



November 2010

MM06-A2

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

Setting the standard for quality in clinical laboratory testing around the world.

The Clinical and Laboratory Standards Institute (CLSI) is a not-for-profit membership organization that brings together the varied perspectives and expertise of the worldwide laboratory community for the advancement of a common cause: to foster excellence in laboratory medicine by developing and implementing clinical laboratory standards and guidelines that help laboratories fulfill their responsibilities with efficiency, effectiveness, and global applicability.

Consensus Process

Consensus—the substantial agreement by materially affected, competent, and interested parties—is core to the development of all CLSI documents. It does not always connote unanimous agreement, but does mean that the participants in the development of a consensus document have considered and resolved all relevant objections and accept the resulting agreement.

Commenting on Documents

CLSI documents undergo periodic evaluation and modification to keep pace with advancements in technologies, procedures, methods, and protocols affecting the laboratory or health care.

CLSI's consensus process depends on experts who volunteer to serve as contributing authors and/or as participants in the reviewing and commenting process. At the end of each comment period, the committee that developed the document is obligated to review all comments, respond in writing to all substantive comments, and revise the draft document as appropriate.

Comments on published CLSI documents are equally essential, and may be submitted by anyone, at any time, on any document. All comments are addressed according to the consensus process by a committee of experts.

Appeals Process

If it is believed that an objection has not been adequately addressed, the process for appeals is documented in the CLSI Standards Development Policies and Process document.

All comments and responses submitted on draft and published documents are retained on file at CLSI and are available upon request.

Get Involved—Volunteer!

Do you use CLSI documents in your workplace? Do you see room for improvement? Would you like to get involved in the revision process? Or maybe you see a need to develop a new document for an emerging technology? CLSI wants to hear from you. We are always looking for volunteers. By donating your time and talents to improve the standards that affect your own work, you will play an active role in improving public health across the globe.

For further information on committee participation or to submit comments, contact CLSI.

Clinical and Laboratory Standards Institute
950 West Valley Road, Suite 2500
Wayne, PA 19087 USA
P: 610.688.0100
F: 610.688.0700
www.clsi.org
standard@clsi.org

ISBN 1-56238-736-7
ISSN 0273-3099

MM06-A2
Vol. 30 No. 22
Replaces MM06-A
Vol. 23 No. 28

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

Volume 30 Number 22

Angela M. Caliendo, MD, PhD
Helen Fernandes, PhD
Christina Egan, PhD, CBSP
Haja Sittana El Mubarak, PhD
Mark J. Espy, MS
Hawazin Faruki, DrPH
David Hillyard, MD
Michael A. Lewinski, PhD, D(ABMM)
Li Li, MS

Phillip T. Moen, Jr, PhD
Dave Petrich, MBA
Mangalathu S. Rajeevan, PhD
Venkatakrishna Shyamala, PhD
Linda D. Starr-Spires, PhD
Yi-Wei Tang, MD, PhD, D(ABMM)
Jan Turczyn, MT(ASCP), CLS
Alexandra Valsamakis, MD, PhD
Belinda Yen-Lieberman, PhD

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.

Clinical and Laboratory Standards Institute (CLSI). *Quantitative Molecular Methods for Infectious Diseases; Approved Guideline—Second Edition*. CLSI document MM06-A2 (ISBN 1-56238-736-7). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2010.

The Clinical and Laboratory Standards Institute consensus process, which is the mechanism for moving a document through two or more levels of review by the health care community, is an ongoing process. Users should expect revised editions of any given document. Because rapid changes in technology may affect the procedures, methods, and protocols in a standard or guideline, users should replace outdated editions with the current editions of CLSI documents. Current editions are listed in the CLSI catalog and posted on our website at www.clsi.org. If your organization is not a member and would like to become one, and to request a copy of the catalog, contact us at: Telephone: 610.688.0100; Fax: 610.688.0700; E-Mail: customerservice@clsi.org; Website: www.clsi.org.



