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CLINICAL AND LABORATORY STANDARDS INSTITUTE®

3rd Edition

MM03

Molecular Diagnostic Methods for Infectious Diseases

This report 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 CLSI report for global application.

MM03, 3rd ed.
February 2015
Replaces MM03-A2

Molecular Diagnostic Methods for Infectious Diseases

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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 document MM03—*Molecular Diagnostic Methods for Infectious Diseases* 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, QA, regulatory issues, and recommendations for manufacturers and clinical laboratories.

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Contents

Contents (Continued)

Foreword

MM03 was originally published as an approved guideline in 1995. It was the first of what was to become many CLSI molecular diagnostics guidelines, and the first molecular microbiology consensus guideline published. Molecular microbiology 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 MM03 and is now an integral part of laboratory medicine.

Overview of Changes

With the change in format and category definitions for all CLSI documents, MM03 has been recategorized as a report and replaces MM03-A2. Although MM03 has been revised for the purpose of keeping information current, the revisions do not significantly affect the scope or purpose of the document, nor do they change the methodology used. Revisions to the document include:

- Formatting and template design have been updated to reflect current CLSI style.
- References to most trademarked products have been deleted.
- CLSI references have been updated to reflect current document numbers and editions.
- International Organization for Standardization definitions and references have been updated to reflect current editions.
- New test descriptions and figures have been added to the text and the appendix in order to reflect current technology.

Note that the trade name TaqMan® is included as a reference to Figure 1 of this document. It is Clinical and Laboratory Standards Institute's policy to avoid using a trade name unless the product identified is the only one available, or it serves solely as an illustrative example of the procedure, practice, or material described. In this case, the working group and consensus committee believe the illustration derived from the published reference is an important descriptive adjunct to the document. In such cases, it is acceptable to use the product's trade name when the illustration is being reprinted from a referenced publication. It should be understood that information on this product in this document also applies to any equivalent products.

Key Words

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

Molecular Diagnostic Methods for Infectious Diseases

Chapter 1: Introduction

This chapter includes:

- Document scope and applicable exclusions
- Background information pertinent to the document content
- Standard precautions information
- "Note on Terminology" that highlights particular use and/or variation in use of terms and/or definitions
- Terms and definitions used in the document
- Abbreviations and acronyms used in the document

1.1 Scope

This document 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
- QA
- Regulatory issues
- Recommendations for manufacturers and clinical laboratories

This document 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. Users are directed to the *Manual of Clinical Microbiology*[1] and *Molecular Microbiology: Diagnostic Principles and Practice*[2] for more information on specific applications.

This document should be used in conjunction with the following related CLSI documents: MM06,[3] MM09,[4] MM10,[5] MM13,[6] MM14,[7] MM17,[8] and MM19.[9]

1.2 Background

Nucleic acid–based methods for detection and identification of microorganisms are commonly used in clinical laboratories. However, the 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, laboratory-developed nucleic acid tests (NATs) have become the standard of care. Due to the number of different laboratory-developed tests (LDTs) 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 recommendations 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 document is also intended to serve as a resource for the relevant regulatory agencies.

1.3 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 known infectious agents and thus are more comprehensive than universal precautions, which are intended to apply only to transmission of bloodborne pathogens. The Centers for Disease Control and Prevention address this topic in published guidelines that address the daily operations of diagnostic medicine in human and animals while encouraging a culture of safety in the laboratory.[10] 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.[11]

1.4 Terminology

1.4.1 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, International Organization for Standardization (ISO), and European Committee for Standardization (CEN) documents; and that legally required use of terms, regional usage, and different consensus timelines are all important considerations in the harmonization process. In light of this, CLSI's consensus process for development and revision of standards and

The Quality Management System Approach

Clinical and Laboratory Standards Institute (CLSI) subscribes to a quality management system (QMS) 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 QMS 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 as follows:

Organization	Personnel	Process Management	Nonconforming Event Management
Quality Customer Focus	Purchasing and Inventory	Documents and Records	Assessments
Facilities and Safety	Equipment	Information Management	Continual Improvement

MM03 addresses the QSE 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 page 92.

