FLOW CYTOMETRY IN CLINICAL DIAGNOSIS

4TH
CONTRIBUTORS

Patricia Aoun, MD
Associate Professor of Pathology
Dept. of Pathology and Microbiology
University of Nebraska Medical Center
Omaha NE

Kenneth A. Auli, MD
Director, Maine Medical Center Research Institute
Scarborough ME

David Barnett, PhD
Consultant Clinical Scientist
Honorary Senior Lecturer
UK NEQAS for Leukocyte Immunophenotyping
Dept. of Haematology
Royal Hallamshire Hospital
Sheffield UK

Robert A. Bray, PhD
Professor of Pathology
Codirector, Histocompatibility and Molecular Immunogenetics Laboratory
Emory University
Atlanta GA

John L. Carey, MD
Head, Division of Immunopathology
Dept. of Pathology
Henry Ford Hospital
Detroit MI

Irene J. Check, PhD
Professor of Pathology
Northwestern University Feinberg School of Medicine
Director, Clinical Pathology Division
Evanston Hospital Corporation
Evanston IL

Bruce H. Davis, MD
President, Trillium Diagnostics, LLC
Director of Hematopathology, US Labs
Brewer ME

Thomas N. Denny, MSc
Associate Professor of Medicine
Chief Operating Officer
Duke Human Vaccine Institute & Center for HIV/AIDS Vaccine Immunology
Director, NIAID Immunology Quality Assessment Program
Duke University Medical Center
Duke University, Durham NC

Howard M. Gebel, PhD
Professor of Pathology
Dept. of Pathology
Codirector, Histocompatibility & Molecular Immunogenetics Laboratory
Emory University Hospital
Atlanta GA

Rebecca L. Johnson, MD
Chair, Pathology and Clinical Laboratories
Berkshire Health Systems
Pittsfield MA

Nitin J. Karandikar, MD, PhD
Assistant Professor of Pathology & Neurology
Director, Division of Hematopathology & Immunology
Medical Director, Flow Cytometry & Cellular Immunology
University of Texas Southwestern Medical Center
Dallas TX

Mike Keeney, ART, FIMLS
Assistant Scientist
Division of Laboratory Hematology
London Health Sciences Centre
London ON

David F. Keren, MD
Medical Director
Wärde Medical Laboratory
Staff Pathologist, St Joseph Mercy Hospital
Adjunct Clinical Professor of Pathology
University of Michigan Medical School
Ann Arbor MI

Steven F. Kroft, MD
Professor of Pathology
Director of Hematopathology
Dept. of Pathology
Medical College of Wisconsin
Milwaukee WI

J. Philip McCoy, PhD
Flow Cytometry Core Facility
National Heart, Lung, and Blood Institute
National Institutes of Health
Bethesda MD

Perran R. McDaniel
General Manager, Flow Cytometry Laboratory
Genoptix, Inc.
San Diego CA

Maurice R. G. O’Gorman, PhD, MSc, MBA
Professor of Pediatric Medicine
Northwestern University Feinberg School of Medicine
Children’s Memorial Hospital
Chicago IL

Teri Oldaker
Genzyme Genetics
Los Angeles CA

Samuel J. Pirruccello, MD
Professor of Pathology
Dept. of Pathology and Microbiology
University of Nebraska Medical Center
Omaha NE

Jeffrey A. Shragger
Assistant Professor, Director of Hematopathology
Dept. of Pathology and Lab Medicine
University of Cincinnati Medical Center
Cincinnati OH

Maryalice Stebler-Stevenson, PhD, MD
Chief, Flow Cytometry Unit
Laboratory of Pathology
National Cancer Institute
National Institutes of Health
Bethesda MD

Elizabeth Stone
Esoterix
Brentwood TN

D. Robert Sutherland
Associate Professor, Dept of Medicine
University of Toronto
Technical Director, Clinical Flow Cytometry Laboratory
University Health Network/Toronto General Hospital
Toronto ON

Jeffrey S. Warren, MD
Alfred S. Warthin Professor of Pathology
Dept. of Pathology
University of Michigan Medical School
Ann Arbor MI

