CLINICAL AND LABORATORY STANDARDS INSTITUTE®

1st Edition

H62

Validation of Assays Performed by Flow Cytometry

This guideline includes validation strategies for cell-based assays performed by flow cytometry. This guideline also includes recommendations for instrument qualification and standardization and assay optimization. It also covers recommended practices for the examination and postexamination phases.

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

Abstract

Clinical and Laboratory Standards Institute guideline H62—*Validation of Assays Performed by Flow Cytometry* focuses primarily on analytical method validation. There are currently no official guidance documents for the validation of assays performed by flow cytometry. Existing guidance for the validation of biochemical methods for quantifying soluble analytes found in plasma, serum, and urine is not fully applicable for quantification and characterization of cellular measurands. Validation of flow cytometry is challenging because the data generated are not derived from a calibration curve and true reference standards are lacking. Additional topics covered in this guideline include instrument qualification and standardization and assay optimization. It also covers recommended practices for the examination and postexamination phases. The recommendations presented in H62 are applicable to a wide range of flow cytometry laboratories, including basic research facilities, biopharmaceutical companies, medical laboratories, and manufacturers. This guideline provides specific recommendations for the appropriate analytical method validation approach based on the intended use of the data and regulatory and accreditation requirements, if any, associated with this use. H62 is designed to assist any laboratory using flow cytometry, as well as manufacturers, in developing, validating, verifying, controlling, analyzing, and implementing fluorescence cell-based assays.

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Foreword

Multiparametric flow cytometry is one of the leading technologies for cellular analysis because it allows simultaneous detection of numerous characteristics of individual cells with relatively high throughput. Although this technology has been a critical component in medical laboratories and drug development for many years, its importance has increased dramatically in the past few years.

In medical laboratories, flow cytometry became an important platform in the mid-1980s when CD4 T-cell counts became critical measurements in the diagnosis and treatment of AIDS. The importance of flow cytometry continued to grow as flow cytometric methods were used to count $CD34^+$ cells for hematopoietic stem cell transplantation and to guide diagnosis and treatment decisions for leukemia and lymphoma as well as other blood diseases, such as paroxysmal nocturnal hemoglobinuria. More recently, flow cytometry has become a critical tool in the assessment of measurable residual disease in leukemia and lymphoma. Flow cytometric analysis provides a tool to effectively evaluate patients with primary immunodeficiencies and to confirm or establish the immune phenotype of a gene mutation.[1]

In the biopharmaceutical industry, this flexible and powerful platform has been important in supporting biomarkers in all phases of drug development for nearly 20 years. More recently, with the introduction of immunotherapeutic agents, novel vaccines, and cell-based therapies, flow cytometry has become a critical tool supporting every aspect from manufacturing to primary end point determinations in medical development. This heightened role for flow cytometry in both laboratory medicine and drug development has resulted in an increased need for high-quality and validated methods, which in turn has created a need for official guidance from regulatory agencies and accreditation organizations regarding the validation of assays performed by flow cytometry. Because no official guidance exists for validation of assays used in flow cytometry, H62 seeks to fill the need for consensus recommendations.

Because data should be reliable, no matter the intended use, the target audience for this guideline includes nonregulated laboratories such as basic science research laboratories, as well as regulated laboratories. As such, a one-size-fits-all approach to analytical method validation is not appropriate. This guideline presents a fit-for-purpose (FFP) approach to analytical method validation, as described in Chapters 3 and 6. Briefly, the concept of FFP method validation was introduced in 2005 by the American Association of Pharmaceutical Scientists.[2] This publication conveyed the message that although some degree of validation should always be conducted to generate reliable data, the level of validation should be tailored to the intended use of the data. If, as is the case in preclinical or nonclinical settings such as drug development and basic research, the intended use of the data changes, additional validation should be conducted to meet the new intended use and regulatory requirements associated with this use. However, in a clinical environment, the intended use of the data from assays used for clinical diagnosis and longitudinal monitoring would not change. The term "FFP method validation" appears in numerous publications, including *Bioanalytical Method Validation: Guidance for Industry,* which was published in 2018 by the Center for Drug Evaluation and Research, for the US Food and Drug Administration,[3] and should not be misinterpreted as a justification for inadequate validation. H62 presents minimal standards for FFP, as well as analytical validation for a wide variety of intended uses (see Table 22 and Appendix A for more information).

