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Charting Uncharted Territory: a Review of the Verification and Implementation Process for Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry (MALDI-TOF MS) for Organism Identification

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Abstract

The advent of matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) for organism identification is changing the paradigm for laboratory diagnosis of infectious diseases. As a result of the improved speed, quality, and affordability provided by MALDI-TOF, laboratories have begun transitioning to this method for routine organism identifications. Currently, there are two manufacturers that produce MALDI-TOF MS systems for organism identification and that are actively pursuing Food and Drug Administration clearance, and both databases are currently labeled research use only. However, there is no formal guidance on what is specifically required to verify the performance of these systems. The goal of this document is to establish some basic guidance for those laboratories seeking to verify MALDI-TOF. To this end, product selection, aspects of verification, and implementation are discussed.

Introduction

The advent of matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) for organism identification is changing the paradigm for laboratory diagnosis of infectious diseases. This technology uses spectrophotometric analysis of whole-cell protein fingerprints to rapidly generate organism identifications (1,2). Results provided by MALDI-TOF have been shown to be very accurate and, on a per test basis, cost only a fraction of what other identification methods do (3,4). Despite a price tag that exceeds several hundred thousand dollars, laboratories can expect a rapid return on investment. For example, a laboratory providing microbiology for an average-size hospital (~500 beds) would likely achieve a return on investment in less than 3 years (unpublished data).

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As a result of the improved speed, quality, and affordability provided by MALDI-TOF, laboratories have begun transitioning to the method for routine organism identifications. Currently, there are two manufacturers that produce MALDI-TOF MS systems for organism identification. Bruker Daltronics, Inc. (Leipzig, Germany), offers the MicroFlex with BioTyper software, and bioMérieux (Marcy-l'Etoile, France) offers the Vitek MS, which is a combination of hardware purchased from AnagnosTec and the Saramis database. Although the companies are actively pursuing Food and Drug Administration (FDA) clearance, both databases are currently labeled research use only (RUO).

Currently, there is no formal guidance on what is specifically required to verify the performance of these systems. The purpose of this review is to discuss the process of validating and implementing MALDI-TOF MS for clinical use to call upon the experiences of those who have been through the process. Ultimately, the goal of this document is to establish some basic guidance for those looking to verify MALDI-TOF and to begin developing a formal guidance document.

Product Selection

As of the writing of this review, there are no FDA-cleared MALDI-TOF products available for bacterial identification. Although the instruments themselves are not specifically labeled, the software packages that generate the identifications are currently labeled RUO. Consequently, the regulatory

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environment, as well as the challenge of validating large organism databases, has thus far prevented widespread adoption of the technology in the United States. Due to a more progressive regulatory environment, MALDI-TOF has been widely adopted in Europe, and numerous publications have demonstrated its utility. As a result, many laboratories in the U.S. are electing to invest in this technology despite the hurdles mentioned above.

As previously mentioned, the two primary manufacturers of MALDI-TOF instruments for bacterial identification are bioMérieux with the Vitek MS and Bruker Daltonik with the Microflex Biotyper. The two instruments are roughly equivalent in cost but differ functionally in some important ways. These differences are not necessarily good or bad but may factor into the decision as to which instrument is best for a given laboratory.

The most obvious difference between the instruments is overall size. The Bruker instrument fits on a benchtop, whereas the bioMérieux instrument is considerably taller but does have a similar footprint. This size difference is a product of the longer flight tube present in the bioMérieux instrument. It is unclear at this point whether the difference in tube length results in performance differences, but the longer tube does result in a longer time to achieve proper vacuum pressure in the bioMérieux instrument. With that said, the inconvenience of the longer pressurization time may be mitigated by the fact that the bioMérieux instrument is capable of being loaded simultaneously with 4 target plates while the Bruker instrument can process only one target plate at a time. The implications of this for workflow may be that one could load four simultaneous targets in the bioMérieux instrument and thus only have to pressurize the instrument once, whereas to run four successive target plates on the Bruker instrument would require four separate pressurizations. Therefore, laboratories may want to consider workflow and volume when selecting an instrument.

The target plates themselves are another difference between the manufacturers. bioMérieux has relied most heavily on a disposable target plate, and Bruker relies on a polished steel reusable plate. The benefit of a disposable plate is that no cleaning is required. Not only is the cleaning process time-consuming (~30 minutes), but it also necessitates the use of hazardous chemicals. Conversely, the disposable targets add to the consumable costs of identification.

At this time, there have not been enough head-to-head comparisons published to really compare the performances of these instruments. The publications that do exist suggest that their performances are roughly equivalent. One study by Cherkaoui and colleagues (3) compared the instruments over 720 continuous clinical isolates and found that both instruments performed very well and were superior to conventional methods. It appeared as though the Bruker instrument produced a greater number of high-confidence identifications (Bruker, 94.4% high-confidence identifications versus bioMérieux, 88.8%). Conversely, the bioMérieux instrument generated only 3 (0.5%) misidentifications whereas Bruker had 6 (0.9%). A more recent study compared the two instruments and found that globally the Bruker instrument was superior to the Saramis database but that for certain organisms, such as differentiating *Salmonella enterica* serovar Typhi, Saramis was superior (5). One other study by Veloo and colleagues (6) compared the efficacies of these instruments for the identification of anaerobic bacteria. They found that overall the instruments performed equally well when isolates not present in the database were excluded from the analysis. When all data were included, bioMérieux's instrument appeared to be superior. Specifically, the bioMérieux instrument performed better for anaerobic Gram-positive cocci, but the Bruker instrument performed better for *Bacteroides fragilis* group organisms. Others have also compared the performance of these instruments for identifying anaerobes (7).

