MALDI-TOF MS for the Diagnosis of Infectious Diseases
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BACKGROUND: First introduced into clinical microbiology laboratories in Europe, MALDI-TOF MS is being rapidly embraced by laboratories around the globe. Although it has multiple applications, its widespread adoption in clinical microbiology relates to its use as an inexpensive, easy, fast, and accurate method for identification of grown bacteria and fungi based on automated analysis of the mass distribution of bacterial proteins.

CONTENT: This review provides a historical perspective on this new technology. Modern applications in the clinical microbiology laboratory are reviewed with a focus on the most recent publications in the field. Identification of aerobic and anaerobic bacteria, mycobacteria, and fungi are discussed, as are applications for testing urine and positive blood culture bottles. The strengths and limitations of MALDI-TOF MS applications in clinical microbiology are also addressed.

SUMMARY: MALDI-TOF MS is a tool for rapid, accurate, and cost-effective identification of cultured bacteria and fungi in clinical microbiology. The technology is automated, high throughput, and applicable to a broad range of common as well as esoteric bacteria and fungi. MALDI-TOF MS is an incontrovertibly beneficial technology for the clinical microbiology laboratory.

MALDI-TOF MS is being rapidly embraced by laboratories around the globe. This review, based in part on previously published work (1, 2), provides a historical perspective on this new technology. Modern applications in the clinical microbiology laboratory are reviewed with a focus on the most recent publications in the field. Identification of aerobic and anaerobic bacteria, mycobacteria, and fungi are discussed, as are applications for testing urine samples and positive blood culture bottles. The strengths and limitations of MALDI-TOF MS applications in clinical microbiology are also addressed.

History and Development of Commercial Systems

The notion of using mass spectrometry for identification of bacteria was proposed in 1975 (3), though at the time it was not possible to analyze intact proteins because they would have been fragmented in the process. Technology for the analysis of intact macromolecules was invented in the 1980s, allowing intact protein analysis. In 1985, Koichi Tanaka described a “soft desorption ionization” method using ultrafine metal powder and glycerol that enabled mass spectrometric analysis of biological macromolecules, for which he was awarded the Nobel Prize in Chemistry (4). Around the same time, Franz Hillenkamp and Michael Karas reported soft desorption ionization using an organic compound matrix (5), for which the term matrix-assisted laser desorption ionization (MALDI) was coined. Advances in information technology/computer science and development of well-validated comprehensive databases of mass spectra representing diverse types of bacteria and fungi ultimately enabled automation of MALDI-TOF MS and associated data analysis, delivering a tool for the clinical microbiology laboratory.

The most recent developments have been the commercialization and regulatory approval of MALDI-TOF MS for routine bacterial and fungal identification in clinical microbiology laboratories. In the past year, 2 systems, the VITEK® MS (bioMérieux Inc.) and the MALDI Biotyper CA System (Bruker Daltonics Inc.), have been cleared by the US Food and Drug Administration (FDA) for identification of cultured bacteria and, in the case of the former system, yeast. Although each includes a mass spectrometer, software, and database, all 3 components, including the list of microorganisms cleared for identification, are unique to each system. Bruker’s mass spectrometer is a desktop instrument, whereas bioMérieux’s is a larger floor model. Numeric scores are reported on different scales and are not directly comparable.

Bruker’s system is the most studied in terms of publications and evaluations in multiple laboratories. Development began in 2005; in 2008, Mellmann et al. pub-
lished a study demonstrating that by constructing their own database of mass spectra, they could accurately identify nonfermenting gram-negative bacterial isolates (6). A year later, Seng et al. published a prospective evaluation of Bruker’s commercial mass spectral database for identification of 1660 bacteria routinely isolated in their laboratory, reporting correct identification of 95% and 84% to the genus and species levels, respectively, in 6 min/isolate (7). Over the past 5 years, upgraded analysis software and increasingly inclusive and better curated mass spectral libraries have become available. Although the FDA-cleared list of organisms is currently limited to gram-negative bacteria, RUO libraries (e.g., RUO MALDI Biotyper Reference Library v3.3.1.2, Security Relevant library v1.0) are available.

