CLINICAL AND LABORATORY STANDARDS INSTITUTE®

1st Edition

MM23

Molecular Diagnostic Methods for Solid Tumors (Nonhematological Neoplasms)

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.

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

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Molecular Diagnostic Methods for Solid Tumors (Nonhematological Neoplasms)

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Abstract

Clinical and Laboratory Standards Institute document MM23—*Molecular Diagnostic Methods for Solid Tumors (Nonhematological Neoplasms)* describes development and implementation of nucleic acid biomarker assays for accurate detection of somatic and germline alterations with applications to clinical decision making for cancer patients with solid tumors. It is intended for molecular diagnostic laboratory directors, industry laboratory professionals, and health care professionals, including anatomic and clinical pathologists. With the exception of cancer predisposition syndromes, the methods and recommendations discussed in this document focus primarily on detection of tumor-specific genetic abnormalities that are acquired during tumorigenesis and are distinct from normal variations in nonmalignant cells of the same tissue.

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Contents

Foreword

With the completion of the sequence of the human genome, researchers have identified opportunities for detecting and defining molecular alterations in the germline, as well as within malignant tumors. Identification of mutations that drive both neoplastic transformation of normal tissue, as well as progression to more advanced disease states, provides insight into the biology of neoplasia and therapies to arrest the disease. The discovery of many somatic alterations in the tumor cell genomes, new forms of regulatory nucleic acids, gene expression profiling, whole exome sequencing, and microRNA profiling are a few of the molecular tests used by the clinical laboratory to support the individualized use of therapies and improve the outcomes of cancer patients. Clinical oncology is moving from treatment selection that is based solely on the tissue of origin to one based on the molecular genetics of the particular cancer and mutation profiling to define optimal patient therapies. Genetic variations may be matched with available drugs that may not have been previously considered, or with drugs that are available through clinical trials. It is essential that these new tests be useful for medical decision-making purposes, and that their utility be evaluated as quickly and efficiently as possible.

The document development committee was formed to address the need for a guideline on the performance and interpretation of molecular assays used to characterize solid tumors. This guideline covers the current state of molecular diagnostic techniques intended for the characterization of solid tumors, as well as a range of clinical applications, including diagnosis, prognosis, monitoring tumor burden, presymptomatic and predisposition testing, and therapeutic response prediction for available drugs, as well as drugs still in clinical trials. This guideline does not include an extensive discussion of inherited cancer syndromes, which are covered in more depth in CLSI documents MM01[1] and MM19.[2] In addition, due to the rapidly changing nature of molecular diagnostics, this document may be incomplete due to the development of new techniques after its publication.

The methods and QC approaches described herein are not absolute or immutable. They represent expert consensus recommendations presented by the document development committee, and are intended for use by diagnostic laboratories. Such use is intended to facilitate both interlaboratory comparisons of results and diagnostic interpretations, as well as to ensure accuracy in diagnosis and tumor characterization.

Key Words

Cancer, carcinoma, companion diagnostic devices, genetics, genomics, genotyping, molecular methods, mutation detection, nonhematological, oncology, polymerase chain reaction, sequencing, solid tumor, somatic variants, targeted therapy

Molecular Diagnostic Methods for Solid Tumors (Nonhematological Neoplasms)

Chapter 1: Introduction

This chapter includes:

- Document scope and applicable exclusions
- Background information pertinent to the document 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 document
- Abbreviations and acronyms used in the document

1.1 Scope

This guideline describes the development and implementation of nucleic acid biomarker assays for accurate detection of somatic and germline alterations with applications to clinical decision making in oncology. With the exception of cancer predisposition syndromes, the methods and recommendations discussed in this document focus primarily on detection of tumor-specific genetic abnormalities that are acquired during tumorigenesis and are distinct from normal variations in nonmalignant cells of the same tissue. Circulating tumor cells (CTCs) of solid tumor origin and circulating cell-free tumor nucleic acid assays are discussed. Distinguishing characteristics of familial cancer syndromes are presented briefly because many diagnostic testing criteria will be similar to those addressed in CLSI document MM01,[1] which describes inherited trait genetic testing. Genetic markers (acquired or inherited) that predict response to anticancer treatments, including targeted therapies, and the role of these markers in personalized medicine are discussed.

