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Quality Assurance of Laboratory Tests for Autoantibodies to Nuclear Antigens: (1) Indirect Fluorescence Assay for Microscopy and (2) Microtiter Enzyme Immunoassay Methods; Approved Guideline—Second Edition

This document addresses the criteria for ANA testing by immunofluorescence and by enzyme immunoassay, including test components, quantification of results, and classification criteria.

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

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Quality Assurance of Laboratory Tests for Autoantibodies to Nuclear Antigens: (1) Indirect Fluorescence Assay for Microscopy and (2) Microtiter Enzyme Immunoassay Methods; Approved Guideline—Second Edition

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Abstract

Clinical and Laboratory Standards Institute document I/LA2-A2, *Quality Assurance of Laboratory Tests for Autoantibodies to Nuclear Antigens: (1) Indirect Fluorescence Assay for Microscopy and (2) Microtiter Enzyme Immunoassay Methods; Approved Guideline—Second Edition* provides guidance for laboratory scientists and manufacturers who perform immunofluorescence tests for autoantibodies to nuclear antigen to detect diseases. Topics addressed include substrate and fixative variations, fluorochrome-labeled conjugates; microscope optics; assay requirements; assay validation; ELISA enzyme labeled conjugates; ELISA detection methods; coating and blocking concentrations; quantitation of antibodies; reference intervals and reporting test results; intralaboratory quality control; and reference preparations.

Clinical and Laboratory Standards Institute (CLSI). *Quality Assurance of Laboratory Tests for Autoantibodies to Nuclear Antigens: (1) Indirect Fluorescence Assay for Microscopy and (2) Microtiter Enzyme Immunoassay Methods; Approved Guideline—Second Edition.* CLSI document I/LA2-A2 (ISBN 1-56238-601-8). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2006.

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Contents

Contents (Continued)

Foreword

Tests for the detection of antinuclear antibodies are helpful in the evaluation of several systemic rheumatic diseases, such as systemic lupus erythematosus (SLE), discoid lupus erythematosus, mixed connective tissue diseases (MCTD), systemic sclerosis, Sjögren's syndrome, polymyositis, dermatomyositis, and rheumatoid arthritis. Identification of autoantibodies has proven to be useful in the diagnosis, management, and treatment of these diseases.

In developing this guideline, the area committee defined quality assurance as the practice that encompasses all procedures and activities directed toward ensuring that a specified quality of product is achieved and maintained. This update/revision provides guidelines that recommend procedures for manufacturers, clinical laboratories, and customers for the assurance of good laboratory practices in the performance of technological advancements in assay detection. The area committee believes that this guideline addresses some of the critical issues related to IF-ANA testing and other methods developed from advances in analytic technologies, including: Part I — criteria for the immunofluorescence ANA test (Section 5); substrate and fixative variations (Section 6); fluorochrome-labeled conjugates (Section 7); microscope optics (Section 8); Part II — ELISA requirements (Section 9); assay validation (Section 10); ELISA fluorochrome-labeled conjugates (Section 11); ELISA detection methods (Section 12); technical considerations (Section 13); alternative, emerging solid-phase technologies (Section 14); Part III — quantitation of antibodies (Section 15); reference intervals and reporting of results (Section 16); intralaboratory quality control (Section 17); reference preparations for ANA tests (Section 18); summary of important quality assurance procedures (Section 19); and special considerations for the use of other laboratory tests for the detection of ANA (Section 20).

A Summary of Consensus Comments on the previous edition of this document (I/LA2-A) has not been included in the current edition, as all comments were editorial in nature.

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, 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, CLSI 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 CLSI's commitment to align terminology with that of ISO, the following terms are used in I/LA2-A2: *Accuracy* is used in this document when referring to the closeness of the agreement between the result of a measurement and a true value of the measurand; *Measurand* is the particular quantity subject to measurement; and *Measuring range* is used when referring to a set of values of measurands for which the error of a measuring instrument is intended to lie within specified limits.

Key Words

ANA reference preparations, antinuclear antibodies, autoantibodies, enzyme immunoassay, indirect immunofluorescence, nuclear antigen, quality assurance

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1 Scope

The ANAs are associated with many immunologic disorders; however, they are the essential hallmark of systemic rheumatic diseases.