In 1998, AnagnosTec began development of a microbial mass spectral database called SARAMIS, used with Shimadzu’s AXIMA Assurance mass spectrometer (Shimadzu). In 2010, bioMérieux acquired AnagnosTec and changed the system’s name to VITEK MS RUO. bioMérieux developed a new database, operating software, and analysis algorithm referred to as VITEK MS. The FDA-cleared list of organisms includes aerobic and anaerobic gram-negative and gram-positive bacteria, as well as yeast. Lastly, bioMérieux developed the VITEK MS Plus system that includes use of the FDA-cleared database in conjunction with the more comprehensive VITEK MS RUO software and database.

MALDI-TOF MS has been rapidly developed for use in clinical microbiology laboratories; iterative improvements continue to improve its performance for microbial identification. These changes can make it confusing to interpret the literature. Software, databases, and mass spectrometers must be considered, along with cutoff values applied for accepting identifications, sample preparations, comparators, and organism types studied.

MALDI-TOF MS systems have, in general, initially focused on identification of commonly isolated gram-negative and gram-positive bacteria; current databases may be less comprehensive vis-à-vis less common organism types, such as certain anaerobes, mycobacteria, and fungi. Efforts are underway to enhance and/or build databases for identification of esoteric organisms, and with some exceptions (8), MALDI-TOF MS is proving useful for their identification. Although databases are becoming progressively more complete, due to changing nomenclature and ongoing description of new species/genera, regular and ongoing database updates will be imperative to provide quality microbial identification into the foreseeable future.

Users have the option to construct their own mass spectral databases by generating mass spectra using locally important strains and/or those not well represented in the commercial databases. User-constructed databases may be searched alongside commercial databases. Academic laboratories have engaged in database building to enhance the performance of commercially available systems. The Mayo Clinic Custom MALDI-TOF MS Library, for example, currently contains 1599 mass spectral entries representing organisms not adequately addressed by commercially available databases.

**How Does MALDI-TOF MS Work?**

In clinical microbiology, there are several preparatory methods for MALDI-TOF MS, including moving an intact bacterial colony onto the MALDI plate (with and without the addition of a formic acid solution), and preparatory protein extraction. The last method was historically used but, compared to direct colony testing, is not as user friendly for clinical microbiology applications and thus is generally reserved for processing hazardous or difficult-to-lyse organisms. Direct colony processing is easiest, fastest, and least expensive. A colony is “picked” from a culture plate to a “spot” on a MALDI-TOF–MS target plate. The addition of a formic acid solution to the MALDI plate may be used to improve the quality of the generated mass spectrum, which may be particularly helpful for certain types of organisms, such as yeasts (Fig. 1). After drying, the target plate is placed in the mass spectrometer’s ionization chamber (Fig. 2).

In MALDI (matrix-assisted laser desorption ionization), a matrix (e.g., α-cyano-4-hydroxycinnamic acid dissolved in 50% acetonitrile and 2.5% trifluoroacetic acid) assists in the desorption and ionization of microbial analytes through the energy of a laser. The matrix isolates and protects them from fragmentation by the laser. As a result of being “shot” by the laser, microbial and matrix molecules are desorbed, with the majority of energy being absorbed by the matrix, converting it to an ionized state. Through random collision in the gas phase, charge is transferred from matrix to microbial molecules. The ionized microbial molecules are accelerated, based on mass-to-charge ratio, into a TOF (time-of-flight) mass analyzer, a tube under vacuum. Ions travel toward an ion detector, with smaller analytes reaching the detector first, followed by progressively larger analytes. A mass spectrum is generated, representing the number of ions of a given mass impacting the ion detector over time.

