

## CONTENTS

**Preface** xi  
Fiona E. Craig

**Modern Flow Cytometry: A Practical Approach** 453  
James W. Tung, Kartoosh Heydari, Rabin Tirouvanziam,  
Bitu Sahaf, David R. Parks, Leonard A. Herzenberg,  
and Leonore A. Herzenberg

The demonstration that CD T-cell counts can be used to monitor HIV disease progression opened the way to the first clinical application for fluorescence activated cell sorting (FACS) technology. Modern FACS methodologies such multicolor staining and sorting has opened the way to new and constructive therapeutic and clinical applications. This article outlines approaches in which current users can use to improve the quality of their FACS work without undue effort. FACS technology development and the emergence of new software support for this technology are cooperating in this effort.

**Optimizing a Multicolor Immunophenotyping Assay** 469  
Yolanda D. Mahnke and Mario Roederer

Flow cytometry-based immunophenotyping assays have become increasingly multiparametric, concomitantly analyzing multiple cellular parameters. To maximize the quality of the information obtained, antibody conjugate panels need to be developed with care, including requisite controls at every step. Such an optimization procedure for multicolor immunophenotyping assays is time consuming, but the value of having a reliable antibody conjugate panel that provides for sensitive detection of all molecules of interest justifies this time investment. This article outlines important

considerations and procedures to undertake for the successful design and development of multicolor flow cytometry panels.

**Flow Cytometric Evaluation of B-cell Lymphoid Neoplasms** 487  
Fiona E. Craig

Evaluation of B-lymphocytes is one of the most well-established clinical applications of flow cytometric immunophenotyping. This article addresses general principles of the flow cytometric evaluation of B-cell lymphoid neoplasms, followed by discussion of how flow cytometric data can assist in determining a list of diagnostic possibilities and directing additional testing.

**Flow Cytometric Assessment of T-cell Chronic Lymphoproliferative Disorders** 513  
Francois M. Cady and William G. Morice

Flow cytometry is frequently used in the evaluation of potential T-cell lineage lymphoproliferative disorders. Although flow cytometry is a useful tool, interpretation of the results can be challenging, because T-cells lack an easily analyzed structural element that can provide a surrogate marker of clonality such as immunoglobulin light chains on B-cells. Thus, routine T-cell phenotyping assays in the clinical laboratory require the comprehensive analysis of several T-cell-associated antigens. Although the detection of aberrant patterns of T-cell antigen expression can be helpful in establishing a diagnosis of T-cell malignancy, these patterns are not always disease specific, and some can overlap significantly with T-cell phenotypes observed in reactive conditions. Thus, arriving at an accurate diagnosis requires correlation of the flow cytometry results with the clinical, morphologic, and molecular results. Furthermore, the integration of these varied pieces of information into a cogent diagnosis requires an understanding of T-cell biology. In this review, the use of flow cytometry to identify T-cell lymphoproliferative disorders, particularly in peripheral blood and bone marrow specimens, is discussed, and a brief overview of T-cell biology to aid the reader in understanding the significance of the flow cytometry results is provided.

**Acute Lymphoblastic Leukemia: Diagnosis and Detection of Minimal Residual Disease Following Therapy** 533  
Joseph A. DiGiuseppe

Flow cytometric immunophenotyping (FCI) is an important diagnostic modality in the evaluation of patients who have suspected or known acute lymphoblastic leukemia (ALL). It enables rapid identification, quantification, and immunophenotypic characterization of leukemic blasts, permitting accurate and timely diagnosis. Beyond facilitating the classification of ALL into fundamental

diagnostic categories, FCI may anticipate recurrent cytogenetic and molecular abnormalities. FCI permits the detection of leukemic blasts after therapy at a level lower than that achievable by conventional microscopic examination. Flow cytometric detection of minimal residual disease is among the strongest prognostic factors in patients who have ALL and may provide an opportunity for more precise risk-adapted therapies.

