CLINICAL AND LABORATORY STANDARDS INSTITUTE®

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Clinical Evaluation of Immunoassays; Approved Guideline—Second Edition

This document addresses the need for clinical evaluation of new immunoassays and new applications of existing assays, as well as multiple assay formats and their uses. As a guide to designing and executing a clinical evaluation, this document will aid developers of "in-house" assays for institutional use, developers of assays used for monitoring pharmacologic effects of new drugs or biologics, and clinical and regulatory personnel responsible for commercializing products.

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

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Clinical Evaluation of Immunoassays; Approved Guideline—Second Edition

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Abstract

Clinical and Laboratory Standards Institute document I/LA21-A2—*Clinical Evaluation of Immunoassays; Approved Guideline—Second Edition* addresses all aspects of the clinical evaluation of immunoassays developed for commercial or in-house use.

Existing CLSI documents provide guidance for assessing analytical performance, methods comparison, and clinical accuracy of laboratory tests. This document focuses on unique characteristics of immunoassays, and provides a guide to designing, executing, and analyzing a clinical evaluation. In addition, this document will aid developers of "in-house" assays for institutional use, developers of assays used for monitoring pharmacologic effects of new drugs or biologics, and clinical and regulatory personnel responsible for commercializing products.

The elements of this guideline include: 1) a development plan for an effective analysis and evaluation; 2) a discussion of the planning and design considerations that are necessary for a successful evaluation; 3) a description of requirements for conducting the evaluation through monitoring and database management; and 4) a brief review of the analytical performance measures that must be in place before testing clinical specimens.

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Contents

Foreword

This document updates previous guidelines describing the requirements for the clinical evaluation of immunoassays. In preparing this guideline, the working group considered three areas of need regarding the clinical use of immunoassays: 1) for laboratories engaged in the development of immunoassays for use within their institutions, this guideline will provide direction in designing an evaluation of the assay's clinical performance; 2) for those scientists involved in evaluating new therapeutic agents, this guideline will provide direction in establishing immunoassays as reliable clinical end points; and 3) for manufacturers of *in vitro* diagnostic assays, this guideline will provide a checklist to review against their approach to addressing regulatory requirements for commercialization of products.

For the purposes of this document, clinical performance refers to correct classification (ie, clinical [diagnostic] sensitivity and specificity) and does not refer to clinical utility, which may include the effects of environment, economy, and patient outcomes. While there is mention of an assay's analytical performance, users should refer to existing CLSI documents (see the Related CLSI Reference Materials section) and to other sources for more detailed information.

Because the scope of this document does not limit its application to industry or to the clinical or research laboratory, the working group has used the term *clinical evaluation* in place of "clinical study" or "clinical trial." While considered interchangeable from the working group's perspective, the reader should use the term that is appropriate for his or her institution.

It should also be acknowledged that there are different types of evaluations for new assays, including comparative and clinical. Comparative evaluations are typically performed when the laboratorian is considering substituting an assay from one manufacturer with another from a different manufacturer. While having its own unique forms of execution and analyses, this evaluation is simply comparing one assay to another without the postulation of any clinical questions. See the related reference for comparative evaluations (see CLSI/NCCLS document EP09).[1] While it may involve a comparative approach, the clinical evaluation is required for the application of a new assay, a new analyte (measurand), or for a new, intended use of an existing analyte (measurand).

In the assay development to implementation/commercialization continuum, this guideline addresses the activities associated with preclinical testing and clinical evaluation requirements, evaluation design, and analysis. While written for immunoassay developers, the information has broad application to other clinical and research assay formats.

This Revision

During the revision process, the working group updated the content and expanded the current document for assessment of immunoassays to include specific details on selection and use of test specimen panels; specimen library collections; reference panels including specimen commutability issues; and sample size considerations for evaluation studies. An appendix was also added to guide the user in sample size selections. Numerous revisions were made to enhance and ensure global applications.

Key Words

clinical evaluation, clinical evaluation investigator, clinical performance characteristics, database management, diagnostic evaluation, diagnostic performance characteristics, evaluation population, informed consent, institutional review board, pilot evaluation, sponsor, statistical tests

Clinical Evaluation of Immunoassays; Approved Guideline—Second Edition

1 Scope

This document provides specific recommendations for the clinical evaluation of new immunoassays and new applications of existing assays, as well as multiple assay formats and their uses. It focuses on unique characteristics of immunoassays, and provides a guide to designing, executing, and analyzing a clinical evaluation.

