Up next for MALDI-TOF mass spec: AFB, molds

Karen Titus

November 2016—Behold the humble API strip, made of plastic, with multiple miniature test chambers, interpreted with the aid of a color chart, and long a mainstay of microbiology laboratories.

And now? "My new technologists don't even know what an API strip is," says Adam Barker, PhD, assistant professor, Department of Pathology, and medical director of the AFB laboratory, ARUP Laboratories. As MALDI-TOF mass spectrometry captures the laboratory world, older techniques and equipment (and, some fear, technologists themselves) seem to be slipping away like so many factory jobs heading overseas.

At ARUP, API strips are no longer used in the yeast lab—"We put everything on a MALDI plate"—so now when Dr. Barker's residents and fellows pass through his laboratory, he has to dig into his budget to buy strips and chrome agars, "at least until I hear from their boards they're no longer going to be testing on that."

Dr. Barker is not lamenting some golden age of microbiology, but when he and others consider the impact of MALDI-TOF, globalization seems like an apt metaphor. Even if the benefits outweigh the drawbacks, the shift hasn't occurred without its growing pains.

Dr. Barker

Dr. Barker and his colleagues implemented MALDI-TOF for acid-fast bacteria about two years ago. "It's definitely been a learning process," Dr. Barker says.

Take building the database. ARUP has a dedicated R&D staff who work out of the microbiology laboratory; whenever MALDI-TOF fails to identify a specimen, they add the MSP, or main spectra profile, to the database. These efforts have paid off. Currently the lab identifies 85 to 95 percent of AFB organisms by MALDI, says Dr. Barker; when the lab first began using MALDI-TOF, the identification rate was 70 to 80 percent.

Second of two parts. Last month: "Labs enter a MALDI-TOF state of mind"

Commercial databases' identification rates at the time were even lower than ARUP's, Dr. Barker says—in the 50 to 60 percent range. Rates have since moved from that nugatory state. Bruker (whose Biotyper is one of two commercially available FDA-approved MALDI-TOF devices in the United States; BioMérieux's Vitek MS is the other) has since released its updated AFB database. "It's very good now," Dr. Barker says. "They've caught up completely to the database that we've created at ARUP. It's safe for the rest of the labs to use just commercial databases."

If ARUP has found database development nettlesome, the task could seem insurmountable for laboratories with fewer resources. Dr. Barker contends that for those considering MALDI-TOF, the database has been, until now, one of three main impediments to bringing it online.

That hesitancy appears to be dwindling, however. Dr. Barker's proof? When a client sends in 60 or 70 isolates for sequencing—and conspicuously doesn't ask for the identifications to be done on MALDI-TOF—"we know that they're validating a new MALDI instrument," Dr. Barker says. That's now happening frequently for AFB; the same

thing happened in bacteriology about four years ago. Like the bit of wisdom that urges do-gooders to teach the hungry how to fish, rather than to hand over a swordfish steak, MALDI is bringing self-sufficiency to laboratories. “At ARUP, MALDI does nothing but take customers away from us.”

For AFB specifically, Dr. Barker says, the daunting extraction process had been, until recently, another barrier to MALDI-TOF implementation. “It was very difficult to get proteins for MALDI,” he says, noting that despite the multiple methods available, no one managed to develop a unified approach. “A lot of people were waiting for Bruker to have a database and an extraction process they could do.” At ARUP the process was easier, since the laboratory learned how to extract proteins in the process of building the database.

The third barrier has been the desire, on the part of many labs, for an FDA-approved test. (Both Bruker’s and BioMérieux’s databases are approved only for bacteria and yeast, says Dr. Barker. He’s currently helping with BioMérieux’s FDA validation process for AFB and molds.)

These aren’t idle concerns, Dr. Barker concedes. “Some labs wanted to be protected from running an LDT versus an FDA-approved test,” he says. “And that does make a big difference for reimbursement and risk assessment.”