Job:E06-96708  Title:FLOW CYTOMETRY-Front Matter
#200_SQ Dtp:119_P  Page:ii
FLOW CYTOMETRY
IN CLINICAL DIAGNOSIS

4th Edition

John L. Carey, MD
J. Philip McCoy, Jr., PhD
David F. Keren, MD

American Society for
Clinical Pathology
Press
Chicago, IL
Notice
Trade names for equipment and supplies described herein are included as suggestions only. In no way does their inclusion constitute an endorsement or preference by the American Society for Clinical Pathology. The ASCP did not test the equipment, supplies, or procedures and therefore urges all readers to read and follow all manufacturers’ instructions and package insert warnings concerning the proper and safe use of products.
## Table of Contents

**Preface**  
John L. Carey, J. Philip McCoy, Jr, and David F. Keren  

### Chapter 1  
**Evolution of Surface Marker Assays and Immunologic Reagents**  
David F. Keren  

- History and Evolution of Surface Marker Assays  
  - T and B Lymphocytes  
  - Problems with Sheep E-Rosette Assays  
  - Problems with sIg Assays  
- Early Markers for Subpopulations of T and B Lymphocytes  
  - C3 and Fc Rosette Assays  
  - Activated T-Cell Rosettes  
- Enzyme Histochemistry and Tm Lymphocytes  
  - T and B Lymphocytes in Tissue Sections  
  - Immunologic Reagents in Flow Cytometry  
- Correlations of Pre-flow Cell Surface Assays with Monoclonal Markers  
  - Applications of Flow Cytometry in Diagnostic Laboratories  
- References
# Table of Contents

## Chapter 2

**Basic Principles in Clinical Flow Cytometry**

*J. Philip McCoy, Jr.*

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Why Flow Cytometry in the Clinical Laboratory?</td>
<td>15</td>
</tr>
<tr>
<td>Basic Concepts of Cellular Analysis</td>
<td>16</td>
</tr>
<tr>
<td>Design and Operation of a Flow Cytometer</td>
<td>16</td>
</tr>
<tr>
<td>Clinical vs Research Flow Cytometers</td>
<td>21</td>
</tr>
<tr>
<td>Cell Sorting</td>
<td>22</td>
</tr>
<tr>
<td>To Sort or Not To Sort in the Clinical Laboratory</td>
<td>22</td>
</tr>
<tr>
<td>Other Types of Clinical Cytometry</td>
<td>22</td>
</tr>
<tr>
<td>Fluorescence and Fluorochromes</td>
<td>23</td>
</tr>
<tr>
<td>Compensation</td>
<td>24</td>
</tr>
<tr>
<td>Quantitative Flow Cytometry</td>
<td>25</td>
</tr>
<tr>
<td>Gating</td>
<td>26</td>
</tr>
<tr>
<td>Considerations in Sample Preparation</td>
<td>26</td>
</tr>
<tr>
<td>Absolute Cell Counts Using Flow Cytometry</td>
<td>28</td>
</tr>
<tr>
<td>Quality Control</td>
<td>28</td>
</tr>
<tr>
<td>DNA Analysis</td>
<td>29</td>
</tr>
<tr>
<td>Safety Considerations</td>
<td>30</td>
</tr>
<tr>
<td>Limitations</td>
<td>30</td>
</tr>
<tr>
<td>The Future</td>
<td>31</td>
</tr>
<tr>
<td>References</td>
<td>32</td>
</tr>
</tbody>
</table>
## CHAPTER 5
### QUALITY CONTROL AND QUALITY ASSURANCE IN CLINICAL FLOW CYTOMETRY
73

