9-9-2019

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Sources of support: Funding was provided by bioMérieux for technical support and supplies at one study site (Massachusetts General Hospital), and technical assistance was provided by Bruker Daltonics to Marshfield Clinic Health System and by bioMérieux to Mass. Gen. Hosp.

Brief title: MALDI-TOF MS for NTM Identification

Key words: nontuberculous mycobacteria; mass spectrometry; identification; MALDI-TOF; comparison; Bruker Biotyper; bioMérieux VITEK MS

ABSTRACT

Objectives: This multi-center study's aim was to assess the performance of two commercially-available matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) systems in identifying a challenge collection of clinically-relevant nontuberculous mycobacteria (NTM).

Methods: NTM clinical isolates (N=244) belonging to 23 species/subspecies were identified by gene sequencing and analyzed using the Bruker Biotyper with Mycobacterial Library v5.0.0 and the bioMérieux VITEK MS with v3.0 database.

Results: Using the Bruker or bioMérieux systems, 92% or 95% of NTM strains, respectively, were identified at least to the complex/group level; 62% and 57%, respectively, were identified to the highest taxonomic level. Differentiation between members of the *M. abscessus*, *M. fortuitum*, *M. mucogenicum*, *M. avium*, and *M. terrae* complexes/groups was problematic for both systems, as was identification of *M. chelonae* for the Bruker system.

Conclusions: Both systems identified most NTM isolates to the group/complex level, and many to the highest taxonomic level. Performance was comparable.

INTRODUCTION

Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) represents a new paradigm in the analysis of microorganisms in the laboratory (1). Initially, MS instruments were used primarily in larger reference laboratories, and the identification capabilities were limited mostly to bacterial species and yeast (2-8). However, in the last decade, the systems have become more widely utilized in routine clinical laboratories worldwide. In response, the Clinical and Laboratory Standards Institute has recently published a guidance document to assist clinical laboratories with implementation and verification of commercial MALDI-TOF MS systems (9). The range of microorganisms identifiable using commercial systems has expanded considerably to now include filamentous fungi and mycobacteria (10-15).

This study describes a multicenter study including two laboratories routinely using MALDI-TOF MS for non-tuberculous mycobacteria (NTM) identification, with each employing a different commercial MALDI-TOF MS system and database. The laboratory at Massachusetts General Hospital (MGH) in Boston, MA used the VITEK MS IVD system with v3.0 Knowledge Base (bioMérieux, Durham, NC) and the laboratory at the Marshfield Clinic Health System (MCHS) in Marshfield, WI used the Bruker Biotyper system with RUO Mycobacteria Library v5.0.0 (Bruker Daltonics, Billerica, MA). Isolates had previously been identified by gene sequencing approaches at the University of Texas Health Science Center at Tyler (UTHSCT) Mycobacteria/Nocardia Research Laboratory, a clinical reference laboratory with expertise in identification of NTM (13, 15). The objective of this multicenter study was to establish and compare the accuracy of each MALDI-TOF MS system for the identification of NTM clinical isolates in a clinical laboratory setting, using gene sequencing as the reference standard. There

are few head-to-head comparisons between these MALDI-TOF MS systems for NTM identification (16, 17); to our knowledge, this is the first such study that is multicenter, and the first to include both the VITEK MS IVD system with v3.0 Knowledge Base and the Bruker Biotyper system with RUO Mycobacteria Library v5.0.0 in the same study.

MATERIALS AND METHODS

The study was approved by the institutional review board (IRB) at MCHS and was deemed exempt from IRB oversight at MGH and the UTHSCT, in accordance with the ethical standards established by each institution. All isolates were de-identified prior to shipment.

Clinical isolates. The isolates (n=244) included 125 rapidly growing mycobacteria (RGM) and 119 slowly growing mycobacteria (SGM) species, identified previously by gene sequencing at the Mycobacteria/Nocardia Research Laboratory at the UTHSCT (Tables 1 and 2). The isolates had been previously stored at -70°C in trypticase soy broth (Remel, Lenexa, KS) with glycerol, and were subcultured onto Middlebrook 7H10 agar plates (Remel) and shipped on 7H10 agar slants (Remel) from UTHSCT to the two sites (MGH and MCHS).

