

# Exploring MALDI-TOF mass spec for mycobacteria

The Food and Drug Administration in 2017 approved MALDI-TOF mass spectrometry for the identification of mycobacteria. What is the protocol? How is workflow affected? Are there cost savings and turnaround time improvements? Omai Garner, PhD, D(ABMM), answered these and other questions and shared his laboratory's validation data in a Dec. 13, 2018 CAP TODAY webinar supported by BioMérieux ([captodayonline.com](http://captodayonline.com)). Here is an edited transcript of what he said.

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March 2019—The family of Mycobacteriaceae includes the genus *Mycobacterium*, which includes more than 190 species. These organisms have an unusual cell wall. Those of us who work in mycobacteria laboratories know that the cell wall has a high lipid content that makes these organisms acid fast. Thus they're acid-fast bacteria.



Dr. Garner

They have to put together this complicated cell wall and thus it can take from two to 60 days post culture in the organism on a plate to get that organism to grow. This is why we divide this group of organisms into slow-growing mycobacteria, which appear in culture after seven days, and this is typically related to solid culture, and rapid-growing mycobacteria, which grow in fewer than seven days.

The most clinically relevant mycobacteria species is *Mycobacterium tuberculosis*, which is a complex of organisms, including of course *M. tuberculosis* but also *M. bovis*/BCG, *M. africanum*, *M. caprae*, *M. canettii*, and others. We separate all the rest of the mycobacteria by calling them nontuberculous mycobacteria, or NTM, and they can be divided further into groups (**Fig. 1**).

These organisms can be in our drinking and bathing water and in water used for recreation, and this is because often they're found in almost all natural water sources. So we are regularly exposed to nontuberculous mycobacteria, but they exclusively or almost exclusively cause infections in immunocompromised patients.

*Mycobacterium* or nontuberculous mycobacterial infections in North America are on the rise. Between 1998 and 2010 the incidence of *M. tuberculosis* declined overall, though there are populations where this is not true, such as in Los Angeles. The opposite is happening for nontuberculous mycobacteria, in part because all hospitals are serving a larger variety of immunocompromised patients.

**Fig. 1. Mycobacteria classification**

<b>Mycobacterium tuberculosis complex</b>	<b>Nontuberculous mycobacteria (NTM)</b>
<i>M. tuberculosis</i> <i>M. bovis</i> /BCG <i>M. africanum</i> <i>M. caprae</i> <i>M. canettii</i> <i>M. microti</i> <i>M. mungi</i> <i>M. orygis</i>	<b>Non-cultivable</b> <i>M. leprae</i> <i>M. lepromatosis</i> <b>Slow-growing – nonchromogens</b> <i>M. avium</i> complex <i>M. haemophilum</i> <b>Slow-growing – photochromogens</b> <i>M. kansasii</i> <i>M. marinum</i> <b>Slow-growing – scotochromogens</b> <i>M. goodii</i> <i>M. xenopi</i> <b>Rapid growing</b> <i>M. chelonae</i> <i>M. abscessus</i> <i>M. fortuitum</i> <i>M. smegmatis</i>

Laboratories are getting better at being able to identify NTM to species. And MALDI-TOF mass spectrometry may make it possible for labs that do not identify NTM to species to be able to do so. From the clinical perspective of whether to treat, it's critically important to identify these organisms to species. Furthermore, some species of NTM require antimicrobial susceptibility testing for appropriate therapy, and AST relies on accurate species identification for interpretive criteria or for the use of breakpoints.

A level one mycobacteria laboratory performs no mycobacteriological procedures and would send the testing out. A level two laboratory would do acid-fast stains of exudates, effusions, and body fluids, and it may inoculate but then refer cultures to a reference laboratory.

Level three laboratories are able to isolate mycobacteria and have identification schemes in place for *M. tuberculosis* complex and perhaps preliminary identification of NTM. It is the level three laboratory that can take most advantage of MALDI-TOF MS identification of mycobacteria.

Level four laboratories are able to do definitive identification of mycobacteria. They can isolate them and identify them to the extent required to establish a correct clinical diagnosis; they may even do some level of susceptibility testing. Our mycobacteria laboratory at UCLA is a level four laboratory. From a level four laboratory perspective, there are distinct advantages to moving to MALDI-TOF.

