AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology®

MYCOBACTERIOLOGY AND AEROBIC ACTINOMYCETES

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

Barbara A. Body,[a]* Melodie A. Beard,[a] E. Susan Slechta,[b] Kimberly E. Hanson,[b,c] Adam P. Barker,[b,c] N. Esther Babady,[d] Tracy McMillen,[d] Yi-Wei Tang,[d,g] Barbara A. Brown-Elliott,[e] Elena Iakhiaeva,[e] Ravikiran Vasireddy,[e] Sruthi Vasireddy,[e] Terry Smith,[e] Richard J. Wallace, Jr.,[e] S. Turner,[f] L. Curtis,[f] Susan Butler-Wu,[f]* Jenna Rychert[b]

[a]Department of Esoteric Microbiology, Lab. Corp. of America Holdings, Burlington, North Carolina, USA
[b]ARUP Laboratories, Salt Lake City, Utah, USA
[c]University of Utah, Salt Lake City, Utah, USA
[d]Memorial Sloan Kettering Cancer Center, New York, New York, USA
[e]University of Texas Health Science Center, Tyler, Texas, USA
[f]University of Washington Medical Center, Seattle, Washington, USA
[g]Weill Medical College of Cornell University, New York, New York, USA

ABSTRACT This multicenter study was designed to assess the accuracy and reproducibility of the Vitek MS v3.0 matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry system for identification of *Mycobacterium* and *Nocardia* species compared to DNA sequencing. A total of 963 clinical isolates representing 51 taxa were evaluated. In all, 663 isolates were correctly identified to the species level (69%), with another 231 (24%) correctly identified to the complex or group level. Fifty-five isolates (6%) could not be identified despite repeat testing. All of the tuberculous mycobacteria (45/45; 100%) and most of the nontuberculous mycobacteria (569/606; 94%) were correctly identified at least to the group or complex level. However, not all species or subspecies within the *M. tuberculosis*, *M. abscessus*, and *M. avium* complexes and within the *M. fortuitum* and *M. mucogenicum* groups could be differentiated. Among the 312 *Nocardia* isolates tested, 236 (76%) were correctly identified to the species level, with an additional 44 (14%) correctly identified to the complex level. Species within the *N. nova* and *N. transvalensis* complexes could not always be differentiated. Eleven percent of the isolates (103/963) underwent repeat testing in order to get a final result. Identification of a representative set of *Mycobacterium* and *Nocardia* species was highly reproducible, with 297 of 300 (99%) replicates correctly identified using multiple kit lots, instruments, analysts, and sites. These findings demonstrate that the system is robust and has utility for the routine identification of mycobacteria and *Nocardia* in clinical practice.

KEYWORDS MALDI-TOF, *Mycobacterium*, *Mycobacterium tuberculosis*, Nocardia, mass spectrometry, mycobacteria

Mycobacterium and *Nocardia* are diverse groups of bacteria increasingly implicated in disease, and yet these pathogens are challenging to identify to the species level for most clinical laboratories (1–6). The *Mycobacterium tuberculosis* complex is a major public health concern that is responsible for significant morbidity and mortality worldwide. The World Health Organization (WHO) recently estimated that as many as 10.4 million new infections and 1.4 million deaths occurred worldwide due to *M. tuberculosis* complex in 2015 (3). Additionally, nontuberculous mycobacteria (NTM)

Received 9 February 2018 **Returned for modification** 8 March 2018 **Accepted** 8 April 2018

Accepted manuscript posted online 11 April 2018

Citation 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 RJ, Jr, Turner S, Curtis L, Butler-Wu S, 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. https://doi.org/10.1128/JCM.00237-18.

Editor Betty A. Forbes, Virginia Commonwealth University Medical Center

Address correspondence to Jenna Rychert, Jennifer.Rychert@aruplab.com.

* Present address: Barbara A. Body (retired), Charlottesville, Virginia, USA; Susan Butler-Wu, Department of Pathology & Laboratory Medicine, University of Southern California, Los Angeles, Los Angeles, California, USA.

(3–5, 7–12) and *Nocardia* (13–15) are important causes of invasive disease, especially in immunocompromised hosts. Our ability to identify these organisms to the species level is important given that susceptibility testing is not widely performed, except in the case of *M. tuberculosis* complex. Thus, physicians often must make decisions regarding therapy on the basis of identification alone (9, 10).

Over the past 50 years, identification of *Mycobacterium* and *Nocardia* has transitioned from phenotypic characterization to mycolic acid analysis using gas-liquid chromatography (GLC) or high-performance liquid chromatography (HPLC) and, most recently, to the use of gene-specific probes or DNA sequencing. The advent of molecular methods has enabled greater taxonomic differentiation of these organisms in a clinically meaningful time frame (7, 10). However, it is well recognized that targeting of a single gene is frequently incapable of discriminating between closely related species within these genera. For example, 16S rRNA gene sequences are highly conserved among mycobacteria. For the *Nocardia*, identification is complicated by the presence of multiple copies of the 16S rRNA gene (13, 15). For these reasons, multilocus sequence analysis using 16S rRNA, *rpoB*, *erm*, *hsp*65, *gyrB*, and/or *sec*A1 genes has been recommended. Unfortunately, the cost and complexity of multilocus sequencing often restrict its use to reference laboratories, thus delaying identification of isolates from hospital-based clinical laboratories.

The application of matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) for the identification of *Mycobacterium* and *Nocardia* began over a decade ago, but significant variability in test methodologies and performance has been observed (16–40). The direct-spotting method typically used for Gram-negative and Gram-positive bacteria and yeast is not adequate for *Nocardia* and *Mycobacterium* species due to the hardiness of their cell wall; rather, mechanical disruption and protein extraction are necessary. These methods can be performed in the presence of ethanol to simultaneously inactivate infectious organisms (22). Recent research has also highlighted the need for well-curated and validated databases that include a sufficient number of isolates of each species to account for the inherent variability in spectra (18, 20, 25, 26, 39, 40). The objective of this multicenter study was to establish the accuracy and reproducibility of the standardized Vitek MS v3.0 system (Vitek) for the identification of commonly encountered mycobacteria and *Nocardia* in the clinical laboratory. This most recent update to the FDA-cleared Vitek system includes 19 *Mycobacterium* and 12 *Nocardia* species that could be expected to be commonly recovered from cultures of "acid-fast bacilli" on solid medium in the clinical laboratory (41).

MATERIALS AND METHODS

Study sites. The performance of the Vitek MS v3.0 system was evaluated at four clinical laboratories within the United States, namely, LabCorp (Burlington, NC), ARUP Laboratories (Salt Lake City, UT), Memorial Sloan Kettering Cancer Center (New York, NY), and the University of Washington Medical Center (Seattle, WA). This study was approved by the human subjects committees at the respective sites, when deemed necessary by their institutional review boards.

