

Evaluation of the Bruker Biotyper and Vitek MS Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry Systems for Identification of Nonfermenting Gram-Negative Bacilli Isolated from Cultures from Cystic Fibrosis Patients

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The Bruker Biotyper and Vitek MS matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (MS) instruments were evaluated for the identification of nonfermenting Gram-negative bacilli (NFGNB) by a blinded comparison to conventional biochemical or molecular methods. Two hundred NFGNB that were recovered from cultures from cystic fibrosis patients in the University of Iowa Health Care (UIHC) Microbiology Laboratory between 1 January 2006 and 31 October 2010 were sent to Mayo Clinic for analysis with the Bruker Biotyper (software version 3.0) and to bioMérieux for testing with Vitek MS (SARAMIS database version 3.62). If two attempts at direct colony testing failed to provide an acceptable MALDI-TOF identification, an extraction procedure was performed. The MS identifications from both of these systems were provided to UIHC for comparison to the biochemical or molecular identification that had been reported in the patient record. Isolates with discordant results were analyzed by 16S rRNA gene sequencing at UIHC. After discrepancy testing, the Bruker Biotyper result agreed with the biochemical or molecular method, with 72.5% of isolates to the species level, 5.5% to the complex level, and 19% to the genus level (3% not identified). The level of agreement for Vitek MS was 80% species, 3.5% complex, 6% genus, and 3.5% family (7% not identified). Both MS systems provided rapid (≤ 3 min per isolate) and reliable identifications. The agreement of combined species/complex/genus-level identification with the reference method was higher for the Bruker Biotyper (97% versus 89.5%, $P = 0.004$) but required an extraction step more often. Species-level agreement with the reference method was similar for both MS systems (72.5% and 80%, $P = 0.099$).

Patients with cystic fibrosis (CF) have mutations in the gene that encodes CF transmembrane conductance regulator (CFTR) protein (21). Defective CFTR protein production causes abnormal ion transport across epithelial cells in multiple organs (22, 27). Thick airway secretions, chronic infection, and inflammation contribute to lung disease that eventually progresses to respiratory failure, the most common cause of death among CF patients (9). *Staphylococcus aureus*, *Haemophilus influenzae*, and two nonfermenting Gram-negative bacilli (NFGNB), *Pseudomonas aeruginosa* and *Burkholderia cepacia* complex, are the pathogenic bacteria most frequently isolated from respiratory cultures from CF patients (6, 14). Determining the clinical significance of other NFGNB (e.g., *Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans*, *Ralstonia* spp., *Pandoraea* spp., and *Burkholderia gladioli*) commonly recovered from CF respiratory cultures can be difficult (11).

Distinguishing among NFGNB using manual and automated commercial identification systems is often challenging due to limited biochemical reactivity (15). The molecular methods required for accurate identification of some NFGNB are expensive and labor intensive (3, 5). Matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (MS) offers the potential for routine identification of bacteria that is faster, more accurate, and less expensive than conventional biochemical methods (2, 24). MALDI-TOF MS analyzes the spectra of bacterial proteins that have been ionized from intact cells (13). An extraction step is sometimes required. Identification is determined by

comparing the spectra (peaks based on mass/charge ratio) to a reference library (13). The objective of this blinded study was to determine the ability of two MALDI-TOF MS devices, the Bruker Biotyper (Bruker Daltonics Inc., Billerica, MA) and Vitek MS (bioMérieux Inc., Durham, NC), to identify NFGNB that were isolated from CF cultures in comparison to conventional biochemical or molecular methods.

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MATERIALS AND METHODS

Two hundred NFGNB from CF patient respiratory cultures performed in the University of Iowa Health Care (UIHC) Microbiology Laboratory between 1 January 2006 and 31 October 2010 were sent to investigators at Mayo Clinic for analysis with the Bruker Biotyper (software version 3.0) and to investigators at bioMérieux for testing with Vitek MS (formerly referred to as Axima@SARAMIS; database version 3.62). Only one isolate representing each NFGNB species was included for each CF patient. Mu-

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coid and nonmucoid *P. aeruginosa* isolates were considered to be separate species.