Reference method for identification. Identification of all study isolates was performed by gene sequencing at the UTHSCT. RGM strains were identified by sequencing the 720 bp region V of the *rpoB* (beta subunit of RNA polymerase) gene as described by Adekambi et al. (18, 19), and for the *Mycobacterium abscessus* complex, the *erm*(41) (erythromycin ribosomal methylase) gene was also sequenced as previously described (20) to aid in the identification of subspecies. Partial 16S rRNA gene sequencing was performed on SGM strains using MicroSeq 500 rDNA PCR and sequencing kits according to the manufacturer's instructions (Life Technologies, Carlsbad, CA). The resulting approximately 500 bp gene sequence was analyzed using software

from RipSeq (Pathogenomix, Santa Cruz, CA). Quality control for gene sequencing included positive control strains (*Mycobacterium fortuitum* ATCC 6841T for RGM and SGM for *rpo*β and 16S rRNA genes respectively, and ATCC 19977T for the *M. abscessus* complex *erm* gene control). Sterile deionized water was also included as a negative control. To help distinguish between *M. marinum* (strains of which were included in this study) and *M. ulcerans* (not included in this study), the sequence data were supplemented with phenotypic data including growth rate. *M. marinum* is an intermediately growing photochromogen (5-7d) , whereas *M. ulcerans* is a very slowly growing nonchromogen (>4 weeks) . Additionally, the *rpo*β gene was sequenced to separate *M. kansasii* (strains of which were included in this study) from *M. gastri* (not included in this study).

MALDI-TOF MS sample extraction and analysis procedures.

BioMérieux VITEK MS IVD system with v3.0 Knowledge Base

Extraction. Inactivation and extraction procedures were performed using an FDA-cleared reagent kit (bioMérieux, Durham, NC), according the manufacturer's instructions. In brief, mycobacterial isolates were subcultured onto Middlebrook 7H11 plates (Remel). When growth was sufficient, a 1 µl sterile loop was used to transfer a loopful of biomass to 0.5mm Glass Bead Tubes (MoBio Laboratories, Inc., Carlsbad, CA) containing 500 µl of 70% ethanol (Sigma-Aldrich, St. Louis, MO). The tubes were then placed for 5 minutes in a bead beater (BioSpec Products, Bartlesville, OK) for mechanical disruption and allowed to incubate at room temperature for an additional 10 minutes to complete the inactivation process. Each tube was then vortexed for 10 seconds, and the suspension was transferred to a micro-centrifuge vial and centrifuged to create a pellet. The ethanol was removed from the pellet, and the pellet was air

dried. Each pellet was re-suspended in 10 µl of 70% formic acid (Fisher Scientific, Waltham, MA) and incubated at room temperature for five minutes. Finally, 10 µl of acetonitrile (Sigma-Aldrich) was added to each vial, mixed, and centrifuged to create a pellet. One microliter of the supernatant (extract) was transferred to spots (in duplicate) on the target slide and allowed to air dry. One microliter of α-cyano-4-hydroxycinnamic acid (CHCA) matrix (VITEK MS-CHCA, bioMérieux) was then placed on each spot and allowed to dry.

MALDI-TOF MS Analysis. An *Escherichia coli* reference strain (ATCC 8739) was placed on control spots on each target slide for instrument calibration. Spectra for each target were obtained using a nitrogen laser at 50 shots per second for ionization and detection of proteins with a mass-to-charge ratio (*m/z*) between 2,000 to 20,000 Da. Samples were analyzed in duplicate, and species identification was assigned using the v3.0 FDA 510(k) cleared database. The VITEK MS system uses a "traffic light" approach to results interpretation: when a single (non-split) identification is achieved with good confidence, the single identification is displayed along with a green light; when there is low discrimination between several possible identifications (i.e., a split identification), a list of 2 to 4 possible identifications is displayed along with a yellow light; when no identification is achieved, a red light is displayed.

