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# Molecular Diagnostic Methods for Genetic Diseases; Approved Guideline—Second Edition

This document provides guidance for the use of molecular biological techniques for clinical detection of heritable mutations associated with genetic disease.

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



*(Formerly NCCLS)*

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*Advancing Quality in Healthcare Testing*

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### Abstract

Clinical and Laboratory Standards Institute document MM1-A2—*Molecular Diagnostic Methods for Genetic Diseases; Approved Guideline—Second Edition* provides general recommendations for all phases of the operation of a molecular genetics diagnostic laboratory. The recommendations cover nomenclature for human pedigrees and the designation of mutations; laboratory safety; and “front-end” activities, such as intake information, specimen identification and accessioning, and sample preparation. Other topics addressed are molecular analytical techniques, test validation and characterization, quality assurance, results reporting, and selection of referral laboratories. The guideline also includes definitions of selected terms commonly used in the theory and practice of molecular genetics.

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## Foreword

This guideline represents an extensive revision and updating of the first edition of MM1, which was published in 2000. It incorporates many newer technologies that have emerged since that time, and retires a number that are now obsolescent. Also, it is recognized that the larger field of molecular diagnostics has matured appreciably in the intervening years, and authoritative technical, regulatory, and quality assurance guidelines have been developed and accepted by other expert bodies during that time. Where relevant, those documents are referenced or incorporated in this new edition.

Molecular genetics has now become firmly entrenched as the third major subdiscipline of clinical laboratory medical genetics, emerging more recently than the other subspecialties, biochemical genetics and cytogenetics. As with any diagnostic method or test, in order to fully benefit the patient, it must be developed and practiced under appropriate conditions. Thus, the purpose of this guideline is to define conditions and principles that will optimize the provision of accurate genetic information, while minimizing potential harm to the patient or family.

### *A Note on Terminology*

Clinical and Laboratory Standards Institute (CLSI) recognizes that harmonization of terms facilitates the global application of standards, and as a matter of organizational policy, is firmly committed to employing terms that are generally used internationally. This initiative includes a mechanism to resolve ISO/CEN/CLSI differences in nomenclature. However, CLSI is also aware 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, ISO, and CEN documents; and that legally required use of terms, regional usage, and different consensus timelines are all obstacles to harmonization. Therefore, implementation of this policy must be an evolutionary and educational process that begins with new projects and revisions of existing documents.

In keeping with CLSI's commitment to align terminology with that of ISO, the following terms are used in MM1: *specimen* is used in this document when referring to the discrete portion of a body fluid or tissue taken for examination, study, or analysis of one or more quantities or characteristics to determine the character of the whole; *sample* is one or more parts taken from a system, and intended to provide information on the system, often to serve as a basis for decision on the system or its production, e.g., a volume of serum taken from a larger volume of serum (ISO 15189)<sup>1</sup>; *verification* is confirmation, through the provision of objective evidence, that specified requirements have been fulfilled (ISO 9000)<sup>2</sup>, i.e., a one-time process completed to determine or confirm test performance characteristics before the test system is used for patient testing; *validation* is confirmation, through the provision of objective evidence, that requirements for a specific intended use or application have been fulfilled (ISO 9000)<sup>2</sup>, i.e., the components of validation are quality control, proficiency testing, validation of employee competency, instrument calibration, and correlation with clinical findings.

### Key Words

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



## **Molecular Diagnostic Methods for Genetic Diseases; Approved Guideline— Second Edition**

### **1 Scope**

The topics covered in this document relate to the use of molecular biological techniques for clinical detection of heritable mutations associated with genetic disease. This detection may be done for purposes of diagnosis, carrier screening, newborn screening, prenatal testing, or presymptomatic/predisposition testing. The mutations associated with genetic disease are considered germline, and thus are to be distinguished from somatic DNA changes associated with cancer, infectious disease, or environmental insults. Even though many of the laboratory techniques for detecting the latter are the same or similar, the applications, interpretation, and ramifications of these tests for heritable disease are quite different and raise a number of unique dilemmas as discussed above.

The methodologies covered in this document are designed to analyze extracted and/or amplified DNA. Molecular assays performed on intact cells or chromosomes (e.g., fluorescence *in situ* hybridization) are not included.

This document is intended for the use of all genetics laboratories performing clinical molecular genetic testing as a reference material source. This document is not intended to serve as a primer on molecular methods for laboratories who are inexperienced in genetic testing.

### **2 Introduction**

#### **2.1 Diagnostic Utility**

The completion of the full human genome sequence, the identification and cloning of numerous genes associated with inherited diseases, and the advent of powerful methods for molecular analysis of these genes in clinical specimens, have revolutionized the practice of medical genetics. With these methods, it has become possible to diagnose disease in at-risk individuals prior to the onset of symptoms, to screen for asymptomatic carriers of recessive traits, and to perform prenatal diagnosis for those diseases which are not expressed *in utero*. In contrast to the other three major areas of clinical molecular biology (i.e., molecular microbiology, molecular oncology, and DNA-based identity testing), where DNA-based techniques supplement or supplant more traditional diagnostic methods, molecular genetic techniques are often the only approach available for the applications cited. As such, they offer a powerful tool for diagnosis, genetic counseling, and prevention of heritable disease.

DNA-based tests represent the most fundamental and definitive approach to the diagnosis of genetic diseases which are, by definition, due to lesions in an individual's DNA, such as nucleotide substitutions, deletions, insertions, duplications, expansions, and inversions. When these lesions are inherited from one or both parents, they are germline defects, present from conception and found in every DNA-containing cell of the body. Unlike molecular diagnosis of neoplasia or infectious disease, which requires DNA sampling of the tumor or infection site, molecular genetic diagnosis can be performed on any accessible body tissue. Even if the biochemical defect is only expressed in a particular inaccessible organ (e.g., the liver), diagnosis can be made using peripheral leukocytes, amniocytes, or any other convenient cells. Moreover, target amplification techniques, such as the polymerase chain reaction (PCR), render even the most minute samples adequate substrates for genetic analysis, 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 circulating in the maternal blood is an area of intense research, and may someday render amniocentesis and chorionic villus