CLINICAL AND LABORATORY STANDARDS INSTITUTE®

2nd Edition

CLSI MM22™

Microarrays for Diagnosis and Monitoring of Infectious Diseases

This guideline provides recommendations 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.

CLSI MM22-Ed2
January 2024
Replaces CLSI MM22-A

Microarrays for Diagnosis and Monitoring of Infectious Diseases

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Abstract

Clinical and Laboratory Standards Institute guideline MM22—*Microarrays for Diagnosis and Monitoring of Infectious Diseases* discusses the wide variety of nucleic acid microarray technologies that a growing number of medical laboratories have adopted for diagnostic testing. The different types of microarrays and their uses in various types of laboratories have grown tremendously. MM22 specifically discusses infectious diseases 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*. 2nd ed. CLSI guideline MM22 (ISBN 978-1-68440-206-9 [Print]; ISBN 978-1-68440-207-6 [Electronic]). Clinical and Laboratory Standards Institute, USA, 2024.

CLINICAL AND LABORATORY STANDARDS INSTITUTE®

Suggested Citation

CLSI. *Microarrays for Diagnosis and Monitoring of Infectious Diseases.* 2nd edition. CLSI guideline MM22. Clinical and Laboratory Standards Institute; 2024.

Previous Edition:
February 2014

CLSI MM22-Ed2

ISBN 978-1-68440-206-9 (Print)

ISBN 978-1-68440-207-6 (Electronic)

ISSN 1558-6502 (Print)

ISSN 2162-2914 (Electronic)

Volume 44, Number 3

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Foreword

Today, a growing number of medical 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. Because the scope of this subject is too large to be covered in a single CLSI document, MM22 was developed to focus on the use of nucleic acid microarrays in medical microbiology and immunology laboratories (eg, pathogen profiling) and specifically discusses detection, identification, and genotyping of pathogens, as well as antimicrobial drug resistance determinants.

Overview of Changes

This guideline replaces MM22-A, published in 2014. Several changes were made in this edition, including:

- Updating platform overview to include newer technologies now in use for microarray tests
- Adding a new subchapter to describe an individualized quality control plan, which permits the laboratory to customize its QC plan
- Reorganizing content to correspond to the path of workflow
- Adding a process flow chart

NOTE: The content of this guideline is supported by the CLSI consensus process and does not necessarily reflect the views of any single individual or organization.

KEY WORDS

clinical diagnostics
infectious diseases
microarray analysis
nucleic acid testing
pathogen detection

Chapter 1

Introduction

Microarrays for Diagnosis and Monitoring of Infectious Diseases

1 Introduction

1.1 Scope

This guideline specifies the requirements and/or recommendations for the use of microarrays for diagnosis and monitoring of microbial infections. It covers infectious diseases pathogen detection and identification, genotyping (strain characterization), and virulence and resistance genetic markers. The intended users of this guideline are clinical and molecular microbiology laboratories, including bacteriology, mycobacteriology, mycology, parasitology, and virology. This guideline may also serve as a reference for government agencies and industry.

MM22 is not intended to provide manufacturing guidelines. Protein microarrays and microarray applications for unknown pathogen discovery, host gene expression profiling, or host genomic polymorphism determination related to microbial infections are not included. Sequencing as a detection and identification method is not covered by this guideline (see CLSI documents MM09[1] and MM18[2]).