Upon receipt at each MS study site, isolates were subcultured on 5% sheep blood agar and incubated for 24 h at 35°C in 5 to 10% CO₂. Isolates were processed for MS analysis within 6 h after removal from the incubator. A thin film of fresh colony growth was applied directly to MALDI-TOF target plates for each of the mass spectrometry systems. For each attempt at direct colony testing, one spot on the target plate was inoculated for isolates tested with the Bruker Biotyper and two spots on the target plate were inoculated for isolates tested with Vitek MS. The double spotting for the Vitek MS was required for the algorithm used by the Durham research laboratory to interpret the output of the SARAMIS database (version 3.62). Each spot was then covered with matrix solution, allowed to dry, and analyzed. If two attempts at direct colony testing failed to provide an acceptable identification, an extraction procedure was performed as follows. Bacteria were suspended in 70% ethanol (EtOH), followed by centrifugation for 2 min. After supernatant removal, the pellet was dried and resuspended in 50 µl 70% formic acid and 50 µl acetonitrile. After centrifugation for 2 min, 1 µl supernatant was applied to a spot (2 spots for Vitek MS) on the MALDI-TOF plate and dried, 1 µl matrix was added and dried, and the target plate was placed in the instrument for analysis.

For the Bruker system, manufacturer-recommended score cutoffs were used to determine genus-level (1.7000 to 1.999) or species-level (≥ 2.000) identification. Additionally, the score of the identification listed as the first choice had to be at least 10% different from the score of the second identification (different species or genus) to be accepted (23). For the Vitek MS, identifications were considered acceptable based on the following confidence scores for each spot: one spot result of $>90\%$ confidence with no conflicting result of $>85\%$, or one spot result of $>85\%$ confidence with the other spot corroborating the result.

Mass spectrometry identifications were provided to UIHC for comparison to the biochemical or molecular identification originally reported in the patient record. The original identification methods for the 200 isolates were conventional biochemicals (oxidase reaction, characteristic colony morphology, diffusible pigment, and grape or corn taco odor for *P. aeruginosa*; $n = 65$), automated systems (Vitek 2 or Phoenix; $n = 59$), API 20 NE ($n = 25$), RapID ANA ($n = 2$), 16S rRNA gene sequencing at UIHC ($n = 31$), and PCR at the Cystic Fibrosis Foundation *Burkholderia cepacia* Research Laboratory and Repository ($n = 18$). As a CF care center, UIHC routinely sends *B. cepacia* complex isolates to the *B. cepacia* Research Laboratory for identification confirmation with molecular assays targeting 16S rRNA and *recA* genes (15–18, 20).

Isolates with MS results discordant to the originally reported patient record result were evaluated by 16S rRNA gene sequencing at UIHC using the MicroSeq 500 16S rDNA bacterial identification kit (Applied Biosystems, Carlsbad, CA) and the SmartGene integrated database network system (IDNS) (SmartGene, Inc., Raleigh, NC) and the Centroid database for organism identification. Final identifications for 16S rRNA gene analysis were assigned according to CLSI interpretive criteria (4). The isolates with discordant results that had only been tested once on an MS system were reanalyzed by MS at the respective laboratory.

Investigators were blinded to the identification generated by other methods or sites. Differences between MS system performances were assessed with Fisher's exact test (two-tailed) using GraphPad software (available at www.graphpad.com).

RESULTS

The agreement of the identifications provided by each MS system in comparison to the reference methods after discrepancy testing is shown in Table 1. The biochemical and molecular methods used for the initial and final reference identification of specific organisms are outlined in Table 2.

The Bruker system provided scores of ≥ 2.000 for 122 isolates with the initial testing (111 species-level identifications and 11

genus-level identifications because of a $<10\%$ difference in scores for different species). An additional 26 isolates had scores of ≥ 2.000 after the second direct colony test (24 species-level and two genus-level identifications). Extraction of the remaining 52 isolates yielded species-level identification for 20 isolates. The 52 isolates requiring extraction included 29 *S. maltophilia*, 9 *Achromobacter* species, and 3 *P. aeruginosa* (2 mucoid) isolates and single isolates of *A. xylosoxidans*, *Burkholderia multivorans*, *Bordetella petrii*, *Capnocytophaga gingivalis*, *Cellulomonas hominis*, a *Chryseobacterium* sp., *Herbaspirillum seropedicae*, a *Pandoraea* sp., *Ralstonia mannitolilytica*, a *Ralstonia* sp., and *Sphingomonas roseiflava*. Bruker system identifications of *Pseudomonas hibiscicola*, *P. beteli*, and *P. geniculata* were interpreted by the site investigators as *S. maltophilia*.

