

Rapid microbial identifications by MALDI-TOF – *Viva la revolution!*



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Although the application of mass spectrometry to bacterial identification was proposed as far back as 1975 by John Anhalt¹, it was not until 1987 when Professor Franz Hillenkamp and Dr Michael Karas first pioneered Matrix Assisted Laser Desorption/Ionisation Time-of-Flight mass spectrometry (MALDI-TOF MS) for truly rapid microbial identification in diagnostic microbiology laboratories². Conventional phenotypic methods of identifying isolates of bacteria, yeasts and filamentous fungi require hours to several days to complete, depending on the type of organism involved, and may be prone to error due to bias or inexperience³. Even the more recent molecular innovations have their limitations that place them outside the scope and budget of many routine laboratories. However, MALDI-TOF MS technology has changed the way we think about microbial identifications and strain differentiation by providing results from plate to name in approximately five minutes for one isolate and around 90 minutes for 60 isolates at under \$2 an identification. It is likely to be particularly helpful clinically for identification of species in positive blood culture broths and for organisms that are hard to identify quickly.

Principle and method

MALDI-TOF identification requires organisms in pure growth from solid media, or more recently from liquid media such as positive blood culture broths. Whole organisms or extracts are applied manually or by machine to a small designated area of a brushed metal target plate and allowed to dry. 1-2µL of matrix solution such as α-cyano-4-hydroxycinnamic acid in 50% acetonitrile and 2.5% tri-fluoracetic-acid is applied to the spot and allowed to air dry (Figure 1). The matrix solution isolates molecules from the bacterial cells facilitating ionisation without fragmentation.

The target plate is then inserted into the instrument (Figures 2 & 3) where the sample spot is subjected to pulses from the 337-nm nitrogen laser. The resulting ions (mass range usually 2-20kDa) are accelerated by a high voltage electric field after which they ‘drift’ along a flight tube. The time taken to reach the recording device at the end of the flight tube is determined by the differing mass/ionic charge (m/z) ratio of each individual ion. Software compares the resultant mass-spectral profiles (Figure 4) of the ionisable moieties of the cells’ surfaces to patterns stored in a database developed from the analysis of known or typed strains.

While MALDI-TOF usually identifies microorganisms by analysing cellular proteins and peptides, the technology may also be used for the analysis of carbohydrates, polymers, oligonucleotides, single nucleotide polymorphisms and metabolites.

Routine laboratory applications

In the clinical field, the benefits to patient care of rapid microbiological results have long been recognised^{4,5}. However,

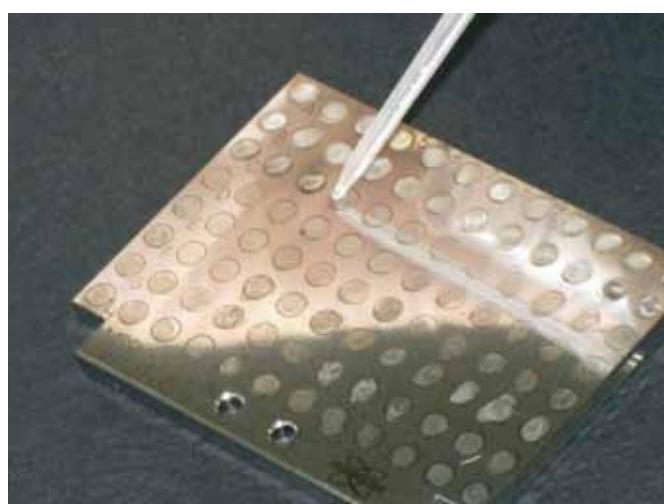


Figure 1. Application of matrix to the MALDI-TOF target plate.



Figure 2. The Bruker Daltonics Microflex Biotyper.

since its inception, diagnostic clinical microbiology has largely been involved in the confirmation of infection and antibiotic prescription. This has been due to the obvious delays inherent in isolation, identification and antimicrobial susceptibility testing. These delays are even more pronounced in the area of infection prevention/control especially when these measures require the assessment of strain relatedness. However, in the mid-1990s



Figure 3. The target plate loaded into the MALDI-TOF.