From what he’s seen the FDA has been surprisingly responsive regarding MALDI validation. As he puts it: “I’m actually blown away.” He’s familiar with the gnashing of teeth that has been the soundtrack to the FDA’s approval process for other devices. While not easing its standards, he says, the agency is “actually being reasonable for organisms that are hard to identify. The FDA is up to speed on the limitations of the instrument, and they’re doing a good job with submissions.”

Still, setting up MALDI-TOF is hardly a ride in the express lane.

As associate director of the ARUP Institute for Clinical and Experimental Pathology, where he works with some 85 scientists and 65 medical directors, Dr. Barker spends considerable time pondering the practicalities of bringing MALDI-TOF into laboratories.

One area they’re looking at now is molds. If MALDI-TOF were a race, “AFB is about two years ahead of molds,” says Dr. Barker. When he and his colleagues did a cost analysis, they discovered that a reference laboratory such as ARUP, with its highly trained and experienced staff, doesn’t benefit as much as a smaller lab might from switching to MALDI-TOF for mold identification. “That was kind of shocking to us.”
Like Trump’s rise to the top of the GOP ticket, it made sense, sort of, after further reflection. “We already get our IDs out pretty fast,” Dr. Barker explains. For molds, TATs will likely improve further as the database gets better, he predicts. “But it’s still not going to be such a big change, like it was for bacteriology and yeast.”

For smaller labs it’s a different story, at least with AFB. Instead of sending their AFB to ARUP, as they did previously, MALDI enables them to run about 85 percent of their AFB identifications in-house. “Those small labs save a lot of money,” says Dr. Barker.

At ARUP, MALDI-TOF for AFB was rolled out indirectly. Wearing yet another hat, Dr. Barker also manages a group called Technical Transfer, which oversees the move from R&D to the lab once a test is considered ready for clinical use.

The laboratory was already comfortable with MALDI-TOF, given its previous experience with mass spectrometry. Or so Dr. Barker thought.

“When we put it in the clinical laboratory, we kind of set it down and said, ‘Okay, here’s a new instrument that you guys are going to be IDing organisms with.’ And to tell that to a clinical microbiologist who’s been there 25, 30 years, who’s used to microscopes and biochemicals and different coloring, was night and day.

“So my lab resisted the MALDI-TOF,” Dr. Barker continues, though not everyone was against it. MALDI was sort of the Bernie Sanders of instruments, as it turned out. “The younger people loved it. They were excited about seeing

something new in the laboratory."

With longtime colleagues, the mistake, he says, was assuming implementation would be easy because the instrument itself is quite easy to run. "Implementation has to be handled correctly."
Another mistake was not reassuring these highly skilled people. Enthusiasm blinded them to technologists' fears. "They thought their jobs were gone," says Dr. Barker. "When we moved it in and I did my first talk on how great it was, their response was, 'Here's the instrument that replaces me.'"

Here's another thing he learned—but only later—from that conversation. When he lauded the instrument's capabilities, some in the laboratory heard a different message: that they themselves weren't good at their work.

Dr. Barker speaks highly of these colleagues, calling them "some of my best people. They're the group that identifies organisms from around the country that no one else can identify. They know their stuff."

But when microbiologist met MALDI, "They didn't want to use it."

Lesson learned. When he implemented a second instrument in the lab, he went about it differently, and it's advice he freely shares when he talks to clients and colleagues at meetings. To prepare his staff for the second go-round, he abandoned the throw-them-into-the-deep-end-of-the-pool approach used earlier. Instead, he and his colleagues led a session about how MALDI works, including its software. They showed the instrument to the staff. And they did extensive training. With any other instrument, he says, training typically might last one or two weeks. With MALDI, they trained for a month and a half. "It does take adjusting for the lab, because they're used to something completely different." In this case, it wasn't even a matter of changing horses midstream; rather, it was more like leaping from saddle to steering wheel.