For discrepancy analysis, repeat Bruker system testing was performed on 15 isolates and the results for three (20%) changed from a species-level to a genus-level identification. A *Pseudomonas pseudoalcaligenes* with an initial MS score of 2.015 for *Pseudomonas mendocina* repeated as *P. mendocina* but with scores <2.000 , so the genus name was assigned. Initial testing of an *Achromobacter* species generated an initial Bruker system identification of *A. ruhlandii* with a score of 2.125 that repeated as *A. ruhlandii* but with scores <2.000 , so the genus name was assigned. Another *Achromobacter* species had an initial Bruker system score of 2.058 for *A. xylosoxidans* that repeated as *A. ruhlandii* (score of 1.976), and the extraction identification of *A. xylosoxidans* (score of 2.014) did not meet the 10% difference criteria for species identification.

The Vitek MS system provided acceptable identifications for 169 isolates after the initial round of duplicate spot testing: 161 isolates to the species level, 5 isolates to the complex level, and 3 isolates to the genus level. Repeat direct colony testing on the remaining 31 isolates yielded an acceptable identification for 8 isolates (4 species-level and 4 genus-level identifications). Extraction performed on 23 isolates provided an acceptable identification for only 2 isolates identified as *Elizabethkingia meningoseptica* and “Family III *Alcaligenaceae*.” For discrepancy analysis, repeat Vitek MS testing was performed on 19 isolates and the results for 1 isolate (5%) changed from species-level (*Ralstonia mannitolilytica*) to genus-level identification (*Ralstonia* spp.).

After discrepancy testing, the Bruker Biotyper result agreed with the reference method for 72.5% of isolates to the species level, 5.5% to the complex level, and 19% to the genus level (3% of isolates were not identified). The level of agreement for Vitek MS with the reference method was 80% species, 3.5% complex, 6% genus, and 3.5% family (7% of isolates were not identified). The level of agreement for identification of specific organisms by each MS system is shown in Table 1.

For discrepancy analysis, 42 isolates were analyzed by 16S rRNA gene sequencing at UIHC (Table 2). The final reference identification was determined using a molecular method for 83 of the 200 isolates.

Errors in genus identifications did not occur for either MS system. The only “errors” were in species identifications that were different or could not be confirmed by the final reference identification. There were discordant Bruker Biotyper results for nine isolates before MS discrepancy testing: *Achromobacter* spp. identified as *Achromobacter insolitus*, *A. xylosoxidans* ($n = 2$), and *A. ruhlandii*; a *Burkholderia* sp. identified as *B. multivorans*; *Pseudomonas* spp. identified as *P. aeruginosa* and *P. mosselii*; *P. pseudoalcaligenes* identified as *P. mendocina*; and a *Ralstonia* sp. iden-

TABLE 1 Agreement of Bruker Biotyper and Vitek MS with conventional biochemical or molecular identifications

Conventional biochemical or molecular method identification	No. of isolates	Bruker Biotyper			No. of isolates with no identification	Vitek MS				No. of isolates with no identification
		Species	Complex	Genus		No. of isolates with agreement with:				
					Species	Complex	Genus	Family		
<i>Achromobacter</i> spp.	12			11 ^a	1		4 ^b	7 ^c	1	
<i>Achromobacter xylosoxidans</i>	3	3					3 ^b			
<i>Burkholderia cepacia</i> complex (BCC)										
<i>B. cenocepacia</i>	4	2	2 ^d		4					
<i>B. multivorans</i>	10	3	7 ^e		5	5 ^f				
Species indeterminate	2		2 ^d			2 ^g				
<i>Burkholderia gladioli</i>	2	2			2					
<i>Burkholderia</i> sp.	1			1 ^h			1 ^h			
<i>Bordetella petrii</i>	1				1				1	
<i>Bordetella bronchiseptica</i>	2			2 ⁱ	2					
<i>Capnocytophaga gingivalis</i>	1				1				1	
<i>Capnocytophaga sputigena</i>	1	1					1			
<i>Cellulomonas hominis</i>	1				1				1	
<i>Chryseobacterium indologenes</i>	2	2							2	
<i>Chryseobacterium</i> sp.	1				1				1	
<i>Elizabethkingia meningoseptica</i>	1	1							1	
<i>Herbaspirillum seropedicae</i>	1			1					1	
<i>Pandoraea</i> sp.	1			1					1	
<i>Pantoea agglomerans</i>	1	1			1					
<i>Pseudomonas aeruginosa</i>	73	73			73					
<i>Pseudomonas aeruginosa</i> , mucoid	16	16			16					
<i>Pseudomonas pseudoalcaligenes</i>	1			1 ^j					1	
<i>Pseudomonas</i> spp.	3			2 ^k	1		2 ^k		1	
<i>Ralstonia mannitolilytica</i>	1			1	1					
<i>Ralstonia</i> spp.	2			2 ^l			1		1	
<i>Roseomonas</i> sp.	1			1 ^m					1	
<i>Sphingomonas roseiflava</i>	1			1					1	
<i>Stenotrophomonas maltophilia</i>	55	41 ⁿ		14 ^o	55					
Total (% agreement to level)	200	145 (72.5)	11 (5.5)	38 (19.0)	6 (3.0)	160 (80.0)	7 (3.5)	12 (6.0)	7 (3.5)	14 (7.0)