This guideline focuses on neoplasms that are neither leukemias nor lymphomas (hematological cancers) because these cancers have been addressed in great detail in CLSI document MM05.[3] This document focuses on the underlying nucleic acid tumor markers and variants, but does not examine cell-surface antigens, immunohistochemistry (IHC), or protein markers. Detailed guidance for the use of nucleic acid sequencing, microarrays, multiplex assays, and quantitative testing are covered in greater detail in CLSI documents MM01,[1] MM05,[3] MM07,[4] MM09,[5] MM12,[6] and MM17.[7]

This document is intended for molecular diagnostic laboratory directors, industry laboratory professionals, and health care professionals, including anatomic and clinical pathologists.

1.2 Background

1.2.1 Tumorigenesis

Tumorigenesis is a multistep process in which initial mutations in some cells confer a selective growth advantage over normal cells. The major steps involved in tumorigenesis include initialization of the growth advantage, establishment and proliferation of clonal populations, invasion of surrounding tissue, and metastasis to other sites in the body.[8] Cells carrying these tumorigenic mutations do not respond normally to signals that restrict proliferation, promote apoptosis, provide immune system surveillance, or drive differentiation (see Figure 1). An increased rate of proliferation or a decreased rate of apoptosis can lead to an accumulation of cells with errors in DNA replication, producing further mutation and/or genomic instability. This process may escalate the expansion of certain clonal populations of tumor cells, which eventually invade surrounding normal tissues. The tumor cells may also metastasize through the blood or lymph systems to other more distant sites of the body and seed other organs (see Figure 2). The size of the tumor mass is a result of the balance between tumor cell proliferation and apoptosis/necrosis when sufficient vascularization and blood supply is available to the tumor mass.