Flow cytometric methods pose unique validation challenges due to the complexity of cellular measurands and the lack of reference materials and because data are not typically derived from a calibration curve. Thus, the existing recommendations for validation of biochemical methods or ligand-binding assays for quantifying soluble analytes found in plasma, serum, and urine cannot be fully applied in the validation of flow cytometric methods for quantifying cellular measurands. In addition to discussing analytical method validation, this guideline provides recommendations for instrument characterization and standardization and assay development and optimization, as well as recommended practices for the examination and postexamination phases. The content is designed to assist laboratories and manufacturers in developing, validating, verifying, controlling, analyzing, and implementing cell-based assays performed by flow cytometry.

NOTE: The content of this guideline is supported by the CLSI consensus process and does not necessarily reflect the views of any single individual or organization.

KEY WORDS

Cell-based assay
Context of use
Fit-for-purpose
Flow cytometry
Laboratory-developed tests
Standardization
Validation
Verification

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Chapter 1

Introduction

This chapter includes:

- Guideline's scope and applicable exclusions
- Standard precautions information
- Terminology information, including:
  - Terms and definitions used in the guideline
  - Abbreviations and acronyms used in the guideline

Validation of Assays Performed by Flow Cytometry

1 Introduction

1.1 Scope

This guideline focuses on the unique requirements for the analytical validation of cell-based assays performed by flow cytometry, which are not covered in other CLSI documents. Although flow cytometry can be used for a wide variety of applications other than cellular analysis, this guideline focuses on cellular analysis; however, the general principles are also applicable to noncellular particles. Recommendations and practical instructions are provided for preexamination phase activities such as sample requirements, reagent optimization evaluation, instrument qualification and standardization, and assay optimization and validation. Guidance for examination phase activities such as instrument monitoring and QC are described, as are recommended practices for postexamination activities, including data review, reporting, storage, and retention. This guideline is intended for use in a flow cytometry environment in which preclinical (or nonclinical) and clinical assessments are conducted, including but not limited to:

- Research laboratories (academic and nonacademic)
- Medical laboratories
- Drug discovery, development, and manufacturing companies
- Reagent, assay, and instrument manufacturers
- Regulatory agencies

This guideline provides general recommendations but does not discuss details of specific applications, such as lymphocyte immunophenotyping or neoplastic cell or erythrocyte analysis, which are covered in CLSI documents H42,[4] H43,[5] and H52.[6] The validation of flow cytometric assays for noncellular measurands or soluble analytes is beyond the scope of this guideline. Software validation is also beyond the scope of this guideline. For more information about software validation, see CLSI document AUTO13.[7] In a regulated setting, it is highly desirable to use software adhering to 21 CFR Part 11 guidelines[8] whenever possible; however, these features are not supported by many flow cytometry software packages. Manual processes must be used to control noncompliant software functionality or to adopt compliant software packages.

1.2 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 bloodborne pathogens. Published guidelines are available that discuss the daily operations of diagnostic medicine in humans and animals while encouraging a culture of safety in the laboratory.[9] For specific precautions required 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.[10]

2 Path of Workflow and Quality System Essentials

2.1 Flow Cytometry Assay Validation Process

Figure 1 depicts the recommended flow cytometry assay validation process. These steps include installation qualification (IQ) (see Chapter 4), assay development and optimization (see Chapter 5), validation planning and implementation (see Subchapters 6.1 and 6.2), and QC verification (see Subchapter 7.4.3). Subchapter 7.4.5 describes sample processing and acquisition.

Abbreviations: EQA, external quality assessment; IQ, installation qualification; OQ, operational qualification; PQ, performance qualification; PT, proficiency testing; QA, quality assurance; QC, quality control.