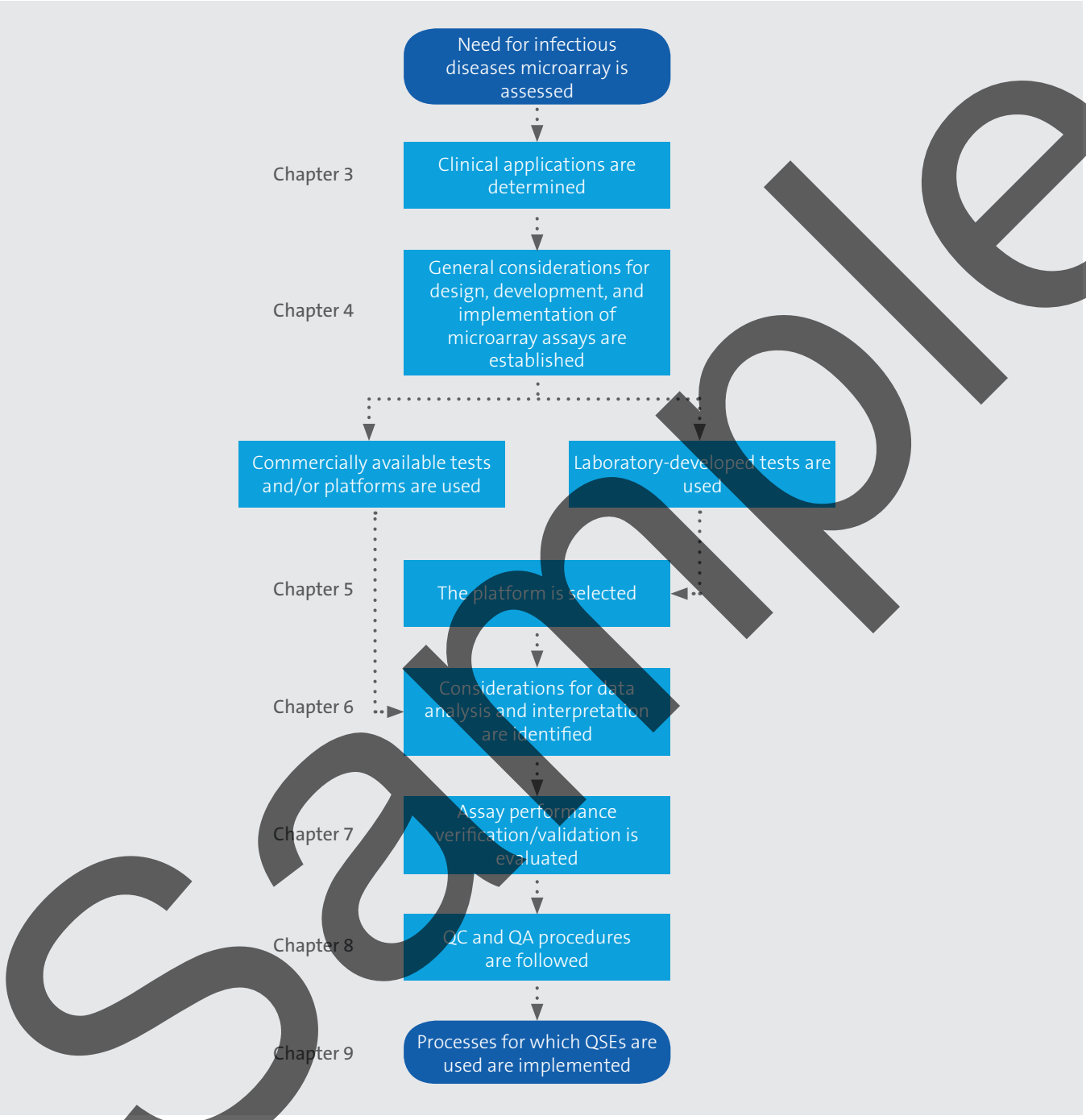
1.2 Background

Molecular detection techniques have been used increasingly by medical microbiology and immunology laboratories. *In vitro* nucleic acid amplification techniques (eg, PCR) have transformed microbial pathogen detection. The continued advancement of molecular infectious diseases diagnostics depends on multiplexing technologies to easily and reliably detect multiple pathogens in a single clinical specimen. Microarray analysis can be used for multiplex detection, characterization, and monitoring of infectious diseases. 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. Advancements in fabrication, robotics, and bioinformatics have improved the efficiency, reproducibility, sensitivity, and specificity of microarray technology. Microarray platforms have expanded to include three-dimensional or suspension arrays. These improvements have transitioned microarrays from strictly research to clinical diagnostic applications, which has led to the optimization of the diagnostic potential of microarrays and to the development of commercially available qualitative and semiquantitative detection platforms. A series of microarray-based diagnostic devices are commercially available, and some have received regulatory agency clearance.

MM22 discusses the variety of nucleic acid microarray technologies that can be used for diagnostic testing. This guideline specifically discusses the clinical application of microarrays, including infectious diseases detection, identification, and genotyping, as well as detection of drug resistance determinants. It also describes types of microarray platforms and general considerations in microarray development. MM22 provides recommendations for validation and verification of microarray performance and quality considerations and discusses parameters for data analysis and interpretation. This guideline also reviews the specifications for laboratories to establish an individualized quality control plan (IQCP).[3]

