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M58

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

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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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

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

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.

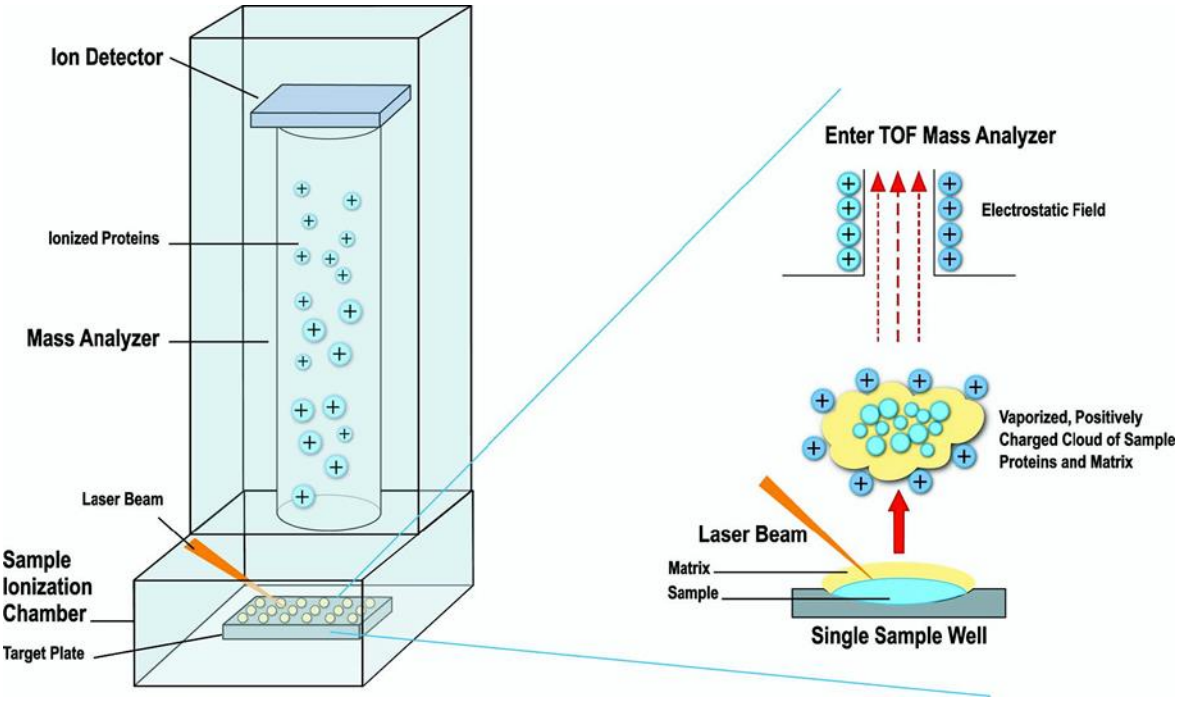
1.2 Background

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

