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Molecular Diagnostic Methods for Infectious Diseases; Approved Guideline—Second Edition

This guideline addresses topics relating to clinical applications, amplified and nonamplified nucleic acid methods, selection and qualification of nucleic acid sequences, establishment and evaluation of test performance characteristics, inhibitors, and interfering substances, controlling false-positive reactions, reporting and interpretation of results, quality assurance, regulatory issues, and recommendations for manufacturers and clinical laboratories.

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

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- the authorization of a project
- the development and open review of documents
- the revision of documents in response to comments by users
- the acceptance of a document as a consensus standard or guideline.

Most documents are subject to two levels of consensus—"proposed" and "approved." Depending on the need for field evaluation or data collection, documents may also be made available for review at an intermediate consensus level.

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Approved An approved standard or guideline has achieved consensus within the healthcare community. It should be reviewed to assess the utility of the final document, to ensure attainment of consensus (i.e., that comments on earlier versions have been satisfactorily addressed), and to identify the need for additional consensus documents.

Our standards and guidelines represent a consensus opinion on good practices and reflect the substantial agreement by materially affected, competent, and interested parties obtained by following CLSI's established consensus procedures. Provisions in CLSI standards and guidelines may be more or less stringent than applicable regulations. Consequently, conformance to this voluntary consensus document does not relieve the user of responsibility for compliance with applicable regulations.

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The comments of users are essential to the consensus process. Anyone may submit a comment, and all comments are addressed, according to the consensus process, by the committee that wrote the document. All comments, including those that result in a change to the document when published at the next consensus level and those that do not result in a change, are responded to by the committee in an appendix to the document. Readers are strongly encouraged to comment in any form and at any time on any document. Address comments to Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087, USA.

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Abstract

Nucleic acid methods for the detection and characterization of microorganisms in clinical specimens are now firmly established in laboratory medicine. These methods offer opportunities for clinical laboratories to provide more rapid and accurate results, and have changed the practice of clinical microbiology and infectious diseases. Clinical and Laboratory Standards Institute (CLSI) document MM3-A2—*Molecular Diagnostic Methods for Infectious Diseases; Approved Guideline—Second Edition* addresses topics relating to clinical applications, amplified and nonamplified nucleic acid methods, selection and qualification of nucleic acid sequences, establishment and evaluation of test performance characteristics, inhibitors, and interfering substances, controlling false-positive reactions, reporting and interpretation of results, quality assurance, regulatory issues, and recommendations for manufacturers and clinical laboratories.

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Foreword

MM3—*Molecular Diagnostic Methods for Infectious Diseases* was published as an approved guideline in 1995. It was the first of what was to be many Clinical and Laboratory Standards Institute (CLSI) guidelines in the area of molecular diagnostics, and the first consensus guideline published in what was then the new field of molecular microbiology. Molecular microbiology, simply put, is the application of nucleic acid methods to the diagnosis and management of patients with infectious diseases. The field has advanced enormously since the publication of the first approved edition of MM3 and is now an integral part of laboratory medicine.

New developments in the enabling technology, increased understanding of its strengths and limitations, and pervasive clinical applications required a complete rewriting of MM3-A. The second approved edition of MM3 bears little resemblance to its predecessor in organization and content, and relies heavily on other relevant, recently published CLSI documents.

Note that the following trade names are included in this document: ATCC®[a] and GenBank®[b]. It is CLSI policy to avoid using trade names unless the products identified are the only ones available, or they serve solely as illustrative examples of the procedure, practice, or material described. In this case, the subcommittee believes the trade names are important descriptive adjuncts to the document.

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 MM3: *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, and *trueness* is used 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*; *repeatability* has replaced the term *within-run precision* where appropriate, when describing the closeness of agreement between results of successive measurements of the same measurand carried out under the same conditions of measurement; *measuring range* has replaced *reportable range* when referring to a set of values of measurands for which the error of a measuring instrument is intended to lie within specified limits. The ISO terms *diagnostic sensitivity* and *diagnostic specificity* have replaced *clinical sensitivity* and *clinical specificity*. In Europe, for the most part, the term *clinical* is applied to the evaluation of medical products used on or in patients, or when referring to clinical studies of drugs, under much more stringent conditions.

[a] ATCC is a registered trademark of the American Type Culture Collection.
[b] GenBank is a registered trademark of the United States Department of Health and Human Services.

Users of MM3-A2 should understand, however, that the fundamental meanings of the terms are similar and to facilitate understanding, terms are defined along with explanatory notes in the guideline's Definitions section.

Key Words

Development, implementation, methods, molecular microbiology, nucleic acid amplification, quality assurance, reporting, verification

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1 Scope

This guideline describes general principles for the development, evaluation, and application of tests designed for direct detection of microorganisms in clinical specimens and for identification of microorganisms grown in culture. The document provides evidence-based recommendations, where appropriate. The following content areas are addressed: clinical applications; amplified and nonamplified nucleic acid methods; selection and qualification of nucleic acid sequences; establishment and evaluation of test performance characteristics, inhibitors, and interfering substances; controlling false-positive reactions; reporting and interpretation of results; quality assurance; regulatory issues; and recommendations for manufacturers and clinical laboratories.

This guideline is intended for use by clinical laboratories, test developers and manufacturers, and regulatory agencies. It is not intended to be a compilation of successful protocols for detection/characterization of microorganisms, but rather to describe general principles for the development, evaluation, and application of these tests. The readers are directed to the *Manual of Clinical Microbiology*, 8th edition, and *Molecular Microbiology: Diagnostic Principles and Practice* from ASM Press, Washington, DC, for more information on specific applications.

This guideline should be used in conjunction with the most current editions of the following related CLSI/NCCLS documents: MM6—*Quantitative Molecular Methods for Infectious Diseases*; MM9—*Nucleic Acid Sequencing Methods in Diagnostic Laboratory Medicine*; MM10—*Genotyping for Infectious Diseases: Identification and Characterization*; MM13—*Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods;* and MM14—*Proficiency Testing (External Quality Assessment) for Molecular Methods.*

2 Introduction

Nucleic acid-based methods for detection and identification of microorganisms are commonly used in clinical laboratories. However, their inherent complexity and unparalleled analytical sensitivity require special attention to the assay design, use of controls, and laboratory practice. The diagnostic industry has not kept pace with the medical demand for these tests, and in many cases, in-laboratory-developed nucleic acid tests have become the standard of care. Due to the number of different in-laboratory-developed assays used, molecular diagnostic methods for infectious diseases often lack standardization.

Although molecular diagnostic methods are becoming more pervasive in clinical laboratories, efforts should continue to increase the understanding of the strengths and limitations of these new methods. These methods often may enhance diagnostic capabilities. However, the results should be interpreted within the clinical context in which they are used, and on the basis of individual laboratory performance.

This document presents consensus guidelines for method development, verification, and validation. It is a guide to practical implementation of molecular tests in the clinical laboratory and to the assessment of their clinical utility. It also provides recommendations to assay developers in clinical laboratories and industry. This guideline is also intended to serve as a resource for the relevant regulatory agencies.

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