

# Applications of MALDI-TOF Mass Spectrometry for the Routine Identification of Microorganisms

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## Abstract

The emerging of MALDI-TOF Mass Spectrometry as a diagnostic tool for the identification of micro-organism has transformed most microbiology laboratories, where phenotypic and cumbersome conventional techniques are still applied currently. Its implementation has allowed high-throughput, rapid, inexpensive and accurate identification of the most clinically relevant bacteria, yeast and mold species [1-5].

The last decade has witnessed how MALDI-TOF MS was increasingly applied for a higher number of microorganisms, available databases grew and sample preparations procedures improved allowing not just the identification of the organism, but also their antibiotic or antifungal susceptibility pattern [6-10] until, recently, several studies have demonstrated MALDI-TOF usefulness for epidemiological studies [11,12].

## Identification of Bacteria

Identification of bacterial isolates using MALDI-TOF MS has become quicker and more accurate as expertise was accumulated, sample proceedings improved and commercial databases updated with more reference protein spectra, allowing a better discrimination from closely-related species. Direct identification of commonly encountered bacteria could be performed from single colonies grown on different agar plates with highly accurate results [1,13]. Besides, the implementation of the on-plate protein extraction step allowed the identification of other groups of microorganisms that pose difficulties for a reliable identification, such as uncommon species or those with special growth requirements [3].

The little amount of bacterial biomass required for MALDI-TOF MS identification is suitable for the rapid identification of those micro-organisms displaying low-growth rate such as the anaerobic bacteria. In this field, great improvements have developed in the last few years. A multicenter study has been published recently showing a high correlation between MALDI-TOF MS and DNA sequencing identification [5]. Besides, the availability of a high number of unusual anaerobic isolates by the researchers within the multicenter project also allowed the improvement of the anaerobic database provided by Bruker Daltonics (Bremen, Germany). Other authors have also reported rapid, accurate and inexpensive identification of a wide variety of anaerobic isolates directly from single colonies and with no need to perform protein extraction [14,16]. This method yields a final identification within minutes and avoids further molecular analysis and can also be performed directly from clinical samples, such as blood cultures [16].

In this regard, several studies have demonstrated that MALDI-TOF MS can be easily and efficiently applied for the identification of microorganisms from blood cultures. Applying a standardized and easy-to-perform centrifuge and wash protocol, microorganisms can be obtained from both types of samples and either directly identified with MALDI-TOF MS by on-plate extraction with formic acid [17,18] or by using a commercial lysis buffer (Sepsityper, Bruker Daltonics, Bremen, Germany) and a standard protein extraction step with formic-acid/acetonitrile [19]. Both approaches have rendered

between 81.4-87.0% blood culture bottles correctly identified at the species level when the score values recommended by the manufacturer are applied. However, the rate of correct identifications increases up to 95% when the cut-off for species-level identification is lowered to 1.8, with no misidentifications detected.

Despite the accuracy, robustness and reduced turnaround time of MALDI-TOF MS, several drawbacks have been described: (1) the capacity of MALDI-TOF MS to detect more polymicrobial infections is limited; (2) yeasts represent a challenge to MALDI-TOF MS when present in blood cultures. In the first case, MALDI-TOF MS has been reported to identify up to two different micro-organisms present in the same sample [18] but it frequently misses the species in lower concentration or fails to identify some of the microorganisms when more than two are present. In the cases when two or more species are expected according to the results of the gram staining, the final identification of the micro-organisms present in a blood culture bottle is delayed 18-24 hours until MALDI-TOF MS can be performed from the colonies grown on agar plates.

When yeasts are suspected in a blood culture sample the implementation of the sample procedure described above yields a very low rate of successful identifications. Thus, either the use of the commercial lysis buffer (Sepsityper, Bruker Daltonics) or detergents such as SDS [20] are recommended for a more effective lysis and identification of these entities.

In the case of urine samples, several studies have been published reporting the efficiency of MALDI-TOF MS for the rapid and accurate identification of microorganisms [21-23]. However, this

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type of sample presents sensitivity issues due to the limited number of bacteria present in a large volume and the already mentioned problems with polymicrobial samples. The hand-on time required for the processing of this kind of sample is usually too long. Thus, the benefit of its implementation is scarce when compared with performing MALDI-TOF MS from single colonies grown on agar plates after 12-hours incubation.

### Identification of Mycobacteria Isolates

For a long time the genus *Mycobacteria* has represented a challenge for MALDI-TOF MS due to the characteristics of their cell wall and the difficulties it pose for an efficient protein extraction. Therefore, successful identification was only achieved when in-house libraries enriched with different *Mycobacteria* species were used [24].