If initial analysis yielded a low discrimination identification, or if no identification was obtained, the VITEK MS analysis was repeated once using the same protein extract. If this did not resolve the issue, the extraction was repeated once.

<u>Bruker MALDI Biotyper 3.3.1.0 with RUO Mycobacteria Library v5.0.0</u>

Extraction. Heat inactivation and extraction procedures were performed according the manufacturer's instructions. Briefly, mycobacterial isolates were subcultured onto Middlebrook

7H11 plates (Remel). When growth was sufficient, a 1 µl sterile loop was used to transfer a loopful of biomass to 1.5 mL Safe Lock microtubes (Eppendorf, Hauppauge, NY) containing HPLC-grade water (Sigma-Aldrich, St. Louis, MO) and heated to 100°C for 30 minutes in a heat block. After cooling absolute ethanol (Sigma-Aldrich) was added to the biomass, vortexed and centrifuged to create a pellet. All of the residual ethanol was removed, and the pellet allowed to dry completely at room temperature. Once dried, a small spatula tip of 0.5mm Zirconia / Silica beads (BioSpec Products, Bartlesville, OK) was added to each tube followed by 10-50 µl acetonitrile (Sigma-Aldrich), based on pellet size, and vortexed for 1 minute. After pellet disruption, equal parts 70% formic acid (Sigma-Aldrich) were added, vortexed briefly and centrifuged to re-pellet. One microliter of the supernatant (extract) was transferred to spots (in duplicate) on a MALDI target plate (Bruker Daltonik GmbH, Bremen, Germany) and allowed to air dry. One microliter of Matrix HCCA (Bruker Daltonik GmbH) was then placed on each spot and allowed to air dry.

MALDI-TOF MS Analysis. A Bacterial Test Standard (typical *Escherichia coli* DH5 alpha; Bruker Daltonik GmbH) was placed on control spots on each MALDI target plate for instrument calibration. Spectra for each target were obtained using a nitrogen laser at 40 shots per second (to total 240 shots) for ionization and detection of proteins with a mass-to-charge ratio (*m/z*) between 2,000 to 20,000 Da. Samples were analyzed in duplicate, and microorganism identification and confidence-level classification (Table 1) were assigned using the Mycobacteria Library v5.0.0 database. For each analysis, the system provides a list of possible identifications, in order of likelihood (with the highest probability identification listed first). Each possible identification is assigned a confidence score as follows:

1.80 to 3.00: High-confidence identification

1.60 to 1.79: Low-confidence identification

0.00 to 1.59: No organism identification possible

Statistical Analysis. Comparisons were made using McNemar's test. P values were 2-tailed and a value of <0.05 was considered significant. All analyses were conducted using GraphPad QuickCalcs (GraphPad Software Inc., La Jolla, CA, USA).

RESULTS

Overall performance in the identification of NTM clinical isolates using MALDI-TOF MS was comparable between the Bruker Biotyper and bioMérieux VITEK systems. The systems identified 92% (225/244) and 95% (231/244) of all isolates to at least the complex/group level, respectively (P=0.18; Tables 1 and 2). The Biotyper system identified 151/244 isolates (62%) to the highest taxonomic level, either the species or subspecies level depending on the organism. The VITEK system identified 138/244 of all isolates (57%) to the highest taxonomic level (P=0.11). The Biotyper system could not identify 12/244 isolates (5%) and misidentified 7/244 isolates (3%); the VITEK system could not identify 11/244 isolates (5%; P=1.0) and misidentified 2/244 isolates (1%; P=0.18). The chief limitations of each system—many of which are common to both—are illustrated in Figure 1.

