EDUCATIONAL COMMENTARY – IMPLEMENTATION OF MALDI-TOF MS

Educational commentary is provided through our affiliation with the American Society for Clinical Pathology (ASCP). To obtain FREE CME/CMLE credits, click on Earn CE Credits under Continuing Education on the left side of the screen.

Learning Objectives

On completion of this exercise, the participant should be able to

- describe the principle of matrix-assisted laser desorption / ionization, time-of-flight mass spectrometry (MALDI-TOF MS) and the use of this technology in clinical microbiology;
- explain the steps involved in setting up a MALDI identification;
- summarize the impact of MALDI on patient care, including the advantages and limitations;
- review the process for implementing MALDI and integrating this testing into the microbiology laboratory; and
- describe the components of MALDI quality management.

Introduction

Traditionally, the clinical microbiology laboratory has relied on an organism’s biochemical properties to aid in identification. There has been significant evolution in this process as testing moved from the conventional test tube to packaged “mini-tube” systems and ultimately to automation. A revolutionary new technology is changing this identification process from biochemical reaction to proteomic analysis, or protein differentiation. Matrix-assisted laser desorption / ionization, time-of-flight mass spectrometry (MALDI-TOF MS, or MALDI) is now being utilized worldwide for the identification of bacteria and fungi. MALDI is only cleared by the U.S. Food and Drug Administration (FDA) for identification of organisms directly from colonies growing on media, although some laboratories are using MALDI in laboratory-developed protocols to identify organisms directly from positive blood culture bottles. This technology is rapid, accurate, and is changing the paradigm of the clinical microbiology laboratory. Advances in the automation of this technology, development of comprehensive databases, and FDA clearance of commercial systems have enabled widespread use in many laboratories.1,2

The MALDI Process (Principle and Setting up a MALDI)

The principle of MALDI technology is based on the irradiation of biomolecules by a laser, which causes them to ionize and accelerate in an electric field. The ionized biomolecules enter a vacuum flight tube
and travel to an ion detector at a speed dependent on their mass to charge ratio, with small ions arriving first, followed by larger ions. The ion detector measures the time of flight and generates a mass spectrum. When a microorganism is tested, the mass spectrum generated provides a proteomic “fingerprint”; this pattern of proteins can then be compared against an extensive database of known microorganisms to identify the organism. The MALDI software then generates a numerical score value that provides information on the accuracy of the identification, known as the identification or confidence percentage. An important element of MALDI-TOF MS is its ability to measure large microbial proteins by keeping them intact during ionization. This is achieved by applying a chemical matrix to the organism and using a soft laser desorption process with gentle ionization to prevent the molecules from being fragmented.1

MALDI identification begins with picking an isolated colony and applying it to a target slide (or target plate, depending on the manufacturer). This is referred to as “spotting” the target slide. The isolate is then overlaid with the matrix solution and allowed to dry. The matrix forms a crystalline lattice with the organism and facilitates the transfer of proteins from the target surface to the gas phase. Organisms with thick cell walls or excess capsular material, such as yeasts, may require an extraction method; a formic acid solution can be added to the organism before overlaying with the matrix solution.3 Once the target slide has been prepared and air-dried, it is placed into the instrument for analysis. See Figure. A single sample can be completed within 5 to 7 minutes, although most laboratories will fill the target slide and run as a batch. A target slide with 96 spots can be completed in approximately an hour.

Impact of MALDI

Advantages
The introduction of MALDI testing into the clinical microbiology laboratory has positively affected patient care in several ways. The greatest advantage to using MALDI is the speed with which organisms can be identified. Biochemical and phenotypic testing often require overnight incubation to arrive at organism identification. MALDI can now identify an organism in a matter of minutes and requires a single colony.3 In addition, MALDI is more successful in the identification of fastidious bacteria which are often biochemically inert. This often saves the laboratory from having to send these organisms to a reference laboratory. Anaerobic bacteria have always been a challenge to identify, often requiring tools such as 16S rRNA gene sequencing or gas or liquid chromatography. MALDI is now the method of choice for the identification of anaerobes.2

For maximum patient benefit, the MALDI information needs to be acted upon. Thus, the laboratory must ensure that the culture report is promptly updated when the MALDI identification becomes available.
Although MALDI only provides identification, and antimicrobial susceptibility testing still needs to be performed, the clinician can initiate empiric therapy via the hospital antibiogram or other references to select the most appropriate treatment. The laboratory must work closely with the antimicrobial stewardship committee when adding MALDI to their testing menu. The quick turnaround time has also been helpful to infection control practitioners, alerting them to pathogens that are a potential threat to public health so they can institute effective measures to prevent spread.