^a All identified by the Bruker system as *Achromobacter* spp. except for two isolates identified as *A. insolitus* (after extraction) and *A. xylosoxidans*.

^b All identified as *Achromobacter denitrificans/xylosoxidans* by Vitek MS.

^c All identified as Family III *Alcaligenaceae* by Vitek MS.

^d *Burkholderia cepacia* complex (*B. cenocepacia* did not meet the 10% difference rule).

^e *B. cepacia* complex (*B. multivorans* did not meet the 10% difference rule).

^f *Burkholderia cepacia/multivorans*.

^g *Burkholderia cenocepacia*.

^h *Burkholderia multivorans*.

ⁱ *Bordetella* spp. (*B. bronchiseptica* did not meet the 10% difference rule).

^j Initial identification, *Pseudomonas mendocina*; final identification after discrepancy testing, *Pseudomonas* spp.

^k One isolate identified as *P. aeruginosa* by both MS systems; other isolate identified as *P. mosselii* by the Bruker system and *P. putida* by Vitek MS.

^l One of the isolates identified as *R. pickettii* and the other as *Ralstonia* spp. by the Bruker system.

^m *Roseomonas mucosa*.

ⁿ Includes 7 isolates reported as *Pseudomonas beteli* ($n = 2$), *P. geniculata* ($n = 3$), or *P. hibiscicola* ($n = 2$) by the Bruker system.

^o These *Stenotrophomonas* species isolates were reported with Bruker system scores <2.000 as *S. maltophilia* ($n = 5$), *P. beteli* ($n = 1$), *P. geniculata* ($n = 1$), or *P. hibiscicola* ($n = 7$).

tified as *R. pickettii*. There were discordant Vitek MS species results for seven isolates before MS discrepancy testing: *Achromobacter* spp. identified as *A. denitrificans/xylosoxidans* ($n = 4$), a *Burkholderia* sp. identified as *B. multivorans*, and *Pseudomonas* spp. identified as *P. aeruginosa* and *P. putida*. After excluding isolates with no identifications, the percentage of these discordant results was similar for each MS system (Bruker Biotyper, 4.6%; Vitek MS, 3.8%; $P = 0.8$).

DISCUSSION

The identifications provided by both MS systems were rapid (≤ 3 min per isolate) and reliable. The agreement of combined species/

complex/genus-level identification with the reference method was higher for the Bruker Biotyper than the Vitek MS (97% versus 89.5%, $P = 0.004$). Species-level agreement for the Vitek MS and Bruker Biotyper was similar at 80% and 72.5%, respectively ($P = 0.099$).

To our knowledge, this is only the third direct comparison of identifications from these two MS systems. Cherkaoui et al. reported correct identifications for 94% of isolates tested with the Bruker system and 89% of isolates tested with Shimadzu, an earlier prototype of the Vitek MS (2). The number of incorrect identifications was <1% for both systems (2). For a study evaluating

