CLSI Releases Revised Guideline for the Use of Quantitative Molecular Methods in the Clinical Laboratory

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Quantitative molecular testing methods have become the standard of care for determining the concentrations of microorganisms in patients. Viral load testing, as it is commonly called, is an essential tool in monitoring a patient’s response to therapies. Because of the constantly changing nature of the field of quantitative molecular methods, it is necessary to provide guidelines for quality assurance and develop a roadmap for laboratories and manufacturers that want to develop assays.

In 2003, the Clinical and Laboratory Standards Institute (CLSI) published MM06-A—Quantitative Molecular Methods for Infectious Diseases; Approved Guideline, which established the original guidelines for laboratory tests that quantified viruses for the purpose of diagnosis and monitoring of infected patients. In November 2010, CLSI released a revised version of that document, entitled MM06-A2—Quantitative Molecular Methods for Infectious Diseases; Approved Guideline—Second Edition, which specifically addressed the changes in technology, performance, assay verification, interpretation, and quality control for quantitative molecular methods.1

Co-Chairholders of the CLSI subcommittee that created the document, Helen Fernandes, PhD, UMDNJ New Jersey Medical School, and Angela M. Caliendo, MD, PhD, Emory University School of Medicine, said, “MM06-A2 is particularly relevant at a time when oversight of laboratory-developed tests is being discussed by regulatory and accrediting agencies. The guideline was written by a diverse group of experts and is designed to be a practical, user-friendly document, applicable to all entities involved in molecular testing.”

MM06-A2 provides a thorough discussion of the issues specific to the quantification of nucleic acid in diagnostic testing and monitoring, such as nucleic acid probes and nucleic acid amplification techniques of the target sequences specific to particular microorganisms. It includes an update on the following:

• new 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
• recommendations for manufacturers and clinical laboratories.1

Drs. Fernandes and Caliendo agree it was important and necessary to revise MM06-A because both the technologies and the clinical uses of quantitative molecular methods tests have progressed since its publication. They note one important advance, the development of real-time polymerase chain reaction, that occurred after the original document was released. This technology is rapid, with a broad linear range, and is now the most commonly used technology in clinical laboratories for this purpose. The revised guideline describes this test and its application.

“Because the field has developed so rapidly, there are a far greater number of people performing this testing than there were 5 years ago. In addition, there are different kinds of tests being used to detect viral load,” Dr. Fernandes said. “In order for the entire field to study the same analyte or answer the same question, it is important to provide guidelines suggesting how one may validate tests and put them into practice.”

The authors of MM06-A2 intended the document to be as user-friendly and flexible as possible to enable laboratories to tailor it toward the validation of their own laboratory-developed tests. Dr. Caliendo explained there are few U.S. Food and Drug Administration-approved quantitative assays measuring virus amounts. Most quantitative assays are laboratory-developed tests. There are often many different parameters to examine before a test is used in a clinical laboratory, and MM06-A2 serves as a single resource that answers and gives guidance on all of the queries one has before bringing a test into the laboratory.

In addition to validation of laboratory-developed tests, MM06-A2 provides guidance on the verification of commercial tests. Verification is a necessary task confirming what different assays are meant to detect. If the manufacturer of a commercial test sets certain specifications, the laboratory verifies those specifications by confirming the test performance characteristics.

At the time MM06-A2 was developed, quantitative molecular assays were primarily applicable to viral diseases. But these methodologies could potentially be applied to other infectious agents and disease processes. “In the document we used viral examples, but it is not limited in its utility to viruses,” Dr. Caliendo explained. “Because the principle is transferable, there may be other applications for this guideline beyond viruses. For example, when studying bacteria that colonize the respiratory tract, one may need a quantitative test to distinguish colonization from true invasive infection.”

One of the strongest features of MM06-A2 is that it was written by professionals with a great deal of experience in the field. The guideline was drafted by clinicians who have personally performed these tests, and the perspective these individuals provide is critical to the development of a workable document. “This document was written by those of us who do the testing,” Dr. Caliendo noted. “We know what the ideal scenarios are, but we are also aware of the constraints of the laboratory. We do not want to lock laboratories down with 1 approach. Although I have worked in this field since
1994, I learned a great deal throughout the drafting of this guideline, which reinforces my belief that people with all levels of experience can benefit from its use.

CLSI has additional reference materials serving as companion documents to MM06-A2. Specifically, MM03-A2—Molecular Diagnostic Methods for Infectious Diseases: Approved Guideline—Second Edition (2006) addresses topics relating to clinical applications; amplified and non-amplified 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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