Copyright ©2010 Clinical and Laboratory Standards Institute. Except as stated below, any reproduction of content from a CLSI copyrighted standard, guideline, companion product, or other material requires express written consent from CLSI. All rights reserved. Interested parties may send permission requests to permissions@clsi.org.

CLSI hereby grants permission to each individual member or purchaser to make a single reproduction of this publication for use in its laboratory procedure manual at a single site. To request permission to use this publication in any other manner, e-mail permissions@clsi.org.

Suggested Citation

CLSI. *Quantitative Molecular Methods for Infectious Diseases; Approved Guideline—Second Edition*. CLSI document MM06-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2010.

Proposed Guideline

December 2001

Approved Guideline

October 2003

Approved Guideline—Second Edition

November 2010

ISBN 1-56238-736-7
ISSN 0273-3099

Committee Membership

Area Committee on Molecular Methods

Roberta M. Madej, MS, MT
Chairholder
Tethys Bioscience
Emeryville, California, USA

Frederick S. Nolte, PhD
Vice-Chairholder
Medical University of South
Carolina
Charleston, South Carolina, USA

Zhimin Cao, MD, PhD
New York State Department of
Health
Albany, New York, USA

Stephen P. Day, PhD
Hologic, Inc.
Madison, Wisconsin, USA

Maurizio Ferrari, MD
H San Raffaele
Milan, Italy

Lisa Kalman, PhD
Centers for Disease Control and
Prevention
Atlanta, Georgia, USA

Uwe Scherf, PhD
FDA Center for Devices and
Radiological Health
Rockville, Maryland, USA

Janet A. Warrington, PhD
Phylotech, Inc.
San Francisco, California, USA

Jean Amos Wilson, PhD, FACMG
Berkeley HeartLab, Inc.
Alameda, California, USA

Emily S. Winn-Deen, PhD
Illumina Inc.
San Diego, California, USA

Advisors

Max Q. Arens, PhD
Washington University School of
Medicine
St. Louis, Missouri, USA

Helen Fernandes, PhD
UMDNJ – University Hospital
Newark, New Jersey, USA

Leslie Hall, MMSc, RM(ASM)
Mayo Clinic
Rochester, Minnesota, USA

Timothy J. O’Leary, MD, PhD
Department of Veterans Affairs
Washington, District of Columbia,
USA

Mario Pazzagli, PhD
University of Florence
Florence, Italy

Cathy A. Petti, MD
Palo Alto Medical Foundation
Palo Alto, California, USA

Carolyn Sue Richards, PhD,
FACMG
Oregon Health Sciences University
Portland, Oregon, USA

Judith C. Wilber, PhD
XDX, Inc.
S. San Francisco, California, USA

Laurina O. Williams, PhD, MPH
Centers for Disease Control and
Prevention
Atlanta, Georgia, USA

Subcommittee on Quantitative Molecular Methods for Infectious Diseases

Angela M. Caliendo, MD, PhD
Co-Chairholder
Emory University School of
Medicine
Atlanta, Georgia, USA

Helen Fernandes, PhD
Co-Chairholder
UMDNJ - University Hospital
Newark, New Jersey, USA

Vladimir V. Cantarelli, PhD
Weinmann Laboratório LTD
Porto Alegre, Brazil

Christina Egan, PhD, CBSP
New York State Department of
Health
Albany, New York, USA

Mark J. Espy, MS
Mayo Clinic
Rochester, Minnesota, USA

Hawazin Faruki, DrPH
Laboratory Corporation of America
Burlington, North Carolina, USA

David Hillyard, MD
ARUP
Salt Lake City, Utah, USA

Li Li, MS
FDA Center for Devices and
Radiological Health
Rockville, Maryland, USA

Dave Petrich, MBA
AcroMetrix Corporation
Benicia, California, USA

Mangalathu S. Rajeevan, PhD
Centers for Disease Control and
Prevention
Atlanta, Georgia, USA

