



H26-A2

Validation, Verification, and Quality Assurance of Automated Hematology Analyzers; Approved Standard—Second Edition

This document provides guidance for the validation, verification, calibration, quality assurance (QA), and quality control (QC) of automated multichannel hematology analyzers for manufacturers, end-user clinical laboratories, accrediting organizations, and regulatory bodies. In addition, end-user clinical laboratories will find guidance for establishment of clinically reportable intervals and for QA for preexamination and examination aspects of their systems.

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

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Validation, Verification, and Quality Assurance of Automated Hematology Analyzers; Approved Standard—Second Edition

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Abstract

Clinical and Laboratory Standards Institute document H26-A2—*Validation, Verification, and Quality Assurance of Automated Hematology Analyzers; Approved Standard—Second Edition* provides guidance for the validation, verification, calibration, quality assurance (QA), and quality control (QC) of automated multichannel hematology analyzers. The intended audience includes manufacturers of such devices, end-user clinical laboratories, accrediting organizations, and regulatory bodies.

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SAMPLE

Foreword

From a medical perspective, the value of an automated hematology analyzer is to provide physicians and other health care providers with reliable hematology data for patient management. From a patient and regulatory perspective, all complete blood count (CBC) results should be statistically and medically comparable on any hematology analyzer.

Reliable data depend on robust system design, which is initially *validated* by the manufacturer using formal study protocols/procedures, and subsequently *verified* by the end-user laboratory. Manufacturers' validations typically include a combination of in-house testing with normal donor blood specimens and evaluations of patient specimens at external practicing clinical laboratories. Verification should focus on the laboratory's specific patient populations.

Because CBC analyses are performed on whole blood, which is a heterogeneous suspension of blood cells, particular attention to various preexamination aspects of specimen collection and handling is critical to success in generating accurate patient results.

With respect to end-user quality control (QC) in the examination phase, the needs of clinical chemistry established the original patterns of internal and external QC methodology and the design of control materials and calibrators. Although automated hematology analyzers share these principles, they also have unique characteristics that require some specialized approaches to QC.

This document replaces and expands on two CLSI hematology documents (H26-A and H38-P) that are no longer in the consensus process. Those documents addressed only calibration and QC of automated analyzers. The present new document adds comprehensive sections for system validation and verification, as well as consideration of preexamination topics. Its approach is more practical than the previous documents, and is focused on specific technical details, with greatly expanded literature references.

Key Words

Analytical measuring interval, automated hematology, carryover, clinically reportable interval, comparability, correlation, imprecision, limit of blank, lower limit of detection, lower limit of quantitation, quality control, reference intervals, repeatability, validation study design, verification study design

Validation, Verification, and Quality Assurance of Automated Hematology Analyzers; Approved Standard—Second Edition

1 Scope

This document covers portions of the life cycle of an automated multichannel hematology system and provides guidance for validation, verification, calibration, quality assurance (QA), and quality control (QC) through standardized approaches to ensure good laboratory science and clinical relevance. The intended audience includes manufacturers of such devices, end-user clinical laboratories, accrediting organizations, and regulatory bodies.

End-user clinical laboratories will also find guidance for establishment of clinically reportable intervals (CRIs) and for QA for preexamination and examination aspects of their systems.

Because current blood cell counters also provide results beyond particle counting and differential separation of cell types, such as expanded platelet and reticulocyte measurements, extended leukocyte differential subtyping, and most recently, proteomic measurements through antigenic identification (ID) using fluorescence measurements, consult the potentially relevant CLSI documents H42,¹ H43,² H44,³ H52,⁴ and I/LA24.⁵

2 Introduction

Historically, each complete blood count (CBC) instrument/reagent manufacturer developed its unique approaches to system validation and performance claims. It is hoped that this document will provide better standardization and transparency across manufacturers. Similarly, a uniform approach to end-user laboratory verification was lacking, and this document should assist that audience in developing consistent testing.

Calibration, internal QC, and external quality assessment (EQA) of hematology analyzers are commonly dependent on stabilized blood products that may contain surrogate particles and nonhuman cells in a nonphysiological fluid. When possible, fresh human blood should be part of an overall QC program, to enhance linkage between QC data and reportable patient results.

In addition to the use of stabilized blood, data generated by statistical analyses of patient assays serve as sources of information for QC, as commonly practiced in the hematology section of the clinical laboratory.

