CLINICAL AND LABORATORY STANDARDS INSTITUTE®

1st Edition

M58

Methods for the Identification of Cultured Microorganisms Using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry

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This guideline includes performance, reporting, and quality assurance recommendations for the identification of cultured microorganisms by medical laboratory professionals using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Recommendations for end-user verification and workflow integration are also included.

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

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Methods for the Identification of Cultured Microorganisms Using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry

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Abstract

Clinical and Laboratory Standards Institute guideline M58—*Methods for the Identification of Cultured Microorganisms Using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry* provides guidance to the end user for adopting matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) in the medical laboratory setting. Included are procedures and guidelines for preparing and analyzing cultured patient isolates, interpreting and reporting results, and troubleshooting. Best practices are described for ensuring quality and safety, and guidelines are provided for the initial introduction of MALDI-TOF MS into an existing laboratory, including method verification, training development and competence assessment programs, and operational considerations.

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Contents

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Foreword

The application of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) to cultured microorganism identification represents a paradigm shift in diagnostic microbiology practices. Compared with conventional phenotypic and biochemical methods, MALDI-TOF MS is frequently faster and more accurate. It has expanded the capabilities of many microbiology laboratories by providing for identification of organisms within certain groups (eg, anaerobes, coagulase-negative staphylococci) that could not otherwise be identified reliably or practically using conventional methods. For larger diagnostic laboratories, the technology reduces the need for referral laboratory testing for identifying agents such as mycobacteria and fungi.

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

Mass spectrometry, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, microbial identification

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Chapter 1: Introduction

This chapter includes:

- Guideline's scope and applicable exclusions
- Background information pertinent to the guideline's 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 guideline
- Abbreviations and acronyms used in the guideline

1.1 Scope

This guideline establishes best practices for applying and integrating matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) technology into the diagnostic microbiology laboratory. It presents preexamination considerations, such as selecting appropriate isolates for analysis and isolate preparation from solid or liquid media, and postexamination activities, such as results interpretation, indications for supplemental testing, and results reporting. Practical recommendations are provided for integrating MALDI-TOF MS into an existing traditional laboratory operation; for establishing QC procedures, safety procedures, and a competence assessment program; and for designing a method verification protocol.

The intended users of this guideline are microbiologists in private, academic, and commercial diagnostic laboratory settings, including public health laboratories and veterinary diagnostic laboratories.

This guideline:

- Is not intended for use in the research setting
- Is not intended to provide guidance pertaining to identifying microorganisms directly from patient specimens (before culture)
- Does not cover antimicrobial susceptibility testing (AST) using MALDI-TOF MS
  - Although several studies have demonstrated that MALDI-TOF MS can be adapted for this purpose, its utility in a diagnostic laboratory setting remains undefined, and the methods are still in development.

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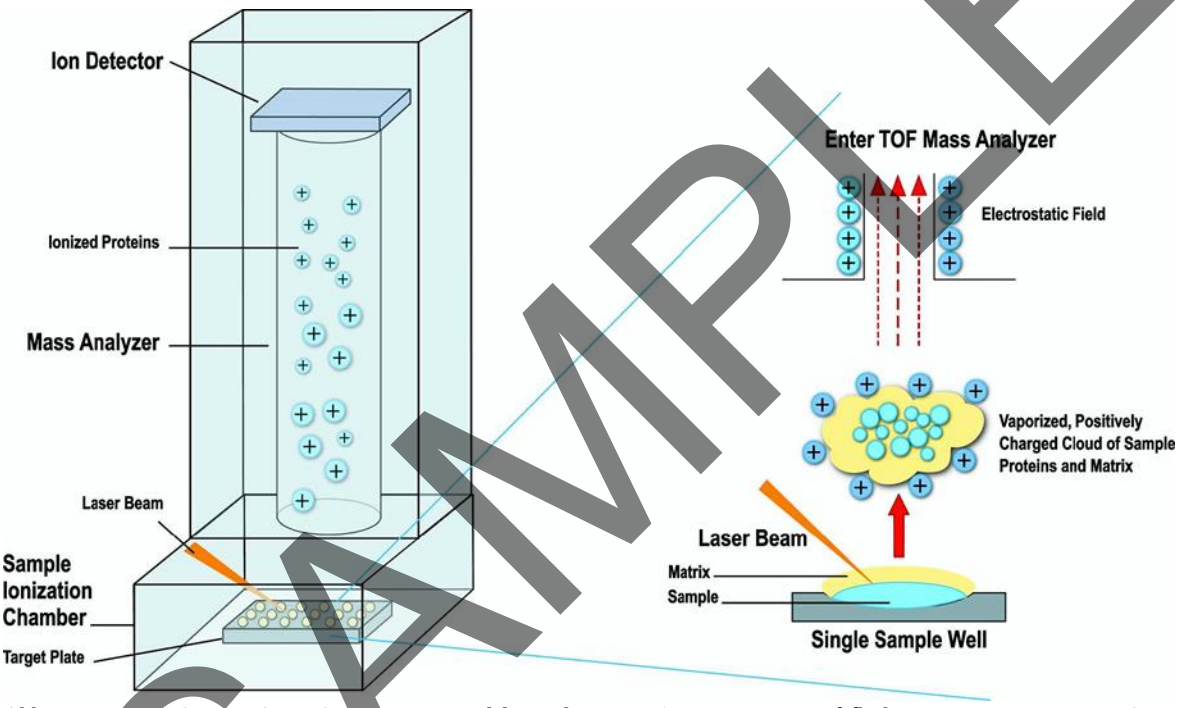
1.2 Background