Organisms. Each study site prospectively collected and tested clinical isolates until they reached a minimum of 10 isolates per species of a predefined list of organisms with the goal of inclusion of at least 30 isolates per species from all sites combined. In the event that a site was unable to obtain 10 unique fresh isolates of a given organism, additional frozen stocks obtained from a culture collection or provided by the sponsor were permitted. In addition to the clinical isolates, a challenge set of 50 well-characterized organisms and a reproducibility panel consisting of five organisms were provided to each laboratory for testing.

Mycobacteria were cultured on one of the following solid media: Coletsos agar, Lowenstein-Jensen agar (L-J), Middlebrook 7H10 agar (7H10), or Middlebrook 7H11 agar (7H11). The cultures were incubated at 35 to 37°C with CO_2 for either 3 to 7 days (rapidly growing mycobacteria [RGM]) or 7 to 28 days (slow growers). The exceptions were *M. haemophilum*, which was preferentially grown on Lowenstein-Jensen agar with 2.5% ferric ammonium citrate at 35 to 37°C with CO_2 for 7 to 28 days or at 25 to 33°C with CO_2 for 7 to 21 days; and *M. xenopi*, which was incubated at 35 to 37°C with CO_2 for 7 to 28 days or at 42°C with CO_2 for 7 to 28 days. *Nocardia* were cultured on buffered charcoal yeast extract agar (BCYE), chocolate agar (CHOC), Columbia blood agar (CBA), or Sabouraud dextrose agar (SDA) under aerobic conditions at 35 to 37°C for 18 to 72 h. Isolates were tested when sufficient growth was observed. The age of the culture and type of medium used were documented for each isolate. No differences were detected in the identification of organisms grown on different medium types or incubated for various

periods of time. This is likely because these were the same culture conditions as were used to develop the v3.0 knowledge base.

Sample preparation. Isolates were prepared for analysis using a *Mycobacterium*/*Nocardia* reagent kit (bioMérieux, Durham, NC) according to the manufacturer's instructions. This included mechanical disruption using 0.5-mm-diameter glass beads and bead beating for 5 min or vortex mixing for 15 min; the two methods have been shown to be equally effective at inactivating pathogens (42). This was followed by a 10-min incubation in 70% ethanol at room temperature and then protein extraction using 70% formic acid and acetonitrile. One microliter of the protein extract was then applied to a single spot on the target slide (Vitek MS-DS; bioMérieux), allowed to dry, and overlaid with 1 μl of α-cyano-4-hydroxycinnamic acid (Vitek MS-CHCA matrix; bioMérieux). Target slides were kept at room temperature and analyzed using Vitek MS v3.0 within 72 h of preparation.

Calibration and quality control. For instrument calibration, an *Escherichia coli* reference strain (ATCC 8739) was transferred to designated wells on the target slide using a 1-μl loop, overlaid with 1 μl of Vitek MS-CHCA matrix, and air dried. Positive-control organisms (*N. farcinica*, ATCC 3308, and *M. smegmatis*, ATCC 19420) were inactivated and extracted using the same protocol as that described above and analyzed alongside a negative control (reagents alone) on each day of testing at each site.

MALDI-TOF analysis for organism identification. The Vitek MS v3.0 system includes an OEM (original equipment manufacturer)-labeled Shimadzu Axima Assurance mass spectrometer linked to a reference database. Spectra are subjected to proprietary processes called "mass binning" and "normalization" and were then queried against the reference database to determine the taxonomic identity of the unknown organism, as previously described (43). Spectra were acquired and analyzed using Myla 4.0 software with database v3.0.

Reference method for organism identification. Reference identification for all study isolates was performed at the University of Texas Health Science Center (Tyler, Texas). Partial 16S rRNA genes and several additional housekeeping genes (the *rpoB* [beta subunit of RNA polymerase], *hsp65* [65-kDa heat shock protein], *secA* [ATPase component of bacterial secretion pathway], internal transcribed spacer [ITS], *gyrB* [B subunit of DNA gyrase], and *erm* [erythromycin ribosomal methylase] genes) were sequenced to identify the isolates. Isolates that could not be resolved to the species or complex level using these gene targets were additionally subjected to 16S rRNA complete gene sequencing. Partial 16S rRNA gene sequencing was performed on the samples using MicroSeq rDNA PCR and sequencing kits according to the recommendations of the manufacturer (Life Technologies, Carlsbad, CA). The resulting ~500-bp gene was analyzed using RipSeq software (Pathogenomix, Santa Cruz, CA). Complete 16S rRNA gene sequencing was performed as previously described by Edwards et al. (44) using slight modifications. Sequencing of region V of the *rpoB* gene (720 bp, excluding the primer regions) was performed on the isolates utilizing primers MycoF and MycoR as described by Adékambi et al. (45). *erm*(41) gene sequencing was performed on *M. abscessus* complex isolates as described previously (46). *Nocardia* isolates were identified by sequencing of a 468-bp fragment of the *secA1* gene as previously described by Conville et al. (47). The 156-amino-acid deduced SecA1 protein sequences were compared to an in-house database of 50 *Nocardia* type strain sequences. Sequences were compared to validated type strains and to all available sequences using RipSeq. Interpretation was in accordance with the Clinical and Laboratory Standards Institute (CLSI) interpretive criteria for DNA target sequencing (48).

Analysis. The Vitek result was considered accurate to the species level if a single identification was given and if it matched the identification obtained by the reference method. It was considered correct to the complex or group level if a single identification or multiple identifications within the same genus were reported and if these results were within the same complex or group as the species identified using the reference method. It was considered incorrect if a single identification was given that did not match the result obtained by sequencing at the species, complex, or group level. In those instances in which no identification was obtained, repeat testing of a single spot was performed using the same extract. If this first repeat failed to result in an identification, a new sample extract was prepared and tested on a single spot. If an identification was still not made, a result of "no identification" was used as the final result. If the identification was made after respotting or spotting a new extract, then it was used as the final result.

Reproducibility testing. Reproducibility testing was performed by two operators at each of three sites. A panel of five organisms (*M. abscessus*, *M. chelonae*, *M. smegmatis*, *N. wallacei*, and *N. otitidiscaviarum*) was tested in duplicate on two runs daily for 5 days. The identity of each organism was blinded with respect to the operators. Testing was performed using three different lots of reagents. The position of each organism on the target slide was predetermined, and the organisms were tested sequentially on one slide and in a randomized manner on a second slide. Sample preparation, organism identification on the Vitek MS v3.0, and result analysis were performed as described above.

