BASIC SCIENCE FOR THE CHEST PHYSICIAN

It's a MALDI but it's a goodie: MALDI-TOF mass spectrometry for microbial identification

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ABSTRACT

The last few years have witnessed a revolution in the diagnostic microbiology laboratory with the emergence of matrix assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) as an indispensible tool in microbial identification. In many laboratories this has superseded biochemical profiling. A mass spectrum is acquired from an unknown micro-organism and this proteomic fingerprint is then compared with a database of reference spectra to ascertain the likely genus and species identity. The reproducibility of this method is facilitated by the analysis of continually produced, highly abundant proteins (mainly ribosomal proteins) in the mass range 2000 to 20 000 Da. MALDI-TOF MS is reliable and rapid and has the ability to determine the identity of an isolate from culture in a matter of minutes rather than the hours or days required by more traditional methods. In addition to microbial identification of cultured isolates, work is underway to extend the utility of MALDI-TOF MS to include bacterial identification directly from clinical samples as well as providing timely information regarding antibiotic resistance and typing of different micro-organisms.

INTRODUCTION

The last few years have witnessed a revolution in the diagnostic microbiology laboratory with the emergence of matrix assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) as an indispensible tool in microbial identification.[1] In many laboratories it has superseded biochemical profiling. A mass spectrum is acquired from an unknown micro-organism and this proteomic fingerprint is then compared with a database of reference spectra to determine the organism identity. MALDI-TOF MS is rapid and reliable, with the ability to accurately determine the identity of a micro-organism from culture in a matter of minutes rather than the hours or days required by more traditional methods.

MALDI-TOF MS

MALDI-TOF MS incorporates MALDI, which is a 'soft ionisation' technique, which gently ionises particles and allows the analysis of larger biomolecules with a time-of-flight analyser to generate a mass spectrum. The reliability of this method relies on the analysis of continually produced, highly abundant proteins (which are mainly ribosomal proteins) in the mass range 2000–20 000 Da. At least as important as the generation of the mass spectrum is the reference database, with which the unknown spectrum is compared in order to determine the identity of a micro-organism. There are a number of commercially available systems that usually make a proprietary reference database available with the mass spectrometer, although inhouse databases can also be developed. A schema of the process of microbial identification using MALDI-TOF MS is presented in figure 1 and can be broadly divided into spectrum acquisition and spectrum analysis.

Spectrum acquisition

A sample of the unknown micro-organism is applied onto a polished steel plate and allowed to dry. This sample may be whole cells (directly transferred from culture) or protein extracts. A matrix solution, which is made up of the matrix (generally a crystalline solid), aqueous acid and organic solvents, is then applied to the sample. During the drying of the matrix solution there is disruption of the cells (if a whole cell sample) and cocrystallisation of the sample and the matrix resulting in the sample embedded within the matrix.

The steel plate is then inserted into the mass spectrometer and a laser is fired in short bursts resulting in vaporisation and desorption of the sample and matrix from the metal plate. In addition to its scaffolding function, the matrix is also important for absorption of the high laser energy and providing the protons for ionisation of the sample proteins. An acceleration voltage is applied to the ionised particles, which then enter a flight tube with a detector placed at the other end. This detector analyses the time of flight of the ions through the flight tube. These charged particles are separated by their mass/charge (m/z) ratio, with smaller proteins travelling more quickly than larger proteins. MALDI generally results in singly charged particles and consequently a spectrum is generated, with peaks representing different masses and the height of the peak representing the intensity of the signal.

Spectrum analysis

The raw mass spectrum generated requires further data processing before the proteomic fingerprint can be compared with the spectra in the reference database. The comparison algorithms and databases used may vary between different systems but in general the principle is similar. A score is generated depending on the number of matching peaks and a list of top matches together with the probability of the identification (from highly probable species identification to no reliable identification) is produced.

