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# Molecular Methods for Clinical Genetics and Oncology Testing; Approved Guideline—Third Edition

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.

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



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*Advancing Quality in Health Care Testing*

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### Abstract

Clinical and Laboratory Standards Institute document MM01-A3—*Molecular Methods for Clinical Genetics and Oncology Testing; Approved Guideline—Third Edition* provides general recommendations for all phases of the operation of a molecular genetics diagnostic laboratory. Clinical molecular testing has application to inherited and acquired medical conditions with genetic etiologies as well as variations associated with drug metabolism. In a clinical molecular laboratory, techniques and practices require strict adherence to quality performance measures. This revised guideline will address the total testing process.

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## Foreword

This document replaces the second edition of the approved guideline, MM01-A2, *Molecular Diagnostic Methods for Genetic Diseases; Approved Guideline—Second Edition*, which was published in June 2006. Several changes were made in this edition; chief among them is the revision of the title and the inclusion of somatic or acquired genetic changes associated with nonhematological cancers, which have not been addressed in other molecular guidelines. The rapid development of new molecular genetic testing methods has prompted the third edition of this guideline to include more current technologies and applications. In addition, this version has been expanded to include topics such as epigenetic testing, mitochondrial disorders, chimerism, and pharmacogenetics. In a rapidly evolving technological area, this document also presents newer molecular methods that are starting to appear in clinical laboratories, such as next-generation DNA sequencing.

## Key Words

Amplification, arrays, gene, genetic disease, genotyping, molecular diagnostic test, mutation detection, nucleic acid, polymerase chain reaction, sequencing, Southern blot

SAMPLE

# Molecular Methods for Clinical Genetics and Oncology Testing; Approved Guideline—Third Edition

## 1 Scope

This revision of MM01 provides guidelines for laboratory testing practices, methods, and technologies for detection of inherited and somatic genetic variation. Because not all genetic testing is done for the purpose of diagnosing disease, the title of this document has been revised to reflect the wide range of indications for molecular genetic testing. This document is intended as a source of reference material for medical genetic testing laboratories performing nucleic acid–based testing. This information includes performance characteristics of the total molecular genetics laboratory testing process, ie, preexamination, examination, and postexamination. This document is intended to provide guidance to experienced laboratory directors, supervisors, and manufacturers involved in assay development, verification, validation, and interpretation of molecular genetic testing.

This document is not intended to serve as an introductory manual for laboratories without experience in molecular genetics. Conversely, the performance characteristics of complex, multivariate diagnostic assays that employ nontransparent, mathematical algorithms to interpret genetic risk or likelihood of drug response are beyond the scope and consideration of this guideline but are addressed in CLSI document MM17.<sup>1</sup> Although some description of pharmacogenetic traits has been included, a full consideration of both targeted therapeutics and inherited variation in drug metabolism and response is beyond the scope of this guideline. This document also does not include molecular virology or molecular microbiology. These topics are addressed in CLSI documents MM03,<sup>2</sup> MM06,<sup>3</sup> MM10,<sup>4</sup> MM11,<sup>5</sup> and MM18.<sup>6</sup>

## 2 Introduction

Molecular genetic testing has become a staple method for the assessment of a growing number of inherited disorders, somatic or acquired diseases with genetic associations, and pharmacogenetic responses. Genotyping can provide useful indicators of disease diagnosis, prognosis, and progression; can be used to guide treatment selection and response; and can interrogate targets for gene-specific therapies. Genetic variations can be used for carrier screening, presymptomatic testing, and predisposition testing, in addition to diagnostic testing, newborn screening, and prenatal testing for heritable disorders.

The purpose of this guidance is to inform laboratories performing nucleic acid–based molecular genetic testing of the appropriate and effective qualities of genetics laboratory operations, assay design and performance characteristics, frequent clinical contexts and applications of these strategies, and the most common molecular methods included in clinical genetic testing practice.

Mutations associated with heritable disorders are detectable in all nucleated cells and thus are considered to be germline or constitutional genetic changes. Somatic genetic changes are characteristic of acquired or sporadic diseases, such as cancer. The molecular biology methods applied to investigate these two scenarios are very similar and focus on detection of DNA and RNA variations. However, the interpretation and utility of the laboratory results may be quite distinct. For example, the impact for screening at-risk family members and predictive risk assessment are important considerations for heritable disease genetic testing, while sensitivity threshold of detection is a defining feature of quantitative testing for the genetic signatures of acquired disease conditions such as cancer.