RESULTS

Overall performance for clinical isolates. A total of 963 clinical isolates, including 651 *Mycobacterium* and 312 *Nocardia* isolates, were tested. For the *Mycobacterium* isolates, 614 (94%) were identified with a single correct identification to the species, group, or complex level (Table 1). Of the remaining isolates, four (<1%) were misidentified and 33 (5%) could not be identified despite repeat testing. For the *Nocardia* isolates, 236 (76%) were correctly identified to the species level, with an additional 44 (14%) identified at the complex level (Table 2). Ten (3%) *Nocardia* isolates were

TABLE 1 Accuracy of identification of mycobacteria[a]

Reference identification	No. (%) of isolates with indicated Vitek MS v3.0 ID result			
	Correct to species level	Correct to complex or group level	Incorrect	No ID
M. tuberculosis complex (n = 45) (includes but does not discriminate among *M. africanum*, *M. bovis*/BCG, *M. canettii*, *M. microti*, *M. pinnipedii*, and *M. tuberculosis*		45 (100)		
NTM slow growers				
M. avium complex				
M. avium (n = 68)	68 (100)			
M. intracellulare (n = 43)	42 (98)	1 (2)		
M. chimaera^N (n = 16)		16 (100)		
MAC-X^N (n = 20) (includes but may not discriminate among *M. arosiense*, *M. bouchedurhonense*, *M. colombiense*, *M. marseillense*, *M. timonense*, *M. vulneris*, and *M. yongonense*)		14 (70)		6 (30)
M. simiae complex				
M. simiae (n = 28)	28 (100)			
M. lentiflavum (n = 37)	33 (89)			4 (10)
M. shigaense^N (n = 3)				3 (100)
M. gordonae (n = 30)	28 (93)			2 (7)
M. gordonae/paragordonae^N (n = 3)		1 (33)	1 (33)	1 (33)
M. haemophilum (n = 18)	17 (94)			1 (6)
M. kansasii (n = 31)	31 (100)			
M. malmoense (n = 4)	4 (100)			
M. marinum (n = 25)	25 (100)			
*M. nebraskense** (n = 1)	1 (100)			
M. paraense^N (n = 1)				1 (100)
M. parascrofulaceum^N (n = 2)			1 (50)	1 (50)
M. paraffinicum^N (n = 9)			2 (22)	7 (78)
M. scrofulaceum (n = 15)	15 (100)			
M. szulgai (n = 24) Does not discriminate from *M. angelicum*[b]	23 (96)			1 (4)
M. xenopi (n = 24)	24 (100)			
Total (n = 402)	339 (84)	32 (8)	4 (1)	27 (7)
NTM rapid growers				
M. chelonae/abscessus group				
M. abscessus complex (n = 40); includes but does not differentiate subspecies *abscessus*, *massiliense*, and *bolletii*		40 (100)		
M. chelonae[c] (n = 29)	28 (97)			1 (3)
M. immunogenum (n = 19)	19 (100)			
M. fortuitum-smegmatis-farcinogenes group				
M. fortuitum group (n = 49); includes but does not discriminate between *M. alvei*, *M. brisbanense*, *M. boenickei*, *M. farcinogenes*,[d] *M. fortuitum*, *M. houstonense*, *M. neworleansense*, *M. peregrinum*, *M. porcinum*, *M. senegalense*, *M. septicum*, *M. setense*		48 (98)		1 (2)
M. smegmatis[e] (n = 21)	21 (100)			
*M. mageritense** (n = 1)[f]	1 (100)			
M. mucogenicum group				
M. mucogenicum (n = 20)[g]	18 (90)			2 (10)
M. phocaicum^N (n = 21)		20 (95)		1 (5)
M. mucogenicum/phocaicum[h] (n = 1)		1 (100)		
*M. neoaurum** (n = 1)	1 (100)			
Total (n = 202)	88 (44)	109 (54)		5 (2)
Mycobacterium species (n = 2)		1 (50)		1 (50)
Total (n = 651)	427 (66)	187 (29)	4 (<1)	33 (5)

[a]ID, identification; ^{N}, species not included in v3.0 database; *, organism included in v3.0 database but not included in FDA claim.
[b]*M. angelicum* has the same first 500-bp rRNA gene sequence as *M. szulgai*; *M. angelicum* was not recognized in the gene sequence database at the time of the study. However, following the study period, all isolates were subjected to *rpoB* gene sequence analysis, which is required to differentiate the species from *M. szulgai*, and no isolates were identified as *M. angelicum*.
[c]Other members of the *M. chelonae* complex, *M. salmoniphilum*, *M. franklinii*, and *M. saopaulense*, were not tested.
[d]*M. farcinogenes* is a slowly growing *Mycobacterium* species.
[e]The other member of the *M. smegmatis* group, *M. goodii*, was not tested.
[f]The other closely related member, *M. wolinskyi*, was not tested.
[g]The other member of the *M. mucogenicum* group, *M. aubagnense*, was not tested.
[h]Most closely related.

TABLE 2 Accuracy of identification of nocardia

Reference identification[a]	No. (%) of isolates with indicated Vitek MS v3.0 ID result			
	Correct to species level	Correct to complex or group level	Incorrect	No ID
N. abscessus (*n* = 16)	15 (94)			1 (6)
N. arthritidis^N (*n* = 6)			1 (17)	5 (83)
N. asiatica^N (*n* = 5)			4 (80)	1 (20)
N. beijingensis^N (*n* = 1)			1 (100)	
N. brevicatena/paucivorans complex				
N. paucivorans (*n* = 18)	18 (100)			
N. nova complex				
N. nova (*n* = 33)		33 (100)		
N. veterana (*n* = 23)	23 (100)			
N. elegans^N (*n* = 2)		2 (100)		
N. transvalensis complex				
N. transvalensis (*n* = 22)	22 (100)			
N. wallacei (*n* = 13)	13 (100)			
N. blacklockiae^N (*n* = 6)		6 (100)		
N. asteroides (*n* = 19)	15 (79)		3 (16)	1 (5)
N. cyriacigeorgica (*n* = 33)	33 (100)			
N. farcinica (*n* = 32)	32 (100)			
N. rhamnosiphila^N (*n* = 2)				2 (100)
N. testacea^N (*n* = 1)				1 (100)
N. brasiliensis (*n* = 37)	35 (95)			2 (5)
N. vulneris^N (*n* = 1)			1 (100)	
N. pseudobrasiliensis (*n* = 13)	12 (92)			1 (8)
N. mexicana^N (*n* = 1)				1 (100)
N. otitidiscaviarum (*n* = 18)	18 (100)			
Nocardia species (*n* = 10)		3 (30)		7 (70)
Total (*n* = 312)	236 (76)	44 (14)	10 (3)	22 (7)

[a]A superscript "*N*" indicates species not included in the v3.0 database.

misidentified, and 22 (7%) were not identified despite repeat testing. Overall, the Vitek system provided a single correct identification to the species, group, or complex level for the majority (894 of 963, 93%) of the *Mycobacterium* and *Nocardia* isolates tested.

