CLINICAL AND LABORATORY STANDARDS INSTITUTE®

2nd Edition

MM17

Validation and Verification of Multiplex Nucleic Acid Assays

This guideline includes recommendations for analytical validation and verification of multiplex assays, as well as a review of different types of biological and synthetic reference materials.

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

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Abstract

Clinical and Laboratory Standards Institute guideline MM17—*Validation and Verification of Multiplex Nucleic Acid Assays* discusses analytical validation and verification of qualitative multiplex nucleic acid assays. Topics covered include sample preparation, a general discussion of multiplex methods and technologies, reference and quality control materials, data analysis, and results reporting. Clinical validity and utility are briefly reviewed. Because of the variety and breadth of multiplex testing, specific protocols for validation and verification are not included. However, detailed recommendations for appropriate analytical validation and verification, based on the most current guidance documents, are provided.

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Foreword

Nucleic acid testing is one of the fastest growing fields in laboratory medicine. First-generation nucleic acid tests concentrated on measuring the presence or quantity of a single target, often using a single internal control. Recently, the multiplex nucleic acid testing field has expanded greatly for both laboratory-developed and marketed tests.

These assays use various platforms and technologies and measure both DNA and RNA targets. Although the chemistry technologies applied to multiplex nucleic assays may be different, sample handling, control strategies, data assessment, and results reporting are independent of any reagent set that might be used. In this guideline, multiplex assays are defined as assays in which two or more targets are simultaneously detected through a common process of sample preparation, amplification (target or signal), detection, and interpretation.

For a multiplex nucleic acid test to reliably achieve its intended use, process control is needed from sample acquisition and nucleic acid preparation for testing to data evaluation and results reporting. The competition among reactions in multiplex assays may necessitate more stringent requirements for sample purity, sample input, reagents, and platforms to avoid nonspecific reactions and background signal. Compared with single measurand assays, multiplex assays need more controls, more complex performance evaluation and data analysis algorithms, and more complex results reporting. Obtaining sufficient and appropriate control and reference materials (RMs) to properly validate and verify multiplex nucleic acid tests is a major challenge.

This guideline is designed to assist laboratories and manufacturers in developing, validating, verifying, controlling, analyzing, and implementing multiplex nucleic acid tests for diagnostic use. It provides recommendations for various aspects of multiplex test validation and verification and also includes a general overview of technologies currently in use for multiplex testing. The types of control and RMs that may be available for validation, verification, and daily quality control testing for multiplex assays are extensively discussed. Evaluation of adequate performance, as well as interpretation and reporting of multiplex testing results, is still evolving, and additional guidance documents from regulatory and standards organizations need to be developed. However, this guideline provides the most up-to-date recommendations currently available.

Overview of Changes

This guideline replaces the previous edition of the approved guideline, MM17-A, published in 2008. Several changes were made in this edition, including:

- Reorganized to fit the CLSI quality management system and path of workflow format
- Moved technologies overview to Appendix A
- Provided detailed, updated information on specimen types
- Added or revised information on RM types and uses
- Included guidance on using an error-based approach to validation and verification

NOTE: The content of this guideline is supported by the CLSI consensus process and does not necessarily reflect the views of any single individual or organization.

Key Words

Genotyping, laboratory-developed test, multiplex, multiplex assay, validation, verification

Validation and Verification of Multiplex Nucleic Acid Assays

Chapter 1: Introduction

This chapter includes:

- Guideline's scope and applicable exclusions
- Background information pertinent to the guideline's 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 guideline
- Abbreviations and acronyms used in the guideline

1.1 Scope

This guideline provides recommendations for qualitative multiplex nucleic acid assay validation and verification. This guideline focuses primarily on analytical validation, analytical verification, and QC and briefly discusses establishing clinical validation and clinical verification for these assays. The intended audience includes laboratory directors, medical microbiologists, laboratory technologists, QA personnel, and assay manufacturers. This guideline is not intended to be regulatory guidance but to provide current best practice recommendations. Additional regulatory and/or accreditation requirements may apply.

The design, acquisition, and appropriate use of different control materials are extensively reviewed. Current assay formats are used to illustrate proper validation and verification protocols, and appropriate data analysis and results reporting for multiplex assays are described. Because traditional single-measurand protocols are difficult or impossible to perform with multiplex assays, an error-based approach to validation and verification is presented. This error-based approach may be applicable to multiplex assays performed with a single test method, for which the performance characteristics for different measurands are expected to be similar.

This guideline describes general considerations and recommendations for multiplex testing platforms but does not discuss some basic technologies covered in detail in other CLSI molecular methods guidelines (eg, this guideline does not specifically discuss many microarray-based detection platforms or next-generation sequencing). Appendix A provides an overview of some currently available multiplex testing technologies. For additional information, see CLSI documents MM01,[1] MM03,[2] MM06,[3] MM09,[4] MM21,[5] MM22,[6] and MM23.[7]

This guideline discusses multiplex assays for genotyping and pathogen detection and excludes gene expression assays. This guideline also does not cover assays measuring individual targets that are then evaluated together to yield a composite score or classifier as a result.

