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The Broad Street Pump

Matrix-assisted laser desorption/ionization – time of flight (MALDI-TOF) mass spectroscopy – bringing clinical microbiology into the new century?

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Automated diagnostics are arriving rapidly in the field of Clinical Microbiology. An approach that has been widely adopted over a short space of time - MALDI-TOF - is described in this issue.

One important reason to discuss this is its place in the rapid diagnosis (e.g. blood culture) of severe infection. Septic shock has a mortality rate of ~38% in Australia[1] and causes more deaths each year than both breast and colon cancer[2]. This compares with best-practice intervention in heart attack, which reduces all-cause mortality from 4.3 to 2.7% and from the best reported non-antibiotic intervention for life-threatening infection (i.e. activated protein C)[6,7], that seem trivial when compared with the reduction in mortality from early antibiotics in septic shock[4,5]. Any microbiological advance that brings us closer to the crucial 12-hour window is very valuable. The deployment of mass spectroscopic methods within an hour of a blood culture signaling positive, which may occur as soon as 9 or 10 hours after inoculation in conventional media, is one such advance.

Like every method, it has weaknesses. Pattern recognition is used to link libraries of representative patterns ('spectra'). These are analysed by machine-associated software and appear fairly robust, but unusual pathogens will often have poor library representation. In addition, mixed cultures or pathogens from which ionisable proteins (e.g. cytoplasmic) are either not readily extracted or do not differentiate key pathogens from close relatives (e.g. *S. pneumoniae* from some other streptococci, *Shigella* from *E. coli*) will always be problematic. Nevertheless, the next decade or so is likely to bring increasing automation to Clinical Microbiology and this particular tool is likely to be a major part of it.

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MALDI-TOF MS; on-going evolution of a revolution in clinical microbiology

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MALDI-TOF instruments comprise three principal components: (1) an ionisation chamber, within which laser-based (usually an ultraviolet nitrogen laser of 337 nm) ionisation and vaporisation of the specimen into the gas phase occurs; (2) a time of flight (TOF) mass analyser that separates ions according to their mass-to-charge ratio (m/z) [1]; and (3) a particle detector (Figures 1 and 2). Matrix characteristics are important determinants of the intensity, size, and quality of MALDI-TOF peaks [2]. In general, MALDI-TOF can directly analyse intact bacteria without pre-treatment because most are lysed following exposure to water, organic solvent or strong acid within the matrix. However, some bacterial species, spores, yeasts and moulds require pre-treatment with stronger organic acids and/or alcohols to release protein [3].

The microbial sample (or analyte) is spotted onto a metal plate (Figure 1) and dried at room temperature prior to matrix addition; alternatively, the sample-matrix mixture can be directly deposited onto the plate. After sample crystallisation within the matrix, the target is loaded into the instrument and pulsed with laser (Figure 2). The matrix absorbs energy, the analyte is ejected (desorbed) and receives protons from matrix molecules, generating gaseous phase cations (matrix-assisted laser desorption and ionisation [MALDI]). The detection of these cations (mass/time of flight) after acceleration generates unique mass spectroscopic signatures that are referenced to a database for microbial identification and strain differentiation. Spectral peaks typically correspond to highly abundant, conserved, house-keeping proteins that are minimally affected by environmental conditions or growth phase (Figure 2) [4]. Spectra within the mass range 3000–20000 Da generally exhibit useful inter-species variability and intra-species similarity [5].

Bruker Daltonics offers a MALDI-TOF instrument with the MALDI Biotyper database, which presently (version 3.2.1.0) contains >82000 spectra from >2000 bacterial and yeast species. More than 100 peaks of minimum quality are used to generate an arbitrary spectral score from <1.7 ("no identification") through 1.7–2.0 ("identification to genus") to ≥2.0 ("identification to species level"). The AXIMA instrument (Shimadzu Corp., Kyoto, Japan) uses the Spectral ARchive and Microbial Identification System (SARAMIS™) database (Anagnostec GmbH, Potsdam, Germany), presently containing >3000 super-spectra from 2000 species [6]. In 2010, Shimadzu Corp. and bioMérieux (Marcy l'Etoile, France) co-developed the VITEK MS instrument incorporating the SARAMIS database. SARAMIS™ utilises peaks shared by a minimum of strains (typically 15) of the same species to build a reference signature ("SuperSpectrum™"); scores >80% define species identification.

Bacteria - Identification from cultures

The performance of MALDI-TOF for bacterial and mycobacterial species identification from colony cultures and directly from clinical specimens has been detailed elsewhere recently [7]. Overall, compared to phenotypic methods, MALDI-TOF may reduce the time required for bacterial identification from up to 48 hours to less than 6 minutes [8]. In one study, 93% of isolates were identified by MALDI-TOF within 24 hours of inoculation, compared with 10% for phenotypic methods [9]. Cost savings range from $1.14-$32.29/bacterial isolate [8,10,11], reduction of overall laboratory costs were $55,138-179,999 per annum ($1USD = €0.73EUR = $0.97AUD) [9,12].

