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Comparison of Two Commercial Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) Systems for Identification of Nontuberculous Mycobacteria.

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1 **Comparison of Two Commercial Matrix-Assisted Laser Desorption/Ionization-Time of**
2 **Flight Mass Spectrometry (MALDI-TOF MS) Systems for Identification of**
3 **Nontuberculous Mycobacteria**

4
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19 Brief title: MALDI-TOF MS for NTM Identification

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

1 **ABSTRACT**

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

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

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

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

INTRODUCTION

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

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

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

5 MATERIALS AND METHODS

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

9 **Clinical isolates.** The isolates (n=244) included 125 rapidly growing mycobacteria (RGM) and
10 119 slowly growing mycobacteria (SGM) species, identified previously by gene sequencing at
11 the Mycobacteria/Nocardia Research Laboratory at the UTHSCT (Tables 1 and 2). The isolates
12 had been previously stored at -70°C in trypticase soy broth (Remel, Lenexa, KS) with glycerol,
13 and were subcultured onto Middlebrook 7H10 agar plates (Remel) and shipped on 7H10 agar
14 slants (Remel) from UTHSCT to the two sites (MGH and MCHS).

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

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

11 **MALDI-TOF MS sample extraction and analysis procedures.**

12 BioMérieux VITEK MS IVD system with v3.0 Knowledge Base

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

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

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

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

20 Bruker MALDI Biotyper 3.3.1.0 with RUO Mycobacteria Library v5.0.0

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

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

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

23 1.80 to 3.00: High-confidence identification

1 1.60 to 1.79: Low-confidence identification

2 0.00 to 1.59: No organism identification possible

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

6 RESULTS

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

17 Among RGM, the Biotyper system identified 59/125 isolates (47%) to the highest
18 taxonomic level, and the VITEK system identified 39/125 isolates (31%) to that level (P=0.004);
19 Table 1). The Biotyper system could not identify 3/125 RGM isolates (2%) and misidentified
20 7/125 RGM isolates (6%); the VITEK system did not fail to identify any RGM isolates (0%;
21 P=0.2) and misidentified 1/125 isolates (1%; P=0.08). Neither system could differentiate among
22 members of the *M. abscessus* complex; both systems identified *M. abscessus* subsp. *abscessus*,

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

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

14 The Biotyper system misidentified 6/15 *M. chelonae* isolates as *M. salmoniphilum*, and
15 an additional isolate (1/15) gave a split identification (*M. chelonae/salmoniphilum*); the
16 remaining 8/15 isolates were identified as *M. chelonae*. In contrast, the VITEK system identified
17 all 15 isolates as *M. chelonae*, the highest taxonomic level. The difference in the number of *M.*
18 *chelonae* misidentifications between the 2 systems (6/15 for the Biotyper system compared with
19 0/15 for the VITEK system) was statistically significant (P=0.04). Both systems reported 11/11
20 *M. mageritense* isolates as *M. mageritense*. The Biotyper and VITEK systems reported 9/9 and
21 8/9 *M. immunogenum* isolates as *M. immunogenum*, respectively. The remaining *M.*
22 *immunogenum* isolate (1/9) was misidentified as *M. abscessus* by the VITEK system.

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

14 Neither system could reliably identify *M. terrae* isolates. The Biotyper system identified
15 1/7 strains to the complex level as *M. terrae* complex; 6/7 isolates were not identified or
16 produced a low confidence identification. The VITEK system produced no identification for 6/7
17 *M. terrae* strains, and misidentified 1/7 strains as *Cronobacter malonaticus*, which is not a
18 *Mycobacterium* species but rather a gram-negative bacillus in the family Enterobacteriaceae.
19 Some members of the *M. avium* complex (MAC) also presented difficulties. Whereas both
20 systems identified 9/9 *M. avium* strains to the highest taxonomic level, and the VITEK system
21 identified 9/9 *M. intracellulare* isolates to that level, the Biotyper system identified 9/9 *M.*
22 *intracellulare* isolates to the group/complex level as *M. chimaera/intracellulare*. In addition, the