At UCLA in our mycobacteria laboratory, we have direct tests so we're able to offer AFB smears and microscopy. We also offer *M. tuberculosis* complex PCR, for which we use GeneXpert (Cepheid). We're able to do culture and identification, including liquid culture and solid media culture. Once that culture is positive, we use a set of DNA probes, a hybridization scheme to be able to immediately identify *M. tuberculosis* or *M. avium* complex.

We also have in-house Sanger sequencing, and this allows us to do gene sequencing for the *rpoB* gene to fully identify nontuberculous mycobacteria to species. In addition, we do in-house some rapid-growing *Mycobacteria* drug susceptibility testing by broth microdilution.

For *M. tuberculosis* complex and *M. avium* complex drug susceptibility testing, we use a reference laboratory. Other nontuberculous mycobacteria that are slow growers are sent to specialized reference laboratories for susceptibility testing, to include even the newest drugs available for mycobacteria treatment.

How would MALDI-TOF fit into and improve this? We receive a number of different specimens for mycobacteria culture as selected by the physician. These diseases can show up anywhere; they are not restricted to pulmonary illnesses. If sterile specimens come in that are non-blood—surgical tissue, bone marrow, sterile fluids—we can grind and vortex and centrifuge and then move on to the direct AFB smear and inoculation of the sample.

If a nonsterile sample comes in, and this is a typical sample for mycobacteria—it could be respiratory swabs, gastric, urine, or stool—we go through the process of *N*-acetyl-L-cysteine-NaOH liquefaction and decontamination. We neutralize and centrifuge, and then move that into the AFB smear process. We do a fluorochrome stain and a Ziehl-Neelsen stain, and then we inoculate solid and liquid media for all specimens. The liquid media system we use is the Mycobacteria Growth Indicator Tube, or MGIT, system.

We do both systems because we look for that liquid media to come up with clinically relevant results possibly faster than the solid media, especially for the slow-growing mycobacteria. But there's still an advantage to using the solid media because potentially you could have polymicrobial mycobacterial infections, and those can be

worked up better on solid media.

Once that liquid culture flags as positive, or we see growth on the primary plates, the next important step is to confirm whether it's acid-fast bacteria. Once we do that, we move into our probes because they can provide a specific answer directly off the liquid media. We run a DNA probe for the *M. tuberculosis* complex and for *M. avium* complex.

If either one of those is positive, we have a confirmed ID and can send it out for susceptibility testing by culturing onto solid media. Additionally, though, if the MGIT cultures are negative and if the DNA probes are negative, we'll subculture to solid media, and all probe-negative organisms that grow on solid media in greater than seven days will be called slow growers. If they grow in fewer than seven days, we'll call them rapid growers. Ultimately, either way, as soon as we get solid media growth, we're able to move on to Sanger sequencing—*rpoB* gene sequencing identifying the region of the *rpoB* gene that can reliably be called to identification for species-level ID of mycobacteria. In our laboratory we are using a greater than 98 percent match to identify these organisms. It is a long, labor-intensive process, but at the end we have an ID that can guide clinical treatment.

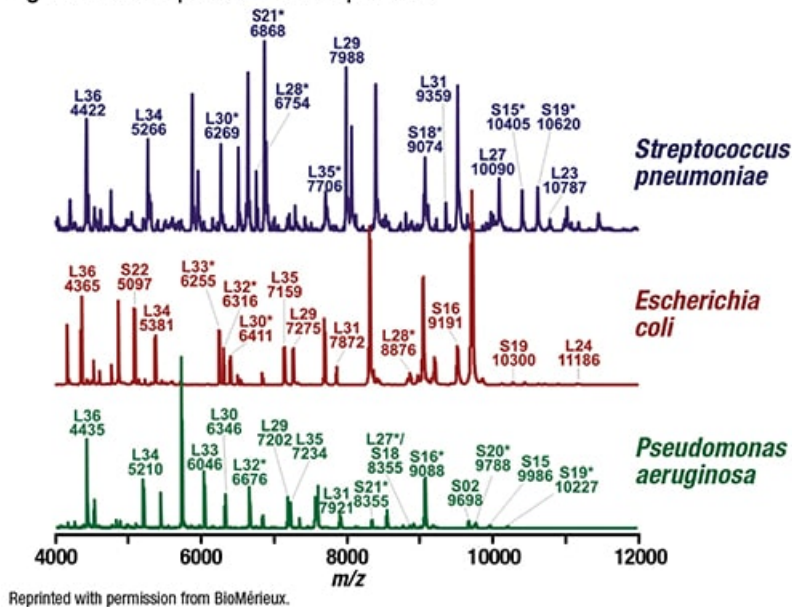
The majority of our isolates that are outside of *M. avium* complex and *M. tuberculosis* are identified by *rpoB* gene sequencing. How long does that take? We looked at the number of days to identification from a pure isolated culture of a *Mycobacterium*, and this is in the most ideal setting, meaning that the *rpoB* gene sequencing works on the first try and there are no repeats. We're able to offer this only once per week because of the strenuousness of the assay, so the turnaround time can be one to eight days post culture for an identification; the average is four days. In microbiology we always ask: Can we do better?