The intended users of this guideline are developers of "in-house" assays for institutional use, developers of assays used for monitoring pharmacologic effects of new drugs or biologics, and clinical and regulatory personnel responsible for commercializing products.

2 Introduction

In vitro diagnostic (IVD) assay development occurs in multiple environments, each with different requirements for the actual use of the assay. Developers that manufacture and market their assays are required to obtain approval in accordance with regulations in countries where the product is being registered or used. (Consult appropriate local regulations and institutional policies for specific applications.) Others may develop assays that do not require the same extent of testing and review. For example, clinical and research laboratories may develop assays for use within their institutions, thereby limiting the assay's use to a select patient population. Another example is the development of research assays for use in monitoring pharmacologic effects or unfavorable reactions. A third example is the development of assays for point-of-care (POC) testing, which require evaluation under conditions in which the assay is performed. This document addresses the need for clinical evaluation of new immunoassays and new applications of existing assays, as well as multiple assay formats and their uses. Whatever the intended use, the assay can only provide medical benefit when it has undergone adequate clinical evaluation.

Separated into four basic sections, this guidance is intended to provide all assay developers with a consistent approach to establishing clinical performance characteristics. First, the immunoassay (preclinical) evaluation is described, including recommendations for establishing analytical performance. Second, the planning and design of the clinical evaluation is discussed. Third, a description of conducting the clinical evaluation is given; and fourth, analysis and summary documentation is reviewed.

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.[2] 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 CLSI document M29.[3]

4 Terminology

4.1 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, the International Organization for Standardization (ISO), and the European Committee for Standardization (CEN) documents; and that legally required use of terms, regional usage, and different consensus timelines are all challenges 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 the context of this document, it is necessary to point out that several terms are used differently in the United States and other countries, notably those in Europe.

In Europe, the term *performance evaluation* is used for the assessment of quality of IVD products both with their analytical and medical (diagnostic) characteristics. *Clinical evaluation* in European terms is applied mostly to the evaluation of medical products, which are used on or in patients or when it refers to clinical studies of drugs, under much more stringent conditions. Appropriately, the US term *clinical evaluation* in the context of this document translates into *diagnostic evaluation* in Europe. Consequently, the terms *diagnostic sensitivity* and *diagnostic specificity* are used in Europe, with the corresponding expressions *clinical sensitivity* and *clinical specificity* in the United States, as they are applied in this document.

In I/LA21, the following terms have been changed to or used parenthetically with their US counterparts to be consistent with accepted international usage: *accuracy* has been changed to *trueness*; *analytical method* has been changed to *measurement procedure*; and *measurand* is used parenthetically with *analyte*.

4.2 Definitions[a]

accuracy (of measurement) – closeness of the agreement between the result of a (single) measurement and a true value of the measurand (analyte) (ISO 17511)[4]; **NOTE:** Accuracy is not a synonym for trueness; but is the combination of trueness and precision (ISO 5725-1).[5]

analyte – component represented in the name of a measurable quantity (ISO 17511)[4]; **NOTE:** Formerly in CLSI documents, analyte was used to describe both a single component (analyte) as well as the analyte in its specific matrix.

analytical sensitivity – see **limit of detection.**

analytical specificity – ability of a measurement procedure to measure solely the measurand (ISO 17511).[4]

area under the [ROC] curve (AUC) – as applied to receiver operating characteristic (ROC) curves, the area subtended by the ROC curve and bounded by the X-axis (false-positive fraction) and the Y-axis (true-positive fraction); **NOTE 1:** By convention, the total area in ROC space is exactly 1 unit. A

[a] These definitions are found in the CLSI harmonized terminology database at www.clsi.org. For complete definitions and detailed source information, please refer to the database.