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

3 DISCUSSION

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

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

16 The Biotyper system with Mycobacteria library v5.0.0 identified significantly more RGM
17 isolates to the highest taxonomic level, compared with the VITEK v3.0 system (47% versus
18 31%; $P=0.004$). Neither system could reliably differentiate among members of the *M. abscessus*
19 complex or the *M. mucogenicum* group. In addition, the VITEK system could not differentiate
20 among *M. fortuitum* group members, and the Biotyper system misidentified nearly half of *M.*
21 *chelonae* isolates. These findings are consistent with the findings of previous studies using the
22 VITEK v3.0 system (17, 26) or earlier versions of the Biotyper Mycobacteria library (11, 12, 17,
23 27, 28), except that using earlier versions of the Biotyper Mycobacteria library the difficulty in

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

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

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

1 Similarly, members of the *M. fortuitum* group are responsible for variable types of
2 clinical disease, most of which, unlike the *M. abscessus* complex, are treated with oral
3 antimicrobials such as quinolones, macrolides and tetracyclines. Some species within the *M.*
4 *fortuitum* (e.g., *M. senegalense*, *M. peregrinum*) lack a functional *erm* gene and show macrolide
5 susceptibility unlike other members (i.e., *M. porcinum* and other former third biovariant species),
6 which harbor a functional *erm* gene rendering them inducibly macrolide resistant. Additionally,
7 members of the *M. fortuitum* group differ in resistance to quinolones and tetracyclines (33, 35-
8 38).

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

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

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

4 Each MALDI-TOF MS system misidentified a small number of isolates (7/244 (3%) for
5 the Biotyper system, 2/244 (1%) for the VITEK system). The difference between the two
6 systems, in number of misidentifications, was not statistically significant. Interestingly, none of
7 the 244 NTM strains included in this study was misidentified on both systems. The most frequent
8 errors were the misidentification of 6/15 *M. chelonae* isolates as *M. salmoniphilum* using the
9 Biotyper system. This is of concern because by gene sequence, the two species are easily
10 separated (*i.e.*, their full 16S rRNA gene sequences differ by 5 bp, their *rpoB* gene sequences
11 differ by 22 bp, and their *hsp65* gene sequences differ by 18 bp), and because *M. salmoniphilum*,
12 unlike *M. chelonae*, has not been associated with human disease. This problem was not apparent
13 with the VITEK platform, which reliably identified *M. chelonae*. The remaining few
14 misidentifications on either system involved a single strain of a given species.

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

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

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

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

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POTENTIAL CONFLICTS OF INTEREST

11
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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	56	85	3	0	7	1	
			P=0.004 ^c		P<0.0001					
<i>M. abscessus</i> complex	38									No differentiation of subspecies by either Biotyper or VITEK
<i>M. abscessus</i> subsp. <i>abscessus</i>	21	--	--	20	21	1	0	0	0	ID as <i>M. abscessus</i> (both Biotyper and VITEK)
<i>M. abscessus</i> subsp. <i>massiliense</i>	12	--	--	11	12	1	0	0	0	ID as <i>M. abscessus</i> (both Biotyper and VITEK)
<i>M. abscessus</i> subsp. <i>bolletii</i>	5	--	--	5	5	0	0	0	0	ID as <i>M. abscessus</i> (both Biotyper and VITEK)
<i>M. chelonae</i>	15	8	15	--	--	1	0	6	0	Biotyper MisID 6/15 as <i>M. salmoniphilum</i>
<i>M. fortuitum</i> group	39		P=0.02						P=0.04	Biotyper ID 1/15 as <i>M. chelonae</i> / <i>salmoniphilum</i> No differentiation of subspecies by VITEK
<i>M. fortuitum</i> subsp. <i>fortuitum</i>	20	20	--	--	20	0	0	0	0	VITEK ID 20/20 as <i>M. fortuitum</i> group
<i>M. neworleansense</i>	1	1	--	--	1	0	0	0	0	VITEK ID 1/1 <i>M. fortuitum</i> group
<i>M. porcinum</i>	10	9	--	--	10	0	0	1	0	Biotyper MisID 1/10 as <i>M. abscessus</i> VITEK ID 10/10 as <i>M. fortuitum</i> group
<i>M. senegalense/conceptionense</i>	8	1		7	8	0	0	0	0	Biotyper ID 7/8 as <i>M. fortuitum</i> complex VITEK ID 8/8 as <i>M. fortuitum</i> group
<i>M. immunogenum</i>	9	9	8	--	--	0	0	0	1	VITEK MisID 1/9 as <i>M. abscessus</i>
<i>M. mageritense</i>	11	11	11	--	--	0	0	0	0	
<i>M. mucogenicum</i> group	13									
<i>M. mucogenicum</i>	5	--	5	5	--	0	0	0	0	Biotyper ID 5/5 as <i>M. mucogenicum/phocaicum</i> group
<i>M. phocaicum</i>	8	--	--	8	8	0	0	0	0	Biotyper ID 8/8 as <i>M. mucogenicum/phocaicum</i> group VITEK ID 8/8 as <i>M. mucogenicum</i>