**How Does the Analysis Work?**

For clinical microbiology applications, highly abundant microbial proteins such as ribosomal proteins are the main contributors to the generated mass spectrum, although specific proteins are not identified and their mass and abundance are merely profiled. In general, mass spectra are unique to individual organism-types, with peaks specific to genera, species, and strains. The mass spec-
trum of the test isolate is compared to a database of reference spectra or deconvoluted spectra to determine relatedness to spectra in the database; the most closely related organisms are identified with a value provided as to the level of confidence in identification. Depending on how high the value is, the organism may be identified at the family, genus, or species level. A variety of algorithms are used for database comparison. For example, the Biotyper system uses pattern recognition and generates a score ranging from 0 to 3.000 on the basis of the presence or absence of mass spectral signals (peaks) in the test isolate matching database entries, and the presence of peaks in the test isolate not present in database entries. According to the manufacturer’s criteria for their RUO application, a score of $\geq 2.000$ represents species-level, a score of $1.700–1.999$ a genus-level, and a score of $\leq 1.700$ no identification. System users have found that lower score cutoffs may be adequate for identification of certain groups of organisms; extensive verification by the end user is required before adopting this approach. For the MALDI Biotyper CA System, a score of $\leq 1.999$ represents no identification. The VITEK MS RUO system database is searched using 1 of 2 algorithms. One is a pattern matching process, referred to as a reference spectra approach. In the second approach,
mass spectral entries are grouped together as “Super-Spectra,” representing spectra of conserved mass signals derived from multiple individual isolates of a particular organism group, with those signals weighted according to their specificity for species, genera, and/or families. The output is a confidence value, ranging from 0% to 100%. With the VITEK MS system, analysis is not directly based on pattern recognition comparing mass spectra. The mass spectral entries of individual isolates are computationally sorted into weighted bin matrices generating “Advanced Spectra Classifiers.” The bin pattern of test isolates is compared against the commercial weighted bin matrix. The weighting algorithm weights bins according to their importance. For example, bins that are highly specific for individual species receive a higher weight than do those that are moderately species specific. All 3 strategies function well. Closely related species with similar patterns and a small number of distinguishing peaks, such as Streptococcus pneumoniae and Streptococcus mitis groups, may be better (though not perfectly) distinguished using the VITEK MS strategy than the others (9, 10). Nevertheless, spectral profiles of some organisms are so similar to one another (e.g., Escherichia coli and Shigella species) that their differentiation using available algorithms is unreliable with any of the aforementioned strategies. This is a particular challenge because E. coli is one of the most frequent organisms encountered in clinical microbiology laboratories; MALDI-TOF MS users must use alternate or additional strategies (e.g., lactose fermentation, quick indole) to distinguish E. coli from Shigella species. MALDI-TOF MS does not currently accurately differentiate all species within certain groups of organisms, such as the Enterobacter cloacae complex (11), Burkholderia cepacia complex, and Streptococcus bovis species group. Likewise, accurate differentiation of all species of Acinetobacter may be challenging. Neisseria polysaccharea may be misidentified as Neisseria meningitidis using the Biotype system (12). It is not yet known whether discrimination of some closely related organisms will be possible with improved databases and/or software. Misidentifications may occur if such details are not considered during data analysis. The aforementioned limitations aside, most organisms are either correctly identified or yield a low score/percent, indicating that identification
has not been achieved; the latter typically implies no “match” in the database, but can occur due to technically poor preparation. In the next section, select studies of MALDI-TOF MS for bacterial and fungal identification are reviewed, with a focus on those recently published.

Performance of MALDI-TOF MS for Identification of Aerobic Bacteria

MALDI-TOF MS performs at least as well as, if not better than, automated biochemical identification for commonly encountered bacteria and yeast. We initially compared the Biotyper system (v2.0 software and library) and the BD Phoenix Automated Microbiology System for identification of 440 common and unusual gram-negative bacilli. The MALDI-TOF MS correctly identified 93% and 82%, whereas the biochemical system correctly identified 83% and 75% of these gram-negative bacilli to the genus and species levels, respectively (13). We further evaluated the Biotyper system (v2.0 software and library) using 217 clinical isolates of staphylococci, streptococci, and enterococci, 98% of which were correctly identified to the genus level (14). McElvania Tekippe et al. evaluated the Biotyper for identification of 239 aerobic gram-positive organisms using direct on-plate testing with formic acid. Using a score cutoff of ≥1.700 for genus-level identification, 92% of the gram-positive organisms were identified (15). Lau et al. evaluated the Biotyper system (v3.0 software and library V.3.1.2.0) for identification of 67 “difficult-to-identify” bacteria; 75% and 45% of the difficult-to-identify bacteria studied were correctly identified to the genus and species levels, respectively, with 4 misidentified (16). Hsueh et al. evaluated the Biotyper system for identification of 147 isolates of aerobically growing gram-positive organisms, including Nocardia species, Listeria monocytogenes, Kocuria species, Rhodococcus species, Gordonia species, and Tsukamurella species (17). All 8 Rhodococcus equi were correctly identified. All 15 Kocuria species yielded a top match to the correct species, either Kocuria kristinae or Kocuria marina, although due to low scores only 27% of Kocuria species were identifiable to the species level. Likewise, all 39 L. monocytogenes isolates studied yielded a top match to L. monocytogenes, but due to low scores only 90% were identifiable to the species level. With the exception of Nocardia nova and Nocardia otitidiscaviarum, Nocardia species were not identified, nor were Tsukamurella or Gordonia species. Schultheiss et al. evaluated the Biotyper system (database v3.1.2.0) for identification of gram-positive bacilli (18). Using the manufacturer’s interpretative criteria and an initial collection of 190 isolates, 85% and 87% of the gram-positive bacilli studied were identified to the genus level with on-plate formic acid testing and formal extraction, respectively, compared to 72% with direct colony testing. For some isolates, multiple species had scores ≥2,000; these included Corynebacterium aurimucosum and Corynebacterium minutissimum, Corynebacterium simulans and Corynebacterium striatum, Lactobacillus casei and Lactobacillus johnsonii, and L. monocytogenes, Listeria ivanovii, and Listeria innocua. Reducing the species cutoff to 1.700 increased the percentage of isolates identified. These investigators also compared identification of 215 clinical isolates of gram-positive bacilli by MALDI-TOF MS using on-plate formic acid testing and a species and genus cutoff of 1.700; 87% and 79% of the gram-positive bacilli studied were identified to the genus and species levels, respectively. The authors proposed an algorithm combining MALDI-TOF MS with nucleic acid sequence analysis for identification of gram-positive bacilli that includes score cutoff values lower than those recommended by the manufacturer and covers the most frequently found genera and species. In our own work, we found that 87% of 92 non-diphtheriae Corynebacterium species could be identified using a species-level cutoff of ≥1.700, with the exception of C. aurimucosum being misidentified as C. minutissimum (19).

