Labs enter a MALDI-TOF state of mind

Karen Titus

October 2016—When MALDI-TOF mass spectrometry enters the microbiology lab, it's a little like watching Sir John Falstaff settle his considerable girth onstage. Things happen. Characters fret and flee, scheme, opine, panic, and, in the case of Prince Hal, ascend to greatness. (And, if we're honest, some just get drunk.) Both, in brief, are an upending presence.

Like Falstaff—also an inspirer of operas and a symphony as well as a beer—MALDI-TOF is complex as well as popular.

Those who add MALDI-TOF mass spec to their microbiology labs, says Dr. John Branda, can use one of two approaches: "Do you want to remove the Band-Aid slowly, or do you want to rip it off?" His lab chose the former but was surprised by the learning curve.

"It's definitely come in to take its place as the preferred method of identification," says Kevin Alby, PhD, assistant director of clinical microbiology, Hospital of the University of Pennsylvania, Philadelphia. "For academic medical centers and large community hospitals, MALDI-TOF is becoming the norm." If such laboratories haven't already begun using MALDI-TOF, they're certainly thinking about it, says Dr. Alby, who is also an assistant professor of pathology and laboratory medicine at Penn's Perelman School of Medicine.

Dr. Alby and several others spoke about MALDI-TOF with CAP TODAY as well as at the ASM Microbe 2016 conference in June, exploring what life has been like with MALDI-TOF front and center in microbiology laboratories.

At his institution, says John Branda, MD, MALDI-TOF has been, among other things, a medical version of *MythBusters.*
To wit:

- Among clinicians—and sometimes even among pathologists and

other lab personnel who work outside of microbiology—there rests the belief that MALDI-TOF can be used to directly identify a microorganism on a primary specimen. Not true, says Dr. Branda, associate director of clinical microbiology, Massachusetts General Hospital, and assistant professor of pathology, Harvard Medical School. “We still have to do a culture and isolate a microorganism as the first step—at least right now.”

- Within the lab, another misconception is that MALDI-TOF is a labor-saving device. Not only will it be a faster method, goes the thinking, but it will also greatly reduce technical hands-on time. “That’s not really how it works,” says Dr. Branda.
- Many laboratory personnel worry that when MALDI-TOF shows up, they, in turn, will be shown the door. “There’s apprehension: *This is your replacement,*” Dr. Branda says. “And that’s definitely not the case.”

When he and his colleagues began implementing MALDI-TOF, about five years ago, they reasonably expected a smooth sail. “On paper,” he says, “the technique of performing the analysis is very simple from a technical standpoint,” despite the instrument’s sophistication.

“For routine bacteria, you just have to find an isolated colony,” either on a primary culture plate or a subculture plate, Dr. Branda says. “You apply it to the target, you overlay it with a matrix, and off you go.” While yeast and mucoid bacteria usually require users to overlay the bugs with formic acid and then add the matrix, “that’s a very quick step.”

Those who add MALDI-TOF to their laboratories can use one of two approaches, he suggests: “Do you want to remove the Band-Aid slowly, or do you want to rip it off?” His lab chose the former, what he calls a staged rollout, moving from one section or group of organisms to another. It made sense, he says, for a complex lab like the one at MGH, and it allowed for incremental training of staff. (The downside, he notes, is that for a time some technologists will be identifying organisms by conventional methods and others will be using MALDI-TOF, which can create inconsistencies in reporting.)

Nevertheless, they were surprised by the learning curve, even with their unhurried approach.

Most of this related to preparing target slides, Dr. Branda says. Applying the isolate to the slide is neither automated nor standardized; the slide needs to contain an adequate amount of material but not so much that it’s counterproductive. “Some techs have a real knack for it and are good at it right from the beginning; other techs struggle to be able to do it reproducibly.”

Because of those difficulties, he recalls, when the lab had trouble with the instrument early on, it was hard to discern whether it was related to the instrument or to the user. “We soon realized that the first thing we needed to do was some troubleshooting with our expert ‘spotter’”—someone who excels at preparing target slides. “That removed a very important variable in the troubleshooting process.”

When the problem is with the instrument, fixes are less easy.