The FDA in 2017 approved MALDI-TOF for the identification of mycobacteria. What's the process? In the bacteriology laboratory, a sample can be taken and put onto a target plate, and the first thing that happens is that matrix is put onto that sample. The matrix is able to, with typical bacteria, kill the bacteria and ionize the proteins to get them ready for the time-of-flight tube.

Then a laser hits on the spot where the bacteria are. The proteins that are ionized within that section desorb or float up above that section where the laser hits. They're then ready to enter the time-of-flight tube, in which they fly to the detector. The smaller proteins will get to the detector first and show up on the spectrum first; the larger proteins from that bacteria will take a longer time to get through and show up later on the spectra. Then you have a proteomic ray of the different sizes of proteins found in that bacteria.

The y-axis of the spectrum is about the quantity of proteins that were there, so not only do you separate proteins by size, but you also get a read of how many proteins are there. That gives you an identifiable spectra that you can compare with other organisms that can lead to an identification.

**Fig. 2. Different species—different patterns**



When I first heard about MALDI mass spec, my questions were what proteins are being analyzed and what proteins are flying through. And what's interesting about MALDI-TOF is that you're only interested in the proteins that identify the difference between, say, *Escherichia coli* and *Staphylococcus aureus*. Those proteins are structural proteins found in the window between 2,000 and 20,000 daltons. The reason we look only in this window for MALDI-TOF is that if you look below this window, those are going to be metabolites and matrices that are not associated with the specific bacteria. Above that window will be the proteins that are enzymes and enzyme complexes.

The challenge of looking at enzymes and enzyme complexes can be laid out in the Gram-negative identification by MALDI-TOF. Let's say I have an *E. coli* growing on a blood agar plate. It's going to produce a certain protein profile of enzymes. If I take that same *E. coli* and grow it on a MacConkey plate, it needs to produce a different set of enzymes to be able to survive the crystal violet and bile salts on that plate. Thus my profile would end up looking very different even though I had the same *E. coli*. That's not good for microbial ID.

Functionally we put almost horse blinders on just to look in this area where you can identify organisms to species, and ultimately it works well. So you see different species, different pattern MALDI-TOF profiles for *Streptococcus pneumoniae*, *E. coli*, and *Pseudomonas aeruginosa* (**Fig. 2**). They're all distinct. It's not a pattern for a technologist to memorize, but it is a pattern that a computer algorithm is able to identify. The variabilities seen in the protein peaks between members of the same species don't fool the MALDI-TOF into calling it something different. That's critical.