Organization	Customer Focus	Facilities and Safety	Personnel	Purchasing and Inventory	Equipment	Process Management	Documents and Records	Information Management	Nonconforming Event Management	Assessments	Continual Improvement
					AUTO01 AUTO02	X AUTO02 AUTO04 C24 EP05 EP12 EP17 EP23 EP24 GP27 MM06 MM09 MM10 MM13 MM14 MM17		AUTO03 AUTO04 AUTO05		GP27	GP27
MM19 QMS01	MM19 QMS01	M29 MM19 QMS01	MM19 QMS01	MM19 QMS01 QMS05	MM19 QMS01	MM19 QMS01	MM19 QMS01 QMS02	MM19 QMS01	MM19 QMS01	MM19 QMS01	MM19 QMS01 QMS06

Path of Workflow

A path of workflow is the description of the necessary processes to deliver the particular product or service that the organization or entity provides. A laboratory path of workflow consists of the sequential processes: preexamination, examination, and postexamination and their respective sequential subprocesses. All laboratories follow these processes to deliver the laboratory's services, namely quality laboratory information.

MM03 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
	X	X	X	X	X		X AUTO03	
				EP23	EP23	EP23		
MM06	MM06	MM06	MM06	MM06	MM06	MM06	MM06	
	MM09	MM09	MM09	MM09	MM09	MM09	MM09	MM09
				MM10	MM10	MM10	MM10	
	MM13	MM13	MM13					MM13
	MM19	MM19	MM19	MM19	MM19	MM19		
QMS01	QMS01	QMS01	QMS01	QMS01	QMS01	QMS01	QMS01	QMS01

Related CLSI Reference Materials*

AUTO01 **Laboratory Automation: Specimen Container/Specimen Carrier. 1st ed., 2000.** This document provides standards for the design and manufacture of specimen containers and carriers used for collecting and processing liquid samples, such as blood and urine, for clinical testing in laboratory automation systems.

AUTO02 **Laboratory Automation: Bar Codes for Specimen Container Identification. 2nd ed., 2005.** This document provides specifications for use of linear bar codes on specimen container tubes in the clinical laboratory and for use on laboratory automation systems.

AUTO03 **Laboratory Automation: Communications With Automated Clinical Laboratory Systems, Instruments, Devices, and Information Systems. 2nd ed., 2009.** This document provides standards to facilitate accurate and timely electronic exchange of data and information between the automated laboratory elements.

AUTO04 **Laboratory Automation: Systems Operational Requirements, Characteristics, and Information Elements. 1st ed., 2001.** This document describes operational requirements, characteristics, and required information elements of clinical laboratory automation systems. This information is used to determine the status of a clinical specimen within the clinical laboratory automation system, as well as the status of the actual components of the clinical laboratory automation system.

AUTO05 **Laboratory Automation: Electromechanical Interfaces. 1st ed., 2001.** This document provides standards for the development of an electromechanical interface between instruments and specimen processing and handling devices used in automated laboratory testing procedures.

C24 **Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions. 3rd ed., 2006.** This guideline provides definitions of analytical intervals, planning of quality control procedures, and guidance for quality control applications.

EP05 **Evaluation of Precision of Quantitative Measurement Procedures. 3rd ed., 2014.** This document provides guidance for evaluating the precision performance of quantitative measurement procedures. It is intended for manufacturers of quantitative measurement procedures for laboratories that develop or modify such procedures.

EP12 **User Protocol for Evaluation of Qualitative Test Performance. 2nd ed., 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.

EP17 **Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures. 2nd ed., 2012.** This document provides guidance for evaluation and documentation of the detection capability of clinical laboratory measurement procedures (ie, limits of blank, detection, and quantitation), for verification of manufacturers' detection capability claims, and for the proper use and interpretation of different detection capability estimates.

EP23™ **Laboratory Quality Control Based on Risk Management. 1st ed., 2011.** This document provides guidance based on risk management for laboratories to develop quality control plans tailored to the particular combination of measuring system, laboratory setting, and clinical application of the test.