MALDI-TOF doesn’t lend itself to tinkering by existing lab staff—if you buy a Bentley, you don’t do your own oil changes. Instead, the manufacturers’ service engineers handle these problems. Unfortunately, Dr. Branda says,

“Those difficulties arise—at this point, anyway—a little more than one would like, ideally.”

Dr. Lau

The problems can be even more complex depending on the target. Anna Lau, PhD, of the National Institutes of Health, is exploring inherent instrument variabilities and settings that labs need to consider when using MALDI-TOF for mold identification. In a study currently wrapping up, she and her colleagues have seen wide variability in instrument performance across eight U.S. academic medical centers. “This is unusual,” says Dr. Lau, co-director of bacteriology, parasitology, and molecular epidemiology, Department of Laboratory Medicine, Clinical Center, NIH. “It’s something that wasn’t seen with bacteria and yeast.” Those bacteria and yeast multicenter studies have shown great reproducibility, inter- and intralaboratory, regardless of the system being used. “So we’re trying to find the answer.”

She suspects instrument settings could be one culprit. “It’s also important to remember that each hospital’s instrument is going to be unique. It’s such a complicated instrument.” One hospital’s laser will be a different age than another’s, for example. “If you have a younger laser, it’s more powerful, so therefore your settings will need to be adjusted.”

When Dr. Branda and colleagues brought on MALDI-TOF at MGH, they discovered the instrument is quite sensitive to temperature. When the temperature exceeds a set point—“We’re talking about a few degrees above a normal room temperature”—the instrument will either stall or start to become unreliable.

Like most laboratories, the one at MGH doesn’t have space to spare. Once in the crowded room with other instruments and equipment, the device created enough heat on its own to lift the room temperature into a troublesome zone, Dr. Branda says. Dealing with that unexpected problem required “a whole lot of engineering work—we had to jury-rig a special exhaust system to siphon away the hot air.” The ultimate solution would have been to upgrade the HVAC system in the room, a massive and expensive undertaking.

Another surprise, as Dr. Branda alluded to, was that MALDI-TOF hasn’t led to labor savings. Technologists are still doing much of the work they previously did, plus the new procedure. In sections of the lab where staffing was already tight, he says, the additional workload required more staff. It’s not something they anticipated, he says, “particularly because there are articles in the literature that suggest the opposite. That just hasn’t been our experience.”

For those in the lab who worried their years of expertise now had a best-by date, Dr. Branda notes that there’s still a need to correlate MALDI-TOF results with colony morphology and Gram-stain morphology to ensure results are accurate. While MALDI-TOF results generally are reliable, he says, the potential for errors exists, as it does with any method. “So you still need a very well-trained, qualified medical technologist to look at the result and make a judgment about whether the result makes sense,” in the context of the specimen itself and the isolate’s phenotypic features.

Not that Dr. Branda and colleagues plan to evict MALDI-TOF from the laboratory. “The results, in general, are at least as accurate, and often more accurate, than the results we would have produced by more conventional means in the recent past,” he says.

But MALDI-TOF, for all its apparent simplicity, can, like a certain fictional fat man, try the patience of those who work in the lab. Knowing what he knows now, what would Dr. Branda do differently? What are he and his colleagues now doing better?

When they first introduced MALDI-TOF, he says, they followed a decentralized plan, in which bench technologists individually prepared their own target slides and target maps and did their own analysis on the instrument. But, like the Founding Fathers' views on the federal government, things evolved in practice. "It turned out to be the wrong approach," says Dr. Branda, "so we opted for a more centralized approach." Now, each day one or two technologists are primarily responsible for the aforementioned tasks and, to some extent, ensuring the results are entered into the laboratory information system.

This has turned out to be much more practical for the large MGH lab, Dr. Branda says, with its many users and one MALDI-TOF instrument. "Also, it was inefficient for all these technologists to be getting up and down and going back and forth to the instrument to do procedures. It turned out to be far more efficient to have someone essentially making rounds to pick up cultures and do the analyses."