TABLE 2 Biochemical and molecular methods used for reference identification

Final identification	No. of isolates	Initial identification method				Final reference identification method						
		Conventional biochemical	Automated system	API 20 NE	16S rRNA gene sequencing	PCR	Conventional biochemical	Automated system	API 20 NE	16S rRNA gene sequencing	PCR	
<i>Achromobacter</i> spp.	12			4 ^a	7	1					12	
<i>Achromobacter xylosoxidans</i>	3			3					3			
<i>Burkholderia cepacia</i> complex (BCC)												
<i>B. cenocepacia</i>	4					4						4
<i>B. multivorans</i>	10					10						10
Species indeterminate	2				1	1				1		1
<i>Burkholderia gladioli</i>	2					2						2
<i>Burkholderia</i> sp.	1			1 ^b							1	
<i>Bordetella petrii</i>	1				1						1	
<i>Bordetella bronchiseptica</i>	2			2					2			
<i>Capnocytophaga gingivalis</i>	1	1 ^c									1	
<i>Capnocytophaga sputigena</i>	1	1 ^c									1	
<i>Cellulomonas hominis</i>	1			1 ^d							1	
<i>Chryseobacterium indologenes</i>	2		1	1				1	1			
<i>Chryseobacterium</i> sp.	1			1 ^e							1	
<i>Elizabethkingia meningoseptica</i>	1			1					1			
<i>Herbaspirillum seropedicae</i>	1				1						1	
<i>Pandoraea</i> sp.	1				1						1	
<i>Pantoea agglomerans</i>	1			1 ^f							1	
<i>Pseudomonas aeruginosa</i>	73	51	4 ^g	5 ^h	13		51		2		20	
<i>Pseudomonas aeruginosa</i> , mucoid	16	14		2			14		2			
<i>Pseudomonas pseudoalcaligenes</i>	1		1 ⁱ								1	
<i>Pseudomonas</i> spp.	3			1 ^j	2						3	
<i>Ralstonia mannitolilytica</i>	1		1					1				
<i>Ralstonia</i> spp.	2				2						2	
<i>Roseomonas</i> sp.	1		1 ^k								1	
<i>Sphingomonas roseiflava</i>	1			1 ^l							1	
<i>Stenotrophomonas maltophilia</i>	55		51	1 ^m	3			39			16	
Total	200	67	59	25	31	18	65	41	11	66	17	

^a API identifications of *A. xylosoxidans* ("excellent"; $n = 2$), *Alcaligenes (Achromobacter)* spp. (*faecalis* 59%/denitrificans 31%; $n = 1$), and *Burkholderia (Ralstonia) pickettii* ("good"; $n = 1$).

^b API "acceptable" identification as *Ralstonia pickettii*.

^c RapID ANA identification as *Capnocytophaga* species.

^d API "very good" identification as *Sphingomonas paucimobilis*.

^e API "good" identification as *Chryseobacterium indologenes*.

^f API "very good" identification as *Pseudomonas luteola*.

^g Four isolates with Vitek 2 identification as *Pseudomonas fluorescens*.

^h Three isolates with API "good" identifications as *Psychrobacter phenylpyruvicus* ($n = 2$) and *Ralstonia pickettii* ($n = 1$).

ⁱ Vitek 2 identification as *Pseudomonas stutzeri*.

^j API "good" identification as *Alcaligenes faecalis*.

^k Vitek 2 identification as *Bordetella bronchiseptica*.

^l API "good" identification as *Sphingomonas paucimobilis*.

^m API "acceptable" identification as *Brevundimonas vesicularis*.

identification of anaerobic bacteria, the Bruker system had more correct species-level identifications than Shimadzu (67% versus 49%), but Shimadzu had fewer incorrect identifications (1.4%) than the Bruker Biotyper (7.9%) (12).

P. aeruginosa is the predominant NFGNB isolated from CF patients, reported in 51.2% of respiratory cultures in the 2010 CF Patient Registry (6). Infections due to *P. aeruginosa* are initially periodic and caused by different strains but then progress to a continuous chronic infection with one mucoid strain (14). The exact role *P. aeruginosa* plays in progression of CF lung disease has not yet been elucidated (11).

The final identifications provided by both MS systems for 89 *P. aeruginosa* isolates (including 16 mucoid strains) were 100% concordant with the biochemical ($n = 69$) or molecular ($n = 20$) methods. Three *P. aeruginosa* isolates (2 mucoid strains) required extraction for identification by the Bruker system, whereas all 89 isolates were identified without extraction by Vitek MS. Degand et al. also found 100% agreement of Bruker system identifications with conventional biochemical ($n = 85$) or molecular ($n = 16$) analysis for 400 *P. aeruginosa* isolates from 101 CF patients (7). Bizzini et