Among RGM, the Biotyper system identified 59/125 isolates (47%) to the highest taxonomic level, and the VITEK system identified 39/125 isolates (31%) to that level (P=0.004); Table 1). The Biotyper system could not identify 3/125 RGM isolates (2%) and misidentified 7/125 RGM isolates (6%); the VITEK system did not fail to identify any RGM isolates (0%; P=0.2) and misidentified 1/125 isolates (1%; P=0.08). Neither system could differentiate among members of the *M. abscessus* complex; both systems identified *M. abscessus* subsp. *abscessus*,

M. abscessus subsp. *massiliense*, and *M. abscessus* subsp. *bolletii* isolates as *M. abscessus*. Similarly, the Biotyper system identified 13/13 *M. mucogenicum* group members (5 *M. mucogenicum* and 8 *M. phocaicum*) as *M. mucogenicum/phocaicum* group; the VITEK system identified 5/5 *M. mucogenicum* isolates as *M. mucogenicum*, but also identified 8/8 *M. phocaicum* isolates as *M. mucogenicum*.

The VITEK system reported 39/39 *M. fortuitum* group isolates as *M. fortuitum* group, whereas the Biotyper system was better able to differentiate among *M. fortuitum* group members. The Biotyper system identified 20/20 *M. fortuitum* subsp. *fortuitum* isolates as *M. fortuitum*, 9/10 *M. porcinum* isolates as *M. porcinum*, 1/1 *M. neworleansense* isolates as *M. neworleansense*, and 1/8 *M. senegalense/conceptionense* isolates as *M. senegalense* (*M. senegalense* and *M. conceptionense* are indistinguishable based on genomic sequencing) (21). However, the Biotyper system misidentified 1/10 *M. porcinum* isolates as *M. abscessus*, and identified 7/8 *M. senegalense* isolates as *M. fortuitum* complex.

The Biotyper system misidentified 6/15 *M. chelonae* isolates as *M. salmoniphilum*, and an additional isolate (1/15) gave a split identification (*M. chelonae/salmoniphilum*); the remaining 8/15 isolates were identified as *M. chelonae*. In contrast, the VITEK system identified all 15 isolates as *M. chelonae*, the highest taxonomic level. The difference in the number of *M. chelonae* misidentifications between the 2 systems (6/15 for the Bioptyper system compared with 0/15 for the VITEK system) was statistically significant (P=0.04). Both systems reported 11/11 *M. mageritense* isolates as *M. mageritense*. The Biotyper and VITEK systems reported 9/9 and 8/9 *M. immunogenum* isolates as *M. immunogenum*, respectively. The remaining *M. immunogenum* isolate (1/9) was misidentified as *M. abscessus* by the VITEK system.

Among SGM, the Biotyper system identified 92/119 isolates (77%) to the highest taxonomic level, and the VITEK system identified 99/119 isolates (83%) to that level (P=0.07; Table 2). The Biotyper system could not identify 9/119 SGM isolates (8%) and did not misidentify any SGM isolates (0%); the VITEK system could not identify 11/119 of SGM isolates (9%; P=0.5) and misidentified 1/119 isolates (1%; P=1.0). Both systems identified nearly all strains of several species to the highest taxonomic level, including *M. simiae* (16/16 strains on both systems), *M. arupense* (20/20 strains on the Biotyper system; 19/20 strains on the VITEK system, with 1 strain not identified), *M. kansasii* (9/9 strains on both systems), *M. xenopi* (9/9 strains on both systems), *M. nebraskense* (10/10 strains on the Biotyper system; 9/10 strains on the VITEK system, with 1 strain not identified), and *M. marinum* (8/8 strains on both systems). Both systems also identified most *M. gordonae* strains to the highest taxonomic level, but the Biotyper system produced 3/14 low confidence identifications, and 3/14 strains were not identified by the VITEK system.

