Briefing Note: MALDI-TOF MS Test Procedure

Last Updated: 22nd January 2024

Background

Matrix assisted laser desorption ionization – time of flight mass spectrometry (MALDI-TOF MS) is a rapid and reliable tool used to analyse a range of specimens brought to the clinical laboratory. For example, it allows for the analysis of biomolecules (e.g. DNA, ribosomal proteins, peptides, sugars) and large organic molecules (e.g. polymers, dendrimers), including the protein composition of a microbial cell. It has therefore emerged as a new technology for species identification.

Extraction and Analysis

There are several extraction methods used for the pre-treatment of clinical specimens / isolates before they are placed on a plate ready for the MALDI-TOF MS machine, however, two main methods are commonly used:

- Pure colonies on appropriate agar medium or plate. It is recommended that freshly grown colonies (grown overnight) should be used, or in the case of slow growing bacteria, grown for several days. A pure bacterial colony (typically single) is picked from a culture plate using a wooden or plastic stick, pipette tip, or loop and then placed onto a spot on a MALDI-TOF MS target plate. This is known as a 'direct' smear application. Most bacteria will be identified readily with a direct smear application (without any requirement for formic acid overlay). The spot on the target plate is then overlaid with 1–2µL of 'matrix'. The matrix involves mixing or coating with a solution of an energy-absorbent material which entraps and co-crystallizes the sample when dried.

- Clinical specimens (e.g. direct blood culture material, urine, cerebrospinal fluid, or protein extract). There are commercial extraction kits for use directly on positive blood culture bottles. All reagents and consumables required for processing blood culture fluid are supplied in the kit.

The molecules of interest are introduced to the ionisation source of the mass spectrometer (ionisation is triggered by a laser beam), where they are ionised to acquire positive or negative charges. The ions then travel through the mass analyser and arrive at different parts of the detector. This depends on their mass / charge (m / z) ratio. A 'mass spectrum' is generated and automatically compared against a database of mass spectra by the software, resulting in identification of the organism (e.g. Figure 1).

It should be noted that antimicrobial susceptibility is not directly determined by this method as the species-specific proteins - to be identified using the MALDI-TOF MS spectra - are largely unaltered by their antimicrobial susceptibility status. An example is the direct discrimination between strains, such as MRSA vs. MSSA.

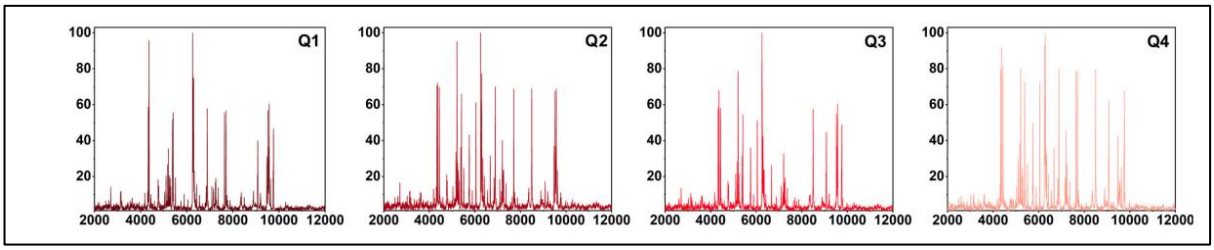

Figure 1: Example of MALDI-TOF MS Mass Spectra of Various Microbes

Advantages

- Compared with other identification methods the results of the analysis are available within minutes, to a few hours, rather than days.
- Minimal consumable costs make this method well suited for routine and high throughput use.
- Requires only a single colony overlaid with a 'matrix' to perform the test in most instances (but not for yeasts or mucoid colonies).
- Exposure risk is very low because samples are often inactivated by extraction before use.
- Useful in identification of bacteria that are difficult to culture such as Mycobacteria, *Bartonella* species, *Legionella* species, etc.

Limitations

Identification using MALDI-TOF MS relies on comparison of the spectra of the specimen / isolate with those of reference databases. For organisms commonly encountered in the laboratory MALDI-TOF MS can accurately identify most closely related species, however, there are some exceptions. For inherently similar organisms it is common to report to the group, complex, or genus level. In cases where differentiation to the species level is clinically necessary, supplemental testing should be performed.

- *E. coli* vs. *Shigella*
- Viridans streptococci vs. pneumococci
- Members of the *Candida albicans* complex
- *Neisseria cinerea* and *Neisseria polysaccharea* vs. N. meningitidis
- Mycobacteria
- *Burkholderia* species
- *Acinetobacter* species
- Corynebacteria streptococci
- β-haemolytic streptococci

Recommended Reading

Rychert J. Benefits and limitations of MALDI-TOF mass spectrometry for the identification of microorganisms. Journal of Infectiology and Epidemiology. 2019 Jul 2;2(4). Available here: Benefits and Limitations of MALDI-TOF Mass Spectrometry for the Identification of Microorganisms (infectiologyjournal.com) – A good overview of what MALDI-TOF MS can do using microbes.

Bruker Daltonics (2017). MALDI TOF Process. https://www.youtube.com/watch?v=0jeFpXHZ8W0 . – A video from one of the manufacturers of the MALDI-TOF machines illustrating how it works.

Clark AE, Kaleta EJ, Arora A, Wolk DM. Matrix-assisted laser desorption ionization–time of flight mass spectrometry: a fundamental shift in the routine practice of clinical microbiology. Clinical microbiology reviews. 2013 Jul;26(3):547-603. Available here: Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry: a Fundamental Shift in the Routine Practice of Clinical Microbiology - PMC (nih.gov) – Very good illustrations to show how cultures / clinical specimens are prepared prior to MALDI-TOF MS process (pages 551, 555, and 586).