They've also improved their technologist training, particularly in target slide preparation. Technologists need to demonstrate a level of success before they're allowed to use the instrument clinically. Moreover, they've learned when to turn to the service engineers for help with troubleshooting. "We kind of know the warning signs when something's not right."

There's one other myth Dr. Branda has seen busted, this one espoused by clinicians.

For the most part, feedback on MALDI-TOF analysis has been positive, he reports. Oftentimes clinicians are seeing identification results more quickly than they would have in the past. Moreover, the laboratory can precisely identify organisms that in the past it might have grouped or "lumped" (to use Dr. Branda's word) together.

But as clinicians' eyes have been opened to more precise identifications, their expectations may have also grown. "They also have misconceptions about the breadth of functionality," Dr. Branda says, diplomatically. There are certain organisms that his lab, at least, has not yet tried to identify by MALDI-TOF, such as molds (though as Dr. Lau and others are showing, that capability certainly exists). "I think some of the clinicians either hear or read about these things and assume as long as you have this instrument that we can do almost anything with it," Dr. Branda says.

The laboratory, for its part, tries not to provide overly precise identification when it's not clinically meaningful, says Dr. Branda. Clinicians can benefit from knowing the identification of subspecies within the Strep bovis group, for example. But with a superficial wound culture, with four or five different commensal flora in the culture, "If you precisely identify each one of those, it signals to the clinician that those are somehow pathogenic or clinically relevant, and it may lead to misunderstanding and overtreatment.

"There are times when we need to pull back," he continues. "Even though we know precisely what all those organisms are in the culture, we still use lumping terms, like 'organisms resembling cutaneous flora.'"

MALDI-TOF identifications can create other muddles, such as split identifications. To sort through that, Dr. Branda asks whether the possible identifications are all from the same genus. If they're not, he says, it's time to troubleshoot and do supplemental testing to determine if the instrument is at fault or if the isolate is mixed or impure.

If there's no identification, Dr. Branda recommends examining the spectrum itself. A poor-quality spectrum is, usually, the result of poor technique. "Spotting doesn't come easily to everyone," he says. "You've either added too much of the organism, or not enough. You forgot to apply the matrix, or you scooped up some medium with it."

And if everything's been handled well but no ID is forthcoming? "Usually that's because the organism is not in your database," he says.

In the future, Dr. Branda expects his lab to expand to mycobacteria, *Nocardia*, and, eventually, mold identification.

Beyond adding more organism classes, the laboratory continues adding to the list of specific organisms it can

identify by MALDI-TOF. “There are a lot of uncommon or rare bacteria or yeasts that are present in the instrument database,” he acknowledges, but he wants the laboratory to gain more experience with MALDI-TOF before relying on results for clinical use.

Even longer term, he predicts a time when MALDI-TOF might be applied to areas that are only being investigated now, including antimicrobial susceptibility testing, strain typing, or even application to primary specimens, without the need for culture.

And in the near future, he notes, there’s other news: A CLSI document, M58, is likely to be published this year, offering guidance on MALDI-TOF use. “It’s a practical guideline for performing MALDI-TOF mass spec for microorganism identification.”

At times, an almost elegiac element slips into the discussions of MALDI-TOF and its role in the laboratory: *Who among you is maintaining the custom of your ancestors?*

Dr. Alby advises labs considering MALDI-TOF mass spectrometry to pay close attention to fit. “You can’t just drop this in like it’s a regular biochemical analyzer,” he says. “You need to adjust your workflow around the technology, and maybe not the other way around.”

When MALDI-TOF arrives in the lab, observers talk about witnessing the loss of classic microbiology—shades of the former Prince Hal, now Henry IV of England, telling Falstaff, “I know thee not, old man.”

At Penn, says Dr. Alby, “Now we have techs and students who have no idea about anything [related to] the biochemical reactions or classic techniques. Which is fine—except for the occasions when the MALDI-TOF is down. It’s an instrument; it’s not always working.” Though Dr. Alby is not advocating a return to days of yore, his point is clear: Biochemical tubes always worked. “You’re not going to have downtime with your isolates.”

Dr. Alby’s laboratory uses a protocol that relies on classical methods “to get us through our work, because if the MALDI is down for two days, we can’t not do microbiology—as much as I think our techs would like that,” he jokes.