These studies raise an important point regarding any analysis of a MALDI-TOF instrument for bacterial identification. The most critical aspect of instrument performance is actually the quality of the database entries. Interestingly, it has comparatively little to do with the hardware. What this means for the consumer is that these products will continue to improve over time as the databases grow and become more robust. Due to this evolution, one must be careful about drawing conclusions from studies using out-of-date databases. It is also worth noting that the bioMérieux product is a combination of a software package purchased from AnagnosTec (Golm, Germany) and hardware purchased from Shimadzu Corporation (Kyoto, Japan). We know very little about the changes that bioMérieux made to the instrument following the acquisition of these two components and the relabeling of them under the bioMérieux title "Vitek MS." It will be important to review future publications, but it is likely that bioMérieux will continue to develop and improve the database going forward. For additional information on these instruments, see references 8-13 and 14-18, which assess the performances of the Bruker and the bioMérieux systems independently.

One important consideration for a MALDI-TOF purchase is the ability to interface with susceptibility software systems and/or laboratory information systems. The bioMérieux Vitek MS is able to interface with the Myla middleware, which in turn interfaces the MALDI-TOF results with a bioMérieux susceptibility result. It is unclear at this time whether the software will allow laboratories using other susceptibility systems to interface the Vitek MS result.

Bruker has developed relationships with Becton Dickinson (BD), as well as Siemens, so it is likely that laboratories will be able to interface the Bruker results with BD's Epicenter software and with the Siemens' Microscan.

With all of that said, there is an interesting and unfortunate twist to the MALDI-TOF interface discussion. Because the software packages that produce identifications on the MALDI-TOF are labeled RUO, some manufacturers may elect to delay interfacing results with their FDA-cleared susceptibility instruments for legal and regulatory reasons. As a result, users may have to wait for the MALDI-TOF software to be FDA cleared before they will be able to interface their systems. In practice, managing MALDI-TOF data manually is doable, but does make for a more cumbersome workflow than many laboratories are accustomed to with automatically interfaced instruments. In addition,

manual data entry introduces a possible opportunity for transcription error.

Verification

Philosophy of verification study design

One of the strengths of MALDI-TOF is that the identification databases include a large number of organism entries. In contrast to growth-based identification systems, new organism entries can be added to databases with ease, allowing tremendous growth and improvement in database quality over time. The high number of entries (thousands) poses a problem for verification, however. It is not practical for laboratories to verify every database entry with multiple replicates, so verification studies should be designed to capture the majority of clinical isolates encountered by the laboratory. Laboratories demonstrating the accurate performance of MALDI-TOF for isolates commonly encountered in their institution should feel confident that they have verified the method for clinical use against those isolates. How laboratories handle isolates not included in the verification study is a matter of personal opinion. Some feel that if the database performed well in the initial verification the method should be considered verified for all isolates. Others feel that isolates not included in the verification study must be verified with alternative methods when encountered during clinical testing. This subject will be discussed in detail below

How many isolates are necessary to verify MALDI-TOF MS for organism identification?

MALDI-TOF instruments are only as good as the databases they use to identify organisms. In the case of both bioMérieux and Bruker, the RUO databases consist of thousands of entries. A common question asked is, "How many isolates are needed to verify MALDI-TOF performance?"

Currently, there is no formal guidance on how to verify MALDI-TOF performance. Therefore, there is no recommendation on the number of isolates that must be tested, and this is left to the discretion of the laboratory's director. It would seem prudent to ensure through verification that isolates from routine culture workups are included in the verification, including Gram-negative and Gram-positive organisms and yeast. Laboratories may wish to further divide the organisms into subcategories, such as Gram-positive rods versus Gram-positive cocci or *Enterobacteriaceae* versus glucose non-fermenters versus fastidious Gram-negative organisms.

A survey of 6 laboratories that have verified MALDI-TOF for clinical use shows that the consensus among them was to include more than 1,000 isolates (Table 1). In all cases, the database verifications were performed and analyzed by organism category. In other words, data for Gram-negative and Gram-positive organisms were considered separate verifications. Although the technical procedures for identifying a Gram-positive versus a Gram-negative bacterium do not differ (i.e., MALDI-TOF does not require prior knowledge of Gram stain morphology to identify an organism), performance has been shown to differ by organism category (19-22). Therefore, verification analysis will be more meaningful if Gram-negative organisms are considered separately from Gram-positive organisms.

Analyzing organism categories separately may also allow laboratories to revise manufacturer-recommended interpretive criteria. For example, the Bruker Biotyper system recommends that organism scores greater than 2.0 are sufficient to report to the species level. Scores between 1.7 and 1.99 provide confidence to the genus level, and scores below 1.7 are not reliable. In our laboratory verification, we found that all scores above 1.7 for Gram-positive cocci, as well as *Candida* spp., were correct to the species level. In contrast, we found significant species level discrepancies with the glucose non-fermenting Gram-negative rods with scores below 2.0. Therefore, we revised our interpretive cutoffs for Gram-positive cocci and yeasts, but not for the Gram-negative rods. A recent publication by Tekippe et al. (23) also found that a high percentage of Gram-positive isolates with MALDI-TOF scores above 1.7 were correct.

The Tekippe study notwithstanding,

Table 1. MALDI-TOF validation approach of 6 U.S. microbiology laboratories

Laboratory	No. of isolates	Evaluate and analyze organism categories separately	Primary comparator method	Discrepant analysis	Database update verification	Extraction method verification	Verify different media	Spot application method
1	>1,000	Yes	Biochemical	16S sequencing	Not done yet	Formic acid overlay	No	Toothpick
2	800	Yes	Biochemical	16S sequencing	Not done yet	Formic acid overlay and full extraction	Yes	Toothpick and swab
3	>1,000	Yes	16S sequencing	NA[a]	Live organism	Full extraction	Yes	Pipette tip and inoculating needle
4	>1,000	Yes	Biochemical	16S sequencing	No response	Formic acid overlay and full extraction	No response	No response
5	>1,000	Yes	Biochemical	16S sequencing	Not done yet	Formic acid overlay and full extraction	Yes	Toothpick
6	>1,000	Yes	Biochemical and 16S sequencing	16S sequencing	Not done yet	Full extraction	Yes	Toothpick

[a]NA, not applicable.