1.2 Background

With the complete sequencing of the human genome, ever increasing numbers of sequenced viral and bacterial genomes, and the development of the associated fields of genomics and pharmacogenomics, there has been a rapid expansion of available genotyping assays. More importantly, genotyping assays are increasingly run as multiplex assays.

Multiplex assays have two or more targets that are detected simultaneously through a common process of sample preparation, target or signal amplification, allele discrimination, and collective interpretation. These assays can be used for many different applications, including:

- Genotyping for common mutations in the Ashkenazi Jewish population
- Detecting and identifying infectious agents (eg, microbial pathogen panels such as respiratory viral pathogen panels)
- Identifying genetic disorders (eg, targeted mutation panels such as cystic fibrosis transmembrane conductance regulator [*CFTR*] mutation analysis or cancer mutation panels)
- Choosing drug therapies and doses (eg, pharmacogenetic panels such as cytochrome P450 genotyping for drug metabolism alleles)
- Assessing disease progression and prognosis

Regardless of the medical use, all multiplex assays present significant challenges in validation and verification, acquisition of appropriate biological control materials, data analysis, and reporting. Laboratories can develop in-house assays (laboratory-developed tests [LDTs]) or use available multiplex assays using various technologies and instrument platforms.

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. Published guidelines are available that discuss the daily operations of diagnostic medicine in humans and animals while encouraging a culture of safety in the laboratory.[8] 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.[9]

1.4 Terminology

1.4.1 A Note on Terminology

CLSI, as a global leader in standardization, is firmly committed to achieving global harmonization whenever 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 different countries and regions and that legally required use of terms, regional usage, and different consensus timelines are all important considerations in

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 that facilitates project management, defines a document structure using 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:

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

MM17 covers 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.

Organization	Customer Focus	Facilities and Safety	Personnel	Purchasing and Inventory	Equipment	Process Management	Documents and Records	Information Management	Nonconforming Event Management	Assessments	Continual Improvement
						X AUTO08 EP05 EP06 EP07 EP10 EP12 EP14 EP15 EP17 EP23 EP25 EP26				EP10	
		M29				MM01 MM03 MM05 MM06 MM09 MM13				MM05	
MM19	MM19	MM19	MM19	MM19	MM19 QMS13	MM19 MM21 MM22 MM23	MM19 QMS02	MM19	MM19	MM19	MM19

Related CLSI Reference Materials*

AUTO08 **Managing and Validating Laboratory Information Systems. 1st ed., 2006.** This document provides guidance for developing a protocol for validation of the laboratory information system (LIS), as well as protocols for assessing the dependability of the LIS when storing, retrieving, and transmitting data.

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 and for laboratories that develop or modify such procedures.

EP06 **Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach. 1st ed., 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 **Interference Testing in Clinical Chemistry. 3rd ed., 2018.** This guideline provides background information, guidance, and experimental procedures for investigating, identifying, and characterizing the effects of interferents on clinical chemistry test results.

EP10 **Preliminary Evaluation of Quantitative Clinical Laboratory Measurement Procedures. 3rd ed., 2014.** This guideline provides experimental design and data analysis for preliminary evaluation of the performance of a measurement procedure or device.

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.

EP14 **Evaluation of Commutability of Processed Samples. 3rd ed., 2014.** This document provides guidance for evaluating the commutability of processed samples by determining if they behave differently than unprocessed patient samples when two quantitative measurement procedures are compared.

EP15 **User Verification of Precision and Estimation of Bias. 3rd ed., 2014.** This document describes the estimation of imprecision and of bias for clinical laboratory quantitative measurement procedures using a protocol that can be completed within as few as five days.

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.

EP25 **Evaluation of Stability of *In Vitro* Diagnostic Reagents. 1st ed., 2009.** This document provides guidance for establishing shelf-life and in-use stability claims for *in vitro* diagnostic reagents such as reagent kits, calibrators, and control products.

EP26 **User Evaluation of Between-Reagent Lot Variation. 1st ed., 2013.** This document provides guidance for laboratories on the evaluation of a new reagent lot, including a protocol using patient samples to detect significant changes from the current lot.

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.

* 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)

MM01 **Molecular Methods for Clinical Genetics and Oncology Testing. 3rd ed., 2012.** This document provides guidance for the use of molecular biological techniques for detection of mutations associated with inherited medical disorders, somatic or acquired diseases with genetic associations, and pharmacogenetic response.

MM03 **Molecular Diagnostic Methods for Infectious Diseases. 3rd ed., 2015.** 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.

MM05 **Nucleic Acid Amplification Assays for Molecular Hematopathology. 2nd ed., 2012.** This guideline addresses the performance and application of assays for gene rearrangement and translocations by both polymerase chain reaction (PCR) and reverse-transcriptase PCR techniques, and includes information on specimen collection, sample preparation, test reporting, test validation, and quality assurance.

MM06 **Quantitative Molecular Methods for Infectious Diseases. 2nd ed., 2010.** 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.

