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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.

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

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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