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H42-A2
Vol. 27 No. 16
Replaces H42-A
Vol. 18 No. 21

Enumeration of Immunologically Defined Cell Populations by Flow Cytometry; Approved Guideline—Second Edition

This document provides guidance for the immunophenotypic analysis of non-neoplastic lymphocytes by immunofluorescence-based flow cytometry; sample and instrument quality control; and precautions for acquisition of data from lymphocytes.

A guideline for global application developed through the Clinical and Laboratory Standards Institute consensus process.

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H42-A2
ISBN 1-56238-640-9
Volume 27 Number 16
ISSN 0273-3099

Enumeration of Immunologically Defined Cell Populations by Flow Cytometry; Approved Guideline—Second Edition

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Abstract

Clinical and Laboratory Standards Institute document H42-A2—*Enumeration of Immunologically Defined Cell Populations by Flow Cytometry; Approved Guideline—Second Edition* was developed to address issues of procedures and quality assurance for clinical applications of flow cytometry. It is designed to aid clinical laboratorians in the development of quality assurance procedures and to establish the foundation for different laboratories using different commercially available instruments to obtain comparable results. 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.

Clinical and Laboratory Standards Institute (CLSI). *Enumeration of Immunologically Defined Cell Populations by Flow Cytometry; Approved Guideline—Second Edition*. CLSI document H42-A2 (ISBN 1-56238-640-9). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2007.

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Suggested Citation

(Clinical and Laboratory Standards Institute. *Enumeration of Immunologically Defined Cell Populations by Flow Cytometry; Approved Guideline—Second Edition.* CLSI document H42-A2 [ISBN 1-56238-640-9]. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2007.)

Proposed Guideline
December 1989

Tentative Guideline
May 1992

Approved Guideline
December 1998

Approved Guideline—Second Edition
May 2007

ISBN 1-56238-640-9
ISSN 0273-3099

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Foreword

Advances in the availability and reproducibility of monoclonal antibody reagents specific for a wide range of cell types, coupled with lower costs for increasingly automated flow cytometers with greater data analysis capabilities, have made flow cytometry the method of choice for immunophenotyping hematopoietic cells in the clinical laboratory. CLSI document H42-A2 represents the effort of the CLSI Working Group on Immunophenotyping of Lymphocytes appointed to establish guidelines for enumeration of lymphocyte subsets and CD34$^+$ hematopoietic stem cells by flow cytometry. In this context, it should be noted that for both types of assays, similar guidelines already have been developed by specific professional organizations or at national levels.[1-10] The current guideline aims to bring the state-of-the-art techniques together in a comprehensive, but readily usable format. It should be recognized that on occasion, national guidelines will override this document where applicable.

H42-A2 is designed to aid clinical laboratorians in the development of quality assurance procedures and to establish the foundation for laboratories using different commercially available instruments to obtain comparable results. This document should help minimize interoperator and interlaboratory variability in the various components 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.

In an effort to create an easy-to-use guideline, the main body of the H42-A2 document was divided into three parts. Part A: *General* includes the Scope, Introduction, Standard Precautions, Overview, Definitions, Safety, and an introductory section on methods for enumeration of absolute cell numbers. Part B: *Enumeration of Lymphocyte Subsets* includes recommendations related to the collection, transport, preparation, and analysis of specimens for enumeration of lymphocyte subsets. Part C: *Enumeration of CD34$^+$ Hematopoietic Stem and Progenitor Cells*, includes specific recommendations for the enumeration of CD34$^+$ stem cells, as well as—for convenience—some of the same general information provided in Part B (e.g., patient information, venipuncture technique, labeling of specimen).

A Note on Terminology

CLSI, as a global leader in standardization, is firmly committed to achieving global harmonization wherever possible. Harmonization is a process of recognizing, understanding, and explaining differences while taking steps to achieve worldwide uniformity. CLSI recognizes that medical conventions in the global metrological community have evolved differently in the United States, Europe, and elsewhere; that these differences are reflected in CLSI, ISO, and CEN documents; and that legally required use of terms, regional usage, and different consensus timelines are all obstacles to harmonization. In light of this, CLSI recognizes that harmonization of terms facilitates the global application of standards and deserves immediate attention. Implementation of this policy must be an evolutionary and educational process that begins with new projects and revisions of existing documents.

In order to align the usage of terminology in this document with that of ISO, the term *accuracy,* in its metrological sense, refers to the closeness of the agreement between the result of a (single) measurement and a true value of a measurand, and comprises both random and systematic effects.

The term *diagnostic sensitivity* is combined with the term *clinical sensitivity,* and correspondingly the term *diagnostic specificity* is combined with the term *clinical specificity*, because in Europe, the term "clinical" often refers to clinical studies of drugs under stringent conditions.

All terms and definitions will be reviewed again for consistency with international use, and revised appropriately during the next scheduled revision of this document.

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Key Words

Autofluorescence, CD system, $CD34^+$ hematopoietic stem cells, color compensation, dual-parameter display, flow cytometry, fluorescein isothiocyanate (FITC), fluorescence, forward angle light scatter, gate, histogram, immunoglobulin, immunophenotyping, linear amplification, logarithmic amplification, low angle light scatter, lymphocyte, lymphocyte subsets, 90° light scatter, phycoerythrin (PE), positive procedure control, single-parameter display (histogram), subclass

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Enumeration of Immunologically Defined Cell Populations by Flow Cytometry; Approved Guideline—Second Edition

PART A: General

1 Scope

The scope of this document is to establish performance guidelines for the identification and enumeration of lymphocyte subpopulations and the enumeration of $CD34^+$ hematopoietic progenitors using immunofluorescence-based flow cytometry (FCM).

The working group recognizes that other, so-called nontraditional methodologies exist or are in development for enumeration of $CD4^+$ T-lymphocytes (e.g., systems using microcapillary sample delivery or nonfluorescent cell detection; see Appendix C). Some of the issues discussed in this document are common to the use of any method for $CD4^+$ T-cell enumeration (e.g., sample collection and transport, safety issues, data reporting, and interpretation). However, issues such as sample preparation, instrument calibration, and quality control differ significantly for nontraditional methodologies and are not discussed in this document.

Presently, there are no universally accepted standards for precision, accuracy, and interlaboratory comparability in lymphocyte enumeration by FCM. General consensus was reached on the basic International Society for Hematotherapy and Graft Engineering (ISHAGE)[9] guidelines for CD34 analysis, and this forms the basis of the technique described herein. It is beyond the scope of this document to establish general performance criteria and reference intervals. Therefore, it is each laboratory's responsibility to establish instrument performance criteria and staining characteristics for its own specific reagents.

2 Introduction

Flow cytometry is an established technology that has moved from the research laboratory into the clinical laboratory. The goal of this document is to establish quality assurance procedures that will help ensure precision and accuracy of flow cytometric results appropriate for their use in the clinical laboratory. Since at present, most assays for lymphocyte subset and hematopoietic progenitor enumeration in clinical laboratories are fluorescence-based, this document is limited to specific issues surrounding the use of such systems. Major points of attention include the following:

- potentially biohazardous procedures and appropriate precautions;
- type and frequency of methodologic controls required;
- 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 intervals.

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