MALDI-TOF can definitively identify most bacteria from blood cultures within 1-2 hours [13], to genus (75.8%-80.5%) and species (59.5%-77.9%) level, using a standardised protocol (e.g. Bruker MALDI-TOF MS with MALDI Sepsityper™ Kit) [14,15]. Problems may be encountered with Gram-positive (e.g. pneumococcal identification), anaerobic and polymicrobial bacteraemias [14,16-18] and specimens from which bacterial proteins are difficult to extract [19]. Direct identification of pathogens from BacT/ALERT®SA/SN blood culture broths may be significantly less than from

BACTEC™ and VERSATREK™ broths (62% vs. 76% and 69% respectively) [18]. Similarly, charcoal broths may interfere with performance (e.g. BacT/ALERT®FA/FN vs SA/SN: 8% vs. 28%) [17].

Access to the most complete MALDI-TOF databases will not resolve some of the inherent limitations in bacterial identification [20], particularly for closely related species that cannot be distinguished from each other such as:

- *S. pneumoniae* & *S. mitis/S. oralis;*
- *S. pyogenes* & group C/G streptococci;
- *Pseudomonas hibiscicola/P. beteli* & *Stenotrophomonas maltophila;*
- *Aeromonas veronii* & *A. caviae*;
- *Neisseria subflava* & *N. sicca;*
- *Listeria monocytogenes* & *L.innocua/L.ivanovii*
- *E. coli* & *Shigella* [6,7,12,13].

Other applications, including the detection of antimicrobial resistance [21-24] and toxins [25-27], are not yet in widespread use.

Fungi - Yeasts

Pooled sensitivities for "species-level" and "genus-level" identification range from 79%-100% and 94%-100% respectively [28-31]. In general, common candida pathogens (e.g. *C. albicans, C. glabrata, C. parapsilosis, C. tropicalis* and *C. krusei*) are reliably identified with good intra-species reproducibility [28,30-32]. The identification of non-candida yeasts including *Trichosporon, Saccharomyces, Geotrichum* and *Rhodotorula* species relies on the supplementation of reference spectra [33,34]. *Cryptococcus* species identification rates range from 33%-50% using Biotyper software [28,31,34]. *C. neoformans* and *C. gattii* were differentiated, as were *C. neoformans* var. *neoformans* and var. *grubii* for 85/86 (99%) isolates [35]. The Biotyper (version 3.0) and SARAMIS™ database systems have comparable sensitivities (97.6% and 96.1% respectively) for yeast identification overall [28]. Growth media, and especially protein extraction considerations are important [33,36]. MALDI-TOF has detected *Candida* species and *C. neoformans* from clinical and simulated blood cultures with sensitivities approaching 100% [37-39]. Turnaround times are approximately 30 minutes in patients with candidaemia [40]. A modified Sepsityper™ extraction protocol enabled identification of *C. albicans, C. parapsilosis, C. tropicalis* and *C. neoformans* in blood cultures (detection limit 10^5 CFU/mL) [38].

Filamentous fungi

There are limited data on species identification of filamentous fungi. Despite poor representation of reference spectra from clinical mould isolates in commercial MALDI-TOF databases, *Aspergillus, Penicillium, Fusarium, Scedosporium* species and dermatophytes have been identified with good accuracy [41-46]. De Carolis *et al.* prospectively evaluated an in-house reference database for the identification of *Aspergillus, Fusarium* and *Mucorales* species [46]. 91/94 (96.8%) isolates were correctly identified to species level and all, to genus level. Theel et al. identified 93% and 59.6% of dermatophytes to genus and species level respectively, but only after database supplementation [47].

Conclusion

MALDI-TOF enables early and accurate identification of bacteria and yeasts, including directly from blood culture broths. It is clearly an invaluable partner to the Gram stain, with major caveats only for polymicrobial bacteraemias and the differentiation of viridans streptococci from *S. pneumoniae*. It is rapidly establishing itself as an essential tool in the modern diagnostic microbiology laboratory.

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Figure 1. Principles of MALDI-TOF. Laser pulses strike the dried sample overlaid with matrix solution on the target plate. Molecules are desorbed and ionised before being accelerated through the field-free vacuum, where they drift across to the particle detector. The time-of-flight is dependent on the mass of the molecules, and spectra are referenced to a database for species identification.