Figure 1. Flow Cytometry Assay Validation Process

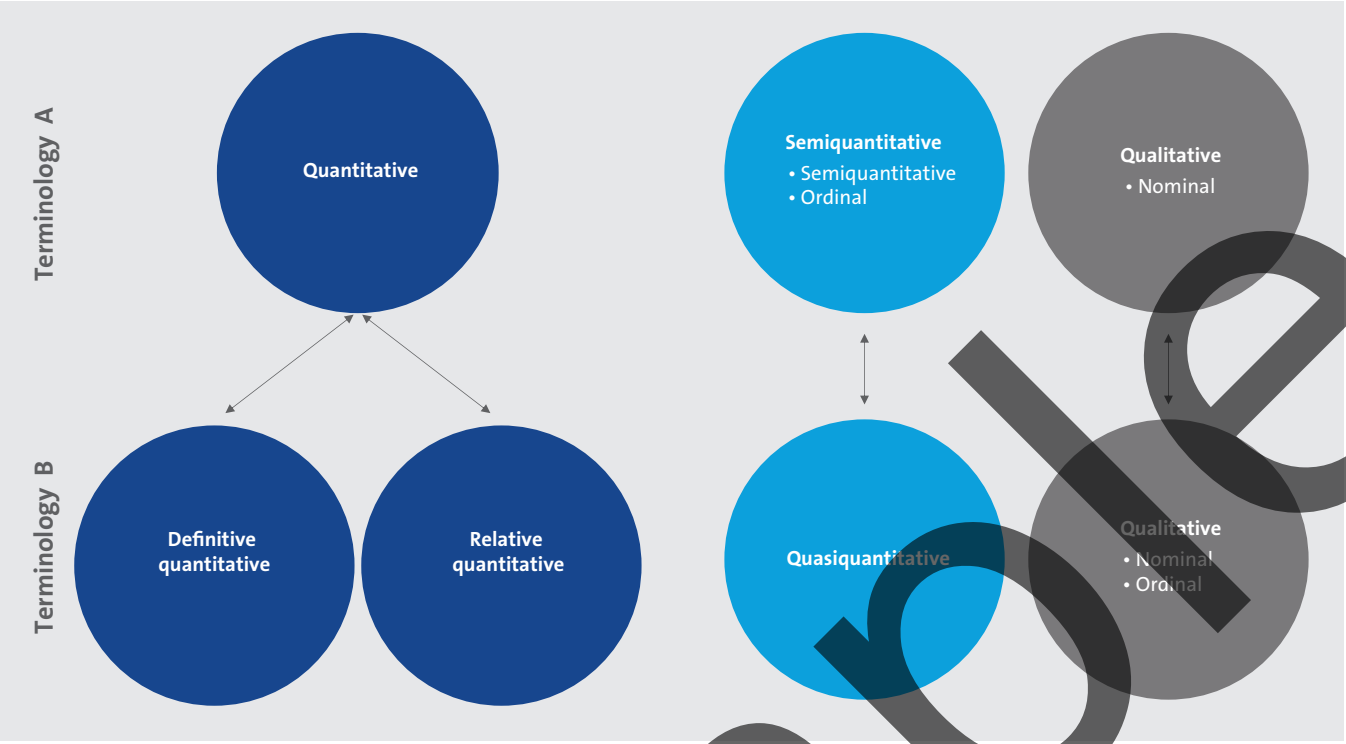

Figure 2. Terminology for Bioanalytical Data Categories. This diagram illustrates the relationship between different terms used to describe bioanalytical data. With terminology A, "quantitative data" is used more broadly, whereas with terminology B, the quantitative data are more granular and include "definitive quantitative" and "relative quantitative" (refer to Subchapters 3.1.1.1 and 3.1.1.2). The definitions for "semiquantitative" and "quasiquantitative" seem to be the same (continuous numeric results expressed in terms that are characteristic of the test samples but are not derived from an authoritative calibration curve or standard reference material), with the exception that with terminology A, semiquantitative data also include ordinal data. With terminology A, qualitative data include only nominal qualitative values. Terminology A is more broadly accepted by ISO and regulatory agencies, whereas terminology B often appears in recommendation papers from the biopharmaceutical biomarker community.[2,21-24,41]

3.1.1.1 Definitive Quantitative Data

Definitive quantitative data are used to determine the absolute quantitative values for unknown samples using well-defined reference standards that are fully representative of the endogenous measurand. Examples of assays that generate definitive quantitative data include pharmacokinetic (PK) and liquid chromatography–mass spectrometry assays. Nonetheless, all laboratory measurements, including those classified as definitive quantitative, are imperfect and have some degree of uncertainty associated with the measurand.