Multiple evaluations of the VITEK MS system (database v2.0) have been published. Richter et al. evaluated 965 routinely encountered Enterobacteriaceae and showed that VITEK MS correctly identified 97% and 84% of the Enterobacteriaceae studied to the genus and species levels, respectively (20). Manji et al. showed that the VITEK MS correctly identified 91% and 78% of 558 non-Enterobacteriaceae gram-negative bacilli to the genus and species levels, respectively (21). Branda et al. evaluated the VITEK MS system for identification of 226 isolates of fastidious gram-negative bacteria; 97% and 96% of the fastidious gram-negative bacteria studied were accurately identified to the genus and species levels, respectively (22). Rychert et al. evaluated the Vitek MS system for identification of 1146 aerobic gram-positive bacteria and showed that 93% were correctly identified to the species level, with an additional 3% identified only to the genus level because of a split identification with multiple matching species within the same genus (including Listeria monocytogenes with matches to L. monocytogenes, Listeria ivanovii, and Listeria welshimeri) (23). Karpanoja et al. compared the MALDI Biotyper and VITEK MS IVD systems for identification of viridans group streptococci using 54 type strains and 97 blood culture isolates (24). The Biotyper and VITEK MS systems yielded correct species-level identification for 94% and 69% of the type strains and correctly classified 89% and 93% of the blood culture isolates to the group level, respectively.

Although MALDI-TOF MS is able to accurately identify Francisella tularensis and Brucella species, the Biotyper library does not contain them and so will not identify these organisms; use of Bruker’s “Security Rele-
tant” database enables Brucella species and F. tularensis identification (8).

Performance of MALDI-TOF MS for Identification of Anaerobic Bacteria

MALDI-TOF MS has become the method of choice for identification of anaerobic bacteria, replacing 16S rRNA gene sequencing and gas–liquid chromatography. Many clinical microbiology laboratories have not had tools available to identify anaerobic bacteria; MALDI-TOF MS provides a tool to do this, which enables increased routine identification of anaerobic bacteria, likely informing interpretation of the clinical significance and predicted susceptibility of anaerobes.