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most publications discussing the performance of the MALDI-TOF databases do not present their data in a way that allows the reader to draw conclusions about whether manufacturer-recommended cutoffs can be adjusted. However, in surveying the 6 laboratories mentioned in Table 1, nearly all have made similar observations and intend to alter manufacturer-recommended cutoffs. It is important to point out that this survey included only those using the Bruker MicroFlex with Biotyper software. No laboratories using the bioMérieux instrument opted to participate in this survey, and no publications have addressed the performance of the manufacturer-recommended interpretive criteria. Thus, no conclusions can be made regarding whether the bioMérieux cutoffs can be revised through verification.

Another consideration when designing the verification study is the diversity of the organism group. Laboratories will likely require more isolates to verify the Gram-negative organisms versus the Gram-positive organisms. This is because there is a greater diversity among clinically relevant Gram-negative organisms, and thus, a greater number of isolates may be required to complete verification of this organism category. Conversely, anaerobic and yeast organisms may not require as many isolates because the isolates encountered in the clinical laboratory are less diverse. An alternative approach would be to simply require that a given organism have a certain number of verification entries before it is accepted for use in the laboratory. This approach is similar to what the FDA is mandating for their studies but could require a number of isolates that would be too much for most laboratories to test. In addition, this approach would be problematic for infrequently encountered isolates. Laboratories validating their MALDI-TOF with this approach are requiring between 20 and 50 isolates for each species and a smaller number for rare organisms.

Laboratories electing not to verify each individual species may want to consider setting organism thresholds for inclusion in the verification process. Ideally, laboratories want their databases to be verified against organisms commonly encountered in their individual patient populations. Databases will therefore be verified against a representative subset of clinical isolates. However, if one were to take all clinical isolates as they were encountered in the laboratory verification, resources would be consumed by testing *Escherichia coli* and *Staphylococcus aureus*, as these are the most commonly encountered clinically relevant isolates. Rather, laboratories should set caps for individual genera and species and move on to other organisms when those thresholds have been met.

Comparator method and discrepant analysis

An important consideration for validating MALDI-TOF is the selection of a comparator method. Gene sequencing (in most cases 16S rRNA gene sequencing) is considered by most to be the gold standard for bacterial identification (Note that there are a number of organisms that the 16S rRNA gene cannot reliably identify, such as *E. coli* and *Shigella*, and *Streptococcus pneumoniae* and the *Streptococcus mitis* group). However, this method is slow and expensive and may not be readily available in all laboratory settings. Most laboratories other than larger reference laboratories have elected to use their current identification systems or techniques as the comparator method for MALDI-TOF verification. If MALDI-TOF and the conventional method agree, no further analysis is warranted and MALDI-TOF is considered to be correct. Discrepant identifications are resolved by a combination of additional biochemical or gene sequencing.

Through numerous publications and the personal experiences of others, it appears that MALDI-TOF is much more accurate than current conventional methods. Laboratories taking on MALDI-TOF verifications may find themselves trusting MALDI-TOF over their conventional methods. Indeed, several studies showed that most widely used commercial biochemical identification systems produce erroneous results for certain organism categories (3,22). This begs the question as to whether conventional methods can be trusted. Most laboratories feel that because these are FDA-cleared instruments, it is appropriate to compare MALDI-TOF results with those obtained by these instruments. Others have relied exclusively on 16S rRNA gene sequencing (Table 1).

Medium verification

Several studies have shown that the type of medium from which an organism is selected for MALDI-TOF identification can impact the quality of the result generated. In many cases, organisms can easily be selected from a blood or chocolate agar plate for identification. These media have been shown to produce very reliable identifications. However, selective and/or differential media are commonly used in the laboratory, and being able to perform identifications directly from these media improves turnaround time. Anderson and colleagues (24) assessed the impacts of solid media on the identification of bacterial isolates and found significant differences among the media and the percent identifications generated from them. For example, 95% of staphylococci were identified from sheep blood agar plates but only 75% from Columbia colistin-nalidixic acid (CNA). Importantly, though, different media were not associated with incorrect identifications, only a lower identification rate.

The Anderson study is the only systematic evaluation of MALDI-TOF performance with different media available in the literature. However, when asked, all surveyed laboratories who are using MALDI-TOF reported they have conducted similar internal studies. One such study tested a wider variety of media but found similar results. Greater than 70% of isolates were identified to the species level when tested from MacConkey agar, chocolate agar, *Burkholderia cepacia* selective agar (BCSA), CNA, mannitol salt agar (MSA), and Hektoen enteric agars. One hundred percent of isolates were identified from CNA, MSA, BCSA, and MacConkey agar (data not shown). Importantly, no incorrect identifications were produced in these studies.

The number of isolates tested in the above-mentioned studies was relatively small; however, the primary goal of these studies was not to reverify the database for each piece of medium. Rather, it was designed to assess the feasibility of attempting identifications from these various medium types. Laboratories performing these studies may want to set thresholds for accepting a particular medium for use in their laboratory. These thresholds should be set with the objective of maximizing a

technologist's efficiency. For example, if a given piece of medium produces an identification only 20% of the time, it is probably more efficient to simply subculture that organism to a blood agar plate and perform an identification the following day. The Anderson study and the unpublished data presented here suggest that most media are acceptable for routine clinical use, but performance should be evaluated in each individual laboratory.