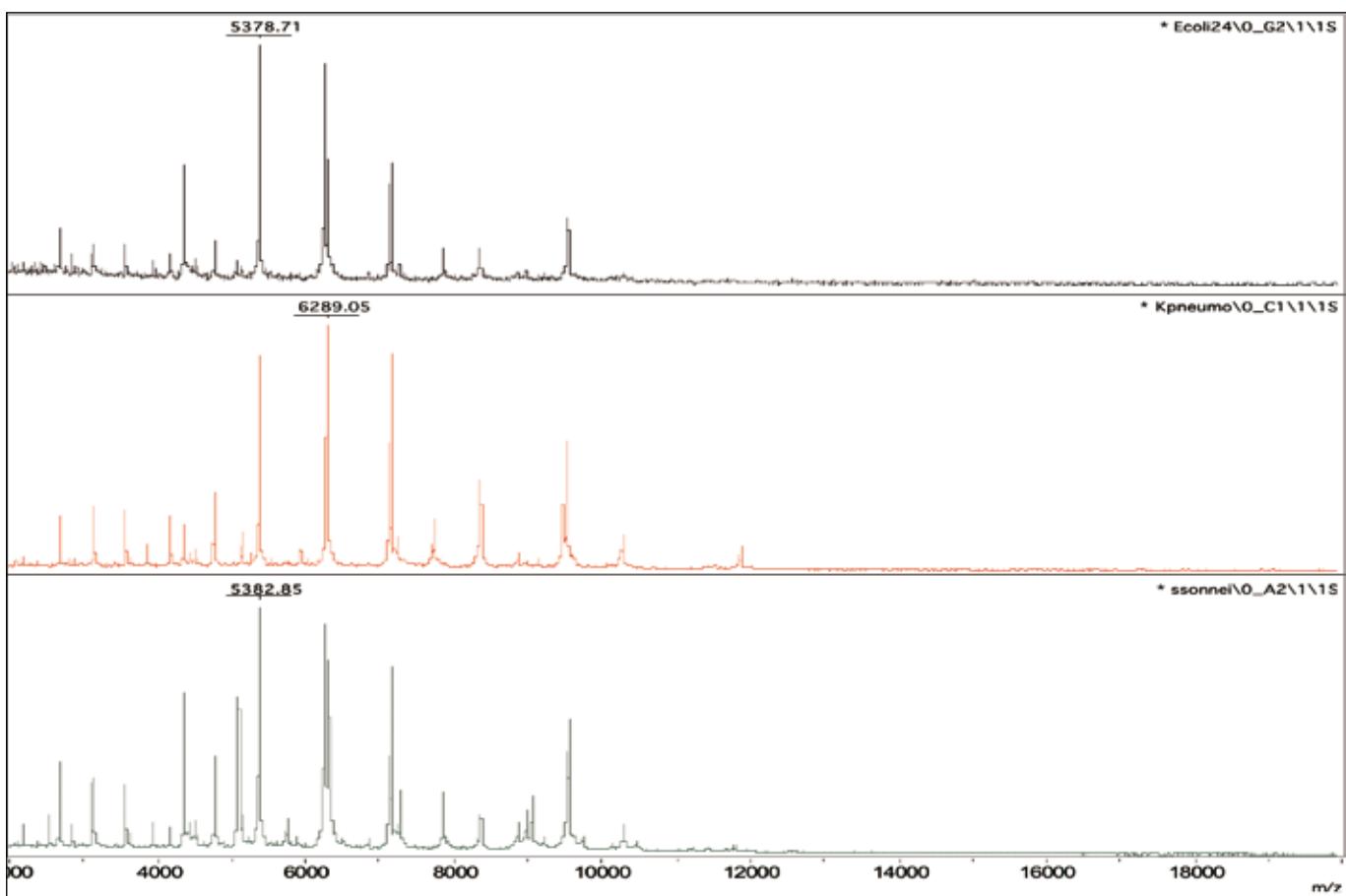


Figure 4. Protein spectra obtained from the analysis of *E. coli* ATCC 25922, *K. pneumoniae* ATCC 13833 and *S. sonnei* ATCC 25931 showing the m/z ratio along the x axis.

laboratories started to look to MALDI-TOF technology to improve the turnaround time and accuracy of organism identification^{6,7}.