Performance for the clinical isolates of mycobacteria. As shown in Table 1, 45 (100%) of the 45 isolates of *M. tuberculosis* complex were correctly identified. Ninety-two percent of the slowly growing NTM and 98% of the rapidly growing mycobacteria (RGM) were correctly identified to the species, complex, or group level. Among the RGM, the Vitek system was able to accurately identify species within the *M. chelonae/abscessus group* as *M. chelonae* (28 of 29 correctly identified to the species level, 1 "no identification"), *M. immunogenum* (19 of 19 correctly identified to the species level), or *M. abscessus* complex (40 of 40 correctly identified). The Vitek system was unable to differentiate the three subspecies of *M. abscessus* (i.e., subspecies *abscessus*, *massiliense*, and *bolletii*). Although the Vitek system cannot differentiate between species within the *M. fortuitum* group, 48 (98%) of 49 isolates tested within this group were identified correctly. Within the *M. mucogenicum* group, 18 of 20 isolates were correctly identified as *M. mucogenicum*. However, *M. phocaicum*, which is not represented in the database, had 20 of the 21 isolates misidentified as *M. mucogenicum*. The remaining *M. phocaicum* isolate was not identified. Additionally, there was one isolate identified by the reference method as being most closely related to *M. mucogenicum/phocaicum* that was identified as *M. mucogenicum* by the Vitek system.

Among the slowly growing NTM, eight species were accurately identified to the species level for every isolate tested; this included 28 of 28 *M. simiae*, 31 of 31 *M. kansasii*, 25 of 25 *M. marinum*, 15 of 15 *M. scrofulaceum*, and 24 of 24 *M. xenopi* isolates. The Vitek system was relatively accurate in identifying the other slowly growing NTM

TABLE 3 Identifications correct to the complex or group level

Reference identification[a]	Vitek MS v3.0 identification(s) (no. of isolates)
Mycobacteria	
M. tuberculosis complex	*M. tuberculosis* complex (45)
M. intracellulare	*M. avium* (1)
M. chimera[N]	*M. avium* (1), *M. intracellulare* (15)
MAC-X[N,b]	*M. intracellulare* (14)
M. gordonae/paragordonae[N]	*M. gordonae* (1)
M. abscessus complex	*M. abscessus* (40)
M. fortuitum group	*M. fortuitum* group (48)
M. phocaicum[N]	*M. mucogenicum* (20)
M. mucogenicum/phocaicum[c]	*M. mucogenicum* (1)
Nocardia	
N. nova	*N. nova/africana* (33)
N. elegans[N]	*N. nova/africana* (2)
N. blacklockiae[N]	*N. wallacei* (6)

[a]A superscript "*N*" indicates species not included in the v3.0 database.
[b]MAC-X includes *M. arosiense*, *M. bouchedurhonense*, *M. colombiense*, *M. marseillense*, *M. timonense*, *M. vulneris*, and *M. yongonense*.
[c]Most closely related.

species, including 28 (93%) of 30 *M. gordonae*, 17 (94%) of 18 *M. haemophilum*, and 23 (96%) of 24 *M. szulgai* isolates. The Vitek system was also able to successfully identify the more commonly encountered members of the *M. avium* complex (MAC), namely, *M. avium* (68 of 68; 100% accurate) and *M. intracellulare* (42 of 43; 98% accurate). There was one isolate of *M. intracellulare* that was misidentified as *M. avium* (Table 3). Other MAC members were misidentified at the species level (Table 3) but correctly identified at the complex level, generally as a result of not being included in the Vitek database. These included 16 *M. chimera* isolates, 1 of which was identified as *M. avium* and 15 of which were identified as *M. intracellulare*. Similarly, 14 "MAC-X" isolates (49) were identified as *M. intracellulare*, while an additional six isolates could not be identified. A result of "no identification" was obtained for all three isolates of *M. shigaense*, the single member of the *M. simiae* complex not represented in the database. There were three additional slowly growing NTM species that were not represented in the Vitek database, including *M. paraense* (one isolate was not identified), *M. parascrofulaceum* (one isolate was not identified and one isolate was misidentified as *M. scrofulaceum*), and *M. paraffinicum* (of nine isolates, seven were not identified and two were misidentified as *M. scrofulaceum*). Considering all of the mycobacterial isolates tested, the Vitek system successfully identified 94% to the species, complex, or group level.

Performance for the clinical isolates of *Nocardia*. A total of 312 isolates representing 21 different *Nocardia* species were tested (Table 2). Of these, 236 (76%) were correctly identified to the species level and another 44 (14%) were correctly identified to the complex level. Twenty-two (7%) isolates could not be identified despite repeat testing. Seven common species (*N. cyriacigeorgica*, *N. farcinica*, *N. otitidiscaviarum*, *N. paucivorans*, *N. transvalensis*, *N. veterana*, *and N. wallacei*) were correctly identified to the species level for every isolate tested (33/33, 32/32, 18/18, 18/18, 22/22, 23/23, and 13/13, respectively). Fifteen (79%) of 19 *N. asteroides* isolates were identified to the species level by the Vitek system. Three *N. asteroides* isolates were misidentified as *N. neocaledoniensis* (Table 4), and one was not identified. While all 23 *N. veterana* isolates were correctly identified to the species level, the Vitek system did not perform as well on other members of the *N. nova* complex. Each of the 33 *N. nova* isolates was given a result consisting of both *N. nova* and *N. africana* identifications (Table 3). This was also the case for the two isolates of *N. elegans*, which, notably, is not represented in the Vitek database (Table 3). Similarly, all 22 isolates of *N. transvalensis* and all 13 isolates of *N. wallacei* were correctly identified to the species level, but the other member of the *N. transvalensis* complex, *N. blacklockiae*, which is not in the database, was consistently (6 of 6, 100%) misidentified as *N. wallacei*. There were six additional species that were not

TABLE 4 Incorrect identifications

Reference identification[a]	Vitek MS v3.0 identification(s) (no. of isolates)
Mycobacteria	
M. gordonae/paragordonae[N]	*M. scrofulaceum* (1)
M. parascrofulaceum[N]	*M. scrofulaceum* (1)
M. paraffinicum[N]	*M. scrofulaceum* (2)
Nocardia	
N. asiatica[N]	*N. abscessus* (1), *N. beijingensis* (2), *N. abscessus/beijingensis* (1)
N. beijingensis[N]	*N. abscessus* (1)
N. asteroides	*N. neocaldonensis* (3)
N. arthritidis[N]	*N. beijingensis* (1)
N. vulneris[N]	*N. brasiliensis* (1)

[a]A superscript "*N*" indicates species not included in the v3.0 database.

represented in the Vitek database. This included two isolates of *N. rhamnosiphila* and one isolate of *N. testacea*, which were not identified; one isolate of *N. asiatica* and one isolate of *N. beijingensis*, which were misidentified as *N. abscessus*; two isolates of *N. asiatica* and one isolate of *N. arthritidis*, which were misidentified as *N. beijingensis*; one isolate of *N. asiatica*, which was misidentified as *N. abscessus*/*N. beijingensis*, and one isolate of *N. vulneris*, which was misidentified as *N. brasiliensis*. Considering all *Nocardia* isolates tested, 280 (90%) were correctly identified to the species or complex level.