For the chemistry and toxicology groups at ARUP, training has been much shorter. "One day and they're ready to go." But with the transition in micro, the failures piled up: Plates were getting stuck, pumps were going down. "They had never worked with mass spec before, and we didn't appreciate that." Mass spectrometry enters the microbiology lab as a parvenu. Dr. Barker draws on his own microbiology training to explain. "Micro labs don't know how to do QC and maintain the instrument properly. We put an instrument in, run it, and if it breaks we call to get it fixed." Think of it as the renter's approach, with an on-site building engineer. But MALDI is more of a homeowner proposition: "You need daily QC, you check the laser power, check the pump, make sure everything's running, every day. That's different than any other instrument we have in the micro group."

The upshot: He's since pulled toxicology people into the group to manage the instrument, or, as he likes to say, "We moved a chemistry test into the micro lab."

On a related note, he acknowledges the importance of service contracts in keeping MALDI viable—and how they differ from what the micro lab is used to, both in terms of cost and amount of time granted to return an instrument to working order. With his laboratory's current contract, he says, the company has three to five days to get the instrument up and running. "In the clinical micro world, that's unheard of," Dr. Barker says. When he's asked, as he frequently is, how his lab manages, his answer is direct: "We bought two MALDI-TOFs to overcome it. When one goes down, we always have a backup."

That may be a costly answer, but so is running the laboratory without MALDI, which would mean sending everything out for sequencing. "Once you move to MALDI, the way to make it cost-effective is to stop purchasing the other materials that MALDI's replacing." The cost savings is huge—an identification requiring sequencing followed by RT-PCR might run $300 to $400. MALDI can get the job done for about $30, Dr. Barker says.

And the 95 percent ID rates are higher than ID rates achieved using older methods. Though he says the clinical microbiologists who trained him get mad at him for saying so, Dr. Barker is adamant: "We're not going back. Yeast is definitely not going back, routine bacteriology is definitely not going back. AFB and molds might be the only areas where I see a possible hole in the MALDI world, because the nomenclature is so complex. But AFB is not going back."

Ultimately, Dr. Barker has concluded, MALDI-TOF is merely an instrument in the microbiology lab. MALDI didn't replace any FTEs (although their jobs have shifted), and API strips are a rapidly receding memory.

For now, though, fellows are still learning the old ways. Dr. Barker notes that as part of their training, they're given a series of 30 unknowns to identify using biochemical techniques. It takes about 2½ weeks to work through all the identifications. Dr. Barker reports that after one fellow completed this task, he then ID'd them all on MALDI. Time elapsed? Ten minutes.

Even as MALDI-TOF works its way into AFB testing with more regularity, it's been difficult to assess the clinical impact.

To cite an example, Dr. Barker talks about turnaround time for non-TB, i.e. rapid grower microorganisms and nontuberculosis mycobacteria. Formerly, once the organism was grown, it would be sent off for sequencing, Dr. Barker says, and it would take two to three days for that turnaround. That could even be followed by another PCR test, since AFB is so similar genetically, and 16S doesn't always differentiate between species.

"All in all, it took about five to six days, once the organism grew, to turn out an ID," Dr. Barker says. Now, the TAT has shrunk to about 24 hours.

"In my lab, four or five days is huge for clinicians," he continues. By rapidly differentiating between species, physicians can change therapy. Take, for instance, *erm*, an inducible resistance marker. CLSI guidelines mandate that labs hold *Mycobacterium abscessu*s and *M. chelonae* for 14 days during susceptibility before doing a read. "Everyone had to buy incubators, and we had to wait 14 days to finalize. And what we found out is that *chelonae* never has *erm*. So right off the bat, if you do a MALDI, you can differentiate those, cut off about 50 percent of your holds, and tell the clinicians what they need 24 hours in."