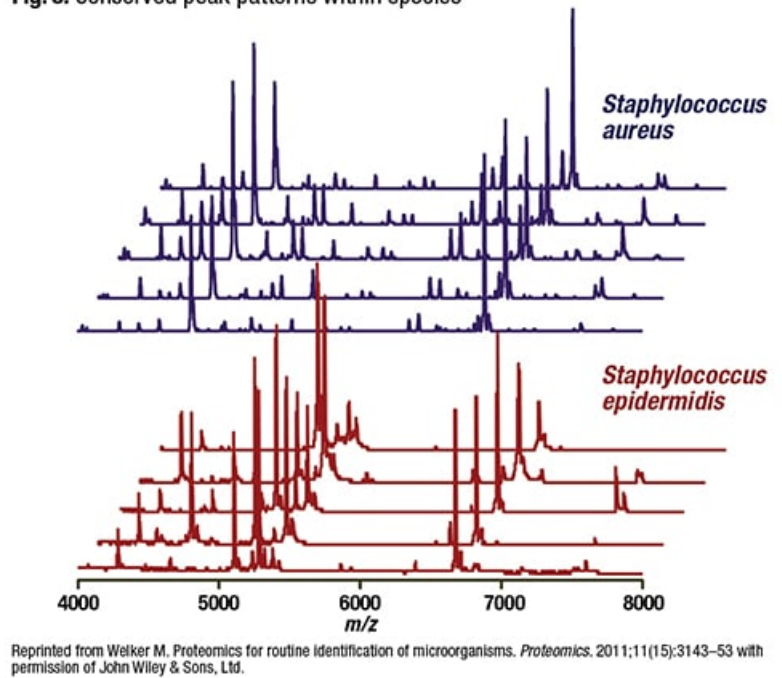
Let's look at two genetically similar organisms of coagulase-negative *Staph* and *S. aureus* (**Fig. 3**). The peaks are sufficiently different to determine the difference between these two, but the small variations seen among the five *S. aureus* isolates still do not make the computer make a mistake and call that *S. aureus* by genus and species something else.

The Vitek MS specifically does this by Bin Matrixing, a methodology proprietary to BioMérieux and the Vitek MS. Bin Matrixing looks at each peak of the spectrum and asks whether the presence or absence of that peak is related to the species ID. If it's peak one and it's a high peak, does that peak relate or not relate to *S. aureus*? And it does that 1,300 times across the spectra, thereby providing a reliable ID if it comes out at the end. So the spectra will be collected and analyzed and then the identification will be delivered, and there will be a competence factor associated with that identification. In addition to providing a reliable ID, MALDI-TOF is a simple workflow.

Let's talk about the difference in protocol. Once the isolate or the extraction is on the MALDI-TOF, everything is similar to what happens in the bacteriology laboratory. Whether or not you're moving from liquid culture or solid culture, there will be three distinct steps: inactivation, cell disruption, and protein extraction. These three steps are very necessary, especially in the mycobacteria laboratory. My MALDI-TOF does not live in my BSL2+ with BSL3 practices area of my laboratory. So it's critically important that the isolate is inactivated, the cells are disrupted, and the proteins are extracted. The organism is no longer alive before I bring it out of the lab.

The steps usually include methanol washes, bead beating for inactivation, and then formic acid and acetonitrile protein extraction and isolation. This does add to the workflow time so it's not as quick as MALDI-TOF for bacteriology. There's about a 90-minute addition to workflow time if you're coming from liquid culture, about 45 minutes if you're coming from solid culture.

**Fig. 3.** Conserved peak patterns within species



For solid culture ID, it starts with a mechanical disruption with glass beads and bead beating for five minutes or vortex mixing for 15 minutes, and then incubation in 70 percent ethanol for 10 minutes, followed by protein extraction using 70 percent formic acid and acetonitrile. At this point it's similar to what goes on in the bacteriology laboratory, where a microliter of that protein extract can be applied to a spot on the target slide, allowed to dry, and then overlaid with one microliter of matrix.

For liquid culture ID, you can take a 3-mL aliquot of liquid culture, centrifuge it for 10 minutes, decant the supernatant to remove the residual media, resuspend in 70 percent ethanol with beads, mix on a horizontal position vortexer for 15 minutes or a bead beater for five minutes, incubate 10 minutes at room temperature, then centrifuge, decant the supernatant, and resuspend the pellet in formic acid and acetonitrile. In addition, you take one microliter of that protein extract, apply it to a spot on the target slide, allow it to dry, and overlay it with one microliter of matrix. From that point on, functionally it looks very much the same.

We don't see all of the more than 190 species of mycobacteria in our patients, and all species do not cause disease. Take note of what FDA cleared on the Vitek MS (**Fig. 4**), meaning what sample identifications can come off and be released without extra testing by the laboratory. I find it to be a fairly comprehensive group of organisms, and I will talk about how the list compares with the UCLA isolates.