Organism application method

The method by which a laboratory applies the test organism to the target plate may vary and should be verified if multiple methods are to be utilized. Common methods employed for application include a toothpick, a swab, a pipette tip, an inoculating loop, and a full ethanol/formic acid extraction. A number of studies have demonstrated the benefit of a full ethanol/formic acid extraction. This technique appears to be particularly useful for identifying mucoid Gram-negative and -positive bacteria and yeast. The method is cumbersome, however, which mitigates the benefit derived from the simplicity of the direct-smear method.

Due to the much simpler workflow of spotting an organism on a target with a toothpick or swab, most laboratories have elected to make these the primary application methods in lieu of full ethanol extractions. Laboratories should be wary of the method they select and should verify that a given technique will not void manufacturer warranties. The MALDI-TOF instrument could be damaged if incorrect materials are used and contaminate the flight tube. For example, MALDI-TOF reagent tubes and pipette tips must all be of a certain caliber of plastic to avoid contamination with plasticizer from the plastic.

The direct smear is the technique that has been most thoroughly evaluated in the literature (25-27). Although these studies show that the direct-smear method is effective, a significant number of Gram-positive bacteria and yeasts fail to be identified. A full ethanol/formic acid extraction has proven to be a very reliable way to identify these organisms, but it is labor-intensive and slower (3,28-30).

A limited number of studies have assessed the utility of a direct smear that is then overlaid with 1 µl of formic acid on the target plate. Once the formic acid has dried, 1 µl of matrix (α-cyano-4-hydroxy-cinnamic acid; Bruker Daltonic Inc., Billerica, MA) is added, as it would be for a normal direct smear. Tekippe and colleagues (23) found that when they analyzed 239 Gram-positive organisms, the formic acid overlay method was superior to the direct-smear method. Others have shown this method to yield similar results (9,10,31,32). For example, in their letter to the editor, Haigh et al. (31) improved the rate of identification by 10% when a formic acid overlay was employed. As the Tekippe (23) and Theel (9) studies showed, this method was particularly beneficial when applied to Gram-positive bacteria and yeast. In addition, Theel found that the more laborious full extraction method did not improve the scores that could be obtained with the direct formic acid overlay technique. This is slightly discrepant with what Matsuda et al. (10) found when they showed that full extraction identified 89.5% of staphylococci, while the on-plate extraction method yielded only 80.8% identification; these findings were statistically significant.

Laboratories will likely need to verify the full ethanol extraction method regardless of which smear method they choose. Despite the benefits of the formic acid overlay technique, there will still be occasional isolates (mucoid Gram-negative and Gram-positive bacteria, yeasts, etc.) that require a full extraction. However, the literature suggests that the formic acid overlay or "on-plate extraction" method is superior to the standard direct smear.

One thing of particular importance is the pattern of organism distribution after spotting. MALDI-TOF performs best when it is used to analyze a relatively smooth layer of evenly distributed organisms. The swab technique makes it very easy to pick a bacterial or yeast colony and place an even layer of the organisms to be identified. If organism chunks are placed on the target, MALDI-TOF may struggle to identify the isolate in question. Figure 1 provides some examples of how the different techniques apply organisms. These images were taken using the camera software present on the Bruker MicroFlex. An *S. aureus* strain was spotted using different techniques and then overlaid with α-cyano-4-hydroxy-cinnamic acid. Figures 1A and B show two different applications of *S. aureus* using a toothpick. Figure 1A is an example of the pattern generated by applying organisms with the side of the toothpick, while Fig. 1B shows the less even application that results when the tip of the toothpick is used. The chunks of organisms seen in Fig. 1B result in a less efficient MALDI-TOF analysis, and therefore, an even distribution is preferred. Figures 1C and D demonstrate two consecutive swab applications. Notice the even distribution of organisms, as well as the dilution effect that occurs with the second spot. This dilution effect can sometimes improve identification scores if the primary spot has too many organisms. Because it can sometimes be difficult to know how many organisms are required, some laboratories choose to spot organisms in duplicate or even triplicate to improve their chances of obtaining a high-quality identification. The risk with duplicate spotting is that MALDI-TOF may then

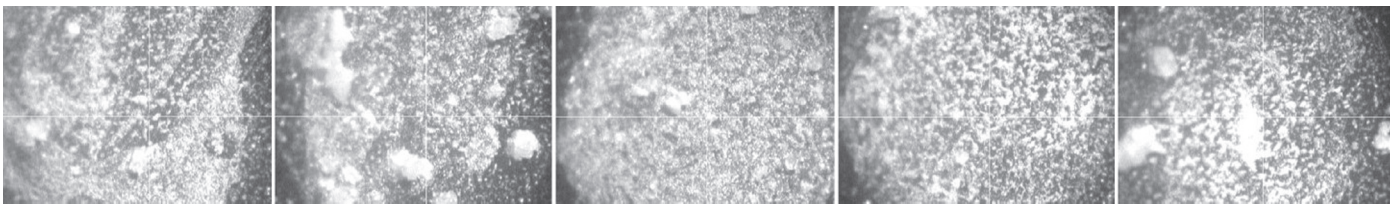

Figure 1. Images taken of *S. aureus* spotted by various application methods. Spotted organisms were overlaid with HCCA matrix. Organisms were applied with the side of a toothpick (A), the tip of a toothpick (B), a swab in a heavy concentration (C), a swab in a light concentration (D), and a pipette tip (E).

produce discrepant results for the same organism. This is rare but can be difficult to interpret. Lastly, Fig. 1E demonstrates organisms applied using a 10-µl pipette tip. The results appear to be very similar to those produced by toothpick application.

The toothpick/pipette tip method makes generating a perfectly smooth layer of organisms slightly more challenging for those who are unfamiliar with the method. However, over time, users become familiar with the technique, and MALDI-TOF run failures as a product of spotting error decrease dramatically (personal observation). There is no consensus as to what the best technique is. Each offers a unique set of advantages. The toothpick method is cheap and easier. There is a brief learning period, but after that period, "no peaks" incidents drop off dramatically. Further, technologists preferred the technique to the swab in our laboratory because they felt they had better control over the toothpick and could more readily select small colonies for identification. Nevertheless, as Fig. 1 demonstrates, the swab technique clearly produces an even layer of organisms.

