CLINICAL AND LABORATORY STANDARDS INSTITUTE®

February 2014

MM22-A

Microarrays for Diagnosis and Monitoring of Infectious Diseases; Approved Guideline

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.

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

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For further information on committee participation or to submit comments, contact CLSI.

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ISBN 1-56238-951-3 (Print)
ISBN 1-56238-952-1 (Electronic)
ISSN 1558-6502 (Print) MM22-A
ISSN 2162-2914 (Electronic) Vol. 34 No. 3

Microarrays for Diagnosis and Monitoring of Infectious Diseases; Approved Guideline

Volume 34 Number 3

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Abstract

Clinical and Laboratory Standards Institute document MM22-A—*Microarrays for Diagnosis and Monitoring of Infectious Diseases; Approved Guideline* discusses the wide variety of nucleic acid microarray technologies that a growing number of clinical laboratories have adopted for diagnostic testing. The different types of microarrays and their uses in various types of laboratories have grown tremendously. MM22 specifically addresses infectious disease detection, identification, and genotyping, as well as drug resistance determinants. This guideline describes types of microarray platforms and general considerations in microarray development. It also provides recommendations for validation and verification of microarray performance and QC and QA considerations, and discusses parameters for data analysis and interpretation.

Clinical and Laboratory Standards Institute (CLSI). *Microarrays for Diagnosis and Monitoring of Infectious Diseases; Approved Guideline*. CLSI document MM22-A (ISBN 1-56238-951-3 [Print]; ISBN 1-56238-952-1 [Electronic]). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2014.

CLINICAL AND LABORATORY STANDARDS INSTITUTE®

Suggested Citation

CLSI. *Microarrays for Diagnosis and Monitoring of Infectious Diseases; Approved Guideline*. CLSI document MM22-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.

Approved Guideline
February 2014

ISBN 1-56238-951-3 (Print)
ISBN 1-56238-952-1 (Electronic)
ISSN 1558-6502 (Print)
ISSN 2162-2914 (Electronic)

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Foreword

At the time of the publication of CLSI document MM12,[1] nucleic acid microarrays were not a major part of the diagnostic test menu in clinical laboratories. Today, a growing number of clinical laboratories have adopted a wide variety of microarray platforms for clinical testing and, as a result, the types and associated purposes of such arrays have grown tremendously.

The scope of this subject is now too large to be covered in CLSI document MM12,[1] and users of CLSI documents would find separate guidance documents on human genetics and oncology and infectious pathogens to be more useful than one large, comprehensive document. Toward that goal, the Consensus Committee on Molecular Methods recommended revision of CLSI document MM12[1] to focus on expression arrays and to be directed toward assay manufacturers.

Two new CLSI documents were proposed for development to provide guidance to clinical laboratories. MM22 focuses on the use of nucleic acid microarrays in clinical microbiology and immunology laboratories (pathogen profiling, etc.) and specifically addresses detection, identification, and genotyping of infectious pathogens, as well as antimicrobial drug resistance determinants. The second document, in development concurrently with MM22, focuses on the use of microarrays for human genetics and oncology.

Key Words

Clinical diagnostics, infectious diseases, microarray analysis, nucleic acid testing, pathogen detection

Microarrays for Diagnosis and Monitoring of Infectious Diseases; Approved Guideline

1 Scope

Microarrays can be distinguished from each other based upon characteristics such as the nature of the probe, the solid surface support used, and the specific method used for probe addressing and/or target detection. MM22 focuses on the use of nucleic acid microarrays in microbiology and immunology laboratories and specifies the requirements and recommendations for the use of microarrays for diagnosis and monitoring of infectious diseases. It also covers infectious disease pathogen detection, identification, and genotyping (strain characterization) as well as virulence and resistance genetic markers.

The intended users of this guideline are clinical laboratories performing qualitative, multiplexed nucleic acid–based testing, which includes, but is not limited to, bacteriology, mycobacteriology, mycology, parasitology, and virology. This guideline is not intended for use by research laboratories, but the research community may use this guideline in order to translate their findings more easily. MM22 is not intended for *in vitro* diagnostic (IVD) device manufacturers or to provide manufacturing guidelines, and does not cover protein microarrays nor address microarray applications for unknown pathogen discovery, host or microbial gene expression profiling, or host genomic polymorphism determination related to microbial infections.

2 Introduction

The use of molecular detection techniques continues to increase in clinical microbiology and immunology laboratories. The implementation of *in vitro* nucleic acid amplification techniques, led by PCR, in diagnostic laboratories has transformed viral detection and specific microbial pathogen detection. The continued advancement of molecular infectious disease diagnostics depends on the ability of multiplexing technologies to easily and reliably detect multiple pathogens in a single clinical specimen. One approach to multiplex detection, characterization, and monitoring of infectious diseases is microarray analysis.

Simply defined, a microarray is a collection of microscopic features (most commonly DNA) that can be probed with target molecules to produce either quantitative (gene expression) or qualitative (detection and identification) data. Largely due to advances in fabrication, robotics, and bioinformatics, microarray technology has continued to improve in terms of efficiency, reproducibility, sensitivity, and specificity. In addition, microarray platforms have expanded to include three-dimensional or suspension arrays. These improvements have allowed for the transition of microarrays from strictly research settings to clinical diagnostic applications. This has led to optimization of the diagnostic potential of microarrays and to the development of commercially available qualitative detection platforms. A series of microarray-based diagnostic devices are commercially available, with some having achieved regulatory agency clearance. Some platforms can achieve multiplex analysis through parallel detection in multiple reaction chambers as well. Thus, a new era in molecular diagnostics has arrived where the use of microarray technology in the clinical microbiology laboratory is a reality.