We looked at the specimens collected between November 2015 and February 2018 and we identified by probe 1,100 mycobacteria isolates. At UCLA the most common isolate is *M. avium* complex, accounting for 55 percent of what we see. We isolate only about four percent *M. tuberculosis* complex. The rapid-growing mycobacteria account for 30 percent of our organisms, and the slow-growing mycobacteria account for another four percent. And these groups are the groups we've been doing *rpoB* gene sequencing on and the groups we want to be able to target when we use MALDI-TOF.



**Fig. 4.** MALDI-TOF mycobacteria species in the FDA-cleared database

Rapid growers	Slow growers	
<i>M. abscessus</i>	<i>M. haemophilum</i>	<i>M. lentiflavum</i>
<i>M. chelonae</i>	<i>M. xenopi</i>	<i>M. simiae</i>
<i>M. immunogenum</i>	<i>M. marinum</i>	<i>M. malmoeense</i>
<i>M. smegmatis</i>	<i>M. goodii</i>	<i>M. scrofulaceum</i>
		<i>M. avium</i>
<b><i>M. fortuitum</i> group = calls as MFG</b>		<i>M. intracellulare</i>
<i>M. alvei</i>	<b><i>M. tuberculosis</i> complex</b>	
<i>M. farcinogenes</i>	<i>M. africanum</i>	
<i>M. fortuitum</i>	<i>M. bovis</i>	
<i>M. fortuitum ssp fortuitum</i>	<i>M. canettii</i>	
<i>M. houstonense</i>	<i>M. tuberculosis</i>	
<i>M. peregrinum</i>	<i>M. szulgai</i>	
<i>M. porcinum</i>	<i>M. kansasii</i>	
<i>M. senegalense</i>		
<i>M. mucogenicum</i>		

If we were using MALDI-TOF for the identification of mycobacteria for all of the mycobacteria we looked at, more than 96 percent of our mycobacteria isolates would be in the FDA-cleared database. Four percent would not be in that database. And any identification of nonclinically validated organisms must be performed with an alternative laboratory method. In our laboratory we will still do *rpoB* gene sequencing on those organisms to be able to confirm the identity that comes off the MALDI-TOF.

Now, a quick look at the data. There are two published papers in the *Journal of Clinical Microbiology*. One is the evaluation of the Vitek MS for MALDI-TOF for identification of *Mycobacterium* and *Nocardia*, and this was on solid media (Body BA, et al. 2018;56[6]:e00237-18). There were four clinical testing laboratories: LabCorp, ARUP, Memorial Sloan Kettering Cancer Center, and the University of Washington Medical Center. They tested mycobacteria from various types of solid media—Lowenstein-Jensen, Middlebrook 7H10 and 7H11, and Coletsos agar—on the Vitek MS and compared it with the gold standard of reference sequencing identification—a combination of 16s rRNA, *rpoB*, and other housekeeping genes.

Their data were strong. Of the 651 clinical mycobacteria isolates tested, 94 percent were correctly identified to the species, complex, or group level, run on the Vitek MS: 100 percent of the MTB, 92 percent of the slow-growing NTM, and 98 percent of the rapid growers. Thirty-three isolates, or five percent, could not be identified, meaning the MALDI-TOF provided no ID. This typically means there weren't enough spectral peaks or there was not enough to compare it with to give a reliable ID in the database.

Of the 33 isolates, there were 27 slow growers. Some of those isolates like *M. paraffinicum* were species that were not in the database. Some of those isolates that belonged to the *M. avium* complex but were not *M. avium* or *M. intracellulare* were also species not found in the database. And there were five rapid growers, two of which were isolates of *M. mucogenicum* that could not be identified.

Four isolates—less than one percent—were misidentified, and the misidentification can be dangerous because if isolates are misidentified as an FDA-approved isolate, then that ID would just go out. They found that the low percentage of organisms that were misidentified were not misidentified on that particular list, so from a solid media perspective the data are good.

Does MALDI-TOF work for a liquid culture? A paper was published on the performance of the Vitek MS v3.0 for identifying species from patient samples by use of automated liquid media systems (Miller E, et al. *J Clin Microbiol*. 2018;56[8]:e00219-18). LabCorp (using VersaTrek mycobacteria bottles) and Memorial Sloan Kettering (using Bactec MGIT 960 tubes) were the testing laboratories. They again tested for mycobacteria identification directly from the liquid media. They used either a seed and recovery or clinical isolate model, and they compared to reference sequencing ID.

