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Continuing medical education: Methods of rapid diagnosis

Fast methods of fungal and bacterial identification. MALDI-TOF mass spectrometry, chromogenic media☆

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ABSTRACT

MALDI-TOF mass spectrometry is now a routine resource in Clinical Microbiology, because of its speed and reliability in the identification of microorganisms. Its performance in the identification of bacteria and yeasts is perfectly contrasted. The identification of mycobacteria and moulds is more complex, due to the heterogeneity of spectra within each species. The methodology is somewhat more complex, and expanding the size of species libraries, and the number of spectra of each species, will be crucial to achieve greater efficiency. Direct identification from blood cultures has been implemented, since its contribution to the management of severe patients is evident, but its application to other samples is more complex.

Chromogenic media have also contributed to the rapid diagnosis in both bacteria and yeast, since they accelerate the diagnosis, facilitate the detection of mixed cultures and allow rapid diagnosis of resistant species.

Métodos rápidos de identificación de bacterias y hongos. Espectrometría de masas MALDI-TOF, medios cromogénicos

Palabras clave:
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RESUMEN

La espectrometría de masas MALDI-TOF es ya una herramienta de trabajo rutinaria en Microbiología Clínica, por su rapidez y fiabilidad en la identificación de microorganismos. Sus resultados están perfectamente contrastados en la identificación de bacterias y levaduras. La identificación de micobacterias y hongos filamentosos presenta mayor complejidad, por la mayor heterogeneidad de espectros dentro de cada especie. La metodología es algo más compleja, y la ampliación del número de especies de referencia, y del número de espectros de cada especie, serán cruciales para alcanzar mayor eficacia. La identificación directa a partir de hemocultivos se ha implantado dada su aportación al manejo de pacientes graves, pero su aplicación a otras muestras es más compleja.

Los medios de cultivos cromogénicos han supuesto también una aportación al diagnóstico rápido tanto en bacterias como en levaduras, ya que aceleran el diagnóstico, facilitan la detección de cultivos mixtos y permiten un diagnóstico rápido de especies resistentes.

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MALDI-TOF mass spectrometry: Origins

The introduction of matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry (MS) represents, in all likelihood, the most important technological change that has occurred in Clinical Microbiology in the last decade. In just a few years it has gone from being a promising novelty to a technology which is fully integrated into daily clinical activity and, in Spain, it is available in the Microbiology Departments of many hospitals.[1] This has required mass spectrometers to undergo a significant technological evolution. In this regard, two breakthroughs turned out to be crucial. First, in 1946, WE Stephens designed the time-of-flight (TOF) system, which made it possible to separate different masses. When ions are accelerated in an electrical field and all acquire the same kinetic energy, the speed that they acquire, and therefore the time that they take to cross the vacuum tube, depends on the mass of the ionised molecule, which may be inferred from the time taken in this crossing.

The second milestone was the development of methods that ionised intact proteins which, due to their size, had not been susceptible to ionisation until then, since the high energy required for ionisation ended up altering or destroying the protein itself. In 1987, Köichi Tanaka presented a new method of analysis (soft laser desorption) which transferred to molecules the energy required to ionise them without breaking their fragile chemical bonds. Tanaka was awarded the Nobel Prize in Chemistry in 2002 for this discovery, together with John B. Fenn, for "the development of methods for identification and structural analyses of biological macromolecules". It was not long before mass spectrometers based on this type of analysis were developed. However, some changes had to be made to the method before MALDI-TOF MS was finally developed. In MALDI-TOF MS, desorption of ions is promoted by a matrix that absorbs laser energy and partially transfers it to the molecules being studied. Although these modifications improved the technique by making it simpler and more sensitive, those responsible for these improvements, Michael Karas and Franz Hillenkamp, were not co-recipients of the Nobel prize. This generated significant controversy. The contributions of these authors enabled macromolecules and biopolymers to begin to be studied using MS. This opened up new fields of application for this technology.

