IMMUNOPHENOTYPING
CYTOMETRIC CELLULAR ANALYSIS

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IMMUNOPHENOTYPING

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Preface

Flow cytometry is a technology that was first developed about 30 years ago to provide a quantitative evaluation of cellular properties based on their light scatter properties and characteristics that could be determined using fluorescent probes. The first flow cytometers were developed for use in research. They were very expensive, quite large, and required a dedicated room and operator.

Immunophenotyping is the identification of cells using fluorochrome-conjugated antibodies as probes to proteins expressed by cells. The flow cytometer was introduced into the clinical laboratory in the early 1980s when monoclonal antibodies became available and instruments were developed that were smaller, less expensive, and user-friendlier. In the last 20 years, there has been a virtual explosion in immunophenotyping applications using flow cytometry in the clinical laboratory. This book provides information for clinicians and laboratorians about the uses of flow cytometers for immunophenotyping.

There are several books that have been published that include methodologies for processing cells. We thought it would be of value to produce a book that describes the development of immunophenotyping and the current state of the art. With more and more clinical information and evaluation demonstrating the value of immunophenotyping, this has become an established clinical procedure. While several new competing technologies offer new diagnostic approaches, whether they are cost-effective or add value to current flow cytometric methods remains unanswered. Accordingly, these do not offer a competitive test with their flow cytometry counterpart at this time, but are more likely to be supplemental to the currently established flow cytometric tests. Nevertheless, there is a need to put a great deal of effort into promoting the value of clinical flow cytometric tests which can be more efficiently performed and provide more definitive results than many of the classical diagnostic procedures. This is especially important in areas of clinical immunology and hematology.

The first four chapters provide a background for clinical immunophenotyping applications. In Chapter 1 we discuss the tools of immunophenotyping by applying the law of mass action to understand the dynamics of antibody binding to cells. This is important when considering such issues as nonspecific binding of antibody to cells and characteristics of antibodies that affects their binding properties. As in all clinical tests, issues regarding quality control and quality assurance are extremely important. Chapter 2 discusses methods used for quality controlling immunophenotyping assays by taking a practical approach to the problem. Since we rely on computers to
accurately analyze data generated using the flow cytometer, it is important to under-
stand what the questions are in order to answer them correctly. Chapter 3 describes
approaches to ask and answer these questions. Recently there has been interest in
using the capability of the flow cytometer to quantitate the fluorescence it measures.
Different approaches to this methodology are described in Chapter 4.

Applications of quantitative fluorescence measurements in identifying abnor-
malities in a wide variety of disorders are described in Chapter 5. Chapter 6 describes the
expression of cellular antigens in normal hematopoiesis, as this is the background on
which one must overlay results from abnormal hematopoietic processes. Immuno-
phenotyping is commonly used in evaluating malignancies of the hematopoietic sys-
tem and its use in various disease states is described in Chapters 7, 8, 9, and 10. The
value and type of information provided by immunophenotyping in these malignan-
cies varies and these chapters outline approaches for clinicians and laboratorians to
follow when reviewing clinical data.

HIV immunophenotyping (Chapter 11) is one common application of flow cy-
tometry in clinical laboratories. This chapter describes immunophenotyping CD4 and
CD8 T cells and introduces new methods for obtaining absolute CD4 and CD8 cell
counts. With the advent of new therapeutics using bone marrow transplantation, ac-
curate enumeration of CD34 stem cells is very important. Issues describing the assay
and its applications are found in Chapter 12. Chapter 13 describes using a new, sen-
sitive flow cytometric immunophenotyping assay for cross-matching in the case of
transplants. Platelets and platelet function tests are discussed in Chapter 14. Flow cy-
tometry is an important tool in assessing platelets in disease states and ought to be-
come even more prominent as its value becomes better known.

The last two chapters describe assays that are routinely done in a few laboratories,
but are not extensively performed in clinical laboratories. It is likely these applica-
tions will become more extensively used as the methodology is reduced to practice.
Examining intracellular antigens using flow cytometry (Chapter 15) provides oppor-
tunities to identify antigens that are often very difficult to accurately and precisely
apply to both solid tissues and hematopoietic cells. The caveats of this method are ex-
tensively discussed in the chapter. Lastly, the ability to detect antigens intracellu-
larly using molecular biology approaches is an exciting new application (Chapter 16) that
offers an extensive repertoire of biological markers. The use of both of these methods
for intracellular antigen or nucleic acid detection combined with surface antigen de-
tection is currently the state of the art in identification of cells and their functions.

We believe the future for immunophenotyping in the clinical arena has matured
and, at least for hematopoietic cells, is a major clinical process for patient manage-
ment. The future for this technology is outstanding because it is the only technology
available today that can both rapidly and accurately measure multiple correlated cell
properties.

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