In our laboratory, we elected to verify a duplicate spotting method for both the direct smear and the direct smear with formic acid overlay. Both the toothpick and swab methods were verified in this way. Our data were consistent with the findings of Tekippe and others in that we observed a significant improvement in identification scores with the addition of a formic acid overlay. Thus, we elected to use a duplicate-spot, formic acid overlay method for clinical testing. After 6 months of clinical use, a retrospective review of our data showed that the second spot was necessary and sufficient to identify approximately 5% of organisms. In other words, had we been using only a single spot (or the so-called "heavy spot"), we would have had to repeat identifications for only 5% of the isolates. We feel that the burden of performing all identifications in duplicate outweighs that of having to repeat identifications for 5% of isolates and plan to transition to single-spot testing (data not shown).

Incubation time and storage conditions

Microbiologists are accustomed to processing organisms for growth-based testing, which can be highly dependent on the growth phase to generate proper results. As a consequence, most manufacturers recommend that identifications be performed from freshly grown overnight cultures. One of the primary benefits of MALDI-TOF is that identification is largely independent of organism growth characteristics. Nonetheless, laboratories should verify that the growth phase does not play an important role in the quality of their MALDI-TOF results. Our laboratory studies demonstrated that MALDI-TOF produced very stable results out to 7 days of incubation. The verification study for incubation time was terminated at 7 days, so it is entirely possible that results would be reliable for longer incubation periods (data not shown). Tekippe and colleagues (23) looked at this question in a different way. They tested the stability of identification scores over several days of subculture and found that scores were lower on subculture days 3, 4, and 5 than they were on day 1.

Additionally, many laboratories store plates for some period of time following final culture results. One of the benefits of MALDI-TOF is that fresh colonies are not required for identification, so technologists can attempt identifications from these stored plates should a physician call and request additional workups. In our laboratory, we have found that this simple feature has greatly improved many technologist-physician interactions. Prior to MALDI-TOF, physicians would have to wait several days for additional identification results. With MALDI-TOF, we are able to satisfy nearly all requests within hours. Extended incubation is something that laboratories need to verify in order to ensure that identification quality is stable over time. Most laboratories store their plates for some time after culture workup is complete. Tekippe et al. (23) looked at MALDI-TOF identification of a subset of isolates stored for 5 days at 35°C, 4°C, and room temperature. They found that the identification efficiency did not differ significantly between the storage conditions and that the average scores for all were well above 2.0. Although not statistically significant, isolates stored at 4°C produced the best results, with >92% generating an acceptable species level identification.

Database update and reverification

Of the laboratories surveyed, only one had verified database updates. The database is the most critical component of MALDI-TOF identification performance. As a result, some form of quality control and reverification should be performed when databases are updated. How this is best accomplished is not established. The single laboratory that had updated their database had elected to reverify with a subset of live organisms. Laboratories may want to selectively choose their reverification panels to suit the changes in each database update. They may also want to select a wide variety of organisms to ensure that the update did not alter performance for organisms not directly present in the update. As with the overall verification process itself, there is no stated number of isolates required to reverify a database.

An alternative to testing live isolates would be to reanalyze previously collected spectra against the new database. Ideally, laboratories would test all spectra included in the original verification study. Although labor-intensive and potentially overkill, this would be the best way to ensure that the performance characteristics established in the original verification were not changed by the database update. The software capability to perform this type of reverification does not currently exist, but perhaps manufacturers will develop a system to automate the analytical process and allow laboratories to more easily accommodate database updates.

Miscellaneous considerations for MALDI-TOF verification study design

Validating a MALDI-TOF as a laboratory-developed test to serve as the primary system for organism identification is a massive undertaking, unlike most other verification studies conducted in the microbiology laboratory. As a result, there are a number of issues that must be considered that are not readily published in the literature. The following is a brief and admittedly data-poor discussion of some of these issues compiled from the experiences of those who have been through the verification process.

Isolate freezing

Unless laboratories intend to resolve discrepant results in real time, they will

want to freeze isolates that are included in the verification study for future testing. This will likely mean the storage of around 1,000 isolates. This requires not only a significant amount of freezer space, but also an organization system that allows easy retrieval of isolates.

Data management

Laboratories will likely verify multiple methods tested in duplicate or quadruplicate. They may wish to capture both primary and secondary scores. All of this adds up to thousands, maybe tens of thousands, of data points. Developing a robust organization system and having the staff to manage these data will be important to a successful verification study.

Consistency

These verification studies take a long time. To ensure consistency over the study, it may be prudent to limit the number of staff involved. This must be weighed against the fact that there is a learning curve for setting up MALDI-TOF targets. Easing staff into MALDI-TOF use prior to going live for clinical use will help to make for a smooth transition. Laboratories may want to consider a "soft start" prior to going live with MALDI-TOF for clinical isolates to give staff not involved in the verification a chance to become comfortable with the new technique.

Use for clinical care prior to verification study completion - In most cases, it becomes very obvious that MALDI-TOF is superior to conventional methods. Laboratories will come to this conclusion long before the verification study is complete but must resist the temptation to use a method that has not been fully verified on patient isolates.

Implementation of MALDI-TOF into Clinical Use

In the U.S., neither the Bruker nor the bioMérieux MALDI-TOF system is FDA cleared for clinical use. As a result, the number of laboratories that have adapted MALDI-TOF is small, and of the laboratories that have implemented MALDI-TOF, most are using the Bruker system. The following discussion will address the challenges laboratories face in implementing MALDI-TOF for clinical use. Unfortunately, there are very few data addressing the actual process of implementing the technology, and therefore, this discussion is based on the consensus of the relative few who have brought MALDI-TOF to clinical practice. Table 2 summarizes some of the pertinent implementation strategies employed by laboratories now using MALDI-TOF for patient testing. Further, the discussion is meant to be generalized to all systems but is based solely on the experiences of those using the Bruker system.

