CLINICAL AND LABORATORY STANDARDS INSTITUTE®

1st Edition

MM21

Genomic Copy Number Microarrays for Constitutional Genetic and Oncology Applications

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.

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

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Genomic Copy Number Microarrays for Constitutional Genetic and Oncology Applications

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Abstract

Clinical and Laboratory Standards Institute document MM21—*Genomic Copy Number Microarrays for Constitutional Genetic and Oncology Applications* discusses nucleic acid microarray technologies for diagnostic testing that a growing number of medical laboratories have adopted. The different types of microarrays and their uses in various types of laboratories have grown tremendously. MM21 specifically addresses 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 discussed.

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Contents

Foreword

MM21 was developed to provide more targeted guidance on molecular microarrays. It focuses on the appropriate performance, validation, verification, and interpretation of nucleic acid microarrays primarily used for cytogenetic applications to measure copy number (CN) imbalances.

When the first guideline on molecular microarrays was published (see CLSI document MM12[1]) nucleic acid microarrays were not a major part of medical laboratory test options. Today, many medical laboratories have adopted a variety of testing platforms and, as a result, the types of arrays and laboratories using arrays have increased tremendously. After reviewing CLSI document MM12,[1] the subject matter experts determined that the document, in its current form, is quite broad in scope. To allow for the most clinically relevant applications to be addressed in greater depth, CLSI document MM12[1] was split into three documents, with each document focusing on a particular field of clinical genetics:

- CN and absence of heterozygosity detection arrays (MM21)
- Expression arrays and methylation profiling (see CLSI document MM12[1])
- Nucleic acid microarrays for use in microbiology/immunology laboratories (eg, pathogen profiling) (see CLSI document MM22[2])

NOTE: Mandates are occasionally allowed in CLSI guidelines, in cases in which the document development committee feels strongly that a particular action is either required or prohibited, or when a guideline addresses provisions based on regulations. Throughout MM21, the use of the term "must" was evaluated by the document development committee and deemed appropriate because the uses are either 1) based on a requirement or 2) indicative of a necessary step to ensure patient safety or proper fulfillment of a procedure.

Key Words

Array-based comparative genomic hybridization, chromosomal microarrays, genomic copy number microarrays, whole genome testing

Genomic Copy Number Microarrays for Constitutional Genetic and Oncology Applications

Chapter 1: Introduction

This chapter includes:

- Document scope and applicable exclusions
- Background information pertinent to the document content
- Standard Precautions information
- Terms and definitions used in the document
- "Note on Terminology" that highlights particular use and/or variation in use of terms and/or definitions
- Abbreviations and acronyms used in the document

1.1 Scope

This guideline provides recommendations for the appropriate performance, validation or verification, and interpretation of nucleic acid microarrays used primarily for cytogenetic applications to measure copy number (CN) imbalances, traditional array-based comparative genomic hybridization (aCGH), and single nucleotide polymorphism (SNP) arrays for CN imbalances and absence of heterozygosity (AOH). Both constitutional (prenatal/postnatal) and oncology applications are addressed.

The intended users of this guideline are clinical genetics laboratorians who perform cytogenetics and molecular genetics testing.

This guideline:

- Is not intended for research laboratorians
- Is not intended to provide guidance to manufacturers
- Does not address methylation arrays, RNA expression microarrays, resequencing and genotyping arrays not intended for CN detection, microarrays for the diagnosis and monitoring of infectious diseases, or non-nucleic acid microarrays (eg, protein arrays)

1.2 Background

Chromosome abnormalities play a significant role in human genetic diseases. Abnormalities may involve chromosome number (eg, aneuploidy characterized by extra or missing chromosomes) and/or chromosome structure (eg, structural aberrations such as deletion, duplication, inversion, translocation, ring chromosome, isochromosome, and marker chromosome). Chromosomal structural defects are balanced (eg, reciprocal translocations, Robertsonian translocation) or, more often, they are unbalanced, associated with CN gains or losses in the DNA. Historically, conventional cytogenetic techniques (eg, G-