Neither system could reliably identify *M. terrae* isolates. The Biotyper system identified 1/7 strains to the complex level as *M. terrae* complex; 6/7 isolates were not identified or produced a low confidence identification. The VITEK system produced no identification for 6/7 *M. terrae* strains, and misidentified 1/7 strains as *Cronobacter malonaticus*, which is not a *Mycobacterium* species but rather a gram-negative bacillus in the family Enterobacteriaceae. Some members of the *M. avium* complex (MAC) also presented difficulties. Whereas both systems identified 9/9 *M. avium* strains to the highest taxonomic level, and the VITEK system identified 9/9 *M. intracellulare* isolates to that level, the Biotyper system identified 9/9 *M. intracellulare* isolates to the group/complex level as *M. chimaera/intracellulare*. In addition, the

Biotyper identified 8/8 *M. chimaera* strains as *M. chimaera/intracellulare*, and the VITEK system identified 8/8 *M. chimaera* strains as *M. intracellulare*.

DISCUSSION

The use of MALDI-TOF MS has increasingly shifted the paradigm for the identification of microorganisms in the clinical laboratory from the limited capabilities of biochemical testing to a proteomic approach with expanded capabilities. MALDI-TOF MS systems were not widely available commercially until the latter 1990s (22-24), and only cleared for routine clinical use by the FDA in 2013. Currently, two commercial MALDI-TOF MS systems, the Bruker Biotyper and the bioMérieux VITEK MS, are being used in U.S. clinical laboratories for the identification of microorganisms, including NTM (13, 14).

The current study showed comparable results between the two commercial MALDI-TOF MS systems for NTM identification, consistent with a recent meta-analysis (25). Although the systems identified 92% (Biotyper) or 95% (VITEK) of all isolates to at least the complex/group level, they only identified 62% or 57% of strains, respectively, to the highest taxonomic level (species or subspecies, rather than complex or group).

The Biotyper system with Mycobacteria library v5.0.0 identified significantly more RGM isolates to the highest taxonomic level, compared with the VITEK v3.0 system (47% versus 31%; P=0.004). Neither system could reliably differentiate among members of the *M. abscessus* complex or the *M. mucogenicum* group. In addition, the VITEK system could not differentiate among *M. fortuitum* group members, and the Biotyper system misidentified nearly half of *M. chelonae* isolates. These findings are consistent with the findings of previous studies using the VITEK v3.0 system (17, 26) or earlier versions of the Biotyper Mycobacteria library (11, 12, 17, 27, 28), except that using earlier versions of the Biotyper Mycobacteria library the difficulty in

identifying *M. chelonae* was described as yielding low-confidence identification scores rather than misidentifications. Also, in some other studies this limitation was not observed (29-31). Notably, in contrast to commercial (500 bp) 16S gene sequencing, both MALDI-TOF MS systems were able to differentiate the *M. abscessus* complex from *M. chelonae,* an important advantage.

For SGM, the trend was reversed, although the differences did not quite meet the threshold for statistical significance: the Biotyper system identified 77% to the highest taxonomic level, versus 83% for the VITEK system (P=0.07). Neither system could reliably differentiate among members of the *M. avium* complex, and neither could reliably identify *M. terrae* isolates. These limitations in SGM identification have also been noted in studies using earlier versions of the Biotyper Mycobacteria library (16, 17, 32), and in studies using the VITEK MS v3.0 system (17).

For some NTM species/groups (e.g., the *M. mucogenicum* group), the inability to distinguish among the members may not be clinically significant, because group members have similar antimicrobial susceptibility profiles, and public health implications (33). However, the situation is different for the *M. abscessus* and *M. avium* complexes, and the *M. fortuitum* group. Among *M. abscessus* complex members, *M. abscessus* subsp. *massiliense* strains typically lack a functional *erm*(41) gene, and are susceptible to macrolides, whereas *M. abscessus* subsp. *bolletii* and subsp. *abscessus* strains typically contain a functional *erm*(41) gene and are macrolide-resistant (34). Thus, a laboratory report of *M. abscessus* complex, which would be necessitated using either MALDI MS system, would not provide all the information to guide initial therapy before antimicrobial susceptibility test results are available.

