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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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Guideline A document developed through the consensus process describing criteria for a general operating practice, procedure, or material for voluntary use. A guideline may be used as written or modified by the user to fit specific needs.

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- the authorization of a project
- the development and open review of documents
- the revision of documents in response to comments by users
- the acceptance of a document as a consensus standard or guideline.

Most documents are subject to two levels of consensus—"proposed" and "approved." Depending on the need for field evaluation or data collection, documents may also be made available for review at an intermediate consensus level.

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Approved An approved standard or guideline has achieved consensus within the healthcare community. It should be reviewed to assess the utility of the final document, to ensure attainment of consensus (i.e., that comments on earlier versions have been satisfactorily addressed), and to identify the need for additional consensus documents.

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VOLUNTEER PARTICIPATION

Healthcare professionals in all specialties are urged to volunteer for participation in CLSI projects. Please contact us at customerservice@clsi.org or +610.688.0100 for additional information on committee participation.

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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.