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I/LA23-A

Assessing the Quality of Immunoassay Systems: Radioimmunoassays and Enzyme, Fluorescence, and Luminescence Immunoassays; Approved Guideline

This guideline addresses components for harmonizing and assessing the quality of immunoassay systems for several commonly used dose-response indicator categories, e.g., radioisotopes, enzymes, fluorescence, luminescence, reagents, and experimental components criteria essential to characterizing an immunoassay.

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

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For further information on committee participation or to submit comments, contact CLSI.

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Assessing the Quality of Immunoassay Systems: Radioimmunoassays and Enzyme, Fluorescence, and Luminescence Immunoassays; Approved Guideline

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Abstract

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

The Area Committee on Immunology and Ligand Assays merged NCCLS documents LA1-A2—*Assessing the Quality of Radioimmunoassay Systems; Approved Guideline—Second Edition* and DI4-T—*Enzyme and Fluorescence Immunoassays; Tentative Guideline* into one document assimilating the residual segments of LA1-A2, and updating information in DI4-T into a more generic model, along with the addition of new information for each topic. I/LA23-A has broader utility and applicability while providing resource information previously available in the other two documents.

This new guideline describes the iterations in the development, performance characterization, and certification from sample collection to method transferability. Specific nuances of each of the different dose-response systems for immunoassays are addressed while placing emphasis on mechanisms to assess the quality of the different immunoassay systems—factors that contribute to reliable and reproducible results. This guideline is particularly useful for specific details on optimization and harmonization of immunoassays, especially for those measurands (analytes) that are measured only by quantitation of antigen-antibody reactions (e.g., protein hormones, IgG, serum specific proteins).

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Foreword

The intended audience for I/LA23-A—*Assessing the Quality of Immunoassay Systems: Radioimmunoassays and Enzyme, Fluorescence, and Luminescence Immunoassays; Approved Guideline*, is manufacturers of assay reagents and kits, regulatory and accrediting bodies, and scientists and healthcare professionals that develop and apply immunoassays for a variety of analytical purposes. The purpose of this guideline is to improve the quality and performance of immunoassays and to enhance laboratory and product comparability by promoting a better understanding of the requirements, capabilities, and limitations of these tests. Immunoassays are unique tests using antibodies of defined specificity to measure analytes. Each assay configuration and detection system has advantages and disadvantages. An understanding of the specific application is essential to assay production and use. The range of applications for immunoassays is extensive. The degree of variations in configurations is large and may involve a hierarchy of antibodies used with different specificities for capture, separation, measurement, and dose amplifications.

A comprehensive coverage of the field of immunoassays is too large for the scope of this document. The area committee, during development of this guideline, focused on the core quality management issues. For detailed information, other publications cited in the general references should be consulted. I/LA23-A replaces NCCLS documents LA1-A2—*Assessing the Quality of Radioimmunoassay Systems; Approved Guideline—Second Edition* and D14-T—*Enzyme and Fluorescence Immunoassays; Tentative Guideline*.

A Note on Terminology

NCCLS, 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. NCCLS 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 NCCLS, ISO, and CEN documents; and that legally required use of terms, regional usage, and different consensus timelines are all obstacles to harmonization. Despite these obstacles, NCCLS recognizes that harmonization of terms facilitates the global application of standards and is an area that needs immediate attention. Implementation of this policy must be an evolutionary and educational process that begins with new projects and revisions of existing documents.

In keeping with NCCLS's commitment to align terminology with that of ISO, the following describes the metrological terms and their uses in I/LA23-A:

The term *accuracy* 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. *Trueness* is used in this document when referring to the "closeness of the agreement between the average value from a large series of measurements and to a true value of a measurand"; the measurement of trueness is usually expressed in terms of *bias*. *Precision* is defined as the "closeness of agreement between independent test/measurement results obtained under stipulated 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 ILA23-A, primarily *repeatability*, i.e., "the closeness of the agreement between results of successive measurements of the same measurand carried out under the same conditions of measurement"; while *reproducibility* describes the "closeness of agreement of results of measurements under changed conditions."