Similarly, members of the *M. fortuitum* group are responsible for variable types of clinical disease, most of which, unlike the *M. abscessus* complex, are treated with oral antimicrobials such as quinolones, macrolides and tetracyclines. Some species within the *M. fortuitum* (e.g., *M. senegalense, M. peregrinum*) lack a functional *erm* gene and show macrolide susceptibility unlike other members (i.e., *M. porcinum* and other former third biovariant species), which harbor a functional *erm* gene rendering them inducibly macrolide resistant. Additionally, members of the *M. fortuitum* group differ in resistance to quinolones and tetracyclines (33, 35-38).

With MAC, the member species do have similar antimicrobial susceptibility profiles, so a laboratory report of *M. avium* complex should not be an impediment to optimal initial drug selection. However, one particular member—*M. chimaera*—has been linked to nosocomial outbreaks traced to heater-cooler units used during cardiothoracic surgery (39). Thus, prompt recognition of this species from non-respiratory sites such as blood, or heart valves (tissue), may help identify outbreaks, and the inability to differentiate this species from other MAC members is a limitation with potential infection control consequences.

One option to mitigate these limitations would be to use the MALDI-TOF MS identification as an initial step, with subsequent augmentation using gene sequencing in situations where further identification of an isolate is needed. For *M. abscessus* complex isolates, this should include sequencing of the *erm*(41) gene as recommended by CLSI (40). Previously, investigators have recommended supplementation of the commercial databases with an in-house database, and this may be a viable approach in some settings, but requires extensive characterization of a large number of isolates, which is often cost- and time-prohibitive in clinical laboratories (11, 13, 41). Still, based on initial studies, improvements in MS databases or

data processing algorithms have the potential to improve performance in identifying closely-related species or subspecies to the highest taxonomic level (42-46), and it may be possible for manufacturers to make such improvements in future system upgrades.

Each MALDI-TOF MS system misidentified a small number of isolates (7/244 (3%) for the Biotyper system, 2/244 (1%) for the VITEK system). The difference between the two systems, in number of misidentifications, was not statistically significant. Interestingly, none of the 244 NTM strains included in this study was misidentified on both systems. The most frequent errors were the misidentification of 6/15 *M. chelonae* isolates as *M. salmoniphilum* using the Biotyper system. This is of concern because by gene sequence, the two species are easily separated (*i.e.*, their full 16S rRNA gene sequences differ by 5 bp, their *rpoB* gene sequences differ by 22 bp, and their *hsp*65 gene sequences differ by 18 bp), and because *M. salmoniphilum*, unlike *M. chelonae*, has not been associated with human disease. This problem was not apparent with the VITEK platform, which reliably identified *M. chelonae.* The remaining few misidentifications on either system involved a single strain of a given species.

Each system also failed to identify (or provided only a low-confidence identification for) a small number of strains (12/244 (5%) for the Biotyper system, 11/244 (5%) for the VITEK system). Here, there was some overlap between the 2 systems. The VITEK system failed to identify 6/7 *M. terrae* strains and misidentified the remaining strain; the Biotyper also failed to identify 6/7 *M. terrae* strains. This species is not present in the VITEK v3.0 database but is represented (as *M. terrae* complex) in the Biotyper Mycobacteria library v5.0.0, which did identify 1/7 strains to the complex level. Species in the *M. terrae* complex (*M. virginiense, M. arupense, M. heraklionense, M. kumamotonense*) are causative agents of tenosynovitis and thus are important to identify (47).

Both systems also failed to identify 3/14 *M. gordonae* strains (the same 3 strains), even though this species is present in both databases. Presumably, the databases lack sufficient strain diversity for this species. Regarding identification of *M. gordonae* isolates, it is important to identify non-pathogenic species as well as pathogens in order to prevent unnecessary treatment.

There are some important limitations to our study, including the limited number of isolates tested for some species. Additionally, no species within the *M. avium* complex other than *M. avium, M. intracellulare,* and *M. chimaera* were included, and no species within the *M. terrae* complex other than *M. terrae* and *M. arupense* were tested. Finally, although differences in the ability to identify certain nontuberculous mycobacteria were observed between the Bruker and bioMérieux MALDI-TOF MS systems, the reason for these differences cannot be determined. Numerous factors known to affect performance vary between the two systems, including the approach to sample preparation, sample analysis, data analysis, and the spectral databases themselves, among many other variables.