In the first test they did, they asked whether the media found in the liquid cultures—and liquid cultures can have various supplements, antimicrobials, residual NALC-NaOH—created interfering peaks. They ran those supplements, and not enough peaks were shown to be able to interfere with identification. Then they did a seeded simulated sputum culture—a seed and recovery—and this is with 383 liquid cultures covering 77 strains and 21 species, and the data were very good. They had 99 percent result in correct identification to the species, complex, or group

level. Only four isolates resulted in a no ID.

What's more interesting in this paper is how this ID system performs out of liquid cultures. They tested 73 clinical liquid cultures, of which 64, or 87.7 percent, were identified correctly to species, complex, or group level. But about 10 percent resulted in a no ID (three *M. avium*, two *M. intracellulare*, one *M. lentiflavum*, one *M. tuberculosis* complex). Thus the data are starting to show that the system seems to work very well out of solid culture but may have some issues in being able to give an identification from liquid culture. But we're still below 10 percent.

Now for the UCLA data. You will recall the importance of inactivation. The MALDI-TOF as an instrument is not in my AFB laboratory, so if I'm going to put something on the MALDI-TOF, I want to be sure everything is nonviable. We ran a test where from solid media we did ethanol, bead beating and inactivation, and extraction, and then cultured that out for six weeks. Six weeks in, of the 35 isolates we tested, there was no growth whatsoever. One hundred percent of the isolates didn't show growth, and we tried to cover as much as possible. We of course ran some MTB, we ran slow growers including *M. avium* complex, and we ran rapid growers. This gave us confidence in the FDA approval, in that if you follow the protocols correctly you will have inactivated and you can use this safely outside of your BSL2 with BSL3 practices or BSL3 facility for identification.

Fig. 5. UCLA validation data

Media—MH711 Biplates (*rpoB* or DNA probe identification)

Species	isolates	% species ID	% complex ID	% No-ID	% Mis-ID	Mis-ID
<b>Total</b>	<b>n=46</b>	<b>93.5% (43)</b>		<b>2.2% (1)</b>	<b>4.4% (2)</b>	
<b>MTBC</b>	<b>n=5</b>					
<i>M. tuberculosis</i> complex	5	—	100 (5)	0 (0)	0 (0)	
<b>Rapid growers</b>	<b>n=19</b>					
<i>M. abscessus</i>	4	100 (4)	—	0 (0)	0 (0)	
<i>M. chelonae</i>	5	80 (4)	—	20 (1) X	0 (0)	
<i>M. neoaurum</i>	1	0 (0)	—	0 (0)	100 (1)	<i>M. arupense</i> X
<i>M. fortuitum</i> group	6	—	100 (6)	0 (0)	0 (0)	
<i>M. mucogenicum</i> complex	2	100 (2)	—	0 (0)	0 (0)	
<i>M. mucogenicum</i>	2	100 (2)	—	0 (0)	0 (0)	
<i>M. smegmatis</i> complex	1	0 (0)	—	0 (0)	100 (1)	<i>Citrobacter koseri</i> X
<i>M. mageritense</i>	1	0 (0)	—	0 (0)	100 (1)	
<b>Slow growers</b>	<b>n=22</b>					
<i>M. avium</i> complex	6	—	100 (6)	0 (0)	0 (0)	
<i>M. goodii</i>	3	100 (3)	—	0 (0)	0 (0)	
<i>M. haemophilum</i>	2	100 (2)	—	0 (0)	0 (0)	
<i>M. szulgai</i>	1	100 (1)	—	0 (0)	0 (0)	
<i>M. arupense</i>	2	100 (2)	—	0 (0)	0 (0)	
<i>M. paraffinicum</i>	1	100 (1)	1	0 (0)	0 (0)	
<i>M. simiae</i> complex	7	100 (7)	—	0 (0)	0 (0)	
<i>M. simiae</i>	3	100 (3)	—	0 (0)	0 (0)	
<i>M. lentiflavum</i>	4	100 (4)	—	0 (0)	0 (0)	

Media—Blood agar (*rpoB* identification)