Laboratory assessments and evaluations of the performance of MALDI-TOF MS are appearing in the scientific literature with increasing frequency involving an equally expanding range of microorganisms. A search of the journals published by the American Society for Microbiology alone yields 1129 entries covering studies on some aspect of the proteomics of such diverse organisms as the Hendra virus, *Treponema pallidum*, *Legionella pneumophila*, *Mycobacterium tuberculosis*, *Aspergillus fumigatus* and *Trypanosoma cruzi*.

However, the most consistent theme throughout the publications produced by laboratories evaluating the routine applications of MALDI-TOF MS is undoubtedly the accuracy, speed and minimal reagent and labour costs of the technology⁸⁻¹⁵. Most of these evaluations compare the identifications obtained by the Bruker Daltonics MALDI-TOF MS instrument to routine biochemical methods (mostly commercially manufactured such as bioMérieux's API and Vitek systems), and using molecular techniques such as 16S rRNA gene sequencing to resolve discrepancies. In these studies MALDI-TOF MS species identification is >90% accurate.

As with any identification system, the major limitation is the size and quality of the database used in the analysis. Current MALDI-TOF MS databases lack spectra of the less commonly isolated streptococci, some anaerobes (most notably *Fusobacterium* spp.) and non-fermentative gram negatives.

MALDI-TOF software allows users to add their own organism profiles, which has been shown to improve the identification of less common species. Optimisation of the database by careful selection of isolates for inclusion may enable the identification and recognition of not only the genus and species involved but even the subtype, while improving the resolving power of the strain recognition software¹⁶⁻¹⁸.

The three studies that analysed consecutive clinical isolates with the Bruker MALDI-TOF MS system showed correct identifications for 95.4%, 95.2% and 92.2% of isolates respectively^{10,14,15}. Bacteria that failed to produce acceptable identifications included *Fusobacterium nucleatum*, *Stenotrophomonas maltophilia* and *Streptococcus pneumoniae* (although our preliminary assays indicate an improvement in the identification of *S. pneumoniae*). In each case, the explanation given is the small number of reference spectra for these organisms in the database at the time of testing. In our own laboratory we have found that *Shigella sonnei* is usually misidentified as *E. coli* as these organisms are genetically and proteonomically almost indistinguishable and thus MALDI-TOF MS cannot differentiate these species. However, it does serve as a reminder that nothing in microbiology is 100% and the obligation always remains with the user to ensure that cultural morphology and additional tests, in this case serological confirmation, are consistent with the identification results. Given *Shigella* spp. are virtually always only isolated from faecal specimens and laboratories utilise other methods of

differentiating *Shigella* spp. from other Enterobacteriaceae, this is unlikely to be a clinical problem. Similarly MALDI-TOF may not accurately speciate "viridans" streptococci¹⁵.

Eigner *et al.* examined over 1100 isolates with the Bruker system, producing a correct identification in 79.7% of non-fermenting gram negatives, 99.5% of staphylococci, 100% of enterococci, 93.7% of streptococci and 95.5% of *Enterobacteriaceae* – although it did struggle to differentiate between *Citrobacter braakii* and *C. freundii* (both in the *C. freundii* complex) plus *Klebsiella oxytoca* and *Raoultella ornithinolytica*¹⁰. These findings were also consistent in the evaluation by Cherkaoui *et al.*, who compared both the Bruker and Shimadzu instruments using 720 clinical isolates. In this study, the Bruker and Shimadzu instruments produced high confidence identifications for 94.4% (99.1% correct) and 88.7% (99.4% correct) of isolates respectively⁸. In other studies looking at specific microorganisms, the results have been equally encouraging, with correct identifications obtained in 97.5% of anaerobes¹³, 92.5% of yeasts¹⁹ and 99.3% of *Staphylococcus* spp.⁹.