Repeat testing. Repeat testing was performed when the Vitek gave an initial result of "no identification" or when the spectra were of poor quality and a result was not provided. In either case, the original extract was respotted as a single replicate. If no identification was made again, a new sample extract was prepared from the same subculture and tested as a single replicate. If the identification could still not be obtained, a result of "no identification" was used as the final result. As shown in Table 5, repeat testing was performed on 103 isolates, resulting in an overall repeat rate of 11% (103 of 963). The repeat rate was slightly lower for the mycobacteria (68 of 651, 10%) than for the *Nocardia* (35 of 312, 11%). Species not represented in the v3.0 database typically had the highest repeat rates. For those species in the database, the repeat testing rate was highest for *M. lentiflavum* (35%). and *N. abscessus* (44%). One isolate was misidentified during repeat testing; an *N. asteroides* was misidentified as *N. neocaledoniensis* after respotting. There were three species, *M. haemophilum*, *M. simiae*, and *M. xenopi*, for which repeat testing was performed solely due to poor-quality spectra as indicated by the software. None of the *Nocardia* isolates required repeat testing as a result of poor-quality spectra.

Challenge set. In addition to the isolates obtained during the course of clinical work, a panel of 50 well-characterized isolates representing 18 *Mycobacterium* and 12 *Nocardia* species were run at each of three sites. As shown in Table 6, 149 150 (99%) of the replicates were correctly identified. There were no misidentifications, but three *N. nova* isolates were identified as *N. nova*/*N. africana* and thus correctly identified to the complex level rather than the species level. One *M. haemophilum* isolate was not identified at a single site.

Reproducibility. Reproducibility was assessed by testing a panel of five organisms (*M. abscessus*, *M chelonae*, *M. smegmatis*, *N. wallacei*, and *N. otitidiscaviarum*) in duplicate on two runs a day for 5 days at each of three sites for a total of 300 replicates. This reproducibility testing produced a single correct identification at the species level for 297 (99%) of the replicates tested. In two instances, *M. smegmatis* was not identified; both cases occurred at a single site. There was also a single instance where *N. wallacei* was not identified at a single site. There were no misidentifications of any organism at any site. There also were no differences in the accuracy of organism identification by site, operator, reagent kit, or day.

TABLE 5 Results of repeat testing

Reference identification[a]	No. of isolates requiring repeat testing (repeat rate, %)	No. of isolates with indicated Vitek MS identification result			
		Respot correct	Reextract correct	Reextract incorrect	Final
Mycobacteria (total tested = 813)					
M. abscessus complex (*n* = 40)	1 (3)	1			
M. avium (*n* = 68)	3 (4)	1	2		
M. chelonae (*n* = 29)	1 (3)				1
MAC-X^N (*n* = 20)	6 (30)				6
M. fortuitum group (*n* = 49)	1 (2)				1
M. gordonae (*n* = 33)	6 (17)	4			2
M. gordonae/paragordonae^N (*n* = 3)	1 (100)				1
M. haemophilum (*n* = 18)	3 (9)	1	1		1
M. kansasii (*n* = 31)	4 (13)	4			
M. lentiflavum (*n* = 37)	13 (30)	4	5		4
M. malmoense (*n* = 4)	4 (12)	2			
M. mucogenicum (*n* = 20)	4 (13)	1	1		2
M. paraense^N (*n* = 1)	1 (100)				1
M. parascrofulaceum^N (*n* = 2)	1 (50)				1
M. paraffinicum^N (*n* = 9)	7 (78)				7
M. phocaicum^N (*n* = 21)	1 (5)				1
M. scrofulaceum (*n* = 15)	3 (10)	1			
M. shigaense^N (*n* = 3)	3 (100)				3
M. simiae (*n* = 28)	1 (3)		1		
M. smegmatis (*n* = 21)	6 (20)	1	3		
M. szulgai (*n* = 24)	2 (6)	1			1
M. xenopi (*n* = 24)	1 (3)		1		
Mycobacterium species^N (*n* = 1)	1 (100)				1
Total	68 (10)	21	14		33
Nocardia (total tested = 417)					
N. abscessus (*n* = 16)	7 (23)		6		1
N. arthritidis^N (*n* = 6)	5				5
N. asiatica^N (*n* = 5)	1				1
N. asteroides (*n* = 19)	2 (6)			1	1
N. brasiliensis (*n* = 37)	2 (5)				2
N. mexicana^N (*n* = 1)	1				1
N. otitidiscaviarum (*n* = 18)	1 (3)	1			
N. pseudobrasiliensis (*n* = 13)	4 (13)	3			1
N. rhamnosiphila^N (*n* = 2)	2				2
N. testacea^N (*n* = 1)	1				1
N. vulneris^N (*n* = 1)	1			1	
N. wallacei (*n* = 13)	1 (3)	1			
Nocardia species^N (*n* = 10)	7				7
Total	35 (11)	5	6	1	22
Total (*n* = 963)	103 (11)	26	20	2	55

[a]A superscript "*N*" indicates species not included in the v3.0 database. Only those species that had at least one isolate that required repeat testing are listed.

DISCUSSION

The use of MALDI-TOF mass spectrometry (MS) to identify commonly encountered isolates of Gram-positive and Gram-negative bacteria and yeasts has revolutionized workflow and improved turnaround time in clinical microbiology laboratories around the world (36, 38). MALDI-TOF MS has previously been applied to *Mycobacterium* and *Nocardia* species with mixed results, raising questions about the utility of this method for these organisms (17–21, 23–28, 32, 34–40). Previous studies have used a range of culture conditions, different biomass volumes, and/or different inactivation and extraction procedures (16, 18, 20, 24, 29–31, 33). Several studies have suggested that manufacturer-derived databases require the addition of custom libraries to enable species-level identification rates of at least 90% (18, 20, 25, 26, 39, 40). Given that custom libraries require extensive development and validation, their use may not be a viable option for many clinical laboratories. These factors have collectively led to an absence of more widely spread use of MALDI-TOF mass spectrometry for the identification of these bacteria.