In summary, our multicenter study is corroborative of other recent studies of NTM identification using each system individually, and also with two single-center studies comparing the two commercial platforms (12-17). Our findings demonstrate that both the Bruker Biotyper system with RUO Mycobacterial Library v5.0.0, and the bioMérieux VITEK MS system with v3.0 database, are useful for the identification of many NTM species, but that supplemental testing would be required (if clinically indicated) to identify some mycobacteria to the highest taxonomic level.

FUNDING

The work at Massachusetts General Hospital was supported by funding from bioMérieux for technical support and supplies, and by support for statistical analysis from Harvard Catalyst | The Harvard Clinical and Translational Science Center (National Center for Advancing Translational Sciences, National Institutes of Health Award UL 1TR002541) and financial contributions from Harvard University and its affiliated academic healthcare centers. The content is solely the responsibility of the authors and does not necessarily represent the official views of Harvard Catalyst, Harvard University and its affiliated academic healthcare centers, or the National Institutes of Health. Funding for the gene sequencing performed at the UTHSCT was from laboratory internal sources.

POTENTIAL CONFLICTS OF INTEREST

JAB received research support from bioMérieux for this study, and has received research support from Zeus, bioMérieux, Immunetics, Alere, DiaSorin, the Bay Area Lyme Foundation, the Lyme Disease Biobank Foundation, and the National Institute of Allergy and Infectious Diseases (Award 1R21AI119457-01) for other research studies, and has served as consultant to DiaSorin, T2 Biosystems, AdvanDx and Roche Diagnostics. BBE, RJW, SV, RV, and EI are employed in a gene sequencing reference laboratory. All other authors: No potential conflicts.

ACKNOWLEDGMENTS

The authors would like to thank the laboratory staff at the University of Texas Health Science Center at Tyler, Massachusetts General Hospital and the Marshfield Clinic Health System for their assistance with this study. We also thank Markita Weaver (Bruker Daltonics) and David Pincus (bioMérieux) for technical assistance, Dr. Hang Lee for assistance with the statistical analysis, and Joanne Woodring for her clerical assistance with the manuscript.

Table 1. Rapidly growing mycobacteria.

Reference Identification	No. of Isolates	ID matches reference ID at highest taxonomic level (species or subspecies)[a]		ID matches reference ID at group/complex level only[a]		No ID or low confidence[b] ID		Misidentification		Summary of misidentifications and other explanatory information
		Biotyper	VITEK	Biotyper	VITEK	Biotyper	VITEK	Biotyper	VITEK	
Rapidly growing mycobacteria	125	59	39 P=0.004[c]	56	85 P<0.0001	3	0	7	1	
M. abscessus complex	38									No differentiation of subspecies by either Biotyper or VITEK
M. abscessus subsp. *abscessus*	21	--	--	20	21	1	0	0	0	ID as *M. abscessus* (both Biotyper and VITEK)
M. abscessus subsp. *massiliense*	12	--	--	11	12	1	0	0	0	ID as *M. abscessus* (both Biotyper and VITEK)
M. abscessus subsp. *bolletii*	5	--	--	5	5	0	0	0	0	ID as *M. abscessus* (both Biotyper and VITEK)
M. chelonae	15	8	15 P=0.02	--	--	1	0	6	0 P=0.04	Biotyper MisID 6/15 as *M. salmoniphilum* Biotyper ID 1/15 as *M. chelonae/ salmoniphilum*
M. fortuitum group	39									No differentiation of subspecies by VITEK
M. fortuitum subsp. *fortuitum*	20	20	--	--	20	0	0	0	0	VITEK ID 20/20 as *M. fortuitum* group
M. neworleansense	1	1	--	--	1	0	0	0	0	VITEK ID 1/1 *M. fortuitum* group
M. porcinum	10	9	--	--	10	0	0	1	0	Biotyper MisID 1/10 as *M. abscessus* VITEK ID 10/10 as *M. fortuitum* group
M. senegalense/conceptionense	8	1		7	8	0	0	0	0	Biotyper ID 7/8 as *M. fortuitum* complex VITEK ID 8/8 as *M. fortuitum* group
M. immunogenum	9	9	8	--	--	0	0	0	1	VITEK MisID 1/9 as *M. abscessus*
M. mageritense	11	11	11	--	--	0	0	0	0	
M. mucogenicum group	13									
M. mucogenicum	5	--	5	5	--	0	0	0	0	Biotyper ID 5/5 as *M. mucogenicum/phocaicum* group
M. phocaicum	8	--	--	8	8	0	0	0	0	Biotyper ID 8/8 as *M. mucogenicum/phocaicum* group VITEK ID 8/8 as *M. mucogenicum*