Modifications and variations to MALDI-TOF

Bizzini reports that the Bruker instrument correctly identified 98.5% of 1371 bacteria commonly isolated in his laboratory (93.2% correct to the species level, 5.3% to the genus level)²⁰. While the majority was identified by the direct application of the organism to the target plate, 25% required extractions before a correct identification could be recorded. There are two versions of the extraction method:

1. Inactivates cells but not spores and uses a mixture of formic acid and acetonitrile.
2. Inactivates cells and spores using 80% tri-fluoracetic-acid. The extraction process is essential whenever relatedness studies and dendograms are required.

Shah has taken this technology even further by combining MALDI-TOF analysis with surface-enhanced laser desorption/ionisation time-of-flight (SELDI-TOF-MS)¹⁸. This technique replaces the steel target plate with a ProteinChip® (Ciphergen Biosystems) array with a different surface chemistry on each chip – chemical targets to capture ionic or hydrophilic molecules or biochemical targets able to capture antibody, receptor or DNA molecules. This approach enables the accurate analysis of bacterial proteins of higher mass (up to 150kDa) than by the MALDI-TOF alone. It also enables the capture and analysis of specific proteins, such as toxins, involved in the disease process.

Clinical impact of MALDI-TOF

Rapid identification of infectious agents directly from clinical specimens will greatly assist patient care^{21,22}. Several publications demonstrate the promise offered by MALDI-TOF MS for identifying bacteria and yeast in positive blood cultures. La Scola and Raoult have demonstrated the positive predictive value of the Bruker instrument to be 99.2% in a series of 322 positive blood culture broths. The only difficulties in identifications were

encountered with the viridans streptococci and polymicrobial infections²³. Other publications support the potential benefits of this application although some modifications to specimen preparation may be required in order to provide the most accurate result²⁴⁻²⁶.

The rapid identification of security sensitive biological agents (SSBAs), such as *Salmonella Typhi* and *Francisella tularensis*, allows rapid diagnosis of potentially lethal infections, assisting patient treatment and public health interventions^{7,27}.

Conclusions

In conclusion, the literature would seem to offer wholehearted support for the application of MALDI-TOF MS to routine clinical microbiology, although the instruments are expensive. However, once acquired and with the promise of direct specimen testing with microorganism identifications in a few minutes coupled with the additional possibility of the rapid recognition of resistance mechanisms such as β-lactamase production in *E. coli*²⁸, and methicillin resistance in *S. aureus*²⁹, in addition to reagent savings of around \$5 to \$10 per isolate⁸ and efficiencies in laboratory work flows – well may we say *Viva la revolution!*

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Biographies

Stephen Neville is a Senior Hospital Scientist in the Department of Microbiology & Infectious Diseases, Sydney South West Pathology Service at the Liverpool Hospital Campus. He has an interest in laboratory management, antimicrobial resistance mechanisms and parasitology. He has been active in the ASM for many years, being the founding convener of the NSW and National Parasitology SIGs and has run the highly successful Short Course in Clinical Parasitology for the NSW branch.

Iain Gosbell is a microbiologist and infectious diseases physician, and was previously the Director of the Department of Microbiology and Infectious Diseases, Sydney South West Pathology Service – Liverpool, and took up the position of Professor of Microbiology and Infectious Diseases in the School of Medicine, University of Western Sydney (SoM, UWS), in late 2009. He has published on antibiotic-resistant bacteria, particularly community methicillin-resistant *Staphylococcus aureus*. Recently in conjunction with Slade Jensen and Björn Espedido he helped co-found the Antibiotic Resistance and Mobile Elements Group (ARMEG) at the SoM, UWS, which will examine resistance elements in multiresistant organism (MROS).