Matrix-Assisted Laser Desorption Time of Flight Mass Spectrometry

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KEYWORDS

- Mass spectrometry
- Matrix-assisted laser desorption time of flight mass spectrometry • MALDI-TOF
- Bacterial identification • Clinical microbiology

KEY POINTS

- Matrix-assisted laser desorption time of flight mass spectrometry (MALDI-TOF MS) is suitable for routine microbial identification in the clinical laboratory, and has quickly become an integral diagnostic tool.
- Rapid results provide microbial identification in support of antimicrobial stewardship programs, infection prevention, epidemiologic surveillance, sepsis campaigns, and other health care initiatives.
- Mechanics and processes underlying MALDI-TOF MS for microbial identification can be simplified for general laboratory understanding.

INTRODUCTION

Rapid and accurate identification of bacteria from clinical specimens is a critical function of the clinical microbiology laboratory. Historically, microbial identification depended on culture, biochemical testing, antigenic assays, or target-specific molecular for definitive identification. Matrix-assisted laser desorption time of flight mass spectrometry (MALDI-TOF MS), due to its high analytical sensitivity, is useful for microbial identification in the clinical laboratory. It exhibits high analytical sensitivity and has quickly become an integral diagnostic tool that drastically reduces the time required for definitive identification of bacteria, fungi, and mycobacteria from clinical specimens. Sample preparation is simple and reproducible, and results can be

Disclosure: The authors have nothing to disclose.

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Clin Lab Med 38 (2018) 471–486
https://doi.org/10.1016/j.cll.2018.05.008
labmed.theclinics.com

interfaced directly to the laboratory information system to provide rapid identification in support of antimicrobial stewardship programs, infection prevention, sepsis campaigns, and other health care initiatives. This article focuses on the technology and processes underlying MALDI-TOF MS for microbial identification and the associated downstream outcomes and improvements in health care related to its use.

HOW DOES MATRIX-ASSISTED LASER DESORPTION TIME OF FLIGHT MASS SPECTROMETRY WORK?

Background

MS has been used in the clinical laboratory for decades, but before the emergence of MALDI-TOF MS for microbial identification, its use had been largely relegated to high-complexity chemical analysis. The earliest attempts to use MS for the identification of bacteria occurred in the 1980s.[1] The MALDI ionization method was first introduced in 1987,[2] and was subsequently reported in similar experiments in 1988.[3] During the past decade, MALDI-TOF MS has become a rapid and highly reliable analytical tool for characterization of diverse microbes, such as bacteria, fungi, and viruses.[4–6] A variety of clinical and research-based laboratory methods and commercial instruments are available and are in widespread use across the globe; we describe the most common methods that are suitable for use in the routine clinical microbiology laboratory.

Sample Ionization in Matrix-Assisted Laser Desorption Time of Flight Mass Spectrometry

Microorganism identification by MALDI-TOF MS is dependent on ionization of proteins within the clinical specimen. MALDI is a type of "soft ionization" mechanism that uses transferred energy from a laser to generate protein ions for analysis. This mechanism is in direct contrast to so-called "hard ionization" techniques that transfer high levels of energy and can lead to fragmentation of the analyte. During MALDI, a chemically saturated solution of a low-mass organic compound (matrix) is added to the clinical sample (analyte). The analyte can be either intact bacterial or fungal cells, as in the case of intact cell MALDI-TOF MS (**Fig. 1**), or can be a protein extract from a designated clinical isolate. In some cases, direct blood culture broth, urine, cerebrospinal fluid, or protein extract are analyzed.[6]

Irrespective of the source, clinical samples are placed onto spots (ie, spotted) on a metal target plate and overlaid with matrix. On drying, the clinical sample and the matrix co-crystallize and form a solid deposit of sample embedded into the matrix. The plate holding the crystallized sample is then loaded into the MALDI-TOF instrument. The sample is exposed to a laser, present within a vacuum, which results in ionization of proteins in the sample (see **Fig. 1**).

The matrix is crucial for the successful ionization of the sample. The matrix acts both as a supplier of protons for ionization of the clinical sample, and as a scaffold, on which ionization can occur. Soft ionization of proteins is crucial when using MALDI-TOF MS for microbial identification, as it allows for the analysis of large biomolecules (ie, proteins) with sizes measuring up to 100 kDa without fragmentation.[2,3,7,8] The laser beam focuses on a small zone (usually 0.05–0.2 mm in diameter) on the metal target plate within the area where the sample/matrix mixture has been spotted and dried (see **Fig. 1**). An ultraviolet N_2 laser beam with a wavelength of 337 nm is used in most commercial MALDI-TOF MS instruments, including the Bruker microflex (Bruker Daltonics, Billerica, MA) and the Vitek MS (bioMérieux, Marcy-l'Étoile, France) (**Fig. 2**).

MALDI ionization is often found to be more sensitive than other ionization techniques. Irradiation occurs in short bursts of the laser to avoid damage or degradation