No., number. ID, identification. MisID, misidentified.

a. To be listed in these columns, the top choice (most likely) identification was required to be high confidence (score ≥1.80) and non-split (Bruker system); or the result was required to be a single (non-split), good confidence (green light) identification (bioMérieux system).

b. A low confidence identification means the top choice identification had a low confidence score of 1.60 to 1.79 or a split identification (Bruker system); or multiple possible identifications were provided with a yellow light (bioMérieux system).
c. P values refer to the difference between the Biotyper result and the VITEK result, and are only provided when $P<0.05$. All other differences were non-significant.

Table 2. Slowly growing nontuberculous mycobacteria.

Reference Identification	No. of Isolates	ID matches reference ID at highest taxonomic level (species or subspecies)[a]		ID matches reference ID at group/complex level only[a]		No ID or low confidence[b] ID		Misidentification		Summary of misidentifications and other explanatory information
		Biotyper	VITEK	Biotyper	VITEK	Biotyper	VITEK	Biotyper	VITEK	
Slowly growing mycobacteria	119	92	99	18	8 P=0.004[c]	9	11	0	1	
M. avium complex	26									
M. avium	9	9	9	--	--	0	0	0	0	
M. chimaera	8	--	--	8	8	0	0	0	0	Biotyper ID 8/8 as *M. chimaera/intracellulare* group
M. intracellulare	9	--	9	9	--	0	0	0	0	VITEK ID 8/8 as *M. intracellulare* Biotyper ID 9/9 as *M. chimaera/intracellulare* group
M. gordonae	14	11	11	--	--	3	3	0	0	
M. kansasii	9	9	9	--	--	0	0	0	0	
M. marinum	8	8	8	--	--	0	0	0	0	Gene sequence ID is *M. marinum/ulcerans* but growth rate excludes *M. ulcerans* 1 isolate was most closely related to *M. nebraskense* by 16S rRNA gene sequence
M. nebraskense	10	10	9	--	--	0	1	0	0	
M. simiae	16	16	16	--	--	0	0	0	0	
M. terrae complex	27									
M. terrae	7	--	--	1	--	6	6	0	1	Biotyper ID 1/7 as *M. terrae* complex VITEK MisID 1/7 as *Cronobacter malonaticus*
M. arupense	20	20	19	--	--	0	1	0	0	
M. xenopi	9	9	9	--	--	0	0	0	0	

No., number. ID, identification. MisID, misidentified.

a. To be listed in these columns, the top choice (most likely) identification was required to be high confidence (score ≥1.80) and non-split (Bruker system); or the result was required to be a single (non-split), good confidence (green light) identification (bioMérieux system).

b. A low confidence identification means the top choice identification had a low confidence score of 1.60 to 1.79 or a split identification (Bruker system); or multiple possible identifications were provided with a yellow light (bioMérieux system).

c. P values refer to the difference between the Biotyper result and the VITEK result, and are only provided when $P<0.05$. All other differences were non-significant.

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Figure Legend

Figure 1. Chief limitations of the Bruker Biotyper system with RUO Mycobacteria Library v5.0.0 and the VITEK MS IVD system with v3.0 Knowledge Base, for the identification of nontuberculous mycobacteria.