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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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Contents

Contents (Continued)

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 a*ccuracy* 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.