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

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.

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

CLINICAL AND LABORATORY STANDARDS INSTITUTE®

Clinical and Laboratory Standards Institute

Advancing Quality in Health Care Testing

Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) is an international, interdisciplinary, nonprofit, standards-developing, and educational organization that promotes the development and use of voluntary consensus standards and guidelines within the health care community. It is recognized worldwide for the application of its unique consensus process in the development of standards and guidelines for patient testing and related health care issues. Our process is based on the principle that consensus is an effective and cost-effective way to improve patient testing and health care services.

In addition to developing and promoting the use of voluntary consensus standards and guidelines, we provide an open and unbiased forum to address critical issues affecting the quality of patient testing and health care.

PUBLICATIONS

A document is published as a standard, guideline, or committee report.

Standard A document developed through the consensus process that clearly identifies specific, essential requirements for materials, methods, or practices for use in an unmodified form. A standard may, in addition, contain discretionary elements, which are clearly identified.

Guideline A document developed through the consensus process describing criteria for a general operating practice, procedure, or material for voluntary use. A guideline may be used as written or modified by the user to fit specific needs.

Report A document that has not been subjected to consensus review and is released by the Board of Directors.

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The CLSI voluntary consensus process is a protocol establishing formal criteria for

- 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.

Proposed A consensus document undergoes the first stage of review by the health care community as a proposed standard or guideline. The document should receive a wide and thorough technical review, including an overall review of its scope, approach, and utility, and a line-by-line review of its technical and editorial content.

Approved An approved standard or guideline has achieved consensus within the health care community. It should be reviewed to assess the utility of the final document, to ensure attainment of consensus (ie, 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.

COMMENTS

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 addressed 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.

VOLUNTEER PARTICIPATION

Health care professionals in all specialties are urged to volunteer for participation in CLSI projects. Please contact US at customerservice@clsi.org or +610.688.0100 for additional information on committee participation.

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

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Abstract

Clinical and Laboratory Standards Institute document MM06-A2—*Quantitative Molecular Methods for Infectious Diseases; Approved Guideline—Second Edition* recognizes the increased use of quantitative molecular methods for determining the concentration of microorganisms in patients. CLSI document MM06 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, and presents recommendations for quality assurance, proficiency testing, and interpretation of results.

Issues specific to the quantification of nucleic acid in diagnostic testing and monitoring, particularly in viral diseases, include an update on technologies used in molecular quantification; specimen handling and preparation; standards, calibrators, and reference materials; analytical and clinical verification/validation; reporting and interpreting results; clinical utility; and recommendations for manufacturers and clinical laboratories.

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Foreword

Quantification of nucleic acids has become the standard of care for the diagnosis and monitoring of a number of infections that are predominantly of viral origin. The measurement of viral load has proven prognostic utility in patients infected with several pathogenic viruses and the clinical utility of others is an area of active investigation. Quantitative tests for the measurement of some of these pathogens have become fully automated, and viral load testing is now performed routinely in a significant number of clinical laboratories.

This document is an update of MM06—*Quantitative Molecular Methods for Infectious Diseases; Approved Guideline* that was published in 2003. MM06 established the original guidelines for laboratory tests that quantified viruses for the purpose of diagnosis and monitoring of infected patients. This guideline is to be used in conjunction with CLSI document MM03.[1] This document constitutes the second edition of MM06 and specifically addresses the changes in technology, performance, assay verification, interpretation, and quality control (QC) for quantitative molecular methods in the diagnosis and monitoring of infectious diseases.

Key Words

Accuracy, amplification, calibrators, dynamic range, infectious diseases, limit of detection, limit of quantification, nucleic acid, precision, probe, quality control materials, quantification, reference materials, signal, standards, target, viral load

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

1 Scope

This guideline is to be used for implementation of tests for diagnostic purposes after the benefits and potential risks associated with the use of the test in clinical practice have been considered. Specimen handling and preparation; standards, calibrators, and reference materials; analytical and clinical verification/validation; reporting and interpreting results; and QC and clinical utility are the focus of this document. This document does not establish a clinically acceptable limit of quantification (LoQ) because consensus for most assays is currently lacking on this issue.