TABLE 6 Accuracy of Vitek MS v3.0 for the challenge set

Organism (no. of isolates)	No. (%) of isolates with indicated Vitek MS identification result			
	Correct to species level	Correct to complex or group level	Incorrect	No ID
M. abscessus complex ($n = 6$)		6 (100)		
M. avium ($n = 9$)	9 (100)			
M. chelonae ($n = 3$)	3 (100)			
M. fortuitum group ($n = 15$)		15 (100)		
M. gordonae ($n = 3$)	3 (100)			
M. haemophilum ($n = 6$)	5 (83)			1 (17)
M. immunogenum ($n = 3$)	3 (100)			
M. intracellulare ($n = 9$)	9 (100)			
M. kansasii ($n = 9$)	9 (100)			
M. malmoense ($n = 3$)	3 (100)			
M. marinum ($n = 3$)	3 (100)			
M. mucogenicum ($n = 3$)	3 (100)			
M. scrofulaceum ($n = 3$)	3 (100)			
M. simiae ($n = 3$)	3 (100)			
M. smegmatis ($n = 3$)	3 (100)			
M. szulgai ($n = 3$)	3 (100)			
M. tuberculosis complex ($n = 15$)		15 (100)		
M. xenopi ($n = 6$)	6 (100)			
N. abscessus ($n = 6$)	6 (100)			
N. asteroides ($n = 6$)	6 (100)			
N. brasiliensis ($n = 3$)	3 (100)			
N. cyriacigeorgica ($n = 3$)	3 (100)			
N. farcinica ($n = 3$)	3 (100)			
N. nova ($n = 3$)		3 (100)		
N. otitidiscaviarum ($n = 3$)	3 (100)			
N. paucivorans ($n = 3$)	3 (100)			
N. pseudobrasiliensis ($n = 3$)	3 (100)			
N. transvalensis ($n = 3$)	3 (100)			
N. veterana ($n = 6$)	6 (100)			
N. wallacei ($n = 3$)	3 (100)			
Total no. of isolates in challenge set ($n = 150$)	110 (73)	39 (26)		1 (1)

This report provides evidence that results obtained using the Vitek system are accurate and reproducible for the identification of *Mycobacterium* and *Nocardia* species. The v3.0 system is an improvement over the previous system in that it uses a simple and optimized extraction procedure specifically designed for the mycobacteria and *Nocardia* and relies on a well-developed database. The optimized extraction procedure has also been shown to be effective at inactivating infectious organisms, such as members of the *M. tuberculosis* complex (22). The ability of the Vitek system to identify *M. tuberculosis* complex with high (100%) accuracy is especially important for patient care and infection control. In this study, there were very few (1%) misidentifications. There was also a relatively low frequency of "no identification" calls (6%) which was similar to the rate seen with other bacterial species tested using this system (range, 1% to 8%) (43, 50–53). In the work presented here, isolates that failed to obtain an identification on the first attempt were respotted and then reextracted before the final result of "no identification" was accepted. Using this strategy, almost half (47%) of the isolates with an initial result of "no identification" were able to be correctly identified. Given that the repeat testing rate was low (11%), this strategy may be more efficient than duplicate spotting during the first attempt at identification.

The strengths of this study were the large number of isolates tested, representing a variety of different taxa; the use of a standardized inactivation and extraction procedure; the availability of a well-curated database; and use of a formalized repeat testing algorithm. One limitation of this study was that some clinically important organisms such as members of the *M. terrae* complex, as well as rarer species within the *M. chelonae* complex, *M. smegmatis*, and *M. mucogenicum* groups, were not thoroughly tested. Another limitation was that all testing was performed using isolates from

growth on solid media. Additional studies evaluating the accuracy of the Vitek system for organisms grown in liquid media are under way.

Although the Vitek MS v3.0 system can differentiate some closely related species, it is unable to do so for others. Members of the MAC, *M. tuberculosis*, and *M. abscessus* complex; the *M. fortuitum* and *M. mucogenicum* groups; and the *N. nova* complex frequently could not be differentiated. Similarly, although the system correctly identified all *M. avium* isolates, other members of the *M. avium* complex could not be reliably differentiated. This can be problematic for species not included in the database that are closely related to those included in the database, such as *M. chimaera*, as they may be misidentified as a similar species rather than not identified. Furthermore, although 41 of 41 *M. abscessus* isolates were correctly identified to the species level, the Vitek MS v3.0 system cannot differentiate between the closely related subspecies within this group, including *M. abscessus* subspecies *abscessus*, *bolletii*, and *massiliense*. These subspecies are of particular concern since they can vary in their levels of resistance to macrolides due to the presence or absence of a functional *erm*(41) gene. Reporting identifications to the complex or group level may be warranted in these cases. Similarly to the mycobacteria, the use of molecular methods has revised the taxonomy of *Nocardia* during the last 20 years, with many new species and complexes being defined (1). Selection of optimal antimicrobial therapy for infections caused by *Nocardia* is dependent on correct identification to the species level. However, identification to the complex level, such as was the case for species within the *N. nova* and *N. transvalensis* complexes, may be sufficient given their similar drug susceptibility profiles (1).

In summary, using standardized methods for growth, sample preparation, and analysis, the Vitek MS v3.0 system was 93% accurate for the identification of *Mycobacterium* and *Nocardia* isolates to the species, group, or complex level. Using this system, it may now be feasible for many clinical laboratories to accurately identify the commonly encountered mycobacteria and *Nocardia*.

ACKNOWLEDGMENTS

We thank Eddie Miranda (MSKCC) for technical assistance and Phyllis Della-Latta (Columbia University Medical Center), Barry Kreiswirth (Rutgers University), and Nancy Wengenack (Mayo Clinic) for providing additional clinical isolates. The day-to-day work would not have been possible without the expertise of Andrew Derylak and Paula Fearrington at LabCorp. We thank the Mycobacteria/Nocardia Laboratory staff (including past members) Megan Ashcraft, Victoria Gee, Amber McKinney, and Nicholas Parodi for their laboratory assistance and Joanne Woodring for helping to type the manuscript.

This study was performed as part of an FDA trial of the Vitek MS v3.0 system and was funded by the device manufacturer (bioMérieux, Inc., Durham, NC). The data analysis described here was performed by us without influence by the device manufacturer. Study activities were performed under contract with the Clinical Trials Covance Division of LabCorp and research agreements with the Memorial Sloan Kettering Cancer Center (SK2013-1773). This study was supported in part by NIH/NCI Cancer Center Support Grant P30 (CA008748). B.A.B. was an officer and employee of LabCorp and is now retired.