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	9	11	0	1	
					P=0.004 ^c					
<i>M. avium</i> complex	26									
<i>M. avium</i>	9	9	9	--	--	0	0	0	0	
<i>M. chimaera</i>	8	--	--	8	8	0	0	0	0	Biotyper ID 8/8 as <i>M. chimaera/intracellulare</i> group
<i>M. intracellulare</i>	9	--	9	9	--	0	0	0	0	VITEK ID 8/8 as <i>M. intracellulare</i> Biotyper ID 9/9 as <i>M. chimaera/intracellulare</i> group
<i>M. goodnae</i>	14	11	11	--	--	3	3	0	0	
<i>M. kansasii</i>	9	9	9	--	--	0	0	0	0	
<i>M. marinum</i>	8	8	8	--	--	0	0	0	0	Gene sequence ID is <i>M. marinum/ulcerans</i> but growth rate excludes <i>M. ulcerans</i> 1 isolate was most closely related to <i>M. nebraskense</i> by 16S rRNA gene sequence
<i>M. nebraskense</i>	10	10	9	--	--	0	1	0	0	
<i>M. simiae</i>	16	16	16	--	--	0	0	0	0	
<i>M. terrae</i> complex	27									
<i>M. terrae</i>	7	--	--	1	--	6	6	0	1	Biotyper ID 1/7 as <i>M. terrae</i> complex VITEK MisID 1/7 as <i>Cronobacter malonicus</i>
<i>M. arupense</i>	20	20	19	--	--	0	1	0	0	
<i>M. xenopi</i>	9	9	9	--	--	0	0	0	0	

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

- 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).
- 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).
- 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.

REFERENCES

1. Greub G. 2010. MALDI-TOF mass spectrometry: the quantum leap. *Clin Microbiol Infect* 16:1603.
2. Branda JA, Rychert J, Burnham CA, Bythrow M, Garner OB, Ginocchio CC, Jennemann R, Lewinski MA, Manji R, Mochon AB, Procop GW, Richter SS, Sercia LF, Westblade LF, Ferraro MJ. 2014. Multicenter validation of the VITEK MS v2.0 MALDI-TOF mass spectrometry system for the identification of fastidious gram-negative bacteria. *Diagn Microbiol Infect Dis* 78:129-131.
3. Rychert J, Burnham CA, Bythrow M, Garner C, Ginocchio CC, Jennemann R, Lewinski MA, Manji R, Mochon AB, Procop GW, Richter SS, Sercia LF, Westblade LF, Ferraro MJ, Branda JA, . 2013. Multicenter evaluation of the VITEK MS MALDI-TOF mass spectrometry system for the identification of gram-positive aerobic bacteria. *J Clin Microbiol* 51:2225-2231.
4. Saffert RT, Cunningham SA, Ihde SM, Jobe KE, Mandrekar J, Patel R. 2011. Comparison of Bruker Biotyper matrix-assisted laser desorption ionization-time of flight mass spectrometer to BD Phoenix automated microbiology system for identification of gram-negative bacilli. *J Clin Microbiol* 49:887-892.
5. Seng P, Drancourt M, Gouriet F, La Scola B, Fournier PE, Rolain JM, Raoult D. 2009. Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. *Clin Infect Dis* 49:543-551.
6. Emonet S, Shah HN, Cherkaoui A, Schrenzel J. 2010. Application and use of various mass spectrometry methods in clinical microbiology. *Clin Microbiol Infect* 16:1604-1613.
7. Lefmann M, Honsich C, Böcker S, Storm N, von Wintzingerode F, Schlötelburg C, Moter A, van den Boom D, Göbel UB. 2004. Novel mass spectrometry-based tool for genotypic identification of mycobacteria. *J Clin Microbiol* 42:339-346.
8. Bizzini A, Greub G. 2010. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry, a revolution in clinical microbial identification. *Clin Microbiol Infect* 16:1614-1619.
9. Clinical and Laboratory Standards Institute. 2017. Methods for the identification of cultured microorganisms using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. 1st ed, CLSI Guideline, M58.
10. Rodríguez-Sánchez B, Ruiz-Serrano MJ, Ruiz A, Timke M, Kostrzewa M, Bouza E. 2016. Evaluation of MALDI biotyper mycobacterial library v3.0 for identification of nontuberculous mycobacteria. *J Clin Microbiol* 54:1144-1147.
11. Buckwalter SP, Olson SL, Connelly BJ, Lucas BC, Rodning AA, Walchak RC, Deml SM, Wohlfiel SL, Wengenack NL. 2016. Evaluation of matrix-assisted laser desorption ionization-time of flight mass spectrometry for identification of *Mycobacterium* species, *Nocardia* species, and other aerobic actinomycetes. *J Clin Microbiol* 54:376-384.
12. Mather CA, Rivera SF, Butler-Wu SM. 2014. Comparison of the Bruker Biotyper and Vitek MS matrix-assisted laser desorption ionization-time of flight mass spectrometry systems for identification of mycobacteria using simplified protein extraction protocols. *J Clin Microbiol* 52:130-138.
13. Body BA, Beard MA, Slechta ES, Hanson KE, Barker AP, Babady NE, McMillen T, Tang Y-W, Brown-Elliott BA, Iakhiaeva E, Vasireddy R, Vasireddy S, Smith T, Wallace Jr. RJ, Turner S, Curtis L, S. B-W, Rychert J. 2018. Evaluation of the Vitek MS v3.0 matrix-assisted laser desorption ionization-time of flight mass spectrometry system for identification of *Mycobacterium* and *Nocardia* species. *J Clin Microbiol* 56:e00237-18.