Another significant advantage of MALDI is cost savings. Although the initial instrument cost is high and maintenance expenses are substantial, reagent costs are low. A 2015 study that compared MALDI with biochemical testing showed that MALDI reduced reagent costs by as much as 87.8%. High throughput and ease of use are other advantages of this technology.

**Limitations**

The benefits of MALDI are numerous, but the laboratory should be aware of some limitations when considering adopting this technology. Occasionally, MALDI results are of a low identification or confidence percentage and retesting using MALDI or a supplemental method may be necessary. The majority of identification failures result from spots that are too thick or too thin. Other reasons for failure...
EDUCATIONAL COMMENTARY – IMPLEMENTATION OF MALDI-TOF MS (cont.)

are that the isolate being tested is not included in the database or that an isolate generates an identification not consistent with the colony morphology.

Some organisms have spectral profiles so similar to one another that they cannot be accurately distinguished. These include *Escherichia coli* and *Shigella* species, which is a significant problem, because *E coli* is one of the most common isolates recovered. *Streptococcus pneumoniae* and *Streptococcus mitis* are other examples of organisms MALDI cannot differentiate. The laboratory will need to add appropriate phenotypic tests to distinguish these isolates.

Other limitations include difficulties identifying tiny or mucoid colonies. MALDI may fail to identify these and the organisms may require gene sequencing. Finally, the high initial cost and the expense of service agreements make MALDI a financial burden early on.

Process for Implementing MALDI

The successful implementation of MALDI requires ample planning and preparation. Implementation includes physical preparation, decisions about the best way to transition from current identification methods, workflow approach, integration with other instrument testing systems, verification studies, and staff training.

Physical Preparation

Physical preparation for the MALDI includes space requirements, electrical requirements, temperature considerations, and safety essentials. When considering a location for the MALDI instrument, the laboratory needs to consider space and electrical requirements. The location of the instrument should provide access to the internet to enable remote access and interfacing of the MALDI middleware with the laboratory information system or other instrument systems. If the instrument is a benchtop model, the bench must be able to support the weight and be free of vibration. As the MALDI instrument generates heat during operation, proper airflow must be provided to ensure there is no temperature buildup, and room temperatures must be able to be adjusted as necessary.

Safety considerations include biohazards and chemical hazards. The microbiology laboratory should follow its own biosafety procedures for the appropriate handling and manipulation of cultured microorganisms. Analysis of unknown mycobacteria or filamentous fungi requires a tube extraction procedure to ensure that the isolate is nonviable before testing on MALDI. Although the microbiology laboratory is used to dealing with potential biohazards, the chemical hazards introduced with MALDI are novel. The chemicals used for sample preparation are irritants and corrosives and must be used and stored appropriately. If the laboratory uses reusable target slides, these must be cleaned using toxic chemicals and should be handled in a chemical fume hood.
Rollout: Stepwise (Staged) vs Laboratory-Wide

When implementing MALDI, it is important to consider how the laboratory will transition from its current identification testing system. There are two approaches: a stepwise or staged rollout and a laboratory-wide rollout. The approach taken often depends on the verification performance. There are advantages and disadvantages to each approach. The staged rollout transitions testing in a stepwise fashion (e.g., gram-negative bacteria first, then gram-positive bacteria). It takes longer to complete the transition with this approach, but it allows for the laboratory to ease into the new method. The downside to the stepwise approach is that for a period of time there are different identification methods in use, which can lead to inconsistencies in reporting. The laboratory-wide approach forces the laboratory to make a clean break from its former method. Because all staff are using the same identification method, this approach eliminates some inconsistencies in reporting. However, the MALDI learning curve can be steep, and this approach can be difficult. Each laboratory must determine the approach that best fits its needs.

Workflow: Centralized vs Decentralized

After choosing a rollout method, the next important decision in integrating MALDI is whether to use centralized or decentralized workflow. A centralized workflow involves a small group of technologists who are responsible for most or all of the MALDI testing: spotting, adding reagents, preparing target maps (identifying the spot location of each patient isolate), running the instrument, and interpreting results. This approach limits the number of technologists who perform MALDI and improves consistency. It also helps maximize resources and minimize costs by batching runs and ensuring that all spots on the target slide are filled before loading. The limitation of a centralized workflow is that batch testing delays the reporting of results. In addition, the passing of culture plates from the bench technologists to the MALDI operators may increase error, especially when mixed cultures are being evaluated. In a decentralized workflow, each technologist prepares his or her own target slides and performs MALDI testing. This approach helps reduce clerical and isolate-testing errors because a single technologist manages every step of the process. A limitation of this approach is that more technologists must be MALDI trained and maintain competence. There is also the potential for throughput issues and wastage if target slides are not filled. Again, each laboratory must determine which approach is best for its own workflow.