REFERENCES

1. Brown-Elliott BA, Brown JM, Conville PS, Wallace RJ, Jr. 2006. Clinical and laboratory features of the Nocardia spp. based on current molecular taxonomy. Clin Microbiol Rev 19:259–282. https://doi.org/10.1128/CMR.19.2.259-282.2006.
2. Conville PS, Brown-Elliott BA, Smith T, Zelazny AM. 26 December 2017. The complexities of nocardia taxonomy and identification. J Clin Microbiol https://doi.org/10.1128/JCM.01419-17.
3. Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F, Holland SM, Horsburgh R, Huitt G, Iademarco MF, Iseman M, Olivier K, Ruoss S, von Reyn CF, Wallace RJ, Jr, Winthrop K; ATS Mycobacterial Diseases Subcommittee; American Thoracic Society; Infectious Disease Society of America. 2007. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. Am J Respir Crit Care Med 175:367–416. https://doi.org/10.1164/rccm.200604-571ST.
4. Kang YA, Koh WJ. 2016. Antibiotic treatment for nontuberculous mycobacterial lung disease. Expert Rev Respir Med 10:557–568. https://doi.org/10.1586/17476348.2016.1165611.
5. Ryu YJ, Koh WJ, Daley CL. 2016. Diagnosis and treatment of nontuberculous mycobacterial lung disease: clinicians' perspectives. Tuberc Respir Dis (Seoul) 79:74–84. https://doi.org/10.4046/trd.2016.79.2.74.
6. WHO. 2016. Global tuberculosis report 2016. World Health Organization, Geneva, Switzerland. http://www.who.int/tb/publications/global_report/en/. Accessed 10/4/2017.

7. Forbes BA. 2017. Mycobacterial taxonomy. J Clin Microbiol 55:380–383. https://doi.org/10.1128/JCM.01287-16.
8. Telenti A, Marchesi F, Balz M, Bally F, Bottger EC, Bodmer T. 1993. Rapid identification of mycobacteria to the species level by polymerase chain reaction and restriction enzyme analysis. J Clin Microbiol 31:175–178.
9. Tortoli E. 2003. Impact of genotypic studies on mycobacterial taxonomy: the new mycobacteria of the 1990s. Clin Microbiol Rev 16:319–354. https://doi.org/10.1128/CMR.16.2.319-354.2003.
10. Tortoli E. 2014. Microbiological features and clinical relevance of new species of the genus Mycobacterium. Clin Microbiol Rev 27:727–752. https://doi.org/10.1128/CMR.00035-14.
11. Wayne LG, Sramek HA. 1992. Agents of newly recognized or infrequently encountered mycobacterial diseases. Clin Microbiol Rev 5:1–25. https://doi.org/10.1128/CMR.5.1.1.
12. Wolinsky E. 1979. Nontuberculous mycobacteria and associated diseases. Am Rev Respir Dis 119:107–159.
13. Conville PS, Witebsky FG. 2005. Multiple copies of the 16S rRNA gene in Nocardia nova isolates and implications for sequence-based identification procedures. J Clin Microbiol 43:2881–2885. https://doi.org/10.1128/JCM.43.6.2881-2885.2005.
14. Kiska DL, Hicks K, Pettit DJ. 2002. Identification of medically relevant Nocardia species with an abbreviated battery of tests. J Clin Microbiol 40:1346–1351. https://doi.org/10.1128/JCM.40.4.1346-1351.2002.
15. McTaggart LR, Richardson SE, Witkowska M, Zhang SX. 2010. Phylogeny and identification of Nocardia species on the basis of multilocus sequence analysis. J Clin Microbiol 48:4525–4533. https://doi.org/10.1128/JCM.00883-10.
16. Adams LL, Dionne K, Fisher S, Parrish N. 2016. A rapid, standardized protein extraction method using adaptive focused acoustics for identification of mycobacteria by MALDI-ToF MS. Diagn Microbiol Infect Dis 86:284–288. https://doi.org/10.1016/j.diagmicrobio.2016.06.001.
17. Balada-Llasat JM, Kamboj K, Pancholi P. 2013. Identification of mycobacteria from solid and liquid media by matrix-assisted laser desorption ionization–time of flight mass spectrometry in the clinical laboratory. J Clin Microbiol 51:2875–2879. https://doi.org/10.1128/JCM.00819-13.
18. Blosser SJ, Drake SK, Andrasko JL, Henderson CM, Kamboj K, Antonara S, Mijares L, Conville P, Frank KM, Harrington SM, Balada-Llasat JM, Zelazny AM. 2016. Multicenter matrix-assisted laser desorption ionization–time of flight mass spectrometry study for identification of clinically relevant Nocardia spp. J Clin Microbiol 54:1251–1258. https://doi.org/10.1128/JCM.02942-15.
19. Buchan BW, Riebe KM, Timke M, Kostrzewa M, Ledeboer 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. https://doi.org/10.1309/AJCPBPUBUDEW2OAG.
20. 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. https://doi.org/10.1128/JCM.02128-15.
21. Clark AE, Kaleta EJ, Arora A, Wolk DM. 2013. Matrix-assisted laser desorption ionization–time of flight mass spectrometry: a fundamental shift in the routine practice of clinical microbiology. Clin Microbiol Rev 26:547–603. https://doi.org/10.1128/CMR.00072-12.
22. Dunne WM, Jr, Doing K, Miller E, Miller E, Moreno E, Baghli M, Mailler S, Girard V, van Belkum A, Deol P. 2014. Rapid inactivation of Mycobacterium and Nocardia species before identification using matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 52:3654–3659. https://doi.org/10.1128/JCM.01728-14.
23. El Khéchine A, Couderc C, Flaudrops C, Raoult D, Drancourt M. 2011. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry identification of mycobacteria in routine clinical practice. PLoS One 6:e24720. https://doi.org/10.1371/journal.pone.0024720.
24. 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. https://doi.org/10.1021/ac049410m.
25. Hsueh PR, Lee TF, Du SH, Teng SH, Liao CH, Sheng WH, Teng LJ. 2014. Bruker Biotyper matrix-assisted laser desorption ionization–time of flight mass spectrometry system for identification of Nocardia, Rhodococcus, Kocuria, Gordonia, Tsukamurella, and Listeria species. J Clin Microbiol 52:2371–2379. https://doi.org/10.1128/JCM.00456-14.
26. Khot PD, Bird BA, Durrant RJ, Fisher MA. 2015. Identification of Nocardia species by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 53:3366–3369. https://doi.org/10.1128/JCM.00780-15.
27. 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. https://doi.org/10.1128/JCM.00006-17.
28. Lotz A, Ferroni A, Beretti JL, Dauphin B, Carbonnelle E, Guet-Revillet H, Veziris N, Heym B, Jarlier V, Gaillard JL, 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. https://doi.org/10.1128/JCM.01397-10.
29. Machen A, Kobayashi M, Connelly MR, Wang YF. 2013. Comparison of heat inactivation and cell disruption protocols for identification of mycobacteria from solid culture media by use of vitek matrix-assisted laser desorption ionization–time of flight mass spectrometry. J Clin Microbiol 51:4226–4229. https://doi.org/10.1128/JCM.02612-13.
30. 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. https://doi.org/10.1128/JCM.01996-13.
31. O'Connor JA, Lynch-Healy M, Corcoran D, O'Reilly B, O'Mahony J, Lucey B. 2016. Improved matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS)-based identification of Mycobacterium spp. by use of a novel two-step cell disruption preparatory technique. J Clin Microbiol 54:495–496. https://doi.org/10.1128/JCM.02998-15.
32. Panagea T, Pincus DH, Grogono D, Jones M, Bryant J, Parkhill J, Floto RA, Gilligan P. 2015. Mycobacterium abscessus complex identification with matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 53:2355–2358. https://doi.org/10.1128/JCM.00494-15.
33. Park JS, Choi SH, Hwang SM, Hong YJ, Kim TS, Park KU, Song J, Kim EC. 2016. The impact of protein extraction protocols on the performance of currently available MALDI-TOF mass spectrometry for identification of mycobacterial clinical isolates cultured in liquid media. Clin Chim Acta 460:190–195. https://doi.org/10.1016/j.cca.2016.06.039.
34. Pignone M, 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. https://doi.org/10.1128/JCM.01959-05.
35. Rodríguez-Sánchez B, Ruiz-Serrano MJ, Ruiz A, Timke M, Kostrzewa M, Bouza E. 2016. Evaluation of MALDI Biotyper Mycobacteria Library v3.0 for identification of nontuberculous mycobacteria. J Clin Microbiol 54:1144–1147. https://doi.org/10.1128/JCM.02760-15.
36. 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. https://doi.org/10.1128/JCM.02135-10.
37. 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. https://doi.org/10.1086/600885.
38. Tran A, Alby K, Kerr A, Jones M, Gilligan PH. 2015. Cost savings realized by implementation of routine microbiological identification by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 53:2473–2479. https://doi.org/10.1128/JCM.00833-15.
39. Verroken A, Janssens M, Berhin C, Bogaerts P, Huang TD, Wauters G, Glupczynski Y. 2010. Evaluation of matrix-assisted laser desorption ionization–time of flight mass spectrometry for identification of Nocardia species. J Clin Microbiol 48:4015–4021. https://doi.org/10.1128/JCM.01234-10.
40. Xiao M, Pang L, Chen SC, Fan X, Zhang L, Li HX, Hou X, Cheng JW, Kong F, Zhao YP, Xu YC. 2016. Accurate identification of common pathogenic Nocardia species: evaluation of a multilocus sequence analysis platform and matrix-assisted laser desorption ionization-time of flight mass spectrometry. PLoS One 11:e0147487. https://doi.org/10.1371/journal.pone.0147487.
41. Girard V, Mailler S, Welker M, Arsac M, Celliere B, Cotte-Pattat PJ,