There have been several recent studies examining MALDI-TOF MS for identification of anaerobic bacteria. Jamal et al. reported species-level identification of 89% and 100% of 274 routinely isolated anaerobic bacteria (enriched in Bacteroides fragilis) using the Biotyper DB Update–v3.3 with direct on-plate testing and the VITEK MS v1 system/v1.1 database, respectively (25). Hsu et al. evaluated the Biotyper (v3.0 software) for identification of 101 anaerobic bacteria and showed that using on-plate formic acid testing and a cutoff of ≥1.700 improved the rate of accurate identification compared to direct on-plate testing and manufacturer-recommended cutoff scores (26). We evaluated a diverse collection of 253 clinical anaerobic isolates using the Biotyper system (v3.0 software and library v3.3.1.0), on-plate formic acid testing, and a user-supplemented database; 92% and 71% of anaerobic bacteria were correctly identified to the genus and species levels, respectively (27). Barreau et al. analyzed 1325 anaerobes using MALDI Biotyper (v3.0 software) and a species cutoff of ≥1.900 with direct on-plate testing and showed that 100% and 93% of the isolates were correctly identified at the genus and species levels, respectively (28). Finally, Garner et al. evaluated the VITEK MS system (database v2.0) using 651 anaerobic isolates and reported that 93% and 91% of anaerobic bacteria were correctly identified to the genus and species levels, respectively (29).

Performance of MALDI-TOF MS for Identification of Mycobacteria

As with identification of anaerobic bacteria, identification of mycobacteria has been a challenge; historically it has been done using biochemical testing, DNA probes, high-performance liquid or gas liquid chromatography, and/or gene sequencing. MALDI-TOF MS is proving a useful tool for identification of mycobacteria with a few limitations, such as the inability to differentiate members of the Mycobacterium tuberculosis complex and some closely related species such as Mycobacterium chimaera and Mycobacterium intracellulare or Mycobacterium mucogenicum and Mycobacterium phocaicum. Although Mycobacterium abscessus and Mycobacterium massiliense may be challenging to differentiate, Teng et al. did so using cluster analysis of spectra generated by the Biotyper system (30). MALDI-TOF MS is easier, less expensive, faster, and more accessible to routine clinical microbiology laboratories than traditional strategies for mycobacterial identification, which will likely make it the method of choice for their identification in the near future.

Testing of mycobacteria by MALDI-TOF MS requires special processing to kill tested organisms, disrupt clumped cells, and break open cell envelopes. Databases for mycobacterial identification are under development. Balada-Llasat et al. evaluated 178 mycobacterial isolates grown on solid and/or broth medium and processed using heat inactivation, treatment with ethanol, and mechanical disruption, as recommended by Bruker, and showed that the Biotyper system (Mycobacteria database v1 and software v3.0) was able to identify 98% and 94% of the Mycobacterium species studied to the genus and species levels, respectively (31). Mather et al. tested 2 protein extraction protocols using vortex mixing with silica beads and heat or ethanol killing preparatory to testing using the MALDI Biotyper and Vitek MS RUO platforms; the Biotyper database was augmented with mass spectral entries from 123 clinical Mycobacterium strains (32). Of 198 clinical isolates tested, 95% of the Mycobacterium species studied were correctly identified with the Biotyper system and augmented database, 79% with the Bruker Mycobacterium Library 2.0 database, and 94% with the Vitek MS RUO system.

Performance of MALDI-TOF MS for Identification of Fungi

In addition to bacteria, MALDI-TOF MS can identify yeasts. Although older studies used preparatory extraction for yeasts, many laboratories use the same direct on-plate formic acid testing strategy used for bacteria (Fig. 1) (33).

Dhiman et al. evaluated the Biotyper system (library v3.0) using a species level cutoff of ≥1.800 for identification of 138 common and 103 unusual yeast isolates and reported 96% and 85% accurate genus- and species-level identification, respectively, using protein extraction (34). Lacroix et al. showed that the Biotyper system with protein extraction and the manufacturer’s species-level cutoff identified 97% of 1383 routinely isolated Candida isolates (35). Westblade et al. evaluated the Vitek MS system (database v2.0) for identification of 852 yeast isolates and found that 97% and 96% were identified to the genus and species levels, respectively (36). Pence et al. compared the VITEK MS (IVD Knowledgebase v2.0) and Biotyper (software v3.1) for identification of 117
yeast isolates using on-plate formic acid testing (37). VITEK MS correctly identified 95% of the isolates, whereas the Biotyper correctly identified 83% of the isolates using a species-level cutoff of ≥1.700. Hamprecht et al. compared the Biotyper (v3.0 software, v3.0.10.0 database, species-level cutoff ≥2.000) and VITEK MS (V2.0 knowledge base) systems for identification of 210 yeasts using on-plate formic acid testing (38). The VITEK MS system identified 96% of the isolates; the Biotyper system identified 91% of isolates. De Carolis et al. created an in-house library using spectra from 156 reference and clinical yeast isolates, generated with a fast sample preparation procedure involving suspending a single colony of yeast in 50 μL of 10% formic acid, vortex mixing, and using 1 μL of the lysate for analysis (39). Using their database and processing, they identified 96% of 4232 routinely isolated yeasts using a species-level cutoff of ≥2.000 and the Biotyper system (software v3.0). Rosenvinge et al. showed that the Biotyper system identified 88% of 200 yeast isolates to the species level using on-plate formic acid testing and a species cutoff of ≥1.700 (40). Mancini et al. compared the Biotyper system (library v3.0) with protein extraction and Vitek MS (v1.2.0) with on-plate formic acid testing for the identification of 197 yeast isolates (41). The rate of correct identifications at the species level was comparable using the commercial databases (90% vs 84%) and improved to 100% using the Biotyper system with an in-house-developed database.

