L. Parker, Rebeca Kawahara, and Morten Thaysen-Andersen*
- 17 The Small-Protein Enrichment Assay (SPEA) for Analysis of Low Abundance Peptide Hormones in Plasma ..... 265  
*Dylan James Harney and Mark Larance*

### PART V ANALYSIS OF EXTRACELLULAR VESICLES FROM BLOOD

- 18 In-Depth Proteomic Analysis of Blood Circulating Small Extracellular Vesicles ..... 279  
*Veronica De Giorgis, Elettra Barberis, Marco Falasca, and Marcello Manfredi*
- 19 Protocol for Plasma Extracellular Vesicle and Particle Isolation and Mass Spectrometry-Based Proteomic Identification ..... 291  
*Amirmohammad Nasiri Kenari, Linda Bojmar, Søren Heissel, Henrik Molina, David Lyden, and Ayuko Hoshino*

- 20 Analysis of Extracellular Vesicle and Contaminant Markers in Blood Derivatives Using Multiple Reaction Monitoring ..... 301  
*Lauren A. Newman, Zivile Useckaite, Ting Wu, Michael J. Sorich, and Andrew Rowland*
- 21 Generation of Red Blood Cell Nanovesicles as a Delivery Tool ..... 321  
*Auriane Drack, Alin Rai, and David W. Greening*

## PART VI TARGETED-BASED MASS SPECTROMETRY

- 22 Progress in Targeted Mass Spectrometry (Parallel Accumulation-Serial Fragmentation) and Its Application in Plasma/Serum Proteomics ..... 339  
*Anqi Hu, Jiayi Zhang, and Huali Shen*
- 23 Offline Peptide Fractionation and Parallel Reaction Monitoring MS for the Quantitation of Low-Abundance Plasma Proteins ..... 353  
*Claudia Gaither, Robert Popp, Vincent R. Richard, René P. Zahedi, and Christoph H. Borchers*
- 24 Profiling Serum Intact N-Glycopeptides Using Data-Independent Acquisition Mass Spectrometry ..... 365  
*Yi Yang and Liang Qiao*

## PART VII ASSAY DEVELOPMENT IN BIOMARKER DISCOVERY AND TRANSLATIONAL PROTEOMICS

- 25 Semi-Automated Lectin Magnetic Bead Array (LeMBA) for Translational Serum Glycoprotein Biomarker Discovery and Validation ..... 395  
*Mriga Dutt, Marisa N. Duong, Scott Bringans, Renée S. Richards, Richard Lipscombe, and Michelle M. Hill*
- 26 Accessing Antibody Reactivities in Serum or Plasma to (Auto-)antigens Using Multiplexed Bead-Based Protein Immunoassays ..... 413  
*Jasmin Huber, Silvia Schönthaler, Manuela Hofner, Yasmin Gillitschka, Regina Soldo, Lisa Milchram, Klemens Vierlinger, Christa Nöhhammer, and Andreas Weinhäusel*
- 27 Absolute Quantitative Targeted Proteomics Assays for Plasma Proteins ..... 439  
*Yassene Mohammed, David Goodlett, and Christoph H. Borchers*

## PART VIII PLASMA-BASED PEPTIDE, LIPID, AND METABOLITE ASSAYS

- 28 Rapid and Quantitative Enrichment of Peptides from Plasma for Mass Spectrometric Analysis ..... 477  
*Amy L. George, Rachel E. Foreman, Mariwan H. Sayda, Frank Reimann, Fiona M. Gribble, and Richard G. Kay*
- 29 Comprehensive Targeted Lipidomic Profiling for Research and Clinical Applications ..... 489  
*Kevin Huynh, Thy Duong, Natalie A. Mellett, Michelle Cinel, Corey Giles, and Peter J. Meikle*

## Contents

30	Multiplexed Bead-Based Peptide Immunoassays for the Detection of Antibody Reactivities . . . . .	505
	<i>Silvia Schönthaler, Jasmin Huber, Manuela Hofner, Yasmin Gillitschka, Regina Soldo, Lisa Milchram, Klemens Vierlinger, Christa Nöhhammer, and Andreas Weinhäusel</i>	
31	Array-Based Multiplex and High-Throughput Serology Assays . . . . .	535
	<i>Jennie Olofsson, Ceke Hellström, Eni Andersson, Jamil Yousef, Lovisa Skoglund, Ronald Sjöberg, Anna Månberg, Peter Nilsson, and Elisa Pin</i>	
PART IX SOFTWARE AND BIOINFORMATICS FOR THE PLASMA PROTEOME		
32	Bioinformatics Tools and Knowledgebases to Assist Generating Targeted Assays for Plasma Proteomics . . . . .	557
	<i>Yassene Mohammed, David Goodlett, and Christoph H. Borchers</i>	
33	Use of Longitudinal Serum Analysis and Machine Learning to Develop a Classifier for Cancer Early Detection. . . . .	579
	<i>Rashmi Madda, Vladislav A. Petyuk, Yi-Ting Wang, Tujin Shi, Craig D. Shriver, Karin D. Rodland, and Tao Liu</i>	
	<i>Index</i> . . . . .	593