1.2.1 Principles of MALDI-TOF MS

MALDI-TOF MS involves:

- Using a laser to vaporize and ionize molecules
- Measuring the molecules' mass-to-charge ratios (*m/z*)
- Generating a mass spectrum using time-of-flight mass spectrometry

When adapted for microbial identification, whole microorganisms are analyzed by this method, with or without a preceding extraction step (see Figure 1). A characteristic mass spectrum is produced from an unknown microorganism, which is then compared to a database of mass spectra generated from known microorganisms, and the unknown strain is identified based on the closest match.



Abbreviations: MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; TOF, time-of-flight.

Figure 1. Technical Description for MALDI-TOF MS for Microbial Identification. (By permission of Mayo Foundation for Medical Education and Research. All rights reserved.) The sample is mixed with a matrix on a conductive target slide or plate. After the matrix material crystallizes, the target slide or plate is introduced into the mass spectrometer's ionization chamber and brief laser pulses are fired at the sample. The desorbed and ionized molecules are accelerated through an electrostatic field and into a vacuum tube, and then make contact with an ion detector, with smaller ions reaching the detector faster than larger ions. By measuring the ions' TOF, their *m/z* values are calculated and the aggregate data are expressed as a mass spectrum composed of *m/z* peaks with varying intensities in proportion to their abundance in the sample. The mass spectrum serves as a microbial signature that is compared with a database of spectra from well-characterized microorganisms for identification.

The mass peaks of interest for microbial identification primarily fall in the range of 2 to 20 kDa (2000 to 20 000 Da). Many of these mass peaks represent small- and large-subunit ribosomal proteins, nucleic acid binding proteins, and heat shock proteins, which are typically well conserved.[1] Additional informative peaks likely represent other abundant housekeeping proteins. A key feature of MALDI-TOF MS is its ability to measure these large proteins by keeping them intact during ionization, using a "soft laser

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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

M58 covers 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.

Organization	Customer Focus	Facilities and Safety	Personnel	Purchasing and Inventory	Equipment	Process Management	Documents and Records	Information Management	Nonconforming Event Management	Assessments	Continual Improvement
QMS01	QMS01	M29 QMS01	QMS01 QMS03	QMS01	QMS01	X EP23 M50 M52 MM18 QMS01 QMS24	QMS01	QMS01	QMS01 QMS11	QMS01 QMS24	QMS01 QMS24

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.

M58 covers the medical 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.

Preexamination				**Examination**			**Postexamination**	
Examination ordering	Sample collection	Sample transport	Sample receipt and processing	Examination	Results review and follow-up	Interpretation	Results reporting and archiving	Sample management
QMS01	QMS01	QMS01	X QMS01	X EP23 QMS01	X EP23 M50 QMS01	X EP23 M50 QMS01	X M50 QMS01	QMS01

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Related CLSI Reference Materials*

EP23™ **Laboratory Quality Control Based on Risk Management. 1st ed., 2011.** This document provides guidance based on risk management for laboratories to develop quality control plans tailored to the particular combination of measuring system, laboratory setting, and clinical application of the test.

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.

M50 **Quality Control for Commercial Microbial Identification Systems. 1st ed., 2008.** This document provides guidance for quality control of commercial systems for microbial identification from culture, including information that pertains to manufacturers, distributors, and laboratory users. The intent is to ensure optimal performance of a microbial identification system in an efficient (streamlined) manner.

M52 **Verification of Commercial Microbial Identification and Antimicrobial Susceptibility Testing Systems. 1st ed., 2015.** This guideline includes recommendations for verification of commercial US Food and Drug Administration–cleared microbial identification and antimicrobial susceptibility testing systems by clinical laboratory professionals to fulfill regulatory or quality assurance requirements for the use of these systems for diagnostic testing.

MM18 **Interpretive Criteria for Identification of Bacteria and Fungi by DNA Target Sequencing. 1st ed., 2008.** Sequencing DNA targets of cultured isolates provides a quantitative metric within which to perceive microbial diversity, and can serve as the basis to identify microorganisms. This document is an effort to catalyze the entry of molecular microbiology into clinical usage by establishing interpretive criteria for microorganism identification.

QMS01 **Quality Management Systems: 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.

QMS03 **Training and Competence Assessment. 4th ed., 2016.** This guideline provides a structured approach for developing effective laboratory personnel training and competence assessment programs.

QMS11 **Nonconforming Event Management. 2nd ed., 2015.** Grounded in the principles of quality management, risk management, and patient safety, this guideline provides an outline and content for developing a program to manage a laboratory's nonconforming events.

QMS24 **Using Proficiency Testing and Alternative Assessment to Improve Medical Laboratory Quality. 3rd ed., 2016.** This guideline describes an approach for a complete proficiency testing (PT) process and provides assistance to laboratories in using PT as a quality improvement tool.

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