“But what we’re finding is now we have techs who didn’t work the benches before MALDI was around,” Dr. Alby

continues. “They don’t have the intrinsic knowledge about what’s PYR positive and what’s PYR negative, and things like that.”

At this transitional point, the laboratory still has technologists who know the classical techniques, but it won’t for much longer, as retirement looms for most of them. “So that’s probably our biggest concern: How do we maintain the knowledge?” Dr. Alby asks.

Adam Barker, PhD, assistant professor, Department of Pathology, and medical director, AFB laboratory, ARUP Laboratories, also bemoans this fading knowledge. “Most laboratories have completely lost the ability to read molds. And most laboratories have completely lost the ability to ID rapid-growing mycobacterium,” apart from TB, he says. He blames budget cuts and current lack of expertise in the field.

Dr. Barker

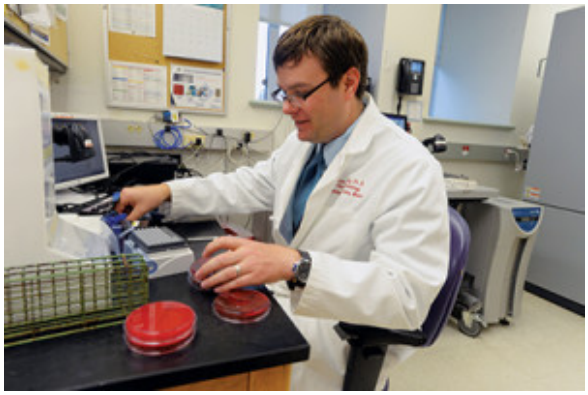
Add to that the aforementioned concern that MALDI-TOF is not accurate 100 percent of the time. Dr. Alby worries about the ability of less-experienced technologists to identify such errors. Will they spot the red flags that indicate when a result doesn’t make sense, the way more experienced technologists can when, say, the colony morphology doesn’t match a MALDI result? “Much to my dismay,” Dr. Alby says with a laugh, “the MALDI is probably right more than it’s wrong when I challenge it. But occasionally I’m smarter than the MALDI.”

Just as technologists have had to make adjustments to their work, the laboratory as a whole has had to reconsider how MALDI-TOF fits in. Simply adding an expensive piece of equipment into the lab, much like the Yankees have added high-priced free agents over the years, is no guarantee of success.

Dr. Alby says he and his colleagues failed to anticipate its impact on workflow. At Penn, the instrument didn’t save time, at least not initially. At the blood culture bench, for example, the laboratory did plate readings on all three shifts but implemented MALDI only on the first shift. That created a different workflow and delayed results slightly.

For those considering MALDI-TOF, Dr. Alby advises paying close attention to fit. “You can’t just drop this in like it’s a regular biochemical analyzer,” he says. “You need to adjust your workflow around the technology, and maybe not the other way around.”

Dr. Alby notes that one of the biggest considerations is how to pay for MALDI-TOF. “For most labs, this is probably the single largest purchase for microbiology they have ever made,” apart from total lab automation.



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It takes considerable cost analysis to convince administrators inside the laboratory and out that spending hundreds of thousands of dollars on equipment in the microbiology lab is a smart move. Academic centers and larger community hospitals have the volumes to help justify the cost, Dr. Alby says, since the savings comes primarily from reagents.

But as Dr. Barker points out, with staff cuts and disappearing expertise, even smaller labs might be able to make an argument for adding MALDI-TOF to their armamentarium as a way to reduce sendouts. “You buy one instrument; you get really good IDs of yeast and bacteria—and maybe 85 percent calls on acid-fast bacteria.” In economic terms, that’s 85 percent of cases labs wouldn’t need to send to ARUP or another reference lab, says Dr. Barker, who is also associate clinical director of the ARUP Institute for Clinical & Experimental Pathology.

Almost any laboratory that does full-service microbiology should consider MALDI-TOF, Dr. Branda suggests. Large, complex labs are unquestionably interested, he says, because of the obvious opportunity to expand their capabilities and to rely less on reference labs. But small hospital labs can benefit as well, he says. It can improve turnaround time substantially as well as provide highly accurate identifications in a lab staffed by technologists with more general, rather than specialized, expertise.