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

I/LA21-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 & Records	Organization	Personnel	Equipment	Purchasing & Inventory	Process Control	Information Management	Occurrence Management	Assessments—External & Internal	Process Improvement	Customer Service	Facilities & Safety
X		X	X C50	H03	X C24, C28, EP05, EP06, EP07, EP09, EP10, EP12, EP14, GP10, H03, H04, I/LA18, I/LA23, I/LA29, I/LA30, M29, X05	X		X EP10	X EP07	I/LA23	X H03 M29

Adapted from CLSI/NCCLS 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/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.

I/LA21-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 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
H03	H03 H04 I/LA23	H03 I/LA23	H03 I/LA23 I/LA29	H03 I/LA23 I/LA29	C50 H03 I/LA23 I/LA29	C50 I/LA23 I/LA29		I/LA23

Adapted from CLSI/NCCLS 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-A2 **How to Define and Determine Reference Intervals in the Clinical Laboratory; Approved Guideline—Second Edition (2000).** This document contains guidelines for determining reference values and reference intervals for quantitative clinical laboratory tests.

C50-A **Mass Spectrometry in the Clinical Laboratory: General Principles and Guidance; Approved Guideline (2007).** This guideline provides a general understanding of mass spectrometry and the principles that dictate its application in the clinical laboratory. It includes guidance, references, and quality assurance markers that will assist with the implementation and correct operation of a mass spectrometry (MS) system for its many applications. Information on maintaining optimum performance, approaches to ensuring accurate and precise mass measurement, verification of methods, quality control of assays within and between instruments, instrument troubleshooting, sample preparation, interpretation of results, and limitations of the technology is included.

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.

EP07-A2 **Interference Testing in Clinical Chemistry; Approved Guideline—Second Edition (2005).** This document provides background information, guidance, and experimental procedures for investigating, identifying, and characterizing the effects of interfering substances on clinical chemistry test results.

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.

EP10-A3 **Preliminary Evaluation of Quantitative Clinical Laboratory Measurement Procedures; Approved Guideline—Third Edition (2006).** This guideline provides experimental design and data analysis for preliminary evaluation of the performance of a measurement procedure or device.

EP12-A2 **User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline—Second Edition (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-A2 **Evaluation of Matrix Effects; Approved Guideline—Second Edition (2005).** This document provides guidance for evaluating the bias in analyte measurements that is due to the sample matrix (physiological or artificial) when two measurement procedures are compared.

GP10-A **Assessment of the Clinical Accuracy of Laboratory Tests Using Receiver Operating Characteristic (ROC) Plots; Approved Guideline (1995).** This document provides a protocol for evaluating the accuracy of a test to discriminate between two subclasses of subjects where there is some clinically relevant reason to separate them. In addition to the use of ROC plots, the importance of defining the question, selecting the sample group, and determining the "true" clinical state are emphasized.

* Proposed-level documents are being advanced through the Clinical and Laboratory Standards Institute 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-A5 **Procedures and Devices for the Collection of Diagnostic Capillary Blood Specimens; Approved Standard—Fifth Edition (2004).** 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.

I/LA18-A2 **Specifications for Immunological Testing for Infectious Diseases; Approved Guideline—Second Edition (2001).** This document addresses specimen collection, handling, and storage, as well as performance criteria for the comparison of immunological test kits and specifications for reference materials.

I/LA23-A **Assessing the Quality of Immunoassay Systems: Radioimmunoassays and Enzyme, Fluorescence, and Luminescence Immunoassays; Approved Guideline (2004).** This guideline addresses components for harmonizing and assessing the quality of immunoassay systems for several commonly used dose-response indicator categories, eg, radioisotopes, enzymes, fluorescence, luminescence, reagents, and experimental components criteria essential to characterizing an immunoassay.

I/LA29-A **Detection of HLA-Specific Alloantibody by Flow Cytometry and Solid Phase Assays; Approved Guideline (2008).** This guideline describes criteria for optimizing methods that utilize flow cytometry and other conventional and multiplex platforms.

I/LA30-A **Immunoassay Interference by Endogenous Antibodies; Approved Guideline (2008).** This guideline discusses the nature and causes of interfering antibodies, as well as their effects on immunoassays and mechanisms by which interference occurs. Methods to identify and characterize the interferences are addressed along with assessment of methods used to eliminate interference.

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.

X05-R **Metrological Traceability and Its Implementation; A Report (2006).** This document provides guidance to manufacturers for establishing and reporting metrological traceability.

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