The NCCLS Harmonization Policy recognizes ISO terms as the preferred terms. When appropriate, alternative terms are included parenthetically to help avoid confusion.

The term *measurand* (a particular quantity subject to measurement) is used in combination with the term *analyte* (component represented in the name of a measurable quantity) when its use relates to a biological fluid/matrix; and the term *measuring range* in combination with *reportable range* when referring to "a set of values of measurands for which the error of a measuring instrument (test) is intended to lie within specified limits."

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.

Users of I/LA23-A should understand, however, that the fundamental meanings of the terms are identical in many cases, and to facilitate understanding, terms are defined in the Definitions section of this guideline.

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

Antibody, assessment, enzyme immunoassay, fluorescence immunoassay, fluorescence system, heterogeneous immunoassay, homogeneous immunoassay, labeling, performance evaluation, quality control, radioimmunoassays, reference materials, separation systems

Assessing the Quality of Immunoassay Systems: Radioimmunoassays and Enzyme, Fluorescence, and Luminescence Immunoassays; Approved Guideline

1 Scope

This document presents guidelines for immunoassays of macromolecular analytes. The factors likely to be important in achieving reliable and reproducible results are emphasized. Use of this document should promote greater reliability and comparability in immunoassay results.

The definitions, information, and procedures necessary to properly assess the quality of immunoassay systems are described. Awareness of the evaluation process allows laboratory personnel to better assess systems that meet the specific needs of the patient population.

Immunoassays are widely used to quantitate specific measurands (analytes) in complex mixtures such as clinical samples. Immunoassays using enzymes or fluorescers as labels are recent developments. Enzyme immunoassays (EIA), fluorescence immunoassays (FIA), and luminescence immunoassays (LIA) were developed to provide a simple, sensitive immunoassay technique that does not use unstable and potentially dangerous radioisotopes. At present, enzyme, fluorescence, and luminescence immunoassays are typically less sensitive than radioimmunoassays (RIA). However, high sensitivity is not necessary in many applications, and there are reasons to expect that sensitivity comparable to radioimmunoassay can and will be achieved by EIA and FIA in the near future. There are no criteria on whether RIA, EIA, FIA, or LIA is the best method for a particular analyte measurement. When radioisotopes cannot be used or when radioisotope decay counters are not available, techniques such as EIA, FIA, or LIA are obligatory. In practice, EIA, FIA, and LIA systems have exhibited other advantages, including high specific activity, reagent stability, and applicability to simple instrumentation. Immunoassays using luminescent technologies are now among the most sensitive, with analytical detection limits as low as one zeptomole (10^{-21} moles).

2 Introduction

Immunoassays have become essential tools for the analytic operation of clinical diagnostic and research laboratories. Numerous advances in immunoassay techniques continue to drive new technologies, especially for application to research in proteomics and the human genome: highly sensitive dose-response indicators, methods for reduction in nonspecific binding and background signal, simultaneous analyte measurements, improved automation, and miniaturized analytic systems.

At the scheduled review of several immunoassay documents, the Area Committee on Immunology and Ligand Assay decided to develop one new document rather than expand the older ones. The area committee combined the most relevant parts of these existing documents on radioimmunoassay and enzyme and fluorescence immunoassays. A new section for luminescence was added to reflect its popularity and wide use by manufacturers of automated instruments. The sections on antigen-antibody components, sample requirements, quality assurance, and assay performance were enhanced for improved utility of the guideline for developers and users of immunoassays. Also, the sections on antibody components were provided in greater detail, because the antibody is probably the most important element in the development and performance of a high-quality and low-bias immunoassay. This guideline will provide information critical to the understanding of immunoassays to the manufacturer, the researcher, and the healthcare professional.

