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CLINICAL AND LABORATORY STANDARDS INSTITUTE®

March 2014

H52-A2

Red Blood Cell Diagnostic Testing Using Flow Cytometry; Approved Guideline—Second Edition

This guideline addresses the diagnostic red blood cell (RBC) assays performed as fluorescence-based assays on a flow cytometry platform; including testing procedures for fetomaternal hemorrhage detection, paroxysmal nocturnal hematuria screening, membrane defect anemia testing for hereditary spherocytosis, and nucleated RBC counting. Points of validation and quality control, and caveats of interpretation are also discussed.

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

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ISBN 1-56238-957-2 (Print)
ISBN 1-56238-958-0 (Electronic)
ISSN 1558-6502 (Print)
ISSN 2162-2914 (Electronic)

H52-A2
Vol. 34 No. 5
Replaces H52-A
Vol. 21 No. 26

Red Blood Cell Diagnostic Testing Using Flow Cytometry; Approved Guideline—Second Edition

Volume 34 Number 5

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Abstract

Clinical and Laboratory Standards Institute document H52-A2—*Red Blood Cell Diagnostic Testing Using Flow Cytometry; Approved Guideline—Second Edition* addresses the diagnostic RBC assays performed as fluorescence-based assays on a flow cytometry platform. Preferred and alternative testing procedures for fetomaternal hemorrhage detection, paroxysmal nocturnal hematuria screening, membrane defect anemia testing for hereditary spherocytosis, and nucleated RBC counting are reviewed. Preferred testing methods, points of validation and QC, and caveats of interpretation are discussed from the perspectives of laboratory practitioners, diagnostic test developers, and regulators. Where appropriate, this guideline integrates current statements of other relevant organizations, such as the International Council for Standardization in Haematology.

Clinical and Laboratory Standards Institute (CLSI). *Red Blood Cell Diagnostic Testing Using Flow Cytometry; Approved Guideline—Second Edition*. CLSI document H52-A2 (ISBN 1-56238-957-2 [Print]; ISBN 1-56238-958-0 [Electronic]). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2014.

Suggested Citation

CLSI. *Red Blood Cell Diagnostic Testing Using Flow Cytometry; Approved Guideline—Second Edition.* CLSI document H52-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.

Previous Editions:
April 2000, January 2001

Reaffirmed:
June 2016

Archived:
January 2021

ISBN 1-56238-957-2 (Print)
ISBN 1-56238-958-0 (Electronic)
ISSN 1558-6502 (Print)
ISSN 2162-2914 (Electronic)

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Acknowledgments

CLSI, the Consensus Committee on Hematology, and the Document Development Committee on RBC Diagnostic Testing Using Flow Cytometry gratefully acknowledge the following volunteers for their important contributions to the development of this document:

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CLSI, the Consensus Committee on Hematology, and the Document Development Committee on RBC Diagnostic Testing Using Flow Cytometry thank James D. Hoyer, MD (Department of Pathology, Mayo Foundation) for undertaking retrospective analysis of the eosin-5-maleimide binding results obtained in one year from hereditary spherocytosis (HS) patients' samples sent to his laboratory. His analysis has calculated the ratios (unpublished data) presented in Table 3 of H52, as well as provided additional support for detecting patients of very mild HS whose RBCs could give fluorescence readings in the gray zone.

Contents

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Foreword

The recommendations contained herein address both methods in daily use in diagnostic clinical flow cytometry (FCM) and methods for verification or calibration of other assays, including automated cell counting instruments.

Presently, there are no universally accepted standards for precision, accuracy, and interlaboratory comparability in FCM. The recommendations provided in this document reflect the committee's understanding of best practices at the time of publication and in accordance with the present guidelines of the International Council for Standardization in Haematology.

This document replaces the first edition of the approved guideline, H52-A, which was published in 2001. Several changes were made in this edition; chief among them is the revision of the document scope from restricted to fetomaternal hemorrhage (FMH) testing methods to a broader scope of all diagnostic assays on RBCs using FCM. These changes reflect both the expansion of diagnostic FCM testing using RBCs and the clinical need to provide guidelines for testing methods not previously covered by CLSI documents. Specifically, this revision expands the discussion of FMH testing to include preferred testing methodology relating to the diagnosis of paroxysmal nocturnal hemoglobinuria and nonimmune membrane-associated hemolytic anemias (hereditary spherocytosis, hereditary pyropoikilocytosis, and ovalocytosis). Additional diagnostic tools to further evaluate anemic conditions by the reliable quantitation of adult F-cells and nucleated RBCs are also included.

Key Words

Anemia, diagnostic testing, erythrocyte, fetomaternal hemorrhage, flow cytometry, hemolytic anemia, red blood cells

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Red Blood Cell Diagnostic Testing Using Flow Cytometry; Approved Guideline—Second Edition

1 Scope

This document establishes performance guidelines for applying the science of flow cytometry (FCM) to RBC diagnostic testing. It provides guidelines for:

- Specimen collection, handling, and storage
- Procedures for calibrating instruments
- Procedures for QC of blood samples

Specific sections are dedicated to:

- Paroxysmal nocturnal hemoglobinuria (PNH)
- Diseases of RBC shape, including hereditary spherocytosis (HS)
- Detection of fetomaternal hemorrhage (FMH)
- Detection of nucleated RBCs (NRBCs)

This document is intended for use by laboratory practitioners, *in vitro* diagnostic (IVD) device manufacturers concerned with quality laboratory medicine practice, and regulators responsible for clearance of new diagnostic devices and quality laboratory medicine practice.

While there are many other RBC applications, particularly in the area of blood transfusion science or immunohematology, they will not be addressed in this guideline. In addition, 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

FCM is an established technology in both the research and clinical laboratory. Recently, several methodologies that allow for precise identification and enumeration of fetal RBCs in the maternal circulation and of membrane surface marker defects in PNH have been introduced into the routine clinical laboratory. The original osmotic fragility (OF) test for the detection of HS has been replaced in many large centers by the simpler and more reproducible eosin-5-maleimide (EMA) binding test. The laborious sugar water and Ham tests have been replaced by direct measurement of decreased or defective phosphatidylinositol-linked proteins by FCM for the diagnosis of PNH. Finally, the detection of NRBCs using a nuclear dye and the pan leukocyte marker CD45 allows accurate enumeration of NRBCs in those samples in which hematology analyzers may have difficulty doing so.

The goal of this document is to describe methodologies and QA procedures that will help ensure precision and accuracy of flow cytometric results appropriate for their use in the clinical laboratory. This document should be used in conjunction with other guidance documents, particularly the 2013 International Council for Standardization in Haematology/International Clinical Cytometry Society (ICSH/ICCS) Validation of Cell-based Fluorescence Assays: Practice Guidelines.[1-5]