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 known 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.⁶ For specific precautions for preventing the laboratory transmission of all known infectious agents from laboratory instruments and materials and for recommendations for the management of exposure to all known infectious diseases, refer to CLSI document M29.⁷

4 Terminology

4.1 A Note on Terminology

CLSI, as a global leader in standardization, is firmly committed to achieving global harmonization whenever 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, International Organization for Standardization (ISO), and European Committee for Standardization (CEN) documents; and that legally required use of terms, regional usage, and different consensus timelines are all important considerations in the harmonization process. In light of this, CLSI's consensus process for development and revision of standards and guidelines focuses on harmonization of terms to facilitate the global application of standards and guidelines.

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 a measured quantity value and a true quantity value of a measurand, and comprises both random and systematic effects. *Trueness* is used in this document when referring to the closeness of agreement between the average of an infinite number of replicate measured quantity values and a reference quantity value; the measurement of trueness is usually expressed in terms of *bias*. *Precision* is defined as the "closeness of agreement between indications or measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions." As such, it cannot have a numerical value but may be determined qualitatively as high, medium, or low. For its numerical expression, the term *imprecision* is used, which is the "dispersion of results of measurements obtained under specified conditions." In addition, different components of precision are defined in H26-A2, primarily *repeatability*, ie, "precision under conditions where independent test results are obtained with the same method on identical test items in the same laboratory by the same operator using the same equipment within short intervals of time," whereas *reproducibility* describes "precision under conditions where test results are obtained with the same method on identical test items in different laboratories with different operators using different equipment."

The term *measurand* (quantity intended to be measured [ISO/IEC Guide 99]), replaces the term *analyte* (component represented in the name of a measurable quantity) when its use relates to a biological fluid/matrix; and the term *measurement procedure* replaces the term *analytical method* for a detailed description of a measurement according to one or more principles and to a given method, based on a model and including any calculation to obtain a result.

The terms *preexamination*, *examination*, and *postexamination* were adopted in place of *preanalytical*, *analytical*, and *postanalytical*, and the term *sample* replaces the term *specimen* where appropriate.

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

The users of H26-A2 should understand that the fundamental meanings of the terms are identical in many cases. The terms in this document are also consistent with those defined in the ISO 15189, ISO 17025, and ISO 9000 series of standards.

4.2 Definitions

accuracy (measurement) – closeness of agreement between a measured quantity value and a true quantity value of a measurand (ISO/IEC Guide 99).⁸

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 CLSI document HS01—*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 health care service’s path of workflow (ie, 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 QSEs are

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

H26-A2 addresses the QSEs indicated by an “X.” For a description of the other documents listed in the grid, please refer to the Related CLSI Reference Materials section on the following page.

Documents and Records	Organization	Personnel	Equipment	Purchasing and Inventory	Process Control	Information Management	Occurrence Management	Assessment—External and Internal	Process Improvement	Customer Service	Facilities and Safety
GP02			H42 H43 I/LA24	H03	X C24 C28 C54 EP05 EP06 EP09 EP15 EP17 EP21 GP27 H03 H04 H20 H42 H43 I/LA24	GP02		GP27 I/LA24	X GP27 I/LA24		H03 M29

Adapted from CLSI document HS01—*A Quality Management System Model for Health Care*.

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 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.

H26-A2 addresses the clinical laboratory path of workflow steps indicated by an “X.” For a description of the documents listed in the grid, please refer to the Related CLSI Reference Materials section on the following page.

Preexamination				Examination			Postexamination	
Examination ordering	Sample collection	Sample transport	Sample receipt/processing	Examination	Results review and follow-up	Interpretation	Results reporting and archiving	Sample management
C54 H03	X H03 H04 H15	C54 H03	H03 H07 H15 H20	X H03 H07 H15 H20	C54 H03 H07 H15 H20			X
H42 H43	H42 H43 H44	H42 H43 H44	H42 H43 H44 H52	H42 H43 H44 H52	H42 H43 H44	H42 H43 H44	H42 H43 H44	H44

Adapted from CLSI document HS01—*A Quality Management System Model for Health Care*.