EP24 **Assessment of the Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristic Curves. 2nd ed., 2011.** This document provides a protocol for evaluating the accuracy of a test to discriminate between two subclasses of subjects when there is some clinically relevant reason to separate them. In addition to the use of receiver operating characteristic curves and the comparison of two curves, the document emphasizes the importance of defining the question, selecting the sample group, and determining the "true" clinical state.

GP27 **Using Proficiency Testing to Improve the Clinical Laboratory. 2nd ed., 2007.** This guideline provides assistance to laboratories in using proficiency testing as a quality improvement tool.

* CLSI documents are continually reviewed and revised through the CLSI consensus process; therefore, readers should refer to the most current editions.

Related CLSI Reference Materials (Continued)

M29 **Protection of Laboratory Workers From Occupationally Acquired Infections. 4th ed., 2014.** 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.

MM06 **Quantitative Molecular Methods for Infectious Diseases. 2nd ed., 2006.** This document provides guidance for the development and use of quantitative molecular methods, such as nucleic acid probes and nucleic acid amplification techniques of the target sequences specific to particular microorganisms. It also presents recommendations for quality assurance, proficiency testing, and interpretation of results.

MM09 **Nucleic Acid Sequencing Methods in Diagnostic Laboratory Medicine. 2nd ed., 2014.** This document addresses diagnostic sequencing using both automated capillary-based sequencers and massively parallel sequencing instruments. Topics include specimen collection and handling; isolation and extraction of nucleic acid; template preparation; sequence generation, alignment, and assembly; validation and verification; ongoing quality assurance; and reporting results.

MM10 **Genotyping for Infectious Diseases: Identification and Characterization. 1st ed., 2006.** This guideline describes currently used analytical approaches and methodologies applied to identify the clinically important genetic characteristics responsible for disease manifestation, outcome, and response to therapy in the infectious disease setting. It also provides guidance on the criteria to be considered for design, validation, and determination of clinical utility of such testing.

MM13 **Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods. 1st ed., 2005.** This document provides guidance related to proper and safe biological specimen collection and nucleic acid isolation and purification. These topics include methods of collection, recommended storage and transport conditions, and available nucleic acid purification technologies for each specimen/nucleic acid type.

MM14 **Design of Molecular Proficiency Testing/External Quality Assessment. 2nd ed., 2013.** This document provides guidelines for a quality proficiency testing/external quality assessment program, including reliable databases; design control in the choice of materials and measurands; good manufacturing processes; documentation procedures; complaint handling; corrective and preventive action plans; and responsive timing of reports.

MM17 **Verification and Validation of Multiplex Nucleic Acid Assays. 1st ed., 2008.** This guideline provides recommendations for analytic verification and validation of multiplex assays, as well as a review of different types of biologic and synthetic reference materials.

MM19 **Establishing Molecular Testing in Clinical Laboratory Environments. 1st ed., 2011.** This guideline provides comprehensive guidance for planning and implementation of molecular diagnostic testing, including strategic planning, regulatory requirements, implementation, quality management, and special considerations for the subspecialties of molecular genetics, infectious diseases, oncology, and pharmacogenetics.

QMS01 **Quality Management System: A Model for Laboratory Services. 4th ed., 2011.** This document provides a model for medical laboratories that will assist with implementation and maintenance of an effective quality management system.

QMS02 **Quality Management System: Development and Management of Laboratory Documents. 6th ed., 2013.** This document provides guidance on the processes needed for document management, including creating, controlling, changing, and retiring a laboratory's policy, process, procedure, and form documents in both paper and electronic environments.

QMS05 **Quality Management System: Qualifying, Selecting, and Evaluating a Referral Laboratory. 2nd ed., 2012.** This guideline provides recommended criteria and easily implemented processes for qualifying, selecting, and evaluating a referral laboratory.

QMS06 **Quality Management System: Continual Improvement. 3rd ed., 2011.** This guideline considers continual improvement as an ongoing, systematic effort that is an essential component of a quality management system. A continual improvement program may consist of fundamental processes and common supporting elements described in this guideline.

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