MALDI-TOF workflow

A significant benefit of MALDI-TOF organism identification is that it can be performed from a single, isolated colony. As a result, microbiologists no longer need to subculture colonies from mixed cultures prior to attempting identification with a growth-based system. This seemingly subtle change in practice in combination with more rapid results has some interesting implications for MALDI-TOF workflow.

First, to ensure that the intended colonies are being analyzed, the technologist who is working up a given culture ought to be responsible for spotting his or her own target plate. This may constitute a significant change for laboratories that have a central technologist dedicated to setting up and processing identification and susceptibility tests. The technologist who is responsible for primary spotting of the target plate, then, must also be responsible for documenting the location of each isolate. Because this process takes place separately from the MALDI-TOF instrument, the data must then be entered a second time into the MALDI-TOF software prior to starting the run. This makes for an inefficient process and one that is prone to clerical error. As Table 2 shows, a common approach is to have plate readers apply the organism to the target plate while having a central processing technologist perform the actual MALDI-TOF run. Ultimately, manufacturers will likely develop satellite software that will allow technologists to remotely enter their target plate data, but currently, this is not a functionality in widespread use.

Second, MALDI-TOF workflow does not lend itself to purity plate streaking in the same way that conventional methods do. This requires a significant adjustment for most microbiologists who are accustomed to having that quality check. Indeed, a weakness of MALDI-TOF is that isolate mismatch is not an uncommon occurrence. Laboratories should take special steps to ensure that isolate locations on target plates are carefully documented. It might also be prudent

Table 2. MALDI-TOF implementation approaches of 4 U.S. microbiology laboratories

Laboratory	Organisms reported	Billing for ID[a]	Spot application method	MALDI runs per day[b]	Person who spots targets	Person who runs MALDI	External QC[c]	No. of spots used for each ID	MALDI interfaced
1	All bacteria, *Candida* spp.	Yes	Toothpick	Multiple	Plate readers	Dedicated person	Positive and negative	2	No
2	All bacteria except anaerobes, yeast	No	Toothpick and swab	2	Plate readers	Dedicated person	Positive	2	No
3	All bacteria, yeast, dermatophytes	No	Pipette tip and inoculating needle	Multiple	Dedicated person	Dedicated person	Positive and negative	1	No
4	All bacteria	No	Toothpick	2	Plate readers	Dedicated person	Positive and negative	2	No

[a] ID, identification.
[b] Number of batched MALDI-TOF runs that are scheduled for a given day. Several target plates may be processed during each run. For example, laboratory 2 runs 4 target plates during each of its two MALDI runs for a total of 8 targets run during a single day.
[c] QC, quality control.

to require some minimal confirmatory testing to ensure organism identifications are valid. Manufacturers are working to provide automated solutions to this problem, but nothing is available at this time.

Third, in a limited review of our identification turnaround times, we found that MALDI-TOF was significantly faster than conventional methods. Our findings were consistent with those of previous publications (3,33). The improved turnaround time means that identifications are available much earlier than susceptibility information. An unexpected problem was observed, though, for susceptibility results generated by identification and susceptibility combination panels. In a number of cases, the susceptibility was delayed by the combination panel's inability to generate a valid identification. Thus, although the susceptibility result had been finalized, the instrument was waiting for the identification to release the results. Ultimately, laboratories utilizing MALDI-TOF for identification will transition away from combination panels, which will eliminate this issue. In the meantime, however, laboratories may want to adopt procedures that allow the MALDI-TOF result to be entered in lieu of a delayed biochemical result.

Quality control

Controlling the quality of MALDI-TOF results is a unique challenge. For the Bruker system, it is recommended that a calibration be performed. Because the quality of MALDI-TOF spectra is dependent on both the time and the space in which an analyte is tested, it is recommended that calibration be performed for each run. Because the calibration standard contains *E. coli*, it can also be used as an internal quality control, and because the calibration standards consist of purified proteins, they are pipetted onto the target and therefore truly represent the identification process. As a result, most laboratories also chose to perform an external positive control. Although the details of how this was performed varied from laboratory to laboratory, most laboratories chose to rotate the quality control organisms on a regular schedule. In addition, some laboratories include a negative control to ensure that reagents have not become contaminated (Table 2).

Resource utilization and staffing

There is no question that in many ways MALDI-TOF is simpler to perform than conventional methods. However, laboratories are unlikely to observe any immediate labor savings or workload reduction following implementation. In some ways, MALDI-TOF actually results in an increased labor demand. For laboratories using combination identification and susceptibility panels, MALDI-TOF requires an extra process. In addition, because they are not FDA cleared, these systems are not easily interfaced with laboratory information systems or susceptibility instruments. This means that data are managed manually, which can be a cumbersome process. In a survey of MALDI-TOF users, no laboratories had interfaced their instrument with a laboratory information system or any other software (Table 2). For laboratories using reusable target plates, routine washing is required, which involves the use of harmful chemicals that must be used within a safety cabinet. In these ways, MALDI-TOF has a deleterious effect on the laboratory workflow.

However, it has a very positive impact in other ways. First, most laboratories find that their dependence on auxiliary test methods decreases dramatically, which may allow the complete discontinuation of their use. Examples from our laboratory and others include the discontinuation of the use of manual biochemical identification strips, such as the API and rapID identification systems. Also, laboratories will no longer have need for organism-specific media, such as *Haemophilus* triplates. 16S rRNA gene sequencing for unusual organisms will be virtually eliminated. While individually these tests are low volume, they all require a certain amount of additional quality control measures and expense (both labor and monetary). In our laboratory, the section that has been most significantly impacted is the quality control bench, because of all the testing that MALDI-TOF has allowed us to discontinue.