3 Standard Precautions

Because it is often impossible to know what might be infectious, all human specimens are to be 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 any pathogen and thus are more comprehensive than universal precautions, which are intended to apply only to transmission of blood-borne pathogens. Standard precaution and universal precaution guidelines are available from the U.S. Centers for Disease Control and Prevention (*Guideline for Isolation Precautions in Hospitals*. Infection Control and Hospital Epidemiology. CDC. 1996;Vol 17;1:53-80), (MMWR 1987;36[suppl 2S]2S-18S), and (MMWR 1988;37:377-382, 387-388). For specific precautions for preventing the laboratory transmission of blood-borne infection from laboratory instruments and materials and for recommendations for the management of blood-borne exposure, refer to the most current edition of NCCLS document M29—*Protection of Laboratory Workers from Occupationally Acquired Infections.*

4 Terminology

4.1 Definitions

Accuracy – Closeness of the agreement between the result of a measurement and a true value of the measurand (VIM93)[1]; **NOTES:** a) "Accepted reference value" may be used in place of "true value"; See **Trueness**, below.

Activity (of a radioactive material) – The number of radioactive transitions taking place in a sample per unit time; **NOTE:** See **Specific activity.**

Adjuvant – A substance admixed with an immunogen to elicit a more marked immune response (RHUD1.7CD).[2]

Affinity – **1)** The force by which atoms, ions, molecules, prosthetic groups, and particles are attracted or held together in chemical compounds; **2)** ***In Immunology***, a measure of the attraction or force of association between a single antigenic site and a single antibody to that site.

Analyte – Component represented in the name of a measurable quantity; **NOTES:** a) In the type of quantity "mass of protein in 24-hour urine," "protein" is the analyte. In "amount of substance of glucose in plasma," "glucose" is the analyte. In both cases, the long phrase represents the **Measurand** (ISO 17511)[3]; b) In the type of quantity "catalytic concentration of lactate dehydrogenase isoenzyme 1 in plasma," "lactate dehydrogenase isoenzyme 1" is the analyte. **(**ISO 18153)[4]; c) In this document, the term **Analyte** is combined with the term **Measurand** when its use relates to a biological fluid/matrix.

Analytical method – Set of operations, described specifically, used in the performance of particular measurements according to a given method (VIM93)[1]; **NOTE:** The term **Analytical method** (U.S.) is equivalent to **Measurement procedure** (Europe).

Analytical specificity – Ability of a measurement procedure to determine solely the measurable quantity it purports to measure; **NOTES:** a) *In quantitative testing*, the ability of a measurement procedure to determine only the component it purports to measure or the extent to which the assay responds only to all subsets of a specified analyte and not to other substances present in the sample; b) *For qualitative or semiquantitative tests*, the method's ability to obtain negative results in concordance with negative results obtained by the reference method; c) *In Immunology*, specificity is an antiserum quality defining its reactivity with defined antigens and lack of specificity is the inaccuracy introduced by cross-reacting and/or interfering substances, because cross-reacting substances compete with the analyte for antibody-binding sites.

The Quality System Approach

NCCLS subscribes to a quality 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 through a gap analysis. The approach is based on the model presented in the most current edition of NCCLS HS1—*A Quality System Model for Health Care.* The quality system approach applies a core set of "quality system essentials (QSEs)," basic to any organization, to all operations in any healthcare service's path of workflow. The QSEs provide the framework for delivery of any type of product or service, serving as a manager's guide. The quality system essentials (QSEs) are:

Documents & Records	Equipment	Information Management	Process Improvement
Organization	Purchasing & Inventory	Occurrence Management	Service & Satisfaction
Personnel	Process Control	Assessment	Facilities & Safety

I/LA23-A addresses the quality system essentials (QSEs) indicated by an "X." For a description of the other NCCLS documents listed in the grid, please refer to the Related NCCLS Publications section at the end of the document.

Documents & Records	Organization	Personnel	Equipment	Purchasing & Inventory	Process Control	Information Management	Occurrence Management	Assessment	Process Improvement	Service & Satisfaction	Facilities & Safety
					X C24 C28 D12 D13 EP6 EP9 I/LA18					X	GP5 M29
I/LA21			I/LA21		I/LA21	I/LA21					

Adapted from NCCLS document HS1—*A Quality 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, GP26-A2 defines a clinical laboratory path of workflow which consists of three sequential processes: preanalytic, analytic, and postanalytic. All clinical laboratories follow these processes to deliver the laboratory's services, namely quality laboratory information.