Species	isolates	% species ID	% complex ID	% No-ID	% Mis-ID	Mis-ID
<b>Total</b>	<b>n=8</b>	<b>87.5% (7)</b>		<b>12.5% (1)</b>	<b>0% (0)</b>	
<b>Rapid growers</b>	<b>n=8</b>					
<i>M. abscessus</i>	2	50 (1)	—	1 (50)	0 (0)	
<i>M. chelonae</i>	2	100 (2)	—	0 (0)	0 (0)	
<i>M. fortuitum</i> group	2	—	100 (2)	0 (0)	0 (0)	
<i>M. mucogenicum</i> complex	2	100 (2)	—	0 (0)	0 (0)	
<i>M. mucogenicum</i>	2	100 (2)	—	0 (0)	0 (0)	

Media—MGIT (6 days post)

Species	isolates	% species ID	% complex ID	% No-ID	% Mis-ID	Mis-ID
<b>Total</b>	<b>n=55</b>	<b>100% (55)</b>		<b>0% (0)</b>	<b>0% (0)</b>	
<b>MTBC</b>	<b>n=4</b>					
<i>M. tuberculosis</i> complex	4	—	100 (4)	0 (0)	0 (0)	
<b>Rapid growers</b>	<b>n=22</b>					
<i>M. abscessus</i>	6	100 (6)	—	0 (0)	0 (0)	
<i>M. chelonae</i>	5	100 (5)	—	0 (0)	0 (0)	
<i>M. fortuitum</i> group	6	—	100 (6)	0 (0)	0 (0)	
<i>M. mucogenicum</i> complex	5	100 (5)	—	0 (0)	0 (0)	
<i>M. mucogenicum</i>	5	100 (5)	—	0 (0)	0 (0)	
<b>Slow growers</b>	<b>n=29</b>					
<i>M. avium</i> complex	6	—	100 (6)	0 (0)	0 (0)	
<i>M. goodii</i>	8	100 (8)	—	0 (0)	0 (0)	
<i>M. arupense</i>	2	100 (2)	—	0 (0)	0 (0)	
<i>M. kansasii</i>	7	100 (7)	—	0 (0)	0 (0)	
<i>M. simiae</i> complex	6	100 (6)	—	0 (0)	0 (0)	
<i>M. simiae</i>	3	100 (3)	—	0 (0)	0 (0)	
<i>M. lentiflavum</i>	3	100 (3)	—	0 (0)	0 (0)	



The UCLA validation data (**Fig. 5**) are based on MH711 biplate media, and our gold standard to compare the identification from the MALDI-TOF was either the DNA probe for *M. tuberculosis* and *M. avium* complex or *rpoB* gene sequencing. Of 46 *M. tuberculosis* isolates tested, we tested five MTB and got 100 percent identification. With the rapid growers, we saw a very high identification to the species or to the complex. We had one *M. chelonae* that gave a no ID, one *M. neoaurum* that was called *M. arupense*, and one *M. smegmatis* complex that was identified as *Citrobacter koseri*. You immediately say, "Wow, a *Citrobacter* when we're working in a mycobacteria laboratory; you're doing an AFB stain." And in doing an AFB stain, you're getting other information. MALDI-TOF is a fantastic tool for the clinical laboratory. It is an identification scheme; it is not there to replace the expertise of the technologists. Just like everything else in the microbiology laboratory, it relies on the expertise of bench technologists to be able to put out correct IDs for patient care. No system is going to be 100 percent.

We did 22 slow growers, and of those 22, we had about 100 percent identification to species or complex.

We see a lot of the rapid growers on blood agar plates, so we did a blood agar plate validation as compared to *rpoB*, and again the data were good. Whether *M. abscessus*, *M. chelonae*, *M. fortuitum* group, or any of these isolates growing on blood agar plate, we had a reliable identification. There was one that gave a no ID.