Dr. Barker says he and his lab colleagues have heard from clients after ARUP moved to MALDI-TOF, pleased with the faster turnaround times and being able to get species, as opposed to simply genus, names. There's even a bit of a "wow" factor, as well as a pie-in-the-sky element. Dr. Barker says clinical colleagues at the University of Utah (which owns the lab) now call and ask for differentiation of subspecies, assuming it's an easy next step. "I think they're excited, too."

In the future, many of these difficulties could start to fade, Dr. Barker suggests. Noting that he's working with biotech companies in the microbiology field, he says they're developing high-resolution mass spectrometry for infectious disease.

In labs now, Dr. Barker says, "We're almost at the saturation level for what MALDI can detect." While some are looking at susceptibility testing, Dr. Barker suggests that's happening simply as a way to further justify the high price tag of the instrument. "You're trying to put as much as you can on it."

High-resolution mass spec could be the holy grail of granularity. "We're using instruments that can go down and directly detect susceptibility proteins," Dr. Barker says. Laboratories would be able to make the call of *Staphylococcus aureus*, and then identify MRSA if present. Moreover, the method should enable labs to look for various resistance markers, since most of the Gram-negative resistant markers will likely be able to be directly detected, he says.

The hope, says Dr. Barker, is that high-resolution mass spec will address current pitfalls in MALDI-TOF. "Although MALDI works great, this will tweak it just enough to get better identification," he says. MALDI currently lacks susceptibility testing. "So even though we get the IDs out very fast, we're still waiting a day or two to get the 'sus' out to the doctor."

How to report unfamiliar identifications

Dr. Branda

Speaking at the ASM Microbe 2016 conference in June, John Branda, MD, cautioned that databases' up-to-date taxonomy means "they're going to report identifications that may be unfamiliar—unfamiliar to you in the laboratory or unfamiliar to the end users."

Labs need to figure out how to address that. Otherwise, "You can create confusion," says Dr. Branda, associate director of clinical microbiology, Massachusetts General Hospital, and assistant professor of pathology, Harvard Medical School.

He suggests using the newest taxon first (for example, *Peptoniphilus asaccharolyticus*) followed by the older, more familiar taxon in parentheses (in this case, *Peptostreptococcus*).

He also suggests creating a translation table. If, for example, a MALDI result identifies *Streptococcus gallolyticus*, subspecies *gallolyticus*, it's critical to report to the subspecies level. "We know that the various subspecies within the *Strep bovis* group have different clinical implications in terms of their likelihood to be related to colonic neoplasms," Dr. Branda says. But in addition to alerting clinicians to the subspecies, "You also don't want the clinician to miss the fact that this is a *bovis*." Since they may be unfamiliar with the new name, Dr. Branda recommends putting "*Strep bovis*" in parentheses. Making note of that in a translation table—"If the MALDI says this, this is how we report it"—can provide uniformity in reporting.

In other cases, however, a confusing name may be nothing more than confusing. In the case of an *Enterobacter kobei*, for instance, it may be reasonable to assume clinicians will not know that the result points to an *Enterobacter cloacae* complex organism. In such a case, providing both the unfamiliar species name and the parenthetical information may be overkill, if members of the broader group carry the same clinical implications. "You may decide that if any of these species is identified, you're going to translate that as *E. cloacae* complex."

A translation table may also help the laboratory deal with pitfalls. In some situations, the MALDI-TOF may not reliably distinguish between two closely related species—say, *Achromobacter xylosoxidans* and *denitrificans*. Given that uncertainty, "We'll just report *Achromobacter* species in most cases," Dr. Branda says. In cases where a more specific ID is critical, such as a patient with cystic fibrosis, the lab will do supplemental testing to distinguish them.—*Karen Titus*

Dr. Lau

Molds are tricky, agrees Anna Lau, PhD. MALDI has been live for molds at the National Institutes of Health

since 2012; its research-use-only database was published in 2013, says Dr. Lau, co-director of bacteriology, parasitology, and molecular epidemiology, Department of Laboratory Medicine, Clinical Center.