Although CLSI document MM05<sup>7</sup> addresses molecular methods for select hematopathology oncology applications (which will not be duplicated in this document), there currently is no guidance document to encompass assays to detect acquired genetic changes for many other tumor types. With increasing use of



tumor genetic profiles to determine treatment selection and efficacy, this revision will include considerations of both acquired and inherited genetic disease testing.

Although sensitivity threshold of detection is a defining feature of quantitative testing for the genetic signatures of acquired disease conditions, the primary focus of this guideline is to address qualitative molecular genetic testing. CLSI guideline MM06<sup>3</sup> includes many discussions of quantitative molecular methods that are applicable to detecting acquired genetic variations in neoplasias, such as linearity and measuring interval, limit of detection (LoD) and limit of quantification (LoQ), results outside the measuring interval, and technologies used in molecular quantification.

When performing a molecular genetic test, laboratories should consider the preexamination, examination, and postexamination issues discussed in this document. In addition, it is important that the test methodology and result interpretation are based on the test indication and application. Ethical issues associated with genetic testing and the privacy of genetic information should also be considered.

Securing qualified and competent personnel at all levels is key to producing accurate and timely medical laboratory test results. Specific qualifications and responsibilities vary somewhat from region to region; however, medical laboratories should have policies and procedures in place governing job descriptions and minimal qualifications to ensure that all personnel have the appropriate skills to perform their jobs competently. For additional information on specific requirements, refer to the International Organization for Standardization (ISO) document ISO 15189<sup>8</sup> and, in the United States, the Clinical Laboratory Improvement Amendments (CLIA).<sup>9</sup> References that provide guidance for personnel management, including CLSI document GP26,<sup>10</sup> are also available.<sup>8,9,11</sup>

## 2.1 Clinical Genetic Testing Applications

The completion of the full human genome sequence, the identification and cloning of numerous genes associated with inherited and acquired conditions and diseases, plus the advent of powerful methods for molecular analysis of these genes in clinical specimens, have revolutionized the practice of molecular genetics and molecular pathology. With these methods, it has become possible to diagnose disease risk in presymptomatic individuals, to screen for asymptomatic carriers of recessive traits, and to perform prenatal diagnosis for diseases that may not manifest *in utero*. Molecular genetic, nucleic acid–based techniques are often the only approaches available for these applications. As such, they offer a powerful tool for diagnosis, genetic consultation, and prevention of heritable diseases. It is the responsibility of the laboratory director to critically monitor the peer-reviewed literature and professional recommendations for each new gene test to avoid implementing tests of uncertain sensitivity, specificity, or clinical utility.

DNA-based tests represent the most fundamental approach to the diagnosis of genetic diseases that are due to mutations in a single gene, such as nucleotide substitutions, deletions, insertions, duplications, expansions, and inversions. These lesions are germline defects because they are inherited from one or both parents, are present from conception, and are contained in every nucleated cell of the body. Molecular genetic diagnosis of heritable disorders can usually be performed on any accessible body tissue. While biochemical deficiencies may be expressed in a particular organ (eg, the liver), DNA-based constitutional changes may be identified using peripheral leukocytes, amniocytes, or any other convenient cells.

Moreover, target amplification techniques, such as the PCR, render adequate substrates for genetic analysis from even the most minute samples, leading to specimen collection techniques even less invasive than simple phlebotomy. Mouthwash, buccal scrapings, random urine collection, dried blood spots, and hair bulb analysis are all viable approaches for obtaining sufficient nucleated cells for mutation detection. Optimization of methods to detect and analyze scarce fetal cells or DNA circulating in the maternal blood, once an area of intense research, is now moving into medical laboratory application.

### 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 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 as follows:

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

MM01-A3 addresses 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, beginning on page 146.