In its nearly 30-year history, MALDI-TOF MS has been used to quantitatively and qualitatively analyse proteins of various origins. Initially, it was applied to previously isolated proteins or small sets of proteins. However, thanks to technical advances in terms of both instrumentation and tools for computerised data analysis, it may now be used to study large groups of proteins.

The possibility of beginning to study complex proteins, not just small peptides, greatly broadened the potential fields of application of MS. One of them, the identification of microorganisms, was quickly applied to clinical practice as the procedure was simplified and the software required to make use of the raw data provided by the spectrometer was improved.

MALDI-TOF mass spectrometry in Clinical Microbiology

Until the introduction of MALDI-TOF MS, bacterial identification, even with significant advances such as the creation of miniaturised identification panels and the automation of their inoculation and reading, continued to draw support from the methods developed by traditional bacteriology. Virtually all identification systems continued to be based on the fermentation of sugars and their detection through the change in pH generated, the metabolism of other substrates and the production of different metabolites and enzyme activities that could be detected by chemical methods. All these methods had several limitations:

- They required bacterial growth, which in most cases meant an incubation period of at least 16–18 h from inoculation to reading.
- They showed problems of identification in all those microorganisms with difficulty growing in the liquid media used for inoculation of these panels, as well as in microorganisms with limited biochemical and enzyme activity.
- It was necessary to consider the margin of error deriving from the fact that individuals from the same species could react differently to different substrates.

These limitations, while known and assumed, were more obvious when, in view of the discrepancies observed in some studies between MALDI-TOF MS and conventional identification, 16 S rRNA sequencing demonstrated that correct identification matched that provided by MALDI-TOF MS in the vast majority of cases.[2]

In this regard, MALDI-TOF MS has obvious advantages:

- Regardless of the potential for identifying microorganisms directly from some samples, which is addressed in another section, even very limited or early plate growth yields reliable identification in a short period of time, thus saving at least those 16–18 h of growth in biochemical identification systems.
- Analysis of the protein profile of the microorganism on the 2–20 kD spectrum, where most ribosomal proteins are located, offers for the vast majority of bacterial species a specific profile, which distinguishes them from all others with a reliability similar to that offered by 16 S rRNA sequencing.

To this must be added the fact that the introduction of this technology has greatly expanded the range of genera and species that may be identified reliably with a method likely to be used routinely. This has even led to a re-evaluation of the role as pathogens of microorganisms that, due to the difficulty of identifying them by classic methods, were very likely underdiagnosed.[3,4]

Anhalt and Fenselau suggested using MS to identify microorganisms as early as 1975.[5] The first study demonstrating the efficacy of MALDI-TOF MS for the identification of microorganisms based on complete cells was published twenty years later.[6]

An article that was likely key to raising awareness of the possibilities of MALDI-TOF MS among specialists involved in the diagnosis of infectious diseases was published in 2009.[7] It studied more than 1600 isolates, including Gram-positive and Gram-negative aerobic and anaerobic bacteria, and obtained a rate of correct identifications of 95.4%. Already this article raised a matter that later proved important for the practical utility of this methodology: the availability of sufficiently extensive microorganism databases, both qualitatively (number of genera and species included) and quantitatively (the authors demonstrated that the likelihood of correct identification was higher for those microorganisms for which at least ten different profiles had been entered in the database).

This marked the start of the rapid expansion of the use of this technology in Clinical Microbiology. The first publication in Spain, in 2010, showed a rate of correlation with the conventional methodology, on a species level, of 100% in Gram-positive bacteria and 87.7% in Gram-negative bacteria.[8] Since then, the number of publications regarding different aspects of the clinical use of MALDI-TOF MS, especially the identification of microorganisms, has risen exponentially.[7–12]

Identification of Gram-negative bacteria using MALDI-TOF

Studies have demonstrated that its efficacy in the identification of enterobacteria and other Gram-negative bacteria is excellent, including both the most common Gram-negative bacteria in clinical practice and other Gram-negative bacteria that are less common or more complicated to identify with the classic methodology