More on liquid culture and a bit about biomass. For MALDI-TOF mass spec to work, there has to be enough organism or enough protein extracted to be able to get a spectral ID, and there cannot be interfering substances. For solid media, the suggestion for *Mycobacterium* is to use a one microliter loopful. We found it works better for a large loopful. For isolations out of liquid, a positive tube test needs to be done as soon as practical. The suggestion is either 1.8 mL or 3 mL. In our liquid culture validation, we got more reliable identification using 6 mL of the liquid culture medium, and then we would let it grow six days post that initial positive. Once we let it grow six days post, that gave us a sufficiently reliable ID. Is that necessary for all mycobacteria? It's hard to know. We looked at that study again, and for the rapid growers we could get it to work much sooner, in the 48-hour range. But when a liquid culture grows in mycobacteria, what you don't know is whether it's a rapid grower or a nonrapid grower. All you know is that it's acid-fast bacteria. For our setting we're going to do six days post liquid culture for identification. This is still a dramatic improvement in time as compared with solid culture identification.

Six days post culture we had reliable identification of *M. tuberculosis* at 100 percent, and across the board we saw 100 percent in the rapid growers and slow growers for identification. You will recall that earlier study and those no IDs—were they a question of biomass, and if they were able to wait a little longer could they functionally get an identification? Our study potentially could show that, and that MALDI-TOF, if your biomass is appropriate, is a reliable system for identification.

How do we then put MALDI-TOF into our system? The MGIT is going to flag positive, you'll see growth on the primary plates, and at UCLA we're going to use the probes because we still have them. Probes can be used day one from liquid culture, so it's going to be our fastest way from liquid cultures to still identify MTB or *M. avium*. If the probes are negative, then we can do the six days post liquid culture MALDI-TOF or directly from solid media. The rapid growers grow up fast on the solid media and then we can run MALDI-TOF. If the MALDI-TOF identification is FDA approved, we can release that result. If it's not FDA approved, then we'll still move to our *rpoB* gene sequencing.

What are the estimated improvements in the laboratory if you're able to put in MALDI-TOF mass spec? The improvements I will point to are specific to UCLA. If we look at the number of days to identification from a pure isolated culture, we estimate that our one- to eight-day range for *rpoB* sequencing would be cut to zero to one day, with an average of a half-day turnaround time, allowing us on average to give an identification of those rapid and slow growers for which we were doing gene sequencing three and a half days earlier than before. This will apply in 96.6 percent of our non-*M. tuberculosis*, non-*M. avium* complex patient isolates.

What do we save in FTE time so technologists can do other important work? *RpoB* gene sequencing is intensive. It's four hours per day and takes two days. At that one test per week it probably "costs" 0.2 FTE. If we put in MALDI-TOF, it's about 1.25 hours. We can run it three times a week and we're at a savings of at least 0.1 FTE in these

general settings. These are estimates only; we have not done a full workup to see what the savings are. There are also potential cost savings in reagents and QC. The savings do not include equipment. If you don't have a MALDI-TOF already, it's going to be expensive to buy one for mycobacteria.

But if you already have a MALDI-TOF and send-out costs are expensive, you can potentially recoup costs. If we look at *rpoB* testing for the year (before mycobacteria), for QC we were spending about \$9,000. For patient testing we're spending about \$18,000—just in reagents. The total is \$27,000. After MALDI-TOF, looking back over that year, if we just did MALDI-TOF, now our *rpoB* gene sequencing has gone way down. Most of these isolates have moved over to MALDI-TOF. We're only spending \$700 there, \$1,500 for QC, for a total of \$2,200. Patient testing on the MALDI-TOF, generally with all the necessary reagents, is about \$9,000. So ultimately this could save UCLA in excess of \$16,000 a year.

The time and cost savings at UCLA are not a guarantee of results at other laboratories, and, again, we haven't done a full cost analysis at UCLA so this isn't even a guarantee of cost savings we could see. But I think we're in the ballpark. As all lab directors know, it's getting harder for us to justify our tests and new testing to the hospital, so these are ways that you can think about it, not even considering shortening patient turnaround time and length of stay. But just in the laboratory how you can accrue savings if you bring this in.

MALDI-TOF for mycobacterial identification will definitely allow for species identification faster. The faster we can get species identification, the higher our clinical impact. We've just begun this and our infectious disease service has already noticed how much faster it's getting identifications for nontuberculous mycobacteria.

MALDI also allows for new opportunities. It is adding more clinically important tests for *Mycobacterium*, and as we have more and more immunocompromised patients who are living longer—thanks to transplants and new cancer therapies—more and more people will be subject to these types of diseases, making it more critical for laboratories to be able to identify them.□