

## Invited Commentary

### Flow Cytometry: New Guidelines to Support Its Clinical Application

Over the last two decades, flow cytometry has advanced from being primarily a research tool in the clinical laboratory to becoming the method of choice for immunophenotyping hematopoietic cells. In addition, the use of flow cytometry is crucial for the diagnosis of hematolymphoid neoplasia. Advances in the field, as well as lower costs for flow cytometers with greater data analysis capabilities, have contributed to the widespread use of flow cytometry in the clinical laboratory. With this increased utilization, laboratorians using flow cytometry need to implement quality assurance procedures to ensure that comparable results are obtained when using different commercially available instruments and reagents.

Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) has produced two new guidelines to support the clinical application of flow cytometry. *Enumeration of Immunologically Defined Cell Populations by Flow Cytometry; Approved Guideline*, 2nd ed (H42-A2) was developed to address issues of procedures and quality assurance for clinical applications of flow cytometry. Specific topics covered include: specimen collection, transport, and preparation; sample quality control and staining procedures; instrument calibration; sample analysis; and data analysis, storage, and reporting [1]. *Clinical Flow Cytometric Analysis of Neoplastic Hematolymphoid Cells; Approved Guideline*, 2nd ed (H43-A2) is a document that builds on H42-A2 and provides guidelines for the appropriate performance of immunophenotyping for the proper diagnosis and management of patients with hematolymphoid neoplasia. Both documents, scheduled for release in March 2007, represent the most up-to-date and useful information in the field of flow cytometry and aim to provide a framework for the development of quality control procedures by laboratorians to improve the standard of care.

#### Guidelines From a Trusted Resource

CLSI is a global, nonprofit, and educational organization that promotes the development and use of voluntary consensus standards and guidelines within the healthcare community. CLSI provides a forum for thousands of volunteer experts to collaborate and share their knowledge by writing documents and developing tools that directly respond to the needs of the healthcare community and help medical professionals achieve effectiveness and efficiency in the workplace. CLSI standards and guidelines represent a consensus opinion on good practices and reflect the input of representatives of the private sector, as well as governmental and accreditation agencies.

#### Flow Cytometry and Its Importance in the Clinical Laboratory

Flow cytometry is an established technology that is important in the clinical laboratory. One example of its importance is in the use of immunofluorescence-based flow cytometry for the identification and enumeration of lymphocyte subpopulations, and the enumeration of CD34<sup>+</sup> stem cells. It is necessary for laboratories to establish quality assurance procedures that will help ensure accurate and reliable results.

There have also been significant advances in the use of flow cytometry in analysis of hematolymphoid neoplasia in recent years. Maryalice Stetler-Stevenson, MD, PhD, National Institutes of Health, explains, "Basically, flow cytometry is vital in the diagnosis of acute leukemia. It has been an important tool in diagnosis and subclassification of lymphoma and chronic lymphoid leukemias. In addition, because flow cytometry is a very sensitive technique, it allows detection of disease at a very low level. The ability to detect the disease at low levels, or minimal residual disease detection, has led to increased sensitivity in monitoring response to treatment. Flow cytometric demonstration of minimal residual dis-

ease at key points in a patient's therapy has been shown to have prognostic importance. Flow cytometry is a technique that has also been applied to new disease categories, such as myelodysplastic syndrome. Myelodysplastic syndrome is a difficult disease to diagnose. Flow cytometric immunophenotyping has been shown to be useful in making this diagnosis and furthermore can be predictive of prognosis."

#### Practical Application

Given the importance of flow cytometry in the clinical laboratory, laboratorians need concise, current, and useful guidelines to ensure that quality procedures are in place. The updated CLSI documents provide this support. Both H42-A2 and H43-A2 reflect the advancements in the field and provide practical resources for day-to-day laboratory procedures.

"The H42-A2 document serves as a reference source for the laboratory itself to develop standard operating procedures. The procedures can then be fine-tuned according to the specific circumstances in the laboratory," says Jan W. Gratama, MD, Erasmus University Medical Center. "This updated edition reflects what is current in the field, such as CD34<sup>+</sup> stem cell enumeration. I recommend this document to laboratories that are developing their own standard operating procedures," he adds.

The H42-A2 document provides information on the set-up of instrumentation and quality control. Examples of con-

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cerns that are addressed in the new H42-A2 guideline include:

- potential biohazardous procedures and appropriate precautions;
- required type and frequency of methodologic controls;
- analysis methods for lymphocyte subset and hematopoietic progenitor identification;
- methods for determination of absolute cell concentrations;
- guidelines for retention of laboratory records; and
- guidelines for definition of laboratory reference ranges [1].

H43-A2 extends the guidelines for immunophenotyping by flow cytometry provided in the previous document to the studies of leukemia and lymphoma. Issues specific to the study of hematolymphoid neoplasia that are covered in the H43-A2 document include:

- processing of multiple types of tissue, including bone marrow, lymph nodes, and solid tissue biopsies;
- methods used to study surface and intracellular antigen expression;
- sample preparation techniques particular to neoplastic specimens;
- reagent panels employed;
- types of methodologic controls required and the necessary frequency of their use;

- rules and precautions followed in acquiring data from neoplastic specimens;
- goals and methods of analysis unique to suspected hematolymphoid neoplasia samples, with emphasis on multiparameter analysis; and
- guidelines for interpreting and reporting data [2].

“H43-A2 provides quality control data that will help in the processing of specimens. For example, the concept of the irreplaceable specimen is discussed, and recommendations are given about specimen viability. There is also an expanded discussion of analysis for lymphoma, including the processing of lymph nodes and other solid tissue,” adds Stetler-Stevenson.

Together, the two documents provide guidelines for the major flow cytometry tests that many laboratories perform. Stetler-Stevenson explains, “The two documents are aligned on quality control procedures. H42-A2 provides extensive detail on instrumentation. H43-A2 provides the information specific to the use of flow cytometry in the analysis of leukemia and lymphoma.”

Both guidelines are designed for ease of implementation by the laboratory and serve as “go-to” references for technologists. Stetler-Stevenson considers the documents to be such references, and says, “We use H43-A2 as a guide for

the medical technologist in the laboratory as to what to do when a problem arises. It has helped to solidify our practices when it comes to anticoagulants, specimen storage, and bone marrow aspirates. We also refer our clinicians to this document and give them excerpts and quotations regarding specimen transport and collection. Therefore, we consider it a useful resource.”

*CLSI welcomes comments and questions about the documents; this feedback serves as the basis for updated document editions. All comments and responses are formally addressed and published in the next edition of the document. For more information about the Clinical and Laboratory Standards Institute references and best practices, visit [www.clsi.org](http://www.clsi.org) or call +610.688.0100.*

#### LITERATURE CITED

1. Clinical and Laboratory Standards Institute. Enumeration of Immunologically Defined Cell Populations by Flow Cytometry; Approved Guideline, 2nd ed. CLSI document H42-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2007.
2. Clinical and Laboratory Standards Institute. Clinical Flow Cytometric Analysis of Neoplastic Hematolymphoid Cells; Approved Guideline, 2nd ed. CLSI document H43-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2007.

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