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.

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.

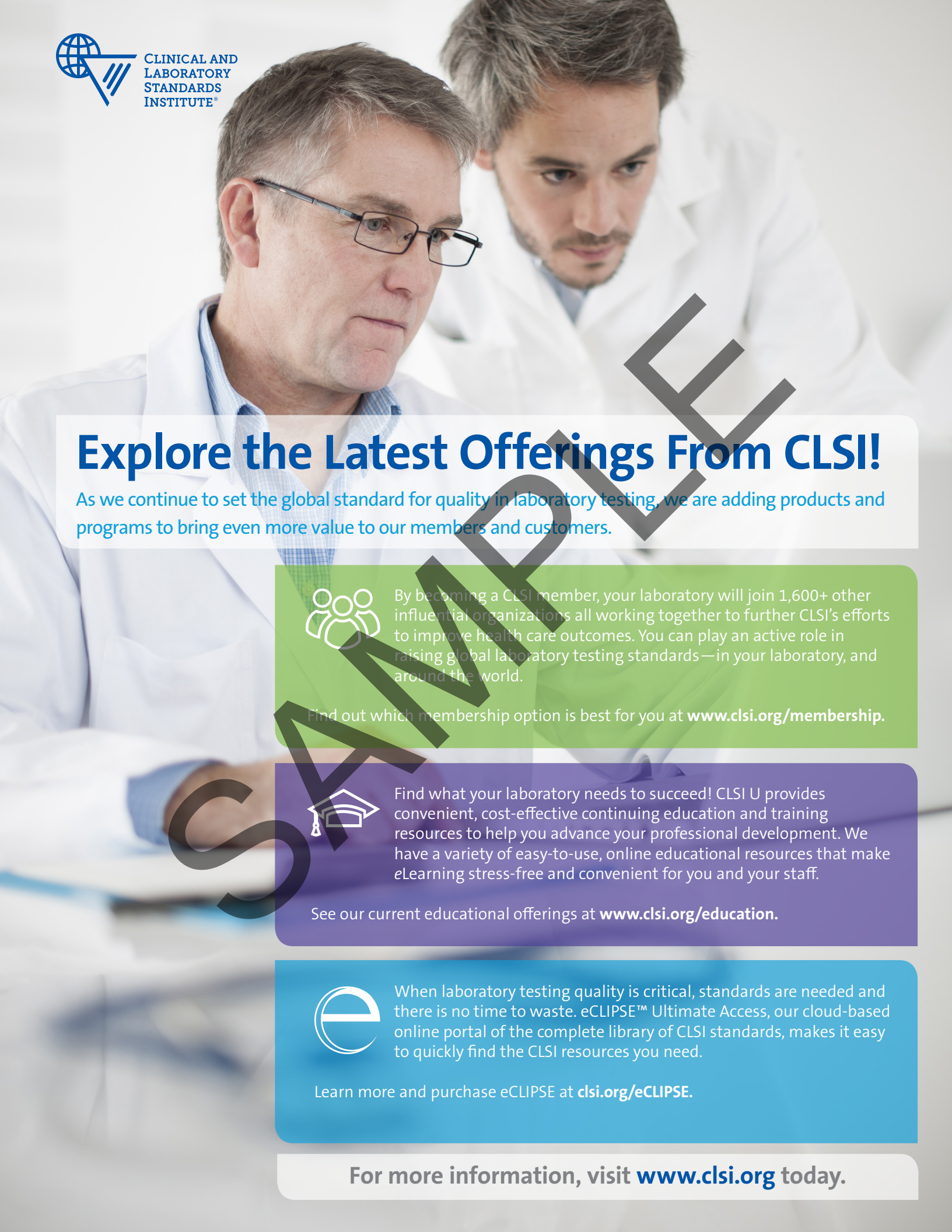
MM21 **Genomic Copy Number Microarrays for Constitutional Genetic and Oncology Applications. 1st ed., 2015.** This guideline provides recommendations for validation, verification, performance, and interpretation of nucleic acid microarrays used for cytogenetic applications to measure copy number imbalances and loss of heterozygosity. Both constitutional and oncology applications are addressed.

MM22 **Microarrays for Diagnosis and Monitoring of Infectious Diseases. 1st ed., 2014.** This document provides guidance for the laboratory development and use of qualitative nucleic acid microarray methods for the diagnosis and monitoring of infectious diseases. It also presents recommendations for validation and verification, quality control, and interpretation of results.

MM23 **Molecular Diagnostic Methods for Solid Tumors (Nonhematological Neoplasms). 1st ed., 2015.** This guideline covers the current state of molecular diagnostic techniques intended for the characterization of solid tumors, and covers a range of clinical applications including diagnosis, prognosis, therapeutic response prediction for available drugs and those still in clinical trials, as well as monitoring and presymptomatic and predisposition testing.

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.

QMS13 **Quality Management System: Equipment. 1st ed., 2011.** This guideline provides recommendations for establishing equipment management processes from selection through decommission of equipment used in the provision of laboratory services.



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