14. Saleeb PG, Drake SK, Murray PR, Zelazny AM. 2011. Identification of mycobacteria in solid-culture media by matrix-assisted laser desorption ionization-time of flight mass spectrometry. *J Clin Microbiol* 49:1790-1794.
15. Miller E, Cantrell C, Beard M, Derylak A, Babady NE, McMillen T, Miranda E, Body B, Tang Y-W, Vasireddy R, Vasireddy S, Smith T, Iakhiaeva E, Wallace Jr. RJ, Brown-Elliott BA, Moreno E, Totty H, Deol P. 2018. Performance of Vitek MS v3.0 for identification of *Mycobacterium* species from patient samples by use of automated liquid medium systems. *J Clin Microbiol* 56:e00219-18.
16. Chen JH, Yam WC, Ngan AH, Fung AM, Woo WL, Yan MK, Choi GK, Ho PL, Cheng VC, Yuen KY. 2013. Advantages of using matrix-assisted laser desorption ionization-time of flight mass spectrometry as a rapid diagnostic tool for identification of yeasts and mycobacteria in the clinical microbiological laboratory. *J Clin Microbiol* 51:3981-3987.
17. Wilen CB, McMullen AR, Burnham CA. 2015. Comparison of sample preparation methods, instrumentation platforms, and contemporary commercial databases for identification of clinically relevant mycobacteria by matrix-assisted laser desorption ionization-time of flight mass spectrometry. *J Clin Microbiol* 53:2308-2315.
18. Adékambi T, Colson P, Drancourt M. 2003. *rpo B*-based identification of nonpigmented and late pigmented rapidly growing mycobacteria. *J Clin Microbiol* 41:5699-5708.
19. Clinical and Laboratory Standards Institute. 2008. Interpretive criteria for identification of bacteria and fungi by DNA target sequencing: Approved Guideline. CLSI document MM18-A.
20. Nash KA, Brown-Elliott BA, Wallace RJ, Jr. 2009. A novel gene, *erm(41)*, confers inducible macrolide resistance to clinical isolates of *Mycobacterium abscessus* but is absent from *Mycobacterium chelonae*. *Antimicrob Agents Chemother* 53:1367-1376.
21. Tortoli E, Fedrizzi T, Meehan CJ, Trovato A, Grottola A, Giacobazzi E, Serpini GF, Tagliazucchi S, Fabio A, Bettua C, Bertorelli R, Frascaro F, De Sanctis V, Pecorari M, Jousson O, Segata N, Cirillo DM. 2017. The new phylogeny of the genus *Mycobacterium*: The old and the news. *Infect Gen Evol* 56:19-25.
22. Hettick JM, Kashon ML, Simpson JP, Siegel PD, Mazurek GH, Weissman DN. 2004. Proteomic profiling of intact mycobacteria by Matrix-Assisted Laser Desorption-Ionization Time-of-Flight Mass Spectrometry. *Anal Chem* 76:5769-5776.
23. Lotz A, Ferroni A, Beretti J-L, Dauphin B, Carbonnelle E, Guet-Revillet H, Veziris N, Heym B, Jarlier V, Gaillard J-L, Pierre-Audigier C, Frapy E, Berche P, Nassif X, Bille E. 2010. Rapid identification of mycobacterial whole cells in solid and liquid culture media by matrix-assisted laser desorption ionization-time of flight mass spectrometry. *J Clin Microbiol* 48:4481-4486.
24. Pignone ML, Greth KM, Cooper J, Emerson D, Tang J. 2006. Identification of mycobacteria by matrix-assisted laser desorption ionization-time-of-flight mass spectrometry. *J Clin Microbiol* 44:1963-1970.
25. Cao Y, Wang L, Ma P, Fan W, Gu B, Ju S. 2018. Accuracy of matrix-assisted laser desorption ionization-time of flight mass spectrometry for identification of mycobacteria: A systematic review and meta-analysis. *Sci Rep* 8:4131.
26. Leyer C, Gregorowicz G, Mougari F, Raskine L, Cambau E, de Briel D. 2017. Comparison of Saramis 4.12 and IVD 3.0 Vitek MS matrix-assisted laser desorption ionization-time of flight mass spectrometry for identification of mycobacteria from solid and liquid culture media. *J Clin Microbiol* 55:2045-2054.
27. Luo LL, Cao W, Chen WW, Zhang RR, Jing LJ, Chen HP, Yu FY, Yue J. 2018. Evaluation of the VITEK MS knowledge base version 3.0 for the identification of clinically relevant *Mycobacterium* species. *Emerg Microbes Infect* 7:114.