**Myeloid Malignancies: Myelodysplastic Syndromes,  
Myeloproliferative Disorders, and Acute Myeloid Leukemia** 551  
Brent L. Wood

As hematopoietic cells proceed in differentiation from stem cells to committed progenitors to later stage mature forms, they undergo a sequence of morphologic, immunophenotypic, and functional changes that are a consequence of interaction between the underlying cellular genetic program and environmental cues, are linear for each cell lineage, and result in a pattern of antigenic expression related to lineage and stage of maturation. The antigenic patterns characteristic of normal maturation have been elucidated systematically and found invariant between individuals. Deviation from this pattern is a hallmark of hematopoietic neoplasia. Application of these principles to myelodysplastic syndromes, myeloproliferative disorders, and acute myeloid leukemia is presented and illustrated.

**The Role of Flow Cytometry in the Diagnosis of Paroxysmal  
Nocturnal Hemoglobinuria in the Clinical Laboratory** 577  
Stephen J. Richards and David Barnett

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare acquired hematopoietic stem cell disorder closely related to aplastic anemia. Hemolytic anemia and life-threatening thromboses are common features in many patients. Rapid diagnosis is highly desirable and flow cytometry plays a key role in the laboratory investigation of PNH. By demonstrating absence of cell membrane glycosylphosphatidylinositol-anchored proteins from granulocytes or red cells, a definitive diagnosis of PNH can be established. This can have a considerable impact on patient management and outcome. As with all rare diseases, internal and external quality assurance is essential for good laboratory practice and to fulfill the requirements of national laboratory accreditation schemes.

**Role of Flow Cytometry in the Diagnosis and Monitoring  
of Primary Immunodeficiency Disease** 591  
Maurice R.G. O’Gorman

This presentation is organized according to the recent classification of primary immunodeficiencies published by the International

Union of Immunological Societies Primary Immunodeficiency meeting. The diseases have been classified into eight groups. After each list, individual diseases that are amenable to assessment by flow cytometry are reviewed with a brief clinical description and a discussion of the appropriate flow cytometry application.

### **Rare-Event Analysis in Flow Cytometry**

627

Albert D. Donnenberg and Vera S. Donnenberg

Technical aspects of rare-event detection are discussed in this article in a practical context, with two real-life examples. A growing number of flow cytometry-based assays depend on rare-event detection for basic science and clinical applications.

### **Cellular Image Analysis and Imaging by Flow Cytometry**

653

David A. Basiji, William E. Orty, Luchuan Liang,  
Vidya Venkatachalam, and Philip Morrissey

Imaging flow cytometry combines the statistical power and fluorescence sensitivity of standard flow cytometry with the spatial resolution and quantitative morphology of digital microscopy. The technique is a good fit for clinical applications by providing a convenient means for imaging and analyzing cells directly in bodily fluids. Examples are provided of the discrimination of cancerous from normal mammary epithelial cells and the high-throughput quantitation of fluorescence in situ hybridization (FISH) probes in human peripheral blood mononuclear cells. The FISH application will be enhanced further by the integration of extended depth-of-field imaging technology with the current optical system.

### **Quality Control in Clinical Flow Cytometry**

671

Teri A. Oldaker

Immunophenotyping by flow cytometry is a robust and highly complex technology used in the enumeration and characterization of leukocytes, including normal lymphocyte subsets and hematologic neoplasms. Samples consist of peripheral blood and many times irreplaceable samples, such as bone marrow and fresh tissue. These samples are collected, transported, prepared, analyzed, and interpreted, resulting in diagnostic and prognostic information. Such information is critical to treatment decisions for patients. In order to obtain accurate and reproducible results, it is essential to have optimized and standardized procedures, rigorous quality control, and assurance programs encompassing preanalytic, analytic, and postanalytic processes.

**More than Just Quality Control**  
John L. Carey and Teri A. Oldaker

687

Providing quality flow cytometric results requires more than monitoring quality control data. Laboratories should standardize all aspects of testing and evaluate each one critically for opportunities to improve. This article discusses a complete quality management system that includes assay validation and change control, specimen collection and delivery, ordering of flow cytometric testing, sample preparation, verification of specimen integrity, flow cytometry data acquisition, analysis and interpretation, reporting of results, document of standard operating procedures, proficiency testing, training, and documentation of ongoing competency.

**Index**

709