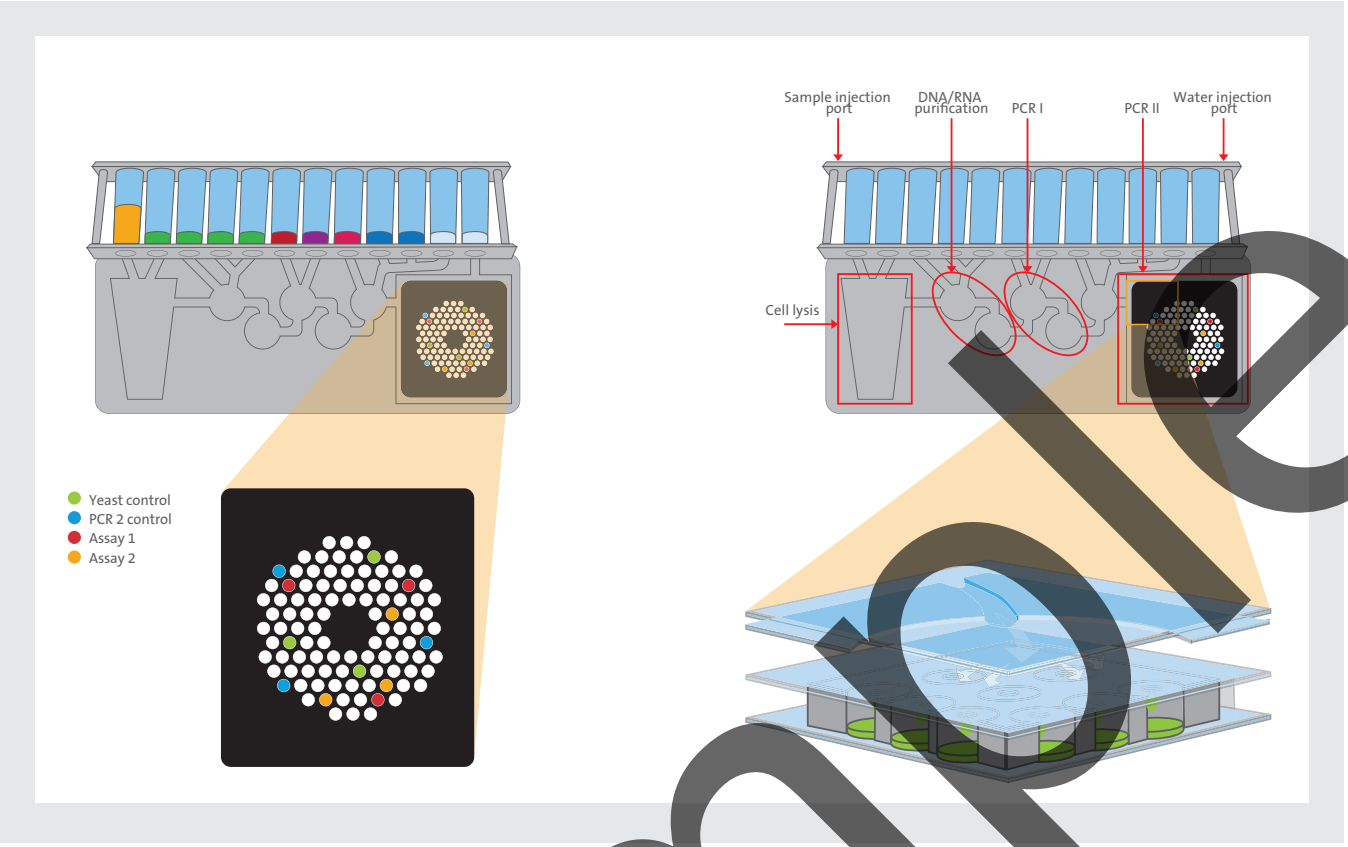
2 Process Flow Chart

Figure 1 shows the process for microarrays of infectious diseases.



Abbreviations: QA, quality assurance; QC, quality control; QSE, quality system essential.
[a] Three basic symbols are used in this process flow chart: oval (signifies the beginning or end of a process), arrow (connects process activities), box (designates process activities).

Figure 1: Process Flow Chart[a]



Abbreviations: DNA, deoxyribonucleic acid; dsDNA, double-stranded deoxyribonucleic acid; PCR, polymerase chain reaction; RNA, ribonucleic acid.

Figure 13. Example of a Nested PCR in a Small-Volume Array.[153] **Top left:** Disposable reagent pouch used for nucleic acid purification and nested multiplex PCR. Colored liquid in the top of the pouch indicates the location of the lyophilized chemicals and enzymes used to carry out the chemistry. **Top right:** Schematic of the pouch showing the different stages of the chemical and enzymatic steps. PCR II indicates the second stage inner PCR, which occurs in an array of 102 1-µL wells. **Bottom left:** Schematic of the array. Primers for different PCR assays are spotted in triplicate across the array. Two control assays (a process control [yeast] and a control for the inner PCR [PCR 2]) and two organism-specific assays are indicated. **Bottom right:** To start the nested PCR reactions in the array, diluted amplicon from the first-stage PCR is mixed with additional DNA polymerase, nucleotides, and a fluorescent dsDNA-binding dye. This mixture is flooded over the top of the array and into the wells through holes in the top of each well. After the wells fill, a clear plastic bladder in the instrument inflates to seal the wells shut. Unique sets of primers in each well enable amplification of specific targets if they are present in the first-stage amplification. (From Poritz MA, Blaschke AJ, Byington CL, et al. FilmArray, an automated nested multiplex PCR system for multi-pathogen detection: development and application to respiratory tract infection. *PLoS One*. 2011;6(10):e26047. doi:10.1371/journal.pone.0026047)

5.6.4 Nanoliter Volume Polymerase Chain Reaction Arrays

Thousands of PCR reactions can be performed in a microarray format that uses "through-holes" in which the PCR primers bind to the sides of the holes in the array.[155] The volume of each reaction is 33 nL, and both probe and dsDNA-binding dye formats are available. Custom arrays with user-chosen formats and primers can be ordered from the manufacturer. The array contains 3072 "through-holes" configured as 48 subarrays of 64 holes that can be injected with 12 to 48 different samples. The amplification reaction occurs in a specialized real-time PCR instrument, but the output is similar to that of a standard real-time PCR instrument.

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PRINT ISBN 978-1-68440-206-9

ELECTRONIC ISBN 978-1-68440-207-6

CLSI MM22-Ed2