3.1.1.2 Relative Quantitative Data

The key differences between definitive and relative quantitative data are how closely the reference standard represents the endogenous measurand and traceability to primary standards. For example, certified consensus reference material is not available[42,43] for most ligand-binding assays (LBAs). In addition, manufacturer-to-manufacturer and lot-to-lot variability with regard to the calibrators and reference standards has been reported.[42,43] Often, the calibrators and reference standards are not identical to the measurand with regard to post-translational modifications and carrier proteins.

Related CLSI Reference Materials[a]

AUTO13 **Laboratory Instrument and Data Management Systems: Design of Software User Interfaces and End-User Software Systems Validation, Operation, and Monitoring. 2nd ed., 2003.** This document identifies important factors that designers and laboratory managers should consider when developing new software-driven systems and selecting software user interfaces. Also included are simple rule to help prepare validation protocols for assessing functionality and dependability of software.

EP05 **Evaluation of Precision of Quantitative Measurement Procedures. 3rd ed., 2014.** This document provides guidance for evaluating the precision performance of quantitative measurement procedures. It is intended for manufacturers of quantitative measurement procedures and for laboratories that develop or modify such procedures.

EP06 **Evaluation of the Linearity of Quantitative Measurement Procedures. 2nd ed., 2020.** This guideline provides information for characterizing the linearity interval of a measurement procedure, validating a linearity interval claim (to be performed by the manufacturer), and verifying an established linearity interval claim (to be performed by the end user).

EP07 **Interference Testing in Clinical Chemistry. 3rd ed., 2018.** This guideline provides background information, guidance, and experimental procedures for investigating, identifying, and characterizing the effects of interferents on clinical chemistry test results.

EP17 **Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures. 2nd ed., 2012.** This document provides guidance for evaluation and documentation of the detection capability of clinical laboratory measurement procedures (ie, limits of blank, detection, and quantitation), for verification of manufacturers' detection capability claims, and for the proper use and interpretation of different detection capability estimates.

EP12 **User Protocol for Evaluation of Qualitative Test Performance. 2nd ed., 2008.** This document provides a consistent approach for protocol design and data analysis when evaluating qualitative diagnostic tests. Guidance is provided for both precision and method-comparison studies.

EP14 **Evaluation of Commutability of Processed Samples. 3rd ed., 2014.** This document provides guidance for evaluating the commutability of processed samples by determining if they behave differently than unprocessed patient samples when two quantitative measurement procedures are compared.

EP23™ **Laboratory Quality Control Based on Risk Management. 1st ed., 2011.** This document provides guidance based on risk management for laboratories to develop quality control plans tailored to the particular combination of measuring system, laboratory setting, and clinical application of the test.

[a] 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)

EP25 **Evaluation of Stability of *In Vitro* Diagnostic Reagents. 1st ed., 2009.** This document provides guidance for establishing shelf-life and in-use stability claims for *in vitro* diagnostic reagents such as reagent kits, calibrators, and control products.

EP28 **Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory. 3rd ed., 2010.** This document contains guidelines for determining reference values and reference intervals for quantitative clinical laboratory tests.

EP37 **Supplemental Tables for Interference Testing in Clinical Chemistry. 1st ed., 2018.** This document includes recommended testing concentrations for analytes and endogenous substances that may interfere in clinical chemistry measurement procedures and is intended for use with the evaluation procedures in the Clinical and Laboratory Standards Institute guideline EP07.

H42 **Enumeration of Immunologically Defined Cell Populations by Flow Cytometry. 2nd ed., 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 **Clinical Flow Cytometric Analysis of Neoplastic Hematolymphoid Cells. 2nd ed., 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.

H52 **Red Blood Cell Diagnostic Testing Using Flow Cytometry. 2nd ed., 2014.** 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.

M29 **Protection of Laboratory Workers From Occupationally Acquired Infections. 4th ed., 2014.** 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.

QMS01 **A Quality Management System Model for Laboratory Services. 5th ed., 2019.** This guideline provides a model for medical laboratories to organize the implementation and maintenance of an effective quality management system.

QMS03 **Training and Competence Assessment. 4th ed., 2016.** This guideline provides a structured approach for developing effective laboratory personnel training and competence assessment programs.

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