28. Buchan BW, Riebe KM, Timke M, Kostrzewa M, Ledebøer NA. 2014. Comparison of MALDI-TOF MS with HPLC and nucleic acid sequencing for the identification of *Mycobacterium* species in cultures using solid medium and broth. *Am J Clin Pathol* 141:25-34.
29. Rodríguez-Temporal D, Perez-Risco D, Struzka EA, Mas M, Alcaide F. 2018. Evaluation of two protein extraction protocols based on freezing and mechanical disruption for identifying nontuberculous mycobacteria by matrix-assisted laser desorption ionization-time of flight mass spectrometry from liquid and solid cultures. *J Clin Microbiol* 56:e01548-17.
30. Mediavilla-Gradolph MC, De Toro-Peinado I, Bermúdez-Ruiz MP, García-Martínez Mde L, Ortega-Torres M, Montiel Quezel-Guerraz N, Palop-Borrás B. 2015. Use of MALDI-TOF MS for identification of nontuberculous mycobacterium species isolated from clinical specimens. *BioMed Res Intern* 2015:1-6.
31. Rodríguez-Sánchez B, Ruiz-Serrano MJ, Marín M, López Roa P, Rodríguez-Créixems M, Bouza E. 2015. Evaluation of matrix-assisted laser desorption ionization-time of flight mass spectrometry for identification of nontuberculous mycobacteria from clinical isolates. *J Clin Microbiol* 53:2737-2740.
32. Lecorche E, Haenn S, Mougari E, Kumanski S, Veziris N, Benmansour H, Baskine L, Moulin L, Cambau E. 2018. Comparison of methods available for identification of *Mycobacterium chimaera*. *Clin Microbiol Infect* 34:409-413.
33. Brown-Elliott BA, Nash KA, Wallace RJ, Jr. 2012. Antimicrobial susceptibility testing, drug resistance mechanisms, and therapy of infections with nontuberculous mycobacteria. *Clin Microbiol Rev* 25:545-582.
34. Brown-Elliott BA, Vasireddy S, Vasireddy R, Iakhiaeva E, Howard ST, Nash KA, Parodi N, Strong A, Gee M, Smith T, Wallace RJ, Jr. 2015. Utility of sequencing the *erm(41)* gene in isolates of *Mycobacterium abscessus* subsp. *abscessus* with low and intermediate clarithromycin MICs. *J Clin Microbiol* 53:1211-1215; ERRATUM *J. Clin. Microbiol.* 54:1172, April 2016.
35. Wallace RJ, Jr., Bedsole G, Sumter G, Sanders CV, Steele LC, Brown BA, Smith J, Graham DR. 1990. Activities of ciprofloxacin and ofloxacin against rapidly growing mycobacteria with demonstration of acquired resistance following single-drug therapy. *Antimicrob Agents Chemother* 34:65-70.
36. Wallace RJJ, Brown-Elliott BA, Brown J, Steigerwalt AG, Hall L, Woods G, Cloud J, Mann L, Wilson R, Crist C, Jost KC, Jr., Byrer DE, Tang J, Cooper J, Stamenova E, Campbell B, Wolfe J, Turenne C. 2005. Polyphasic characterization reveals that the human pathogen *Mycobacterium peregrinum* type II belongs to the bovine pathogen species *Mycobacterium senegalense*. *J Clin Microbiol* 43:5925-35.
37. Brown-Elliott BA, Wallace RJ, Jr., Crist CJ, Mann L, Wilson RW. 2002. Comparison of in vitro activities of gatifloxacin and ciprofloxacin against four taxa of rapidly growing mycobacteria. *Antimicrob Agents Chemother* 46:3283-5.
38. Brown BA, Wallace RJ, Jr., Onyi GO. 1996. Activities of the glycylicyclines N, N-dimethylglycylamido-minocycline and N, N-dimethylglycylamido-6-demethyl-6-deoxytetracycline against *Nocardia* spp. and tetracycline-resistant isolates of rapidly growing mycobacteria. *Antimicrob Agents Chemother* 40:874-878.
39. Schreiber PW, Hasse B, Sax H. 2018. *Mycobacterium chimaera* infections after cardiac surgery – lessons learned. *Clin Microbiol Infect* 24:1117-1118.
40. Clinical and Laboratory Standards Institute. 2018. Susceptibility testing of mycobacteria, *Nocardia* spp., and other aerobic actinomycetes. 3rd ed, CLSI Standard Document, M24.
41. Murugaiyan J, Lewin A, Kamal E, Bakula Z, van Ingen J, Ulmann V, Unzaga Barañano MJ, Humiecka J, Safianowska A, Roesler UH, Jagielski T. 2018. MALDI spectra database for rapid discrimination and subtyping of *Mycobacterium kansasii*. *Front Microbiol* 9:587.