Yi-Wei Tang, MD, PhD,
D(ABMM)
Vanderbilt University Medical
Center
Nashville, Tennessee, USA

Alexandra Valsamakis, MD, PhD
The Johns Hopkins Hospital
Baltimore, Maryland, USA

Advisors

Cafer Eroglu, MD
Ondokuz Mayıs University School
of Medicine
Samsun, Turkey

Michael A. Lewinski, PhD,
D(ABMM)
UCLA Medical Center Clinical
Laboratories
Los Angeles, California, USA

Advisors (Continued)

Jan Turczyn, MT(ASCP), CLS
Siemens Healthcare Diagnostics
Berkeley, California, USA

Belinda Yen-Lieberman, PhD
Cleveland Clinic
Cleveland, Ohio, USA

Staff

Clinical and Laboratory Standards
Institute
Wayne, Pennsylvania, USA

Lois M. Schmidt, DA
*Vice President, Standards
Development*

Marcy Hackenbrack, M(ASCP),
MCM
Staff Liaison

Melissa A. Lewis, ELS
Editorial Manager

Megan P. Larrisey, MA
Assistant Editor

Acknowledgment

CLSI and the Area Committee on Molecular Methods gratefully acknowledge the following individuals for their help in preparing the approved-level, second edition of this document:

Haja Sittana El Mubarak, PhD
OVID/DMD
FDA Center for Devices and Radiological Health
Silver Spring, Maryland, USA

Phillip Moen, Jr, PhD
IntelligentMDx, Inc.
Cambridge, Massachusetts, USA

Venkatakrishna Shyamala, PhD
Consultant, Molecular Diagnostics
North Potomac, Maryland, USA

Linda Starr-Spires, PhD
Sanofi Pasteur, Inc.
Swiftwater, Pennsylvania, USA

Contents

Abstract.....i

Committee Membership..... iii

Foreword..... vii

1 Scope.....1

2 Introduction.....1

3 Standard Precautions.....1

4 Terminology.....1

 4.1 A Note on Terminology1

 4.2 Definitions2

 4.3 Abbreviations and Acronyms8

5 Update on Molecular Quantification Technologies9

 5.1 Amplification With End-point Analysis9

 5.2 Amplification With Real-time Analysis10

 5.3 Signal Intensity Measurement Without Nucleic Acid Amplification12

 5.4 Future Developments.....12

6 Specimen Handling and Preparation for Molecular Quantification.....12

 6.1 Specimen Collection13

 6.2 Specimen Processing14

 6.3 Nucleic Acid Extraction.....14

7 Reference Materials, Standards, Calibrators, and Quality Control Materials.....16

 7.1 Reference Materials16

 7.2 Standards/Secondary Reference Materials.....19

 7.3 Calibrators.....20

 7.4 Quality Control Materials21

8 Analytical Verification and Validation22

 8.1 General Considerations.....22

 8.2 Precision.....23

 8.3 Measuring Range and Linearity25

 8.4 Trueness27

 8.5 Limit of Detection and Limit of Quantification.....29

 8.6 Analytical Specificity30

 8.7 Interfering Substances.....31

 8.8 Method Comparison32

 8.9 Multiplex Quantification.....32

 8.10 Genetic Variability.....32

9 Clinical Validity and Clinical Utility33

 9.1 Introduction.....33

 9.2 Clinical Validation.....33

 9.3 Clinical Utility35

Contents (Continued)

10 Reporting, Interpretation, and Limitations of Results.....39

 10.1 Introduction.....39

 10.2 Result Reporting40

 10.3 Result Interpretation40

 10.4 Result Limitations.....42

11 Continuing Quality Assurance.....43

 11.1 Introduction.....43

 11.2 Preexamination Phase Quality Control.....43

 11.3 Examination Phase Quality Control.....44

 11.4 Postexamination Phase Quality Control49

References.....51

Appendix. Timing of Specimen Collection54

Summary of Delegate Comments and Subcommittee Responses.....56

The Quality Management System Approach60

Related CLSI Reference Materials62

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