This document is intended for manufacturers or laboratories that develop tests, laboratories that perform or intend to implement such tests, clinicians that use the results to diagnose or manage patients, and agencies that regulate their use.

2 Introduction

Nucleic acid testing for infectious agents poses unique issues; quantification introduces additional complexity. With the advent of standardized quantitative kits and the increase in quantitative laboratory-developed testing, a guideline for the development, verification, validation, and implementation of these assays is warranted. At the time of the development of this guideline, the clinical use of quantitative molecular assays was primarily applicable to viral diseases. This document addresses assays used to identify clinical disease and monitor disease progression and prognosis, therapeutic efficacy, and the emergence of active disease in chronic viral infections. In principle, the methodologies can also be applied to other infectious agents and disease processes.

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 blood-borne pathogens. Standard and universal precaution guidelines are available from the US Centers for Disease Control and Prevention.[2] For specific precautions for preventing the laboratory transmission of all known infectious agents from laboratory instruments and materials and for recommendations for the management of exposure to all known infectious diseases, refer to CLSI document M29.[3]

4 Terminology

4.1 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, 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

The Quality Management System Approach

Clinical and Laboratory Standards Institute (CLSI) subscribes to a quality management 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. The approach is based on the model presented in the most current edition of CLSI document HS01—*A Quality Management System Model for Health Care.* The quality management system 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

Documents and Records	Equipment	Information Management	Process Improvement
Organization	Purchasing and Inventory	Occurrence Management	Customer Service
Personnel	Process Control	Assessments—External and Internal	Facilities and Safety

MM06-A2 addresses the QSEs 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.

Documents and Records	Organization	Personnel	Equipment	Purchasing and Inventory	Process Control	Information Management	Occurrence Management	Assessments—External and Internal	Process Improvement	Customer Service	Facilities and Safety
					X C24 C28 EP05 EP06 EP07 EP09 EP10 EP14 EP15 EP17 EP21 GP10				EP07		
				H03	H03			EP10			H03 M29
MM07		MM07	MM07		M50 MM03 MM07 MM09 MM13 MM16 MM17			MM03 MM07			MM07

Adapted from CLSI document HS01—*A Quality Management 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, CLSI document GP26—*Application of a Quality Management System Model for Laboratory Services* defines a clinical laboratory path of workflow, which consists of three sequential processes: preexamination, examination, and postexamination. All clinical laboratories follow these processes to deliver the laboratory's services, namely quality laboratory information.

MM06-A2 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 H03 MM07	X H03 MM03 MM07 MM09 MM13	X H03 MM03 MM07 MM09 MM13	X H03 MM03 MM07 MM09 MM13	X H03 MM03 MM07 MM09	X H03 M50 MM03 MM07 MM09	X M50 MM07 MM09	X M50 MM03 MM07 MM09	 MM07 MM09 MM13

Adapted from CLSI document HS01—*A Quality Management System Model for Health Care*.

Related CLSI Reference Materials

C24-A3 **Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions; Approved Guideline—Third Edition (2006).** This guideline provides definitions of analytical intervals, planning of quality control procedures, and guidance for quality control applications.

C28-A3c **Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline—Third Edition (2008).** This document contains guidelines for determining reference values and reference intervals for quantitative clinical laboratory tests.

EP05-A2 **Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline—Second Edition (2004).** This document provides guidance for designing an experiment to evaluate the precision performance of quantitative measurement methods; recommendations on comparing the resulting precision estimates with manufacturers' precision performance claims and determining when such comparisons are valid; as well as manufacturers' guidelines for establishing claims.

EP06-A **Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline (2003).** This document provides guidance 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.

EP07-A2 **Interference Testing in Clinical Chemistry; Approved Guideline—Second Edition (2005).** This document provides background information, guidance, and experimental procedures for investigating, identifying, and characterizing the effects of interfering substances on clinical chemistry test results.