However, two events rendered MALDI-TOF MS a robust and accurate tool for the identification of *Mycobacterium* spp.: the use of mechanical disruption of the cell wall with glass beads followed by a standard protein extraction step and the improvement of the available database [25]. Both factors have allowed the routine identification of *Mycobacterial* isolates, especially those species grouped as NonTuberculous *Mycobacteria* (NTM) since discrimination within the *Mycobacterium tuberculosis* complex has not been achieved yet.

Currently, the identification of *Mycobacterial* isolates using MALDI-TOF is widely spread, with a good number of research groups publishing high rates of correct species assignment for an increasing number of NTM species [26-28]. A recent multicenter study has demonstrated the high rate of accuracy displayed by MALDI-TOF MS, with close to 100% successful identification at the species level [29]. Besides, the implementation of sonication increased the score values obtained and allowed a reliable identification of NTM isolates. The high correlation of MALDI-TOF MS identification with reference molecular methods, robustness, accuracy and its low price per sample make this technology highly suitable for routine identification of NTM isolates. Its identification requires little hands-on time and allows a rapid and reliable identification of the isolates. This is a key factor for the early onset of the correct antibiotic therapy when required, improving the management of infected patients.

### Identification of Yeasts and Filamentous Fungi

As explained above for the *Mycobacterium* genus, yeasts and filamentous fungi have also represented a challenging taxonomic group for MALDI-TOF MS. After years of experience with this technology and with the available updated databases, most yeasts are currently represented, even *Candida auris* [30]. Nowadays, most commonly encountered yeast species are easily identified with MALDI-TOF MS, even directly from blood cultures, as mentioned before (20) and they are routinely identified using this methodology [31,32].

On the other hand, filamentous fungi have been identified with MALDI-TOF MS after a long and difficult process of sample preparation improvement and in-house database building. This explains why still very few laboratories have applied this technology for the identification of filamentous fungi [31,33,34].

However, the improvement of sample preparation and the good results obtained have encouraged different research groups to build their own in-house databases with well-characterized, local isolates

or even to share them [35] so that identification of filamentous fungi develop an easier task to be carried out with MALDI-TOF MS and, even, to implement this methodology in the routine of the clinical microbiology laboratory [36,37].

### Susceptibility Testing

The huge potential of protein spectra analysis by MALDI-TOF MS has, so far, only started to show. Besides identification of microorganisms, providing reliable information about their susceptibility profile could shorten the time until a directed antibiotic/antifungal treatment is implemented. This fact has often been correlated with a better outcome of the patient and reduced hospital costs [38].

Several authors have reported promising results on this regard. A methodological paper was published in 2012 showing how susceptibility to different beta lactam antibiotics [6]. The protocol consists of incubating the bacterial pellet in the presence of the antibiotic tested and a peak shift is seen due to the breakage of the beta lactam ring when beta-lactamases are present. This study has represented a framework for further development and implementation of susceptibility testing using MALDI-TOF MS. Together with the identification of microorganisms, combination of both tests carried out with the same instrument- have shown to provide useful information in a fast, inexpensive and reliable manner. Estimated turnaround time for identification and susceptibility profile is 30-45 min.

The development of this methodology has allowed different authors the identification of resistance mechanisms from bacteria present in blood culture [39,40]. Identification of the microorganisms present in blood samples and susceptibility testing has been demonstrated to be feasible with MALDI-TOF MS and a high correlation with other reference techniques has been shown.

The emerging of carbapenem-resistant isolates has also become a global health problem. The implementation of MALDI-TOF MS for the detection of this type of resistance mechanisms has also been shown for different bacterial genera, with promising results [40-42]. Recently, a commercial kit has also been commercialized for the easy and standardized detection of imipenem-resistant strains (MBT STAR-Carba IVD Kit -STAR – Selective Testing of Antibiotic Resistance, Bruker Daltonics, Bremen, Germany).

Finally, antifungal susceptibility testing has also been performed with MALDI-TOF MS [43-45]. Different resistance mechanisms have been assayed and their results demonstrate once again the capacity of MALDI-TOF MS to detect them in a quick, efficient and inexpensive manner.

### Conclusions

MALDI-TOF MS technology has emerged as a rapid, reliable and robust tool for the identification of microorganisms. Its user-friendly technology that requires very little training hands-on time to be skillfully performed has rendered it as a must-have instrument in most clinical microbiology laboratories. Once users have started to accumulate expertise on its application to different bacterial species and sample types, it has been implemented for the identification of a wide range of bacteria, mycobacteria, yeasts or molds. Besides, other applications such as susceptibility testing or typing have also been feasible with the same technology. This fact has shown the great

flexibility of MALDI-TOF MS and its potential for future applications. The revolution we have witness in the last decade will probably keep on going for a little while as MALDI-TOF MS unfolds its endless purposes.

## Competing Interests

The authors declares that they have no competing interests.

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