Teri Oldaker and Elizabeth Stone

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regulations, Guidelines, and References</td>
<td>73</td>
</tr>
<tr>
<td>Validation of New Assays</td>
<td>74</td>
</tr>
<tr>
<td>Assay Optimization</td>
<td>76</td>
</tr>
<tr>
<td>Validation Protocol</td>
<td>77</td>
</tr>
<tr>
<td>Initial Assay Launch Preparation</td>
<td>82</td>
</tr>
<tr>
<td>Quality Control</td>
<td>83</td>
</tr>
<tr>
<td>Process/Assay QC</td>
<td>89</td>
</tr>
<tr>
<td>Specimen Quality and Quantity</td>
<td>90</td>
</tr>
<tr>
<td>Proficiency Testing (PT)</td>
<td>91</td>
</tr>
<tr>
<td>Quality Audits</td>
<td>92</td>
</tr>
<tr>
<td>Training</td>
<td>92</td>
</tr>
<tr>
<td>Competency Assessment</td>
<td>93</td>
</tr>
<tr>
<td>Quality Improvement</td>
<td>93</td>
</tr>
<tr>
<td>Quality Programs</td>
<td>93</td>
</tr>
<tr>
<td>Relevant Web Sites</td>
<td>95</td>
</tr>
<tr>
<td>Glossary/Definitions</td>
<td>95</td>
</tr>
<tr>
<td>References</td>
<td>96</td>
</tr>
</tbody>
</table>

## CHAPTER 6
### ECONOMIC AND BUSINESS ASPECTS OF FLOW CYTOMETRY
99

Rebecca L. Johnson

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alternatives to Implementing a Flow Cytometry Service</td>
<td>99</td>
</tr>
<tr>
<td>Implementing a Flow Cytometry Service</td>
<td>100</td>
</tr>
<tr>
<td>Summary</td>
<td>105</td>
</tr>
<tr>
<td>References</td>
<td>105</td>
</tr>
</tbody>
</table>
## Table of Contents

### CHAPTER 9

**FLOW CYTOMETRIC ANALYSIS IN THE DIAGNOSIS AND PROGNOSIS OF LYMPHOMA AND CHRONIC LEUKEMIAS** 129  
Maryalice Stetler-Stevenson and Jeffrey A. Schrager

- Technical Aspects 129  
- Data Analysis 131  
- Flow Cytometry and Diagnosis of Mature B-Cell Malignancies 132  
- Flow Cytometry and Diagnosis of Mature T-Cell and NK-Cell Malignancies 141  
- Flow Cytometric Prognostic Indicators in Mature Lymphoproliferative Disorders 148  

- References 150  
- Case ALCL 157  
- Case ATL 159  
- Case GD 161  
- Case HCL 164

### CHAPTER 10

**FLOW CYTOMETRIC ANALYSIS OF ACUTE LEUKEMIAS, MYELODYSPLASTIC SYNDROMES, AND MYELOPROLIFERATIVE DISORDERS** 168  
Steven H. Kroft and Nitin J. Karandikar

- General Approach 168  
- Antibody Selection and Combinations 169  
- Acute Lymphoblastic Leukemia (ALL) 173  
- Acute Myelogenous Leukemia 192  
- MDSs and Chronic MPDs 201  
- Acute Leukemias of Ambiguous Lineage 204  

- References 207
## CHAPTER 14

**CLINICAL UTILITY OF FLOW CYTOMETRY IN ALLOGENEIC TRANSPLANTATION**

*Robert A. Bray and Howard Gebel*

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Historical Perspective</td>
<td>275</td>
</tr>
<tr>
<td>Flow Cytometry Crossmatching</td>
<td>276</td>
</tr>
<tr>
<td>Panel-Reactive Antibody (PRA) Analysis</td>
<td>280</td>
</tr>
<tr>
<td>Future Applications in Clinical Transplantation</td>
<td>291</td>
</tr>
<tr>
<td>Summary</td>
<td>291</td>
</tr>
<tr>
<td>References</td>
<td>292</td>
</tr>
</tbody>
</table>

## CHAPTER 15

**CLINICAL ASSAYS OF RED CELLS, PLATELETS, AND PHAGOCYTES**

*Bruce H. Davis and Kenneth A. Ault*

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC Analysis in Immunohematology</td>
<td>295</td>
</tr>
<tr>
<td>Fetomaternal Hemorrhage and F-Cell Detection</td>
<td>296</td>
</tr>
<tr>
<td>Paroxysmal Nocturnal Hemoglobinuria</td>
<td>298</td>
</tr>
<tr>
<td>Evaluation of Erythropoietic Activity</td>
<td>299</td>
</tr>
<tr>
<td>Parasite Infections</td>
<td>301</td>
</tr>
<tr>
<td>Evaluation of Hemolytic Anemias</td>
<td>301</td>
</tr>
<tr>
<td>Neutrophil CD64 Quantitation for Detection of Infection/Sepsis</td>
<td>301</td>
</tr>
<tr>
<td>Monocyte Activation Changes in Sepsis</td>
<td>305</td>
</tr>
<tr>
<td>Flow Cytometry of Bronchoalveolar Lavage Specimens</td>
<td>305</td>
</tr>
<tr>
<td>Platelets</td>
<td>306</td>
</tr>
<tr>
<td>References</td>
<td>312</td>
</tr>
</tbody>
</table>
APPENDIX