Chatellier S, Durand G, Beni AM, Schrenzel J, Miller E, Dussoulier R, Dunne WM, Jr, Butler-Wu S, Saubolle MA, Sussland D, Bell M, van Belkum A, Deol P. 2016. Identification of Mycobacterium spp. and Nocardia spp. from solid and liquid cultures by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS). Diagn Microbiol Infect Dis 86:277–283. https://doi.org/10.1016/j.diagmicrobio.2016.07.027.

42. Totty H, Miller E, Moreno E, Dunne WM, Jr, Deol P. 2016. Comparison of mechanical disruption techniques for rapid inactivation of Mycobacterium and Nocardia species before identification using matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry. J Clin Microbiol 54:2626–2627. https://doi.org/10.1128/JCM.01096-16.
43. Rychert J, Burnham CA, Bythrow M, Garner OB, Ginocchio CC, Jennemann R, Lewinski MA, Manji R, Mochon AB, Procop GW, Richter SS, Sercia L, Westblade LF, Ferraro MJ, Branda JA. 2013. Multicenter evaluation of the Vitek MS matrix-assisted laser desorption ionization–time of flight mass spectrometry system for identification of Gram-positive aerobic bacteria. J Clin Microbiol 51:2225–2231. https://doi.org/10.1128/JCM.00682-13.
44. Edwards U, Rogall T, Blocker H, Emde M, Bottger EC. 1989. Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA. Nucleic Acids Res 17:7843–7853.
45. Adékambi T, Colson P, Drancourt M. 2003. rpoB-based identification of nonpigmented and late-pigmenting rapidly growing mycobacteria. J Clin Microbiol 41:5699–5708. https://doi.org/10.1128/JCM.41.12.5699-5708.2003.
46. Brown-Elliott BA, Vasireddy S, Vasireddy R, Iakhiaeva E, Howard ST, Nash K, 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. https://doi.org/10.1128/JCM.02950-14.
47. Conville PS, Zelazny AM, Witebsky FG. 2006. Analysis of secA1 gene sequences for identification of Nocardia species. J Clin Microbiol 44:2760–2766. https://doi.org/10.1128/JCM.00155-06.
48. CLSI. 2008. MM18-A: interpretive criteria for identification of bacteria and fungi by DNA target sequencing, 1st ed. CLSI, Wayne, PA.
49. Viljanen MK, Olkkonen L, Katila ML. 1993. Conventional identification characteristics, mycolate and fatty acid composition, and clinical significance of MAIX AccuProbe-positive isolates of Mycobacterium avium complex. J Clin Microbiol 31:1376–1378.
50. 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. https://doi.org/10.1016/j.diagmicrobio.2013.08.013.
51. Garner O, Mochon A, Branda J, Burnham CA, Bythrow M, Ferraro M, Ginocchio C, Jennemann R, Manji R, Procop GW, Richter S, Rychert J, Sercia L, Westblade L, Lewinski M. 2014. Multi-centre evaluation of mass spectrometric identification of anaerobic bacteria using the VITEK(R) MS system. Clin Microbiol Infect 20:335–339. https://doi.org/10.1111/1469-0691.12317.
52. Manji R, Bythrow M, Branda JA, Burnham CA, Ferraro MJ, Garner OB, Jennemann R, Lewinski MA, Mochon AB, Procop GW, Richter SS, Rychert JA, Sercia L, Westblade LF, Ginocchio CC. 2014. Multi-center evaluation of the VITEK(R) MS system for mass spectrometric identification of non-Enterobacteriaceae Gram-negative bacilli. Eur J Clin Microbiol Infect Dis 33:337–346. https://doi.org/10.1007/s10096-013-1961-2.
53. Richter SS, Sercia L, Branda JA, Burnham CA, Bythrow M, Ferraro MJ, Garner OB, Ginocchio CC, Jennemann R, Lewinski MA, Manji R, Mochon AB, Rychert JA, Westblade LF, Procop GW. 2013. Identification of Enterobacteriaceae by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry using the VITEK MS system. Eur J Clin Microbiol Infect Dis 32:1571–1578. https://doi.org/10.1007/s10096-013-1912-y.