Organization	Customer Focus	Facilities and Safety	Personnel	Purchasing and Inventory	Equipment	Process Management	Documents and Records	Information Management	Nonconforming Event Management	Assessments	Continual Improvement
GP26	GP26	GP26 M29 MM07	GP26 MM07	GP26	GP26 MM07	X C28 EP05 EP06 EP07 EP09 EP12 EP15 EP17 EP18 GP26 GP27 GP29 MM03 MM05 MM06 MM07 MM09 MM10 MM11 MM12 MM13 MM16 MM17 MM18	GP02 GP26 MM07	GP02 GP26	EP18 GP26	EP18 GP26 GP27 GP29 MM03 MM05 MM06 MM07	EP07 EP18 GP26 GP27

## Related CLSI Reference Materials\*

- 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. A CLSI-IFCC joint project.
- 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.
- EP12-A2** **User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline—Second Edition (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.
- 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 utilizing 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. An NCCLS-IFCC joint project.
- EP18-A2** **Risk Management Techniques to Identify and Control Laboratory Error Sources; Approved Guideline—Second Edition (2009).** This guideline describes risk management techniques that will aid in identifying, understanding, and managing sources of failure (potential failure modes) and help to ensure correct results. Although intended primarily for *in vitro* diagnostics, this document will also serve as a reference for clinical laboratory managers and supervisors who wish to learn about risk management techniques and processes.
- GP02-A5** **Laboratory Documents: Development and Control; Approved Guideline—Fifth Edition (2006).** This document provides guidance on development, review, approval, management, and use of policy, process, and procedure documents in the medical laboratory community.
- GP26-A4** **Quality Management System: A Model for Laboratory Services; Approved Guideline—Fourth Edition (2011).** This document provides a model for medical laboratories that will assist with implementation and maintenance of an effective quality management system.
- GP27-A2** **Using Proficiency Testing to Improve the Clinical Laboratory; Approved Guideline—Second Edition (2007).** This guideline provides assistance to laboratories in using proficiency testing as a quality improvement tool.

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\* 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)

- GP29-A2** **Assessment of Laboratory Tests When Proficiency Testing Is Not Available; Approved Guideline—Second Edition (2008).** This document offers methods to assess test performance when proficiency testing (PT) is not available; these methods include examples with statistical analyses. This document is intended for use by laboratory managers and testing personnel in traditional clinical laboratories as well as in point-of-care and bedside testing environments.
- 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.
- 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.
- MM05-A2** **Nucleic Acid Amplification Assays for Molecular Hematopathology; Approved Guideline—Second Edition (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-A2** **Quantitative Molecular Methods for Infectious Diseases; Approved Guideline—Second Edition (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.
- 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.
- MM10-A** **Genotyping for Infectious Diseases: Identification and Characterization; Approved Guideline (2006).** This guideline describes currently used analytical approaches and methodologies applied to identify the clinically important genetic characteristics responsible for disease manifestation, outcome, and response to therapy in the infectious disease setting. It also provides guidance on the criteria to be considered for design, validation, and determination of clinical utility of such testing.
- MM11-A** **Molecular Methods for Bacterial Strain Typing; Approved Guideline (2007).** This guideline examines the biology behind molecular strain typing and the process of characterizing and validating typing systems. The prevalent methods are described with particular attention to pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST).
- MM12-A** **Diagnostic Nucleic Acid Microarrays; Approved Guideline (2006).** This guideline provides recommendations for many aspects of the array process including: a method overview; nucleic acid extraction; the preparation, handling, and assessment of genetic material; quality control; analytic validation; and interpretation and reporting of results. A CLSI-IFCC joint project.

**Related CLSI Reference Materials (Continued)**

- 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. A CLSI-IFCC joint project.
- 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. A CLSI-IFCC joint project.
- MM17-A**      **Verification and Validation of Multiplex Nucleic Acid Assays; Approved Guideline (2008).** This guideline provides recommendations for analytic verification and validation of multiplex assays, as well as a review of different types of biologic and synthetic reference materials.
- MM18-A**      **Interpretive Criteria for Identification of Bacteria and Fungi by DNA Target Sequencing; Approved Guideline (2008).** Sequencing DNA targets of cultured isolates provides a quantitative metric within which to perceive microbial diversity, and can serve as the basis to identify microorganisms. This document is an effort to catalyze the entry of molecular microbiology into clinical usage by establishing interpretive criteria for microorganism identification.

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