REFERENCE RANGES 353

Cord Blood—Percentages: 95% Confidence Interval 353

Pediatric Peripheral Blood—Percentages (and Absolute Counts):
  95% Confidence Interval 353

Neonatal Peripheral Blood—Percentages (and Absolute Counts):
  10th and 10th Percentiles 353

Pediatric Peripheral Blood—Percentages (and Absolute Counts):
  5% and 95% Percentiles 354

Pediatric Peripheral Blood—Percentages (and Absolute Counts):
  95% Confidence Interval 355

Adult Peripheral Blood—Percentages (and Absolute Counts) 355

Pediatric Bone Marrow Aspirates—Percentages 355

Pediatric Bone Marrow Biopsies—Percentages: 25th and 75th Percentiles 356

Adult Peripheral Blood and Bone Marrow—Percentages: 95% Confidence Interval 357

Lymph Node—Percentages: 95% Confidence Interval 357

Characterization of Cluster Designation Antigens 359

INDEX 375
This 4th edition of *Flow Cytometry in Clinical Diagnosis* has been extensively revised to reflect recent advances in clinical flow cytometry. These include both technical advances (e.g., immunophenotyping with five or more markers and multiplex bead array assays) as well as new applications of existing assays (e.g., flow cytometry cross-match in transplantation; immunophenotypic analysis in the diagnosis of myelodysplasia or B CLL prognosis). The emphasis of this text continues to be clinically relevant applications of flow cytometry for the individual patient.

To realize these goals, we have added 10 new authors, in addition to 10 returning contributors from the 3rd edition. The 1st section of the book (chapters 1 through 7) provides basic technical and managerial information about flow cytometry (both traditional cellular assays and newer bead-based assays), monoclonal antibodies, comprehensive quality control, flow cytometry software, and the economics of flow cytometry. The second section in chapters 8 through 10 reviews the general and specific issues regarding flow immunophenotypic evaluation of hematolymphoid neoplasia, in the context of modern classification schemes. In addition to the written material, several dozen cases complete with list mode data files and corresponding expert interpretation are included on a companion CD-ROM.

The remainder of the book discusses the clinical applications of flow cytometry in a wide variety of settings. Chapters 11 and 12 review applications of flow cytometry to the diagnosis of congenital immunodeficiencies of both the adaptive and innate immune systems, while chapter 13 reviews advances in qualitative and quantitative flow analysis of lymphoid cells in HIV-infected individuals. Chapter 14 provides an expert review of the applications of flow cytometry of anti-HLA antibodies and HLA antigens in the evaluation of organ transplant recipients and donors. Closely related is the review of methodologies for stem cell quantitation, and possible new assays in chapter 15. Chapter 16 reviews a mixture of other assays of non-neoplastic hematolymphoid cells (e.g., neutrophil activation; platelet function, fetal red cell quantitation), and, chapter 17 presents the clinical state of DNA analysis of tumors. Lastly, we have compiled an extensive appendix of normal ranges for a wide variety of common antigens. These include 95th percentile confidence intervals for the most common markers for cord, neonatal, pediatric and adult peripheral blood samples, pediatric and adult bone marrows, and non-neoplastic (reactive) lymph nodes.

As long-term practitioners of clinical flow cytometry and contributing authors to the previous three editions of this book, we continue to be encouraged by the expansion of flow cytometry into clinical medicine. We hope that this latest edition continues to serve as a practical guide to effective use of this technology in the clinical laboratory.

*John L. Carey, MD*

*J. Philip McCoy,* Jr., PhD

*David F. Keren, MD*

*Dr McCoy’s chapters were written in a personal capacity and do not represent the opinions of the NIH, DHHS, or the Federal Government.*