For laboratories that plan to use MALDI-TOF for molds, a little education is in order, says Dr. Lau. “In mycology, we’re used to seeing common organisms like *Aspergillus* and *Penicillium*,” she says. With MALDI-TOF identification, “Now we’re seeing a lot of the strange, or rare, organisms.” An added complication is the constantly changing taxonomy and nomenclature brought about by advances in sequencing technology. Laboratories adopting species-specific identification should include comments, where relevant, in the clinical report, Dr. Lau suggests. For example, after the new organism name is listed, note, in brackets, “previously known as” (or words to that effect) so clinicians will know what to refer to in the literature in terms of therapy guidance. “Or add a comment such as: ‘This organism is related to this particular complex, with demonstrated resistance to a particular drug,’” she says.

Dr. Lau says any laboratory with a mass spectrometry instrument should be able to adopt MALDI-TOF for mold identification. Whether smaller labs will is, for now, up in the air. “I would love for this to be universal,” Dr. Lau says. But not every laboratory is the NIH. Those without large resources will find it much easier to use an FDA-approved database—a step that hasn’t yet happened—than develop their own. “Mold databases from manufacturers are certainly available for purchase as a research-use-only tool,” she says. Alternatively, in-house–developed databases such as the NIH mold database are freely available through transfer agreements.

With molds, fears of abandonment—of old ways as well as employees—might be less acute than they’ve been with yeast or AFB, at least at the NIH. Dr. Lau and her colleagues still correlate MALDI results with the morphological or phenotypical findings. “Mass spec isn’t always perfect,” she says. But it has major advantages in being able to provide identification for sterile molds; that is, those that refuse to produce any morphologically identifiable structures.

Because mass spec provides results faster, “Our staff is freed up to explore other avenues of testing including research and expansion of the test menu. It depends on the institution,” she says.

What has to happen for Dr. Lau’s dream of universality to come to pass?

Clinical validation, for starters. Only one such study has looked at the Vitek system comprehensively for molds. “So there has to be a lot more performance reviews in the clinical setting.”

Even though the Biotyper has been the subject of more clinical validation studies, Dr. Lau notes the paucity of data on following the manufacturer’s guidelines and instrument performance. “On the Biotyper, most of the data has been based on in-house–developed databases. As beautiful as that is for clinical mycology in terms of moving diagnostics forward,” that doesn’t address two problems, she says: lack of standardization and lack of availability of those research databases.

And with two systems available, a comparison study might be in order. “There’s only been one study to date that looked at a large number of organisms, but very few of these test organisms were molds. And so a rigorous comparison in the clinical setting of both systems” would be helpful, she says.

Mold identification, moreover, has some technical challenges. Extraction methods are particularly complicated, Dr. Lau says, and can vary according to media (liquid or solid), sample type (entire mold versus spores), and so on. All give different spectra, Dr. Lau says, and therefore different levels of performance, which in turn are dependent on the database and its coverage.

Dr. Lau is finishing up a large study comparing the NIH and Bruker mold databases across eight U.S. academic medical centers. The results are somewhat disorienting, she says, with wide variability in performance, which has not been the case with bacteria and yeast.

In trying to pluck answers from the data, Dr. Lau hints at several possibilities.

Instrument settings and variability certainly have something to do with it, she says. But the fact that other

organisms haven't been affected by these differences means there's likely even more going on. Dr. Lau suspects that the answer has something to do with the concentration of protein in fungi. "It's harder to extract proteins for them," she says. "The extraction process is so much more complex than with yeasts."

Despite these mysteries, Dr. Lau remains optimistic about the potential for using MALDI-TOF for mold identification. The NIH database has been shared with about 82 laboratories worldwide so far. "The feedback that we're getting from some of the labs that are now using it routinely, in a day-to-day setting, has been great, and we continue to expand the database with new MSPs."

"I'm hoping that more labs will soon have the capacity to bring this in-house and make it a real-time clinical test," she says.
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