MALDI-TOF also allows laboratories to identify rare species. That can be a blessing and curse financially as well as clinically.

Once labs start using MALDI-TOF, Dr. Alby says, it’s possible to see a sharp rise in reports of rare species causing infections. It’s not that rare species are necessarily taking off. “We just didn’t know what they were before,” he says.

In the pre-MALDI days, says Dr. Alby, hard-to-differentiate specimens, such as Gram-positive rods, created a dilemma, since they were difficult to differentiate without sequencing. Now, instead of describing them all as diphtheroid-like rods, the lab can provide genus- and species-level identifications.

“That’s useful, but it’s also potentially harmful,” Dr. Alby says. “We’re changing how we report things to the clinical staff.” Now that the laboratory is providing identification of Corynebacterium species, the requests for susceptibility testing have risen. Because MALDI-TOF enables the laboratory to add a species name, the inference among clinicians is that the test results are more significant.

In some cases, the additional information can be helpful. In cases involving a prosthetic joint infection, for example, being able to identify Gram-positive rods on three different tissue samples is helpful—it tells clinicians the likely causative agent in the infection, Dr. Alby says. There’s a similar scenario with enterococcal bacteremia.

MALDI-TOF can differentiate between Enterococcus faecium and Enterococcus faecalis, which has treatment implications.

But it's difficult, at least right now, to parse out the cost savings attributable to better therapeutic interventions. "It's hard for us to dive into that," Dr. Alby says, pointing to changing volumes resulting from mergers with other health systems and shifts in outreach business. Studies that look at extrapolating cost per isolate might be of more use in helping to justify a MALDI-TOF purchase, he adds.

His laboratory has attempted to study MALDI-TOF outcomes related to bacteremia, by defining patient characteristics and analyzing antibody use. The data haven't been all that helpful. "Depending on your baseline, you may or may not see improvement in utilization [although that was the case with enterococcal bacteremia, as noted] or length of stay, because we still don't have susceptibility information."

One pleasant surprise, says Dr. Alby, has been the ability to identify interesting organisms. "You find trends with bacteria or fungi that might be associated with an outbreak. You see the same species over and over, and it may be a species that you never saw before." It's also been helpful in tackling organisms like nonfermenting Gram-negative rods and Gram-positive rods that are more difficult to identify by traditional methods.

The laboratory also sees organisms it wouldn't have known were there. "Some of the veterinary coagulase-positive Staphylococcus, the *Staph intermedius* group, for example—we would call it *Staph aureus*. Every now and then they'll pop up, and it's interesting to see how it plays into the clinical story," Dr. Alby says. "Those are always fun to find."

It has also, Dr. Alby observes, enhanced the lab's standing in some quarters. "The infectious disease group definitely have an appreciation for our ability to merely pronounce the names of these different bacteria," he says. Occasionally even the lab will have to turn to the textbooks to figure out exactly what it's dealing with.

Next month: Dr. Lau and Dr. Barker talk about MALDI-TOF databases, acid-fast bacteria and mold identification, nomenclature, results reporting, and more.

Dr. Barker reports a similar response from some of his clinical colleagues at the University of Utah. "We get calls on the urine bench especially," he says. "We used to just gross ID, and now we're giving out names that none of us have ever heard of."

And as Dr. Lau notes, "The beauty of mass spec is that it is giving us a level of detail in fungal identification that we just didn't have before."

Fungal identifications are challenging because of look-alike organisms, she explains. A mold that looks like a common variety such as *Aspergillus fumigatus* may turn out to be something completely different once it's undergone analysis via MALDI-TOF mass spec. "You'll find that it may be one of the organisms within the *Aspergillus fumigatus* complex, such as *Aspergillus lentulus*," says Dr. Lau. "There've been quite a few publications that show that these cryptic species, or look-alike species, are either more pathogenic, more virulent, or they're more resistant to antifungals. And so mass spec is really giving us now a level of identification to improve clinical care, at the level of specificity associated with that."

Even Falstaff might drink to that.
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