Maldi-tof advantages and disadvantages

Abstract

Rapid diagnosis of microbial pathogens or infections in clinical laboratories is decisive to guarantee accurate therapy and efficient patient care. Although the conventional method, which is mostly based on biochemical testings and long incubation procedures, are precise and sensitive; they are rather slow. Until recently, matrix-assisted laser desorption-ionization-time of flight (MALDI-TOF) has emerged as a powerful technique for identification of microorganisms in clinical microbiology. MADLI-TOF has the advantage of identifying bacterial or fungal species directly on the culture plates as fast as 10 to 15 minutes in a few simple steps. The principle behind MALDI-TOF is based on mass spectrometry and “ soft” ionization technique. Depending on the time of flight of each pathogen, the characteristic spectrum will be analyzed and displayed via the inbuilt software. This review describes the advantages and limitations of MALDI-TOF. It also compares the identification efficacy of bacteria between MALDI-TOF and the culture methods. There are several comparative studies between the conventional techniques and the MALDI-TOF systems in terms of reliability and accuracy of their methods.

Keywords: MALDI-TOF, diagnostic microbiology, spectrometry

Introduction

Microbiological identification of pathogenic bacteria and fungi has used to be performed by conventional methods which involved long process of culturing and biochemical/phenotypic testings. Although cultures are powerful methods in retrieving pathogens, multiplying a single viable pathogen in an appropriate medium logarithmically, it is time consuming and the phenotypic

tests could delay over 24 to 48 hours. In the circumstances, such as bacteremia, which requires a rapid diagnosis and treatment, delay in adequate management could cause mortality to rise by 10 to 20% as shown by Kumar et al.. Length of hospital stay and the price of admission equally decrease with early identification of the etiology of sepsis. New diagnostic methods have been developed, and they do not depend on bacterial or fungal growth and are effective even when the pathogens are not viable. The methods employing nucleic acids are already in clinical use; however, although faster than cultures, they demand technical time and at least 6 to 8 hours of work by a dedicated professional. A major advance is the use of proteomic studies for rapid diagnosis – as fast as 5 to 15 minutes – of etiology of infections, and it is represented by MALDI-TOF.

THE IMPORTANCE OF MALDI-TOF

Mass Spectrometry

Mass spectrometry is an analytical technique that measures ionized chemical compounds based on their mass to charge (m/z) ratio (). By combining the technique of ionization and biomolecular detection from mass spectrometry (), matrix assisted laser desorption ionization-time of flight (MALDI-TOF) was developed and is widely used in microbiological diagnosis (). There are three main units that compose the MALDI-TOF spectrometer: ion source, mass analyzer, and detection device (). The purpose of the ion source is to ionize molecules thus transferring them into the subsequent gas phase (). The mass analyzer unit aids in ion separation based on mass to charge ratio (). Lastly, detection device is there for monetization of separated ions ().

Principles and methodologies

In order for MALDI-TOF to begin processing the biological material (ex: a colony or a blood culture concentrate), sample is placed on a plate containing polymeric matrix (). Next, irradiation occurs through the medium of a laser (). During the process, laser would also vaporize and ionize molecules within the sample (). Afterwards, aspiration of those molecules into the vacuum tube will transport them to the detection device (). Depending on which bacterial or fungal specie is being examine, the time of flight will also be different (). Lastly, the computerized database of MALDI-TOF will generate a chart with different peaks-providing results and interpretations all very quickly ().

Advantages

A major advantage of MALDI-TOF is its rapid turnaround time (<10 mins) and an overall 95% accuracy at the species level, enabling a faster, correct treatment for the patients (). Another benefit of the MALDI-TOF in microbiological diagnostic is its low-cost in supplies and technician processing time (). In addition, the application of “ soft” ionization in MALDI-TOF enables observation of ionized molecules with little to no fragmentation due to the fact that formed ions have low internal energy (). Moreover, MALDI-TOF has been successfully applied in microbial typing and identification at the subspecies level (). This demonstrates the potential of MALDI-TOF being an alternative for identification of resistance mechanisms such as the production of carbapenemases, and assisting with epidemiologic studies and taxonomical classification ().

Limitations

The greatest limitation of MALDI-TOF include low analytical sensitivity without prior culture and the discrimination of phyletically related microorganisms such as Shigella and Escherichia coli. Consequently, MALDI-TOF is not a tool suitable to detect a low amount of bacteria potentially presented in sterile samples such as cerebrospinal fluids and further standardization and optimization will be needed.

APPLICATIONS OF MALDI-TOF IN MICROBIOLOGICAL DIAGNOSTICS

Detection and Identification of Clinical Samples

As previously mentioned, the development of MALDI-TOF has revolutionized the routine identification of microorganisms in clinical microbiology. MALDI-TOF has introduced a more rapid, accurate, cost-savings, high throughput and efficient way in the identification techniques for both bacterial and fungal strains. It is capable of measuring species level of most gram-positive and gram-negative bacterial strains and offers routine identification of yeast isolates in a reliable and much quicker way than the conventional techniques.

Bacterial

Using the conventional diagnostic methods, biochemical and metabolic profiling of the bacterial infections in the body fluids would required one to two days to be completed. In the meantime, patients are administered broad-spectrum antibiotics, which could increase the prevalence of bacterial resistance and sometimes, an inappropriate treatment. Thus, a rapid,

reliable and cost effective methods for the diagnosis of potential microbial pathogens is needed in order to initiate an early and appropriate antimicrobial therapy. Many studies have shown that MALDI-TOF is the best diagnostic methods for early detection of bacteria in blood cultures, urinary tract infections (UTIs), cerebrospinal fluids, respiratory tract infections, and stool samples. In a comparison study with the conventional methods, MALDI was able to detect two and more uropathogens in the minimal processing time for the identification of urinary tract pathogens for diagnosis of UTIs. It is a costly and time consuming process for the diagnosis of enteric bacterial pathogens in the laboratory as stool sample culture and identification usually take up to 3-5 days.

Comparative study performed by He et al. (2010) found that the procedure of identifying stool pathogens by MALDI-TOF, from smear preparation to reporting of the final results, was completed within 30 mins. Thus, turnaround time is improved by shortening 2-3 days. Another clinical emergency that is vital for rapid and early diagnosis is bacterial meningitis. Direct detection of bacteria causing meningitis in cerebrospinal fluids are increasingly being performed with MALDI-TOF in a routine setting with good results (Segawa et al., 2014). It has also been used for rapid identification of atypical, Gram-negative environmental organisms and respiratory tract pathogens which chronically infect patients with cystic fibrosis (Alby et al., 2013 ; Baillie et al., 2013). Recently, a very novel application of MALDI-TOF MS was shown by Guembe et al. (2014) who reported that MALDI-TOF MS can perform better than conventional culture methods in diagnosis of catheter-related bloodstream infections.