H43-A2

Clinical Flow Cytometric Analysis of Neoplastic Hematolymphoid Cells; Approved Guideline—Second Edition

This document provides performance guidelines for the immunophenotypic analysis of neoplastic hematolymphoid cells using immunofluorescence-based flow cytometry; for sample and instrument quality control; and precautions for acquisition of data from neoplastic hematolymphoid cells.

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

The importance of immunophenotyping for the proper diagnosis and management of patients with hematolymphoid neoplasia necessitates the development of guidelines for the appropriate performance of these techniques in the clinical laboratory. CLSI document H43-A2—Clinical Flow Cytometric Analysis of Neoplastic Hematolymphoid Cells; Approved Guideline—Second Edition addresses issues of safety, specimen collection and transportation, sample preparation, immunofluorescent staining, instrument quality control, data acquisition, and data storage for the application of flow cytometry to the immunophenotypic analysis of these disorders. This document builds on CLSI document H42—Enumeration of Immunologically Defined Cell Populations by Flow Cytometry.


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Foreword

H43-A2 establishes performance guidelines for the immunophenotyping of specimens potentially harboring neoplastic hematolymphoid cells by flow cytometry. It is designed to help clinical laboratories using different commercially available instruments and reagents obtain comparable results, and to aid these laboratories in the development of quality assurance procedures that are specifically applicable to such cases.

This document follows a related document, H42—Enumeration of Immunologically Defined Cell Populations by Flow Cytometry. In some respects, some sections of the current document—particularly those that cover specimen collection and transportation, safety, instrument quality control (QC), and data storage—are similar to those in H42. However, issues specific to the study of samples of leukemia and lymphoma are covered in sections on sample preparation, sample staining and QC procedures, data acquisition, data analysis, and result reporting and interpretation.

There has been a substantial expansion of the application of flow cytometry (FCM) in hematolymphoid neoplasia since the previous publication of this approved guideline as H43-A in 1998. Instrumentation has improved, routine use of four or more color FCM has expanded, and new applications in assessment of hematolymphoid malignancies are under constant development. Current classification systems rely upon immunophenotype for diagnosis, increasing the importance of clinical FCM in analysis of hematolymphoid neoplasia. In addition, the clinical utility of flow cytometric analysis in new disease categories, such as myelodysplastic syndrome, was established and prognostic immunophenotypic markers have been described. Flow cytometric immunophenotyping also plays a vital role in evaluation of patients for monoclonal targeted therapies. The document has therefore been revised to reflect these advances.

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 (International Organization for Standardization), and CEN (European Committee for Standardization) 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.
Key Words

Acute leukemia, autofluorescence, B-cell, chronic leukemia, cluster differentiation system, color compensation, erythrocyte lysing, flow cytometry, fluorescence intensity, fluorochrome, forward angle light scatter (FSC), gate, hematolymphoid neoplasia, hematopathology, histogram, immunophenotyping, list mode, lymphoma, monoclonal antibody, multiparameter display, myelodysplastic syndrome, NK-cell, procedural control, quality control, side scatter (SSC), T-cell, tandem conjugate, viability
Clinical Flow Cytometric Analysis of Neoplastic Hematolymphoid Cells; 
Approved Guideline—Second Edition

1 Scope

This document establishes performance guidelines for the flow cytometric immunophenotypic analysis of samples from patients with known or suspected hematolymphoid neoplasia. The World Health Organization (WHO) classification system relies upon morphology, clinical history, immunophenotype, and cytogenetics for diagnosis of hematolymphoid neoplasia. Therefore, immunophenotypic analysis of hematolymphoid neoplasia is crucial for the accurate diagnosis and classification of these complex malignancies. It is not within the scope of this document to recapitulate the criteria used to diagnose leukemias and lymphomas. Readers are referred to *Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues* (Jaffe ES, Harris N, Stein H, Vardiman JW, eds. Lyon, France: IARC Press; 2001). Because this document is intended primarily for laboratory workers in FCM, it cannot describe all the possible clinical situations in which flow cytometric analysis of leukemia or lymphoma is or is not appropriate.

This document includes guidelines for phenotyping cases of acute and chronic leukemias, non-Hodgkin’s lymphomas, plasma cell neoplasms, and myelodysplastic syndromes. The subcommittee recognizes that most of the principles used to approach chronic lymphoid leukemias can also be applied to the study of lymphomas, so these problems are considered together. Special problems unique to the study of non-Hodgkin’s lymphomas are treated separately.