42. Epperson LE, Timke M, Hasan NA, Godo P, Durbin D, Helstrom NK, Shi G, Kostrzewa M, Strong M, Salfinger M. 2018. Evaluation of a novel MALDI biotyper algorithm to distinguish *Mycobacterium intracellulare* from *Mycobacterium chimaera*. *Front Microbiol* 9:3140.
43. Pranada AB, Witt E, Bienia M, Kostrzewa M, Timke M. 2017. Accurate differentiation of *Mycobacterium chimaera* from *Mycobacterium intracellulare* by MALDI-TOF MS analysis. *J Med Microbiol* 66:670-677.
44. Kehrmann J, Wessel S, Murali R, Hampel A, Bange FC, Buer J, Mosel F. 2016. Principal component analysis of MALDI TOF MS mass spectra separates *M. abscessus* (sensu stricto) from *M. massiliense* isolates. *BMC Microbiol* 16:24.
45. Fangous MS, Mougari F, Gouriou S, Calvez E, Raskine L, Cambau E, Payan C, Héry-Arnaud G. 2014. Classification algorithm for subspecies identification within the *Mycobacterium abscessus* species, based on matrix-assisted laser desorption ionization-time of flight mass spectrometry. *J Clin Microbiol* 52:3362-3369.
46. Tseng SP, Teng SH, Lee PS, Wang CF, Yu JS, Lu PL. 2013. Rapid identification of *M. abscessus* and *M. massiliense* by MALDI-TOF mass spectrometry with a comparison to sequencing methods and antimicrobial susceptibility patterns. *Future Microbiol* 8:1381-1389.
47. Vasireddy R, Vasireddy S, Brown-Elliott BA, Wengenack NL, Eke UA, Benwill JL, Turenne C, Wallace RJ, Jr. 2016. *Mycobacterium arupense*, *Mycobacterium heraklionense*, and a newly proposed species, "*Mycobacterium virginense*" sp. nov., but not *Mycobacterium nonchromogenicum*, as species of the *Mycobacterium terrae* complex causing tenosynovitis and osteomyelitis. *J Clin Microbiol* 54:1340-1351.

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.