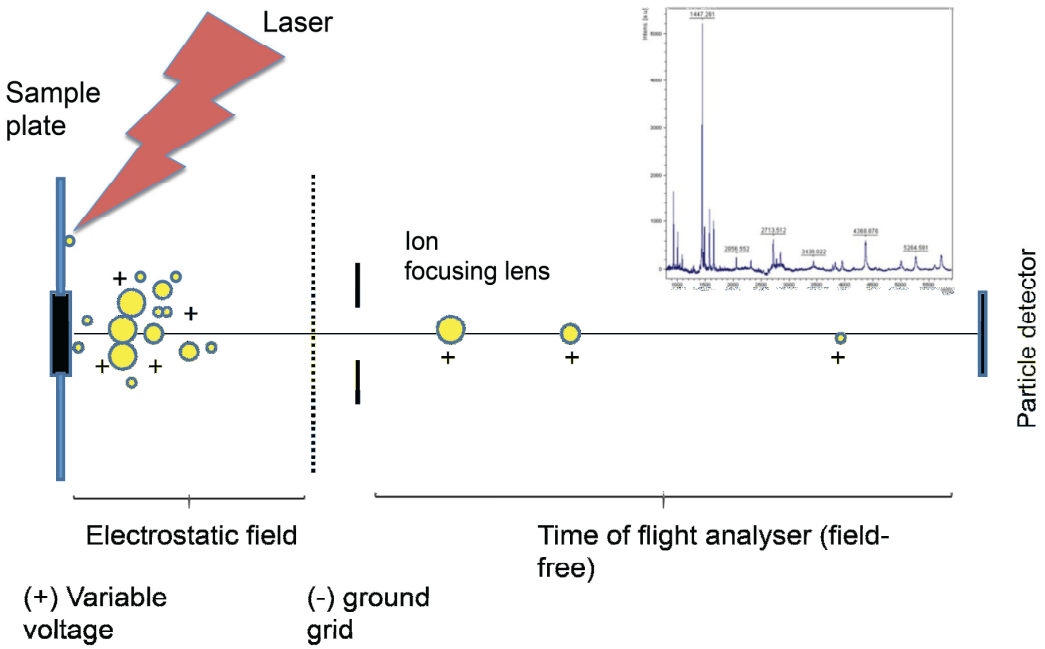
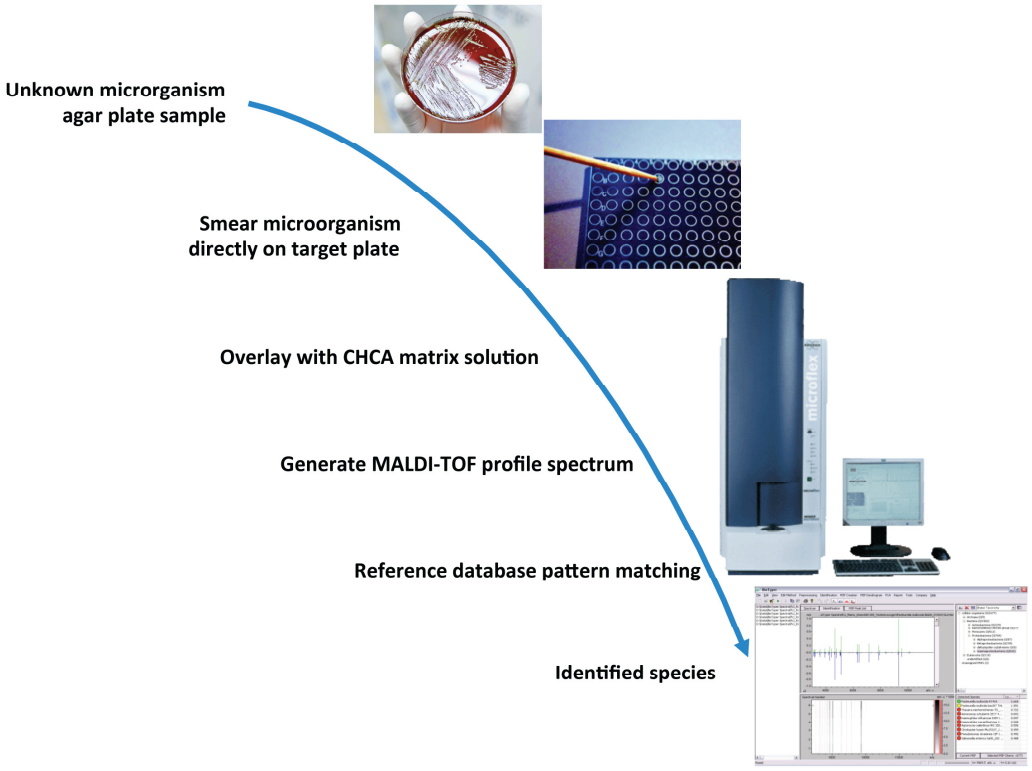


Figure 2. Sample preparation and workflow



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