At present, there are few agreed-upon standards for precision, accuracy, and interlaboratory comparability of leukemia analysis by FCM. Therefore, it is each laboratory’s responsibility to establish instrument performance criteria and staining characteristics for its own specific reagents.

2 Introduction

This document represents the efforts of the CLSI Subcommittee on Flow Cytometry of Leukemic Cells to extend the guidelines for immunophenotyping by FCM to studies of leukemia and lymphoma. To this end, it builds upon the guidelines established in CLSI document H42—*Enumeration of Immunologically Defined Cell Populations by Flow Cytometry*, and several sections of this document are similar to those in H42. However, the subcommittee recognizes that the use of FCM to characterize cases of hematolymphoid neoplasia presents several specific problems that are not covered in H42. Issues specific to the study of hematolymphoid neoplasia that are covered in this document include:

- patient groups included;
- 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 acquisition of data from neoplastic specimens;
- goals and methods of analysis unique to suspected hematolymphoid neoplasia samples, with emphasis on multiparameter analysis; and
- guidelines for interpretation and reporting of data.

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An overview of the differences between phenotyping neoplastic and non-neoplastic samples is provided in Section 4.

3 Standard Precautions

Because it is often impossible to know what isolates or specimens might be infectious, all patient and laboratory specimens are treated as infectious and handled according to “standard precautions.” Standard precautions are guidelines that combine the major features of “universal precautions and body substance isolation” practices. Standard precautions cover the transmission of all infectious agents and thus are more comprehensive than universal precautions, which are intended to apply only to transmission of blood-borne pathogens. Standard and universal precaution guidelines are available from the US Centers for Disease Control and Prevention (Garner JS, Hospital Infection Control Practices Advisory Committee. Guideline for isolation precautions in hospitals. Infect Control Hosp Epidemiol. 1996;17:53-80). For specific precautions for preventing the laboratory transmission of all infectious agents from laboratory instruments and materials and for recommendations for the management of exposure to all infectious disease, refer to the most current edition of CLSI document M29—Protection of Laboratory Workers From Occupationally Acquired Infections.

4 Overview

In the previous edition, the original subcommittee outlined general principles that are useful for performing FCM on blood samples. Some of these principles are directly applicable to the study of neoplastic hematolymphoid samples. Because neoplastic cells differ in many ways from normal cells, and because the overall goals of phenotyping neoplastic hematolymphoid cells are different from those of lymphocyte subset enumeration, special procedures are necessary. This section outlines the most important differences; detailed information is then presented in appropriate sections to follow.

4.1 Goals

The principal goal of neoplastic hematolymphoid phenotyping is to determine the expression of lineage-differentiation and tumor-associated antigens on neoplastic hematolymphoid cells to aid in diagnosis and subclassification. This differs from that of blood lymphocyte phenotyping, whose aim is to enumerate antigenically defined lymphocyte subsets. Phenotypic characterization aids in the recognition and classification of the hematolymphoid malignancy. In some circumstances, the goal of FCM is to detect minimal involvement with hematolymphoid neoplasia for the purposes of staging or minimal residual disease (MRD) detection or disease prognosis. In these situations, sample preparation, data acquisition, gating strategies, and data interpretation are often different.

4.2 Quality Control Procedures

Instrument setup for phenotyping of hematolymphoid neoplasms is generally similar to that used for lymphocyte enumeration. However, in the case of hematolymphoid neoplasms, it is often extremely important to distinguish dim reactivity from either background autofluorescence or nonspecific staining. Section 11 addresses the importance of appropriate control reagents and also points out how to construct a panel of test reagents to include reagents that can also potentially serve as negative controls. Section 12 describes procedures for ensuring that the instrument is capable of resolving low-intensity fluorescence.