I/LA23-A addresses the clinical laboratory path of workflow steps indicated by an "X." For a description of the other NCCLS documents listed in the grid, please refer to the Related NCCLS Publications section at the end of the document.

Preanalytic					**Analytic**		**Postanalytic**	
Patient Assessment	Test Request	Specimen Collection	Specimen Transport	Specimen Receipt	Testing Review	Laboratory Interpretation	Results Report	Post-test Specimen Management
		X H3	X H3	X	X	X		X

Adapted from NCCLS document HS1—*A Quality System Model for Health Care*.

Related NCCLS Publications*

C24-A2 **Statistical Quality Control for Quantitative Measurements: Principles and Definitions; Approved Guideline—Second Edition (1999).** This guideline provides definitions of analytical intervals, plans for 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 provides guidance for determining reference values and reference intervals for quantitative clinical laboratory tests.

DI2-A2 **Immunoprecipitin Analyses: Procedures for Evaluating the Performance of Materials—Second Edition; Approved Guideline (1993) (Reaffirmed 1999).** This guideline provides a description of and procedures for evaluating the performance of materials used in immunoprecipitin analyses. It also includes a discussion on specificity.

DI3-A **Agglutination Analyses: Antibody Characteristics, Methodology, Limitations, and Clinical Validation; Approved Guideline (1993) (Reaffirmed 1999).** This guideline describes the specificity of antibodies and antigens for agglutination techniques, guidance labeling information, and characteristics and limitations of agglutination methods.

EP6-A **Evaluation of the Linearity of Quantitative Measurement Procedure: A Statistical Approach; Approved Guideline (2003).** This document provides guidelines 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.

EP9-A2 **Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline—Second Edition (2002).** This document addresses procedures for determining relative bias between two clinical methods or devices; and for the design of a method comparison experiment using split patient samples and data analysis.

GP5-A2 **Clinical Laboratory Waste Management; Approved Guideline—Second Edition (2002).** Based on U.S. regulations, this document provides guidance on safe handling and disposal of chemical, infectious, radioactive, and multihazardous wastes generated in the clinical laboratory.

HS1-A **A Quality System Model for Health Care; Approved Guideline (2002).** This document provides a model for healthcare service providers that will assist with implementation and maintenance of effective quality systems.

H3-A5 **Procedure for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved Standard—Fifth Edition (2003).** This document provides procedures for the collection of diagnostic specimens by venipuncture, including line draws, blood culture collection, and venipuncture in children. It also includes recommendations on order of draw.

I/LA18-A2 **Specifications for Immunological Testing for Infectious Diseases; Approved Guideline – Second Edition (2001)**. This guideline outlines: specimen requirements; performance criteria; algorithms for the potential use of sequential or duplicate testing; recommendations for intermethod comparisons of immunological test kits for detecting infectious disease; and specifications for development of reference materials.

I/LA21-A **Clinical Evaluation of Immunoassays; Approved Guideline (2002).** This guideline provides recommendations on designing trials that are appropriate for evaluating both the safety and effectiveness of immunoassays. It is a valuable resource in determining the necessary steps in designing an evaluation for new methods, new applications for existing methods, or variations on existing methods.

M29-A2 **Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline—Second Edition (2001).** This document provides guidance on: the risk of transmission of hepatitis viruses and human immunodeficiency viruses in any laboratory setting: specific precautions for preventing the laboratory transmission of blood-borne infection from laboratory instruments and materials; and recommendations for the management of blood-borne exposure.

NRCSL13-A **The Reference System for the Clinical Laboratory: Criteria for Development and Credentialing of Methods and Materials for Harmonization of Results; Approved Guideline (2000).** This document provides procedures for developing and evaluating methods and materials to provide a harmonized clinical measurement system.

* Proposed- and tentative-level documents are being advanced through the NCCLS consensus process; therefore, readers should refer to the most recent editions.

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