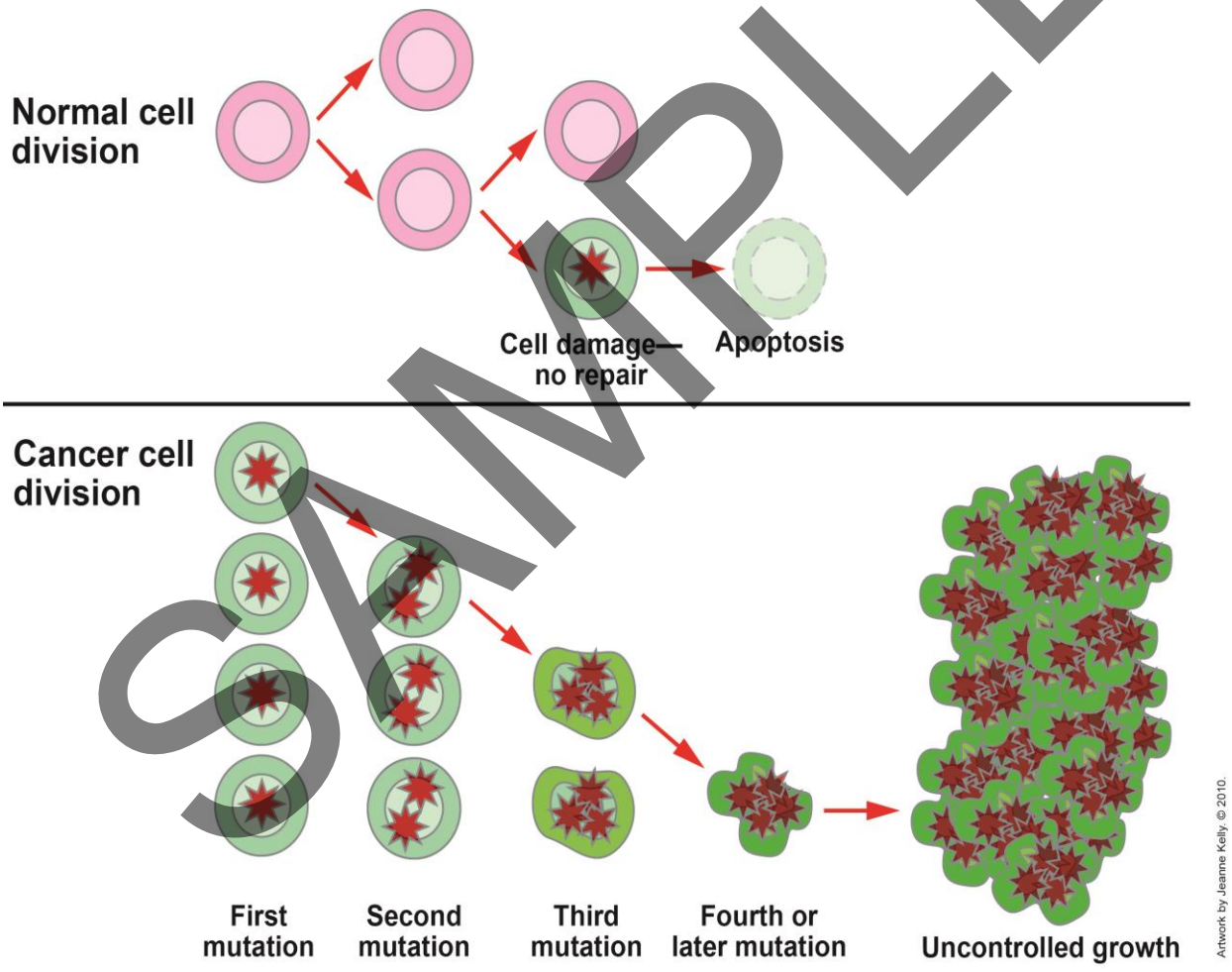

Figure 1. Cancer Can Arise Through the Loss of Control Over Cell Growth and Proliferation.[9] (Artwork originally created for the National Cancer Institute. Reprinted with permission of the artist, Jeanne Kelly. Copyright 2010.)

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, which facilitates project management; defines a document structure via 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 as follows:

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

MM23 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 114.

Organization	Customer Focus	Facilities and Safety	Personnel	Purchasing and Inventory	Equipment	Process Management	Documents and Records	Information Management	Nonconforming Event Management	Assessments	Continual Improvement
		M29				X EP05 EP07 EP12 EP15 EP17 MM01 MM05 MM06 MM07 MM09 MM12 MM17				MM05	
MM19	MM19	MM19	MM19	MM19	MM19	MM19	MM19 QMS02	MM19	MM19	MM19	MM19

Path of Workflow

A path of workflow is the description of the necessary processes to deliver the particular product or service that the organization or entity provides. A laboratory path of workflow consists of the sequential processes: preexamination, examination, and postexamination and their respective sequential subprocesses. All laboratories follow these processes to deliver the laboratory's services, namely quality laboratory information.

MM23 addresses the clinical laboratory path of workflow steps indicated by an "X." For a description of the other documents listed in the grid, please refer to the Related CLSI Reference Materials section on the following page.

Preexamination				**Examination**			**Postexamination**	
Examination ordering	Sample collection	Sample transport	Sample receipt/processing	Examination	Results review and follow-up	Interpretation	Results reporting and archiving	Sample management
X	X		X	X	X	X	X	
MM01	MM01	MM01	MM01	MM01	MM01	MM01	MM01	MM01
MM05			MM05	MM05	MM05	MM05	MM05	MM05
MM06	MM06	MM06	MM06	MM06	MM06	MM06	MM06	
			MM07	MM07	MM07	MM07	MM07	
	MM09	MM09	MM09	MM09	MM09	MM09	MM09	MM09
		MM12	MM12	MM12	MM12	MM12	MM12	MM12
	MM19	MM19	MM19	MM19	MM19	MM19		

Related CLSI Reference Materials*

EP05 **Evaluation of Precision Performance of Quantitative Measurement Methods. 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.

EP07 **Interference Testing in Clinical Chemistry. Approved Guideline. 2nd ed., 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.

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.

EP15 **User Verification of Performance for 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.

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.

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.

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.

MM07 **Fluorescence *In Situ* Hybridization Methods for Clinical Laboratories. 2nd ed., 2013.** This document addresses fluorescence *in situ* hybridization 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.

* 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)

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.

MM12 **Diagnostic Nucleic Acid Microarrays. 1st ed., 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.

MM17 **Verification and Validation of Multiplex Nucleic Acid Assays. 1st ed., 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.

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.

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.

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