Schulthess et al. evaluated Bruker’s Filamentous Fungi Library 1.0 (42). Molds were grown in a broth medium for 24–48 h and subjected to protein extraction. First, they studied a clinical strain collection of 83 nondermatophyte, nondematous molds and showed that 78% and 54% were identified to the genus and species levels, respectively. Reducing the species cutoff to ≥1.700 improved identification to the species level to 71%. They then prospectively tested 200 consecutive clinical mold isolates and were able to identify 84% and 79% to the genus and species levels, respectively, using a species cutoff of ≥1.700. Lau et al. developed an alternate extraction procedure for molds and constructed their own database comprising 294 individual isolates representing 76 genera and 152 species (43). To extract proteins, a small piece of mold was excised and subjected to protein extraction with the addition of zirconia-silica bead processing. They then challenged their database with 421 clinical isolates using the Biotyper software, and demonstrated accurate genus- and species-level identifications in 94% and 90% of isolates, respectively, with no misidentifications.

Theel et al. developed a dermatophyte library and showed that when used in conjunction with the Biotyper library v3.0 and protein extraction, it identified 93% and 60% of 171 isolates to the genus and species levels, respectively (44). De Respinis et al. developed an in-house database and performed MALDI-TOF MS with an AXIMA Confidence mass spectrometer (Shimadzu Biotech) and MALDI-TOF MS Launchpad 2.8 software (Shimadzu Biotech) for identification of dermatophytes; of 141 clinical isolates tested, 96% were correctly identified (45).

**Laboratory Work Flow and Cost Associated with MALDI-TOF MS**

In the past, identification of bacteria and fungi has been a challenging, multistep process, individualized by organism type. Students of clinical microbiology have been meticulously trained to interpret colony and Gram-stain morphology of bacteria growing on solid media as a prelude to selecting appropriate further testing using quick biochemical tests such as catalase and oxidase, manual biochemical tests, automated biochemical tests, and broad-range sequencing. With MALDI-TOF MS, colonies are accurately identified in minutes, without a priori knowledge of microorganism type. And because it doesn’t matter whether a bacterium or yeast is tested, with the exception of safe handling of microorganisms that can be hazardous in the laboratory, the complex decision-making process classically surrounding identification of bacteria or fungi growing on solid media is obviated. Because only a small amount of organism is required, testing can be performed from single colonies on primary culture plates. Tests for screening for some enteric pathogens may be eliminated. Gram-staining colonies may not always be needed because this information is no longer required. For some organisms (e.g., *Staphylococcus aureus*), rapid tests performed at the bench, will remain. DNA sequencing expenses are avoided for enteric organisms, waste disposal is decreased, and QC and laboratory technologist training and labor for replaced and retired tests eliminated.

Seng et al. recently published over a decade of experience in routine identification of clinical bacterial isolates, including 40 months using MALDI-TOF MS and 91 months using phenotypic identification (46). MALDI-TOF MS and phenotypic identification identified 36 and 19 species/10000 isolates, respectively. Additional phenotypic identification was required for 4.5 and 35.2/10000 isolates with MALDI-TOF MS and primary phenotypic identification, respectively. Additionally, compared with phenotypic identification and sequencing, MALDI-TOF MS reduced the time for identification by 55- and 169-fold and cost by 5- and 96-fold, respectively.