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 blood-borne pathogens. The Centers for Disease Control and Prevention address this topic in published guidelines that address the daily operations of diagnostic medicine in human and animal medicine while

encouraging a culture of safety in the laboratory.[2] 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.[3]

4 Terminology

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 for development and revision of standards and guidelines focuses on harmonization of terms to facilitate the global application of standards and guidelines.

In keeping with CLSI's commitment to align terminology with established ISO standards, the following terms are used in MM22: *measurand* (quantity intended to be measured) is used in combination with the term *analyte* (component represented in the name of a measurable quantity) when its use relates to a biological fluid/matrix. *Trueness* is used in this document when referring to the closeness of agreement between the average of an infinite number of replicate measured quantity values; the measurement of trueness is usually expressed in terms of *bias*. *Measurement procedure* has replaced the term *analytical method* for a detailed description of a measurement according to one or more measurement principles and to a given measurement method that is based on a measurement model and includes any calculation used to obtain a measurement result. The term *diagnostic sensitivity* is combined with the term *clinical sensitivity* because in some jurisdictions, the term "clinical" often refers to clinical studies of drugs under stringent conditions. For this document, *validation* is primarily a manufacturer's responsibility to ensure that design goals are met and performance claims are stated for a commercially developed assay or device and a laboratory's responsibility for a laboratory-developed test (LDT). *Verification* is an end-user (clinical laboratory) responsibility to confirm that manufacturer's claims are met on the specific device in use by the laboratory and that medical needs are met. Validation is the establishment of analytical and clinical performance characteristics whereas verification is the confirmation of previously established performance characteristics.

In order to align the usage of terminology in this document with that of ISO and CLSI document QMS01,[4] *preexamination, examination,* and *postexamination* have replaced *preanalytical, analytical,* and *postanalytical,* respectively, when referring to the testing phases within the laboratory path of workflow.

4.2 Definitions

accreditation – procedure by which an authoritative body gives formal recognition that an organization or person is competent to carry out specific tasks (modified from ISO/IEC 17000).[5]

accuracy (measurement) – closeness of agreement between a measured quantity value and a true quantity value of a measurand (JCGM 200:2012)[6]; **NOTE:** The concept "measurement accuracy" is not a quantity and is not given a numerical quantity value. A measurement is said to be more accurate when it offers a smaller measurement error (JCGM 200:2012).[6]

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

MM22-A 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 82.

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 EP05 EP06 EP07 EP09 EP12 EP15 EP17 EP24 EP28 GP27 GP29 MM03 MM05 MM06 MM09 MM10 MM12 MM13 MM17				GP27 GP29 MM05	GP27
		M29									
MM19 QMS01	MM19 QMS01	MM19 QMS01	MM19 QMS01 QMS03	MM19 QMS01	MM19 QMS01 QMS13	MM19 QMS01	MM19 QMS01	MM19 QMS01	MM19 QMS01	MM19 QMS01	MM19 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.

MM22-A 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	
	MM03	MM03	MM03	MM03	MM03		MM03	
MM05			MM05	MM05	MM05	MM05	MM05	MM05
MM06	MM06	MM06	MM06	MM06	MM06	MM06	MM06	
	MM09	MM09	MM09	MM09	MM09	MM09	MM09	MM09
				MM10	MM10	MM10	MM10	
		MM12	MM12	MM12	MM12	MM12	MM12	MM12
	MM13	MM13	MM13					MM13
	MM19	MM19	MM19	MM19	MM19	MM19		
QMS01	QMS01	QMS01	QMS01	QMS01	QMS01	QMS01	QMS01	QMS01

Related CLSI Reference Materials*

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-A3 **Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline—Third Edition (2013).** This document addresses the design of measurement procedure comparison experiments using patient samples and subsequent data analysis techniques used to determine the bias between two *in vitro* diagnostic measurement procedures.

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-A2 **Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline—Second Edition (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.

EP24-A2 **Assessment of the Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristic Curves; Approved Guideline—Second Edition (2011).** This document provides a protocol for evaluating the accuracy of a test to discriminate between two subclasses of subjects when there is some clinically relevant reason to separate them. In addition to the use of receiver operating characteristic curves and the comparison of two curves, the document emphasizes the importance of defining the question, selecting the sample group, and determining the "true" clinical state.

EP28-A3c **Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline—Third Edition (2010).** This document contains guidelines for determining reference values and reference intervals for quantitative clinical laboratory tests. A CLSI-IFCC joint project.

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.

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.

* 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)

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.

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.

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.

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.

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.

MM19-A **Establishing Molecular Testing in Clinical Laboratory Environments; Approved Guideline (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.

Related CLSI Reference Materials (Continued)

QMS01-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.

QMS03-A3 **Training and Competence Assessment; Approved Guideline—Third Edition (2009).** This document provides background information and recommended processes for the development of training and competence assessment programs that meet quality and regulatory objectives.

QMS13-A **Quality Management System: Equipment; Approved Guideline (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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PRINT ISBN 1-56238-951-3

ELECTRONIC ISBN 1-56238-952-1