Related CLSI Reference Materials*

- C24-A3** **Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions; Approved Guideline—Third Edition (2006).** This guideline provides definitions of analytical intervals, planning of quality control procedures, and guidance for quality control applications.
- C28-A3** **Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline—Third Edition (2008).** This document contains guidelines for determining reference values and reference intervals for quantitative clinical laboratory tests.
- C54-A** **Verification of Comparability of Patient Results Within One Health Care System; Approved Guideline (2008).** This document provides guidance on how to verify comparability of quantitative laboratory results for individual patients within a health care system.
- EP05-A2** **Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline—Second Edition (2004).** This document provides guidance for designing an experiment to evaluate the precision performance of quantitative measurement methods; recommendations on comparing the resulting precision estimates with manufacturers' precision performance claims and determining when such comparisons are valid; as well as manufacturers' guidelines for establishing claims.
- EP06-A** **Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline (2003).** This document provides guidance for characterizing the linearity of a method during a method evaluation; for checking linearity as part of routine quality assurance; and for determining and stating a manufacturer's claim for linear range.
- EP09-A2** **Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline—Second Edition (2002).** This document addresses procedures for determining the bias between two clinical methods, and the design of a method comparison experiment using split patient samples and data analysis.
- EP15-A2** **User Verification of Performance for Precision and Trueness; Approved Guideline—Second Edition (2005).** This document describes the demonstration of method precision and trueness for clinical laboratory quantitative methods utilizing a protocol designed to be completed within five working days or less.
- EP17-A** **Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline (2004).** This document provides guidance for determining the lower limit of detection of clinical laboratory methods, for verifying claimed limits, and for the proper use and interpretation of the limits.
- EP21-A** **Estimation of Total Analytical Error for Clinical Laboratory Methods; Approved Guideline (2003).** This document provides manufacturers and end users with a means to estimate total analytical error for an assay. A data collection protocol and an analysis method that can be used to judge the clinical acceptability of new methods using patient specimens are included. These tools can also monitor an assay's total analytical error by using quality control samples.
- GP02-A5** **Laboratory Documents: Development and Control; Approved Guideline—Fifth Edition (2006).** This document provides guidance on development, review, approval, management, and use of policy, process, and procedure documents in the medical laboratory community.
- GP27-A2** **Using Proficiency Testing to Improve the Clinical Laboratory; Approved Guideline—Second Edition (2007).** This guideline provides assistance to laboratories in using proficiency testing as a quality improvement tool.

* CLSI documents are continually reviewed and revised through the CLSI consensus process; therefore, readers should refer to the most current editions.

Related CLSI Reference Materials (Continued)

- H03-A6** **Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved Standard—Sixth Edition (2007).** This document provides procedures for the collection of diagnostic specimens by venipuncture, including line draws, blood culture collection, and venipuncture in children.
- H04-A6** **Procedures and Devices for the Collection of Diagnostic Capillary Blood Specimens; Approved Standard—Sixth Edition (2008).** This document provides a technique for the collection of diagnostic capillary blood specimens, including recommendations for collection sites and specimen handling and identification. Specifications for disposable devices used to collect, process, and transfer diagnostic capillary blood specimens are also included.
- H07-A3** **Procedure for Determining Packed Cell Volume by the Microhematocrit Method; Approved Standard—Third Edition (2000).** This document describes a standard microhematocrit method for determining packed cell volume; specifications for recommended materials and information on potential sources of error are also included.
- H15-A3** **Reference and Selected Procedures for the Quantitative Determination of Hemoglobin in Blood; Approved Standard—Third Edition (2000).** This document describes the principle, materials, and procedure for reference and standardized hemoglobin determinations. It includes specifications for secondary hemiglobin cyanide (HiCN) standards.
- H20-A2** **Reference Leukocyte (WBC) Differential Count (Proportional) and Evaluation of Instrumental Methods; Approved Standard—Second Edition (2007).** This document is a reference method for the evaluation of automated differential counters, based on the visual differential count.
- 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.
- H43-A2** **Clinical Flow Cytometric Analysis of Neoplastic Hematolymphoid Cells; Approved Guideline—Second Edition (2007).** 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.
- H44-A2** **Methods for Reticulocyte Counting (Automated Blood Cell Counters, Flow Cytometry and Supravital Dyes); Approved Guideline—Second Edition (2004).** This document provides guidance for the performance of reticulocyte counting by flow cytometry. It includes methods for determining the trueness and precision of the reticulocyte flow cytometry instrument and a recommended reference procedure.
- H52-A** **Fetal Red Cell Detection; Approved Guideline (2001).** This document provides guidance for the quantitation of fetal red blood cells in blood and other biologic fluids. The performance characteristics of various flow cytometric and microscopic assays are reviewed, recommendations are made for control usage, and principles for distinction of F cells and fetal red cells are discussed.
- 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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