The significance of ANAs is as follows:

- Useful for screening and diagnostic evaluation of systemic rheumatic diseases. A negative test result is helpful in ruling out the possibility of SLE.
- Some of these diseases have distinct profiles of ANA. Significant changes in the levels of certain specific ANA, such as antibodies to ds-DNA, are useful in following the causes of the diseases and their responses to therapy. Levels of ANA do not necessarily correlate with severity of disease or response to therapy.
- ANAs can be useful as experimental reagents in the isolation of nuclear antigens, especially nonhistone antigens or in basic studies in cell biology.

Indirect immunofluorescence and immunoenzyme tests were commonly used for ANA screening, because these procedures are practical, sensitive, primary antigen–antibody reactions. Since 1996, advances in technology have provided new approaches to improve the accuracy and specificity in detection methods. Many laboratories now use enzyme-linked immunosorbent assays (ELISA) and other newly developed immunological tests that have been demonstrated to be suitable as preliminary screening tests to identify antinuclear antibodies. Differences in the data set derived from these assays may modify the interpretation of assay results; therefore, quality assurance information for ELISA-specific methods is included.

2 Introduction

The rheumatic diseases are characterized by the presence of one or more autoantibodies that react with components of the nucleus, cytoplasm, or surface of cells. The rheumatic diseases listed below vary with the type of autoantibodies and the extent and severity of lesions in the various organ systems[1-6]:

- systemic lupus erythematosus (SLE);
- discoid lupus erythematosus (DLE);
- drug-induced lupus erythematosus (LE);
- mixed connective tissue disease (MCTD);
- Sjögren's syndrome;
- scleroderma/CREST (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly telangiectasia) syndrome;
- rheumatoid arthritis;
- dermatomyositis and polymyositis; and

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- other connective tissue disease syndromes that have been poorly defined as to clinical category, including syndromes associated with infectious diseases (such as Lyme disease), tumors, and drug reactions.

Over the past ten years, there has been a progressive characterization of the immunochemical and molecular nature of various autoantigens. An increased number of antigen–antibody systems associated with specific diseases have been identified. The terms "autoantibodies to nuclear antigens" or "antinuclear antibodies" (ANAs) have gained widespread use as generic descriptions of a group of autoantibodies. Several features of ANAs and their relationship to the rheumatic diseases have been reported.[1-6] Some of the ANAs have been used as diagnostic markers — such ANAs include antinative DNA and anti-Sm in SLE; anti-Scl-70 in diffuse scleroderma with lung disease; anticentromere in CREST; and anti-tRNA synthetases in dermatomyositis and polymyositis. Other ANAs are also found in several of the other diseases, such as hepatic disorders, tumors, vasculititis, etc., and can differ markedly in prevalence from the systemic rheumatic diseases. Such ANAs include antihistones in SLE and drug-induced lupus; anti-U1 ribonucleoprotein (RNP) in SLE and MCTD; and anti-SS-A/Ro and anti-SS-B/La in SLE and Sjögren's syndrome.

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 U.S. Centers for Disease Control and Prevention (Garner JS, Hospital Infection Control Practices Advisory Committee. Guideline for isolation precautions in hospitals. *Infect Control Hosp Epidemiol*. 1996;17(1):53-80). 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 the most current edition of CLSI document M29—*Protection of Laboratory Workers From Occupationally Acquired Infections.*

4 Terminology

4.1 Definitions

accuracy (of measurement) – closeness of the agreement between the result of a measurement and a true value of the measurand (VIM93).[7]

analytical specificity – ability of a measurement procedure to measure solely the measurand (ISO 17511)[8]; **NOTE:** ***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.

antinuclear antibodies – as used in this guideline, immunoglobulins detected by immunochemical staining of cells that bind specifically to cell nuclei or certain antigens in the cytoplasm, and immunoglobulins that bind specifically to certain purified nuclear or cytoplasmic antigens detected by binding reactions in gel precipitation assays, ELISA, and other assay methods such as protein microarrays.

biological reference interval – central 95% interval of the distribution of reference values; **NOTE 1:** This supersedes such incorrectly used terms as "normal range"; **NOTE 2:** It is an arbitrary but common

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Related CLSI/NCCLS Publications*

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.

M29-A3 **Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline—Third Edition (2005).** 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.

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

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