EP09-A2-IR **Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline—Second Edition (Interim Revision) (2010).** This document addresses procedures for determining the bias between two clinical methods, and the design of a method comparison experiment using split patient samples and data analysis.

EP10-A3 **Preliminary Evaluation of Quantitative Clinical Laboratory Measurement Procedures; Approved Guideline—Third Edition (2006).** This guideline provides experimental design and data analysis for preliminary evaluation of the performance of a measurement procedure or device.

EP14-A2 **Evaluation of Matrix Effects; Approved Guideline—Second Edition (2005).** This document provides guidance for evaluating the bias in analyte measurements that is due to the sample matrix (physiological or artificial) when two measurement procedures are compared.

EP15-A2 **User Verification of Performance for Precision and Trueness; Approved Guideline—Second Edition (2006).** This document describes the demonstration of method precision and trueness for clinical laboratory quantitative methods using a protocol designed to be completed within five working days or less.

EP17-A **Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline (2004).** This document provides guidance for determining the lower limit of detection of clinical laboratory methods, for verifying claimed limits, and for the proper use and interpretation of the limits.

EP21-A **Estimation of Total Analytical Error for Clinical Laboratory Methods; Approved Guideline (2003).** This document provides manufacturers and end users with a means to estimate total analytical error for an assay. A data collection protocol and an analysis method, which can be used to judge the clinical acceptability of new methods using patient specimens, are included. These tools can also monitor an assay's total analytical error by using quality control samples.

GP10-A **Assessment of the Clinical Accuracy of Laboratory Tests Using Receiver Operating Characteristic (ROC) Plots; Approved Guideline (1995) (Reaffirmed 2001).** This document provides a protocol for evaluating the accuracy of a test to discriminate between two subclasses of subjects where there is some clinically relevant reason to separate them. In addition to the use of ROC plots, the importance of defining the question, selecting the sample group, and determining the "true" clinical state are emphasized.

Related CLSI Reference Materials (Continued)

H03-A6 **Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved Standard—Sixth Edition (2007).** This document provides procedures for the collection of diagnostic specimens by venipuncture, including line draws, blood culture collection, and venipuncture in children.

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

M50-A **Quality Control for Commercial Microbial Identification Systems; Approved Guideline (2008).** This document provides guidance for quality control of commercial systems for microbial identification from culture, including information that pertains to manufacturers, distributors, and laboratory users. The intent is to ensure optimal performance of a microbial identification system in an efficient (streamlined) manner.

MM03-A2 **Molecular Diagnostic Methods for Infectious Diseases; Approved Guideline—Second Edition (2006).** 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.

MM07-A **Fluorescence *In Situ* Hybridization (FISH) Methods for Medical Genetics; Approved Guideline (2004).** This document addresses FISH methods for medical genetic determinations, identification of chromosomal abnormalities, and gene amplification. Recommendations for probe and assay development, manufacture, qualification, verification, and validation; instrument requirements; quality assurance; and evaluation of results are also included.

MM09-A **Nucleic Acid Sequencing Methods in Diagnostic Laboratory Medicine; Approved Guideline (2004).** This document addresses automated, PCR-based, dideoxy-terminator, and primer extension sequencing done on gel- or capillary-based sequencers. Topics covered include specimen collection and handling; isolation of nucleic acid; amplification and sequencing of nucleic acids; interpretation and reporting of results; and quality control/assessment considerations as appropriate.

MM13-A **Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods; Approved Guideline (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.

MM16-A **Use of External RNA Controls in Gene Expression Assays; Approved Guideline (2006).** This document provides protocols supporting the use of external RNA controls in microarray and QRT-PCR-based gene expression experiments, including preparation of control transcripts, design of primers and amplicons, quality control, use in final experimental or clinical test application, and analysis and interpretation of data obtained.

MM17-A **Verification and Validation of Multiplex Nucleic Acid Assays; Approved Guideline (2008).** This guideline provides recommendations for analytical verification and validation of multiplex assays, as well as a review of different types of biological and synthetic reference materials.

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

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