Several reagents used for phenotyping of hematolymphoid neoplasms may not react with normal blood lymphocytes, and special procedures for verifying their reactivity may have to be instituted.
The Quality Management System Approach

Clinical and Laboratory Standards Institute (CLSI) subscribes to a quality management system approach in the development of standards and guidelines, which facilitates project management; defines a document structure via a template; and provides a process to identify needed documents. The approach is based on the model presented in the most current edition of CLSI/NCCLS document HS1—A Quality Management System Model for Health Care. The quality management system approach applies a core set of “quality system essentials” (QSEs), basic to any organization, to all operations in any healthcare service’s path of workflow (i.e., operational aspects that define how a particular product or service is provided). The QSEs provide the framework for delivery of any type of product or service, serving as a manager’s guide. The quality system essentials (QSEs) are:

- Documents & Records
- Organization
- Personnel
- Equipment
- Purchasing & Inventory
- Process Control
- Information Management
- Occurrence Management
- Assessments—External and Internal
- Process Improvement
- Customer Service
- Facilities & Safety

H43-A2 addresses the quality system essentials (QSEs) indicated by an “X.” For a description of the other documents listed in the grid, please refer to the Related CLSI/NCCLS Publications section on the following page.

### Path of Workflow

A path of workflow is the description of the necessary steps to deliver the particular product or service that the organization or entity provides. For example, CLSI/NCCLS document GP26—Application of a Quality Management System Model for Laboratory Services defines a clinical laboratory path of workflow, which consists of three sequential processes: preexamination, examination, and postexamination. All clinical laboratories follow these processes to deliver the laboratory’s services, namely quality laboratory information.

H43-A2 addresses the clinical laboratory path of workflow steps indicated by an “X.” For a description of the other documents listed in the grid, please refer to the Related CLSI/NCCLS Publications section on the following page.

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Adapted from CLSI/NCCLS document HS1—A Quality Management System Model for Health Care.
Related CLSI/NCCLS Publications*

**GP5-A2**  Clinical Laboratory Waste Management; Approved Guideline—Second Edition (2002). Based on US regulations, this document provides guidance on the safe handling and disposal of chemical, infectious, radioactive, and multihazardous wastes generated in the clinical laboratory. While a valuable resource for a wider audience, it is intended for use primarily in the United States.

**H1-A5**  Tubes and Additives for Venous Blood Specimen Collection; Approved Standard—Fifth Edition (2003). This document contains requirements for venous blood collection tubes and additives, including technical descriptions of ethylenediaminetetraacetic acid (EDTA), sodium citrate, and heparin compounds used in blood collection devices.

**H3-A5**  Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved Standard—Fifth Edition (2003). This document provides procedures for the collection of diagnostic specimens by venipuncture, including line draws, blood culture collection, and venipuncture in children.

**H18-A3**  Procedures for the Handling and Processing of Blood Specimens; Approved Guideline—Third Edition (2004). This document includes criteria for preparing an optimal serum or plasma sample and for the devices used to process blood specimens.

**H20-A2**  Reference Leukocyte (WBC) Differential Count (Proportional) and Evaluation of Instrumental Methods; Approved Standard—Second Edition (2007). Discusses automated differential counters and establishes a reference method based on the visual (or manual) differential count for leukocyte differential counting to which an automated or manual test method can be compared, and an experiment to carry out the comparison. Describes procedures for collecting specimens; preparing blood films and requirements for acceptable wedge and spun films; Romanowsky staining; the formed elements; variant leukocyte forms; and a protocol for examining blood films. Details procedures for determining inaccuracy, and within-run and between-run imprecision; procedures for determining sensitivity/specificity/predictive value of flags; and statistical methods for determining inaccuracy and imprecision.

**H26-A**  Performance Goals for the Internal Quality Control of Multichannel Hematology Analyzers; Approved Standard (1996). This document addresses performance goals for analytical accuracy and precision for multichannel hematology analyzers; the relationship of these goals to quality control systems and medical decisions; and recommendations for minimum calibrator performance and the detection of measurement errors.

**H42-A2**  Enumeration of Immunologically Defined Cell Populations by Flow Cytometry; Approved Guideline—Second Edition (2007). 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.

**I/LA24-A**  Fluorescence Calibration and Quantitative Measurement of Fluorescence Intensity; Approved Guideline (2004). This guideline describes the basic principles, reference materials, and laboratory procedures upon which quantitative fluorescence calibration is based.

**M29-A3**  Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline—Third Edition (2005). Based on US regulations, this document provides guidance on the risk of transmission of infectious agents by aerosols, droplets, blood, and body substances in a laboratory setting; specific precautions for preventing the laboratory transmission of microbial infection from laboratory instruments and materials; and recommendations for the management of exposure to infectious agents.

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* Proposed-level documents are being advanced through the Clinical and Laboratory Standards Institute consensus process; therefore, readers should refer to the most current editions.
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