Our experience has been that following implementation of MALDI-TOF MS into our reference laboratory for bacterial identification, we eliminated automated biochemical-based microbial identification.
and have so far reduced the number of tubed biochemical set–based identifications from 4668 to 987/year. To date, we have halved the number of isolates requiring 16S rRNA gene sequencing; through progressive database improvements, this number continues to decrease. Following implementation of MALDI-TOF MS for yeast identification, the associated supply costs decreased from $30 525 to $5400/year as a result of the elimination of germ tube and rapid assimilation of trehalose and API (analytical profile index) strip tests; this was associated with a decrease in turnaround time for identification by 1–5 days, depending on the species. Implementation of MALDI-TOF MS also reduced the number of tests on which our staff must maintain competency and therefore our yearly competency evaluation burden. Following implementation of MALDI-TOF MS for dermatophyte identification, the associated supply costs decreased from $20020 to $2340/year, with a turnaround time savings of 1.5 days.

Antimicrobial Susceptibility Testing Using MALDI-TOF MS

Antimicrobial susceptibility is not directly determined with the above-described strategy. By rapid organism identification, intrinsic antimicrobial resistance characteristics of particular species (or typical susceptibility of the identified species based on local antibiograms) may guide therapy. Because some resistance–associated factors (e.g., β-lactamases) are proteins, and MALDI-TOF MS detects proteins, it might be intuited that antimicrobial resistance–associated proteins would be detected directly. Unfortunately, at least as currently performed, this has been challenging. For example, although β-lactamases are highly active, they are expressed at low concentrations; further, their molecular weights are similar to those of other bacterial proteins and there are hundreds of types of β-lactamases of similar masses. Because MALDI-TOF MS may provide insight into strain typing, and some strains are more or less likely to be resistant to certain antimicrobial agents, strain typing may infer an association with antimicrobial resistance. MALDI-TOF MS may be applied as part of a functional assay to measure β-lactam degradation by β-lactamases; in this case, it is not proteins but antimicrobial compounds and their chemically modified counterparts that are measured (47). This approach requires specific system configuration and incubation to allow antibiotic hydrolysis, and it applies only to resistance mechanisms associated with antibiotic degradation (48–50). Another strategy to detect antimicrobial resistance is to grow an organism for a short period of time (e.g., ≤3 h) in the presence of an antibiotic of interest and isotopically labeled amino acids (51). If the organism is resistant to the antibiotic, it will incorporate isotopically labeled amino acids, increasing protein masses and leading to mass shifts of their corresponding peaks in the profiled spectra (52). Further details on detection of antibiotic resistance using MALDI-TOF MS can be found in the recent review by Hrabak et al. (53).

Direct Testing of Clinical Samples

Although direct testing of clinical samples is not generally feasible because of the high limit of detection of MALDI-TOF MS, urine may be tested directly owing to the high numbers of bacteria present in clinically infected urine (54). Urine flow cytometry may be used to screen out negative urines, with MALDI-TOF MS reserved to test the positive urine samples (55, 56). Urine must be processed before MALDI-TOF MS testing. Limitations include the inability to reliably identify polymicrobial infection (55) and impairment of database matching caused by urinary proteins such as α-defensins (57). Demarco et al. recently described a dialfiltration method using desalting, fractionation, and concentration for preparing urine samples before MALDI-TOF MS analysis (58). Other sample types, such as cerebrospinal fluid (59, 60), may also be tested directly.

Testing of Positive Blood Culture Bottles

MALDI-TOF MS can be used for rapid identification of microorganisms growing in blood culture bottles. The positive bottles may be subcultured to solid media and growth tested by MALDI-TOF MS after a short incubation period (e.g., 2–4 h). Alternatively, blood culture bottles may be tested directly. This application requires preparatory processing because blood culture bottles contain macromolecules from blood and growth media. Processing may be accomplished using differential centrifugation and washings, selective lysis of blood cells, serum separator tubes, or filtration; commercial processing using the Sepsityper (Bruker Daltonics) is available (61–64). Although results are valid when obtained, yield is generally not as good as direct colony testing, with a higher percentage of gram-negative than gram-positive organisms being identified in most studies (65). As with direct testing of urine, not all organisms present in polymicrobial infections are necessarily identified (66). Both the Biotyper and VITEK MS systems have been applied to positive blood culture bottles. Chen et al. used the Sepsityper to evaluate the Vitek MS IVD and Biotyper systems for bacterial identification in 181 monomicrobial blood culture bottles (67). Genus/species level identifications were provided using the Biotyper and VITEK MS systems in 98%/82% and 93%/81% of cases, respectively. Twenty-one blood cultures were composed of 2 bacterial species. The Vitek MS IVD system identified only the majority species in all 21, whereas the Biotyper system identified both of the 2 species...
with >1.6 scores in 5 mixed cultures in the “Top 10 matched pattern choices” (67).