Integration with Other Instruments (Antimicrobial Susceptibility Testing)

Another consideration for implementation of MALDI is integrating the test results with other instrument systems, especially automated antimicrobial susceptibility testing (AST) instruments. This may involve linking the MALDI instrument with the AST instrument. If this is not possible, the laboratory may interface the MALDI with the laboratory information system. The laboratory may also decide to set up autoverification for isolates with a high confidence value. This will speed up the turnaround time and
EDUCATIONAL COMMENTARY – IMPLEMENTATION OF MALDI-TOF MS (cont.)

allow for the release of the identification as soon as MALDI produces the results. The laboratory must use this tool carefully with strict protocols to prevent the release of erroneous results.³

Verification Studies
The laboratory must perform a verification study before reporting any results from the MALDI instrument. The purpose of the verification study is to substantiate the manufacturer’s performance specifications, including accuracy and precision. Accuracy is confirmed by comparing the identification results from MALDI with those obtained by a reference method. The laboratory will run the MALDI in parallel with the existing identification system and compare the results. It is recommended that a minimum of 30 isolates per organism group be used. While these should consist primarily of fresh patient isolates, quality control (QC) strains can be used as a supplement. Precision testing includes testing 10 isolates, three times a day for 3 days to demonstrate reproducibility.³ The laboratory should formulate its verification plan and establish the protocol, acceptance criteria, and how discrepancies will be resolved. After the study is completed, the results must be reviewed with calculations of accuracy and precision. The laboratory needs to determine the acceptability of their results. If the data are not within the acceptability limits, the laboratory will need to expand or repeat a portion of the study.

Training Staff
Staff training will depend on whether the laboratory uses a centralized or decentralized workflow. Initially, a few key staff might be trained and responsible for performing the verification study. This will ensure that the protocol, documentation, and data generation are consistent. Performing MALDI is not a complex process, but there is a learning curve for new technologists, especially for spotting. This technique requires practice to achieve the appropriate application of organism to generate a successful analysis; poor technique can lead to calibration failure and thus increase instrument downtime. New users will often apply an isolate to more than one spot until they have mastered their technique.

Quality Management
Once MALDI testing has been implemented in the laboratory, a quality management program should be developed to ensure a platform for continuous improvement. This includes QC procedures, instrument maintenance, staff competency, and participation in an external proficiency testing program.

Quality control testing with MALDI includes internal QC using a calibration standard specified by the manufacturer. A calibrator must be included with each run to check specific parameters; it must result in correct organism identification when compared with the reference database. If the calibration standard does not meet the necessary specifications, the run will not continue. External QC is also performed: positive and negative controls are tested each day of patient testing.³ ⁶ Positive controls are specific
strains recommended by the manufacturer; negative controls contain reagents only spotted directly on the target slide. Any QC failures (internal or external) must be documented and investigated.

Another aspect of QC relates to the reporting of the information from the MALDI. The laboratory must determine the level of identification that it will provide—genus, species, or complex level. This level of reporting may vary depending on the specimen source or clinical relevance of the isolate. It is important for the laboratory to develop guidelines for the bench technologists to ensure consistency in reporting.

Instrument maintenance for MALDI is similar to that for other automated instruments used in the clinical laboratory. The laboratory needs to develop a maintenance program that includes daily checks recommended by the manufacturer as well as preventive maintenance and software updates. A plan must be in place for backup methods to be used during downtimes. The laboratory must maintain records of all services performed.

Ongoing quality assurance activities guarantee that the MALDI system will continue to perform at a satisfactory level. These actions include ongoing QC with regular supervisory review, competency assessment exercises for staff, investigating unusual results to determine if the MALDI has produced an error, correlating identification with clinical findings, responding to any provider concerns, and participating in an external proficiency testing program.

**Conclusion**

MALDI-TOF MS is a revolutionary technology in clinical microbiology for rapid identification of bacterial and yeast isolates. This technology is less labor-intensive than current identification methods and provides accurate results in minutes from a single isolated colony. Although the initial capital investment is high, this technology provides significant cost savings for the laboratory owing to its ease of use, rapid turnaround time, and minimal cost of testing each sample.

The future of MALDI looks bright! Although this technology is FDA cleared for the identification of bacteria and yeasts from culture media, testing is under way to analyze specimens directly. Some laboratories have developed testing directly on positive blood culture bottles to provide rapid identification of sepsis-causing organisms. Additional applications for MALDI include detection of antimicrobial resistance. MALDI is also being incorporated into total laboratory automation systems that consist of automated sample processing, plating, incubation, culture reading with digital imaging, and spotting to MALDI slides. MALDI is proving to be an excellent tool to accelerate diagnosis and improve patient care.
References


4. MALDI-TOF: Advancements in Technology for the Clinical Microbiology Laboratory. API/ASCP Educational Commentary. API 2014 3rd Test Event.


© ASCP 2018