The reduced dependence on these auxiliary methods signifies a more streamlined identification process. As Desai et al. (33) showed, MALDI-TOF provides very reliable identifications for the often challenging glucose nonfermenting organisms isolated from cystic fibrosis patients. Although the labor savings from this improved efficiency can be difficult to quantify, the impact on the respiratory bench workload will be noticeable.

Billing

MALDI-TOF for organism identification is not currently FDA approved for clinical use. The database is labeled RUO, necessitating that laboratories verify the assay as a laboratory-developed test. Whether these identifications can be billed to patients is a matter of considerable debate. Ultimately, whether a laboratory bills for MALDI-TOF identifications is a decision that each institution must make in consultation with their compliance and legal advisers. Of the surveyed laboratories currently using MALDI-TOF for clinical testing, only one laboratory was billing for identifications (Table 2). Most of the others intend to bill but are awaiting either compliance office approval or FDA clearance. Interestingly, few the systems currently using the RUO database intend to adopt the FDA-cleared platforms when they become available.

For those that are billing or plan to bill for MALDI-TOF identifications, there are a few CPT codes that may apply. There is no consensus, because no specific CPT code for MALDI-TOF organism identification currently exists. The most common CPT code for MALDI-TOF billing appears to be 87077, for aerobic identifications, while others plan to use 87158. Some laboratories are using a combination of 87158 with 87076 for anaerobic identifications, with 87077 for aerobic identifications, and with 87106 for yeast identifications.

Summary

In conclusion, laboratory verification of an RUO database for use as the primary organism identification system is unprecedented, yet the speed, cost savings, and improved quality of MALDI-TOF has inspired many laboratories to conduct the massive verification studies required**.** There is no consensus process for validating such a system, but specific guidance is sorely needed. This review draws from the experiences of a select few laboratories that have completed MALDI-TOF verification studies. While each laboratory conducted their studies differently, there are some commonalities that can provide general guidance.

There is no magic number of isolates required to verify a MALDI-TOF system. All six laboratories surveyed here tested close to or well over 1,000 distinct isolates. A variety of comparator methods were used, but all resolved discrepancies with 16S rRNA gene sequencing. All verification studies analyzed organism categories separately. At a minimum, it would seem wise to analyze Gram-positive organisms separately from Gram-negative organisms. It is unclear whether the Gram-positive rods and cocci warrant separate analysis. The literature does suggest that differences may exist for MALDI-TOF identification of enteric Gram-negative rods versus glucose non-fermenting organisms. As a result, verification studies may need to consider them separate entities.

One important question remains unanswered: is it necessary to verify each individual species in the database? There are precedents on both sides of this argument. The conservative approach would be to require individual verification for each species with some defined number of isolates. Indeed, this has been the precedent set by the FDA for previously cleared devices and appears to be the standard MALDI-TOF systems will be held to. Others would argue that MALDI-TOF is a method that can be verified and that the performance of its database can be inferred from a subset analysis. Powerful precedent has been set for this approach, as well. 16S rRNA gene sequencing for organism identification is based on comparing a sequence to publicly available databases that are in many cases not curated and have not been fully verified, yet the method has become the gold standard for organism identification. Interestingly, it will likely be the gold standard comparator method required by the FDA for MALDI-TOF clinical trials. Based on this example, one could conclude that it is not necessary to verify every species in the database.

Lastly, implementation of MALDI-TOF in clinical practice is a challenge equal to that of validating the system itself. For laboratories choosing to employ the "verify the method and not the entire database" approach, it may be advisable to have special procedures in place to guide the workup of unusual organisms not encountered during the verification study. Perhaps by employing a rolling verification laboratories can implement MALDI-TOF in a timely and cost-effective manner while ensuring the highest-quality result for the patient.