banded karyotyping) were the gold standard for the diagnosis of numerical and structural chromosomal aberrations. These techniques are restricted to relatively large genomic imbalances (eg, larger than at least three to five megabases [Mb]); therefore, they have only limited diagnostic yield. In addition, karyotyping is based on a subjective assessment of banding patterns and is prone to considerable interpersonal and interlaboratory bias. Introduction of FISH improved the resolution of cytogenetic testing, but was limited to only the genomic regions targeted by the FISH probes. It was not until introduction of chromosomal (or cytogenomic) microarrays (CMAs), also known as molecular karyotyping, aCGH, or genomic microarrays, that more precise and comprehensive whole genome analysis of copy number variants (CNVs) provided an enhanced diagnostic yield than is available with conventional cytogenetic diagnostics. Based on their design, microarrays may be able to detect CN imbalances down to single exon resolution in some genomic regions.

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. The Centers for Disease Control and Prevention addresses this topic in published guidelines that address the daily operations of diagnostic medicine in humans and animals while encouraging a culture of safety in the laboratory.[3] 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.[4]

1.4 Terminology

1.4.1 A Note on Terminology

CLSI, as a global leader in standardization, is firmly committed to achieving global harmonization wherever 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 the United States, Europe, and elsewhere; that these differences are reflected in CLSI, International Organization for Standardization (ISO), and European Committee for Standardization (CEN) documents; and that legally required use of terms, regional usage, and different consensus timelines are all important considerations in the harmonization process. In light of this, CLSI's consensus process focuses on harmonization of terms to facilitate the global application of standards and guidelines.

The understanding of a few terms has changed during the last decade as the concepts have developed. Particularly, trueness (measurement trueness) is defined as expressing the closeness of agreement between the average of an infinite number of replicate measurements and a reference value; and precision (measurement precision) is defined as closeness of agreement between indications or measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions. Consequently, accuracy (measurement accuracy) is the closeness of agreement between a measured value and a true quantity value of a measurand. Thus, this concept comprises both trueness and precision, and applies to a single result.

Please note that MM21 retains the use of "reportable range" over "measuring interval," as the internationally accepted definition for "measuring interval" does not apply to MM21. "Reportable range" is defined below.

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 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 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

MM21 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 on the following page.

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 GP27				GP27	GP27
		M29									
						MM01 MM05 MM09 MM12 MM13 MM17				MM05	
MM19 MM20	MM19 MM20	MM19 MM20	MM19 MM20	MM19	MM19	MM19 MM20 MM22	MM19 MM20	MM19 MM20	MM19 MM20	MM19 MM20	MM19 MM20
QMS01	QMS01	QMS01	QMS01	QMS01	QMS01	QMS01	QMS01 QMS02	QMS01	QMS01	QMS01	QMS01

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.

MM21 addresses the clinical laboratory path of workflow processes 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	
MM01 MM05	MM01	MM01	MM01 MM05	MM01 MM05	MM01 MM05	MM01 MM05	MM01 MM05	MM01 MM05
	MM09	MM09	MM09	MM09	MM09	MM09	MM09	MM09
		MM12	MM12	MM12	MM12	MM12	MM12	MM12
	MM13	MM13	MM13					MM13
	MM19	MM19	MM19	MM19	MM19	MM19		
MM20	MM20	MM20	MM20 MM22	MM20 MM22	MM20 MM22	MM20 MM22	MM20 MM22	MM20
QMS01	QMS01	QMS01	QMS01	QMS01	QMS01	QMS01	QMS01	QMS01

Related CLSI Reference Materials*

GP27 **Using Proficiency Testing to Improve the Clinical Laboratory. 2nd ed., 2007.** This guideline provides assistance to laboratories in using proficiency testing as a quality improvement tool.

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.

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.

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.

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.

MM20 **Quality Management for Molecular Genetic Testing. 1st ed., 2012.** This document provides guidance for implementing international quality management system standards in laboratories that perform human molecular genetic testing for inherited or acquired conditions.

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.

QMS01 **Quality Management System: A Model for Laboratory Services. 4th ed., 2011.** This document provides a model for medical laboratories that will assist with implementation and maintenance of an effective quality management system.

* 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)

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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