MALDI-TOF MS performed on positive blood culture bottles can rapidly identify probable contaminants or suggest complementary diagnostic testing in the case of pathogen detection (68). By testing of positive blood culture bottles using MALDI-TOF MS, time to organism identification is reduced by a day or more (69). A limitation is that antimicrobial susceptibility is not provided. To overcome this limitation, MALDI-TOF MS has been tied with direct antimicrobial susceptibility testing of positive blood culture bottles (70), improving time to optimal therapy (71). MALDI-TOF MS on positive blood culture bottles is rapid (estimated at 30–45 min (72, 73) albeit not as fast as direct colony testing); such testing is typically batched with testing performed on a limited number of occasions throughout the day.

Typing Using MALDI-TOF MS

Because MALDI-TOF MS can provide accurate species-level identification, using it for subspecies level identification, and therefore strain typing, has been proposed. For example, Mencacci et al. used MALDI-TOF MS for typing of Acinetobacter baumannii (74), and Josten et al. used MALDI-TOF MS for typing of S. aureus (75). Such applications remain to be fully explored.

Limitations of MALDI-TOF MS

MALDI-TOF MS has limitations. Unlike publically available sequence databases such as GenBank, MALDI-TOF MS databases are proprietary. Low identification percentages for some organisms may be improved by addition of mass spectral entries of underrepresented species or strains (to cover intraspecies variability), but doing so may be beyond the capability of some laboratories. Because of low scores/percentages, repeat testing may be needed. Growth on some media may be associated with low scores/percentages. Tiny or mucoid colonies may fail identification; tiny colonies may be more rapidly identified using 16S rRNA gene sequencing than MALDI-TOF MS. Refined criteria are needed to distinguish closely related species and differentiate them from the next best taxon match. For certain species, genus- or species-specific (including lowered) cutoff values may be appropriate. Newer mass spectrometry methods may enable separation of microorganisms at deeper taxonomic levels than MALDI-TOF MS (76). Laboratory errors may occur as a result of colony inoculation in erroneous target plate locations, testing of impure colonies, smearing between spots, failure to clean target plates, or erroneous data entry into laboratory information systems. There is a learning curve involved in applying ideal colony amounts to target plates. Instrument cost must be considered; laser, software, hardware, or mass spectrometer failure can occur, necessitating an appropriate service plan. Although MALDI-TOF MS is generally reproducible, there are sources of variability, including the mass spectrometer (different types as well as individual instruments), matrix and solvent composition, preparation methods, technologist training and competence, culture conditions (such as media, colony age, and temperature), and biological variability; QC strategies are being defined. Well-isolated colonies must be tested; if colonies are not well isolated, they may represent more than one organism, and the minority species may be undetected. A CLSI guideline on MALDI-TOF MS is being prepared. Because of the ease of use of this technology, technologists may be tempted to work up everything, including clinically nonsignificant isolates, leading to confusion on the part of clinicians receiving such reports and the potential for inappropriate patient treatment. At the same time, because of the ease of use of MALDI-TOF MS, technologists may lose their skills in visually identifying colonies. Because MALDI-TOF MS may yield different (generally more accurate) identification than current systems, reporting of organisms with which healthcare providers are unfamiliar may lead them to ignore potentially clinically significant results or to over treat clinically insignificant results.

Future Perspectives

MALDI-TOF MS is enabling adoption of total laboratory automation in clinical microbiology laboratories. Total laboratory automation features automated sample processing, plating, incubation, plate reading using digital imaging, and spotting to MALDI-TOF MS plates. Early growth detection by digital imaging combined with MALDI-TOF MS results in faster detection of microorganisms compared to conventional techniques (77). We can anticipate new applications of MALDI-TOF MS, such as using antibodies to capture analytes of interest (78). Finally, MALDI-TOF MS will have profound effects on microbiology education of medical technologists, clinical microbiology laboratory directors, and other management staff, medical students, residents, and fellows. For medical students, residents, and fellows who don’t practice in the laboratory, it is time to deemphasize conventional biochemical-based microbial identification and to focus instead on interpretation of results of MALDI-TOF MS-based identification alongside quick biochemical tests and biochemical reactions important to microbial pathogenesis.

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