References

1. Kliem, M. and S. Sauer. 2012. The essence on mass spectrometry based microbial diagnostics. Curr. Opin. Microbiol. 15:397-402.
2. Drake, R.R., S.R. Boggs, and S.K. Drake. 2011. Pathogen identification using mass spectrometry in the clinical microbiology laboratory. J. Mass. Spectrom. 46:1223-1232.
3. Cherkaoui, A. et al. 2010. Comparison of two matrix-assisted laser desorption ionization-time of flight mass spectrometry methods with conventional phenotypic identification for routine identification of bacteria to the species level. J. Clin. Microbiol. 48:1169-1175.
4. Bizzini, A. and G. Greub. 2010. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry, a revolution in clinical microbial identification. Clin. Microbiol. Infect. 16:1614-1619.
5. Martiny, D. et al. 2012. Comparison of the Microflex LT and Vitek MS systems for routine identification of bacteria by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J. Clin. Microbiol. 50:1313-1325.
6. Veloo, A.C. et al. 2011. Comparison of two matrix-assisted laser desorption ionisation-time of flight mass spectrometry methods for the identification of clinically relevant anaerobic bacteria. Clin. Microbiol. Infect. 17:1501-1506.
7. Jamal, W.Y., M. Shahin, and V.O. Rotimi. 14 December 2012. Comparison of two matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry methods and API 20AN for identification of clinically relevant anaerobic bacteria. J. Med. Microbiol. [Epub ahead of print.] doi:10.1099/jmm.0.053256-0.
8. Schmitt, B.H. et al. 2013. Identification of anaerobic bacteria by Bruker Biotyper Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry with on-plate formic acid preparation. J. Clin. Microbiol. 51:782-786.
9. Theel, E.S. et al. 2012. Formic acid-based direct, on-plate testing of yeast and *Corynebacterium* species by Bruker Biotyper matrix-assisted laser desorption ionization-time of flight mass spectrometry. J. Clin. Microbiol. 50:3093-3095.
10. Matsuda, N. et al. 2012. Evaluation of a simple protein extraction method for species identification of clinically relevant staphylococci by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J. Clin. Microbiol. 50:3862-3866.
11. Alatoom, A.A. et al. 2012. Identification of non-*diphtheriae Corynebacterium* by use of matrix-assisted laser desorption ionization-time of flight mass spectrometry. J. Clin. Microbiol. 50:160-163.
12. Alatoom, A.A. et al. 2011. Comparison of direct colony method versus extraction method for identification of Gram-positive cocci by use of Bruker Biotyper matrix-assisted laser desorption ionization-time of flight mass spectrometry. J. Clin. Microbiol. 49:2868-2873.
13. Bizzini, A. et al. 2010. Performance of matrix-assisted laser desorption ionization-time of flight mass spectrometry for identification of bacterial strains routinely isolated in a clinical microbiology laboratory. J. Clin. Microbiol. 48:1549-1554.
14. Harris, P. et al. 2012. Comparison of Vitek MS (MALDI-TOF) to standard routine identification methods: an advance but no panacea. Pathology 44:583-585.
15. Fang, H. et al. 2012. Evaluation of species-specific PCR, Bruker MS, VITEK MS and the VITEK 2 system for the identification of clinical *Enterococcus* isolates. Eur. J. Clin. Microbiol. Infect. Dis. 31:3073-3077.
16. Marko, D.C. et al. 2012. Evaluation of the Bruker Biotyper and Vitek MS matrix-assisted laser desorption ionization-time of flight mass spectrometry systems for identification of nonfermenting gram-negative bacilli isolated from cultures from cystic fibrosis patients. J. Clin. Microbiol. 50:2034-2039.
17. Iriart, X. et al. 2012. Routine identification of medical fungi by the new Vitek MS matrix-assisted laser desorption ionization-time of flight system with a new time-effective strategy. J. Clin. Microbiol. 50:2107-2110.
18. Dubois, D. et al. 2012. Performances of the Vitek MS matrix-assisted laser desorption ionization-time of flight mass spectrometry system for rapid identification of bacteria in routine clinical microbiology. J. Clin. Microbiol. 50:2568-2576.
19. Buchan, B.W. and N.A. Ledeboer. 2013. Advances in the identification of clinical yeast isolates using matrix-assisted laser desorption ionization-time of flight mass spectrometry. J. Clin. Microbiol. [Epub ahead of print.] doi:10.1128/JCM.03105-12.
20. Biswas, S. and J.M. Rolain. 2013. Use of MALDI-TOF mass spectrometry for

  0196-4399/00 (see frontmatter)

identification of bacteria that are difficult to culture. J. Microbiol. Methods 92:14-24.

21. Khot, P.D. et al. 2012. Optimization of matrix-assisted laser desorption ionization-time of flight mass spectrometry analysis for bacterial identification. J. Clin. Microbiol. 50:3845-3852.
22. Saffert, R.T. et al. 2011. Comparison of Bruker Biotyper matrix-assisted laser desorption ionization-time of flight mass spectrometer to BD Phoenix automated microbiology system for identification of gram-negative bacilli. J.Clin. Microbiol. 49:887-892.
23. Tekippe, E.M. et al. 2013. Optimizing identification of clinically relevant Gram-positive organisms using the Bruker Biotyper MALDI-TOF MS System. J. Clin. Microbiol. [Epub ahead of print.] doi:10.1128/JCM.02680-12.
24. Anderson, N.W. et al. 2012. Effects of solid-medium type on routine identification of bacterial isolates by use of matrix-assisted laser desorption ionization-time of flight mass spectrometry. J. Clin. Microbiol. 50:1008-1013.
25. van Veen, S.Q., E.C. Claas, and E.J. Kuijper. 2010. High-throughput identification of bacteria and yeast by matrix-assisted laser desorption ionization-time of flight mass spectrometry in conventional medical microbiology laboratories. J. Clin. Microbiol. 48:900-907.
26. Risch, M. et al. 2010. Comparison of MALDI TOF with conventional identification of clinically relevant bacteria. Swiss Med. Wkly. 140:w13095.
27. Bizzini, A. et al. 2011. Matrix-assisted laser desorption ionization-time of flight mass spectrometry as an alternative to 16S rRNA gene sequencing for identification of difficult-to-identify bacterial strains. J. Clin. Microbiol. 49:693-696.
28. Szabados, F. et al. 2010. Identification of molecularly defined *Staphylococcus aureus* strains using matrix-assisted laser desorption/ionization time of flight mass spectrometry and the Biotyper 2.0 database. J. Med. Microbiol. 59:787-790.
29. Stevenson, L.G. et al. 2010. Evaluation of matrix-assisted laser desorption ionization-time of flight mass spectrometry for identification of clinically important yeast species. J. Clin. Microbiol. 48:3482-3486.
30. Dhiman, N. et al. 2011. Performance and cost analysis of matrix-assisted laser desorption ionization-time of flight mass spectrometry for routine identification of yeast. J. Clin. Microbiol. 49:1614-1616.
31. Haigh, J. et al. 2011. Improved performance of bacterium and yeast identification by a commercial matrix-assisted laser desorption ionization-time of flight mass spectrometry system in the clinical microbiology laboratory. J. Clin. Microbiol. 49:3441.
32. Van Herendael, B.H. et al. 2012. Verification of a modified algorithm for the identification of yeast isolates using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS). Eur. J. Clin. Microbiol. Infect. Dis. 31:841-848.
33. Desai, A.P. et al. 2012. Use of matrix assisted laser desorption ionisation-time of flight mass spectrometry in a paediatric cystic fibrosis patients. J. Clin. Pathol. 65:835-838.