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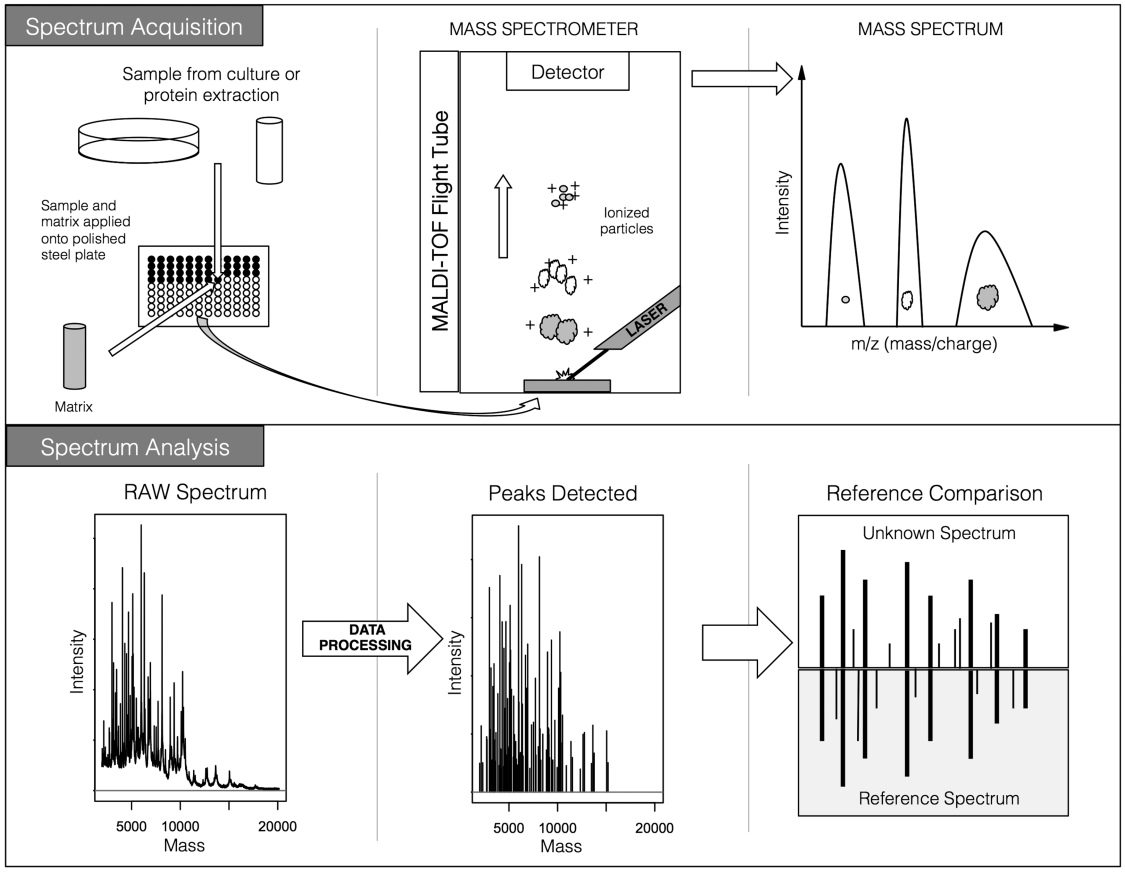

Figure 1 Schema of matrix assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) for the identification of an unknown micro-organism.

BENEFITS AND LIMITATIONS

MALDI-TOF MS is robust, cost-effective and able to provide rapid and accurate identification of micro-organisms from culture despite the potential spectral variation introduced by different culture conditions, protein extraction methods and different laboratory processes. The rapidity of identification has the greatest potential impact on the clinical management of patients allowing earlier decision-making regarding the tailoring of antimicrobial treatment. It is a sensitive technique able to generate mass spectra from samples of $<10^4$ micro-organisms and has the added benefit of not requiring a predefined target (in contrast with PCR-based methods of identification). However, a culture of the micro-organism is currently still required and it is unable to deal with mixed cultures. Additionally, the quality and taxonomic breadth of the reference database is critical, as an accurate identification is only possible if the micro-organism in question is in the database. There is also difficulty in distinguishing between some organisms that are closely genetically related, for example, *Streptococcus pneumonia* and some of the viridans group *Streptococci*, and further testing to confirm identification may be required.

FURTHER MALDI-TOF MS APPLICATIONS

Further research is underway to extend the utility of MALDI-TOF MS, which is now ubiquitous in diagnostic microbiology laboratories. In particular, to expand the number of micro-organisms identified, to identify micro-organisms directly from clinical specimens, to detect antibiotic resistance and to aid in epidemiological investigation. Increasing the range of micro-organisms identified, including routine identification of mycobacteria and fungi, will require extension of reference databases and standardisation and validation of identification protocols. The identification of bacteria directly from positive blood cultures[2] holds the promise of reducing the identification time even further, however there are still challenges to be overcome if reliable identification of micro-organisms directly from other clinical samples, for example, urine and cerebrospinal fluid is to be achieved. MALDI-TOF MS has also been used to determine antimicrobial resistance by detecting the degradation products of antibiotics broken down by bacterial enzymes.[3] From an epidemiological point of view, the development of MALDI-TOF MS for strain typing,[4] which is still in its infancy, would be a significant boon for infection control and public health.

CONCLUSIONS

MALDI-TOF MS has already become an integral tool in the diagnostic microbiology laboratory for microbial identification, resulting in faster accurate identification of micro-organisms, which allows earlier treatment decisions to be made. Ongoing development of this technology and integration with more traditional and molecular microbiological techniques promises to improve the services offered by the laboratory further benefiting the clinical management of patients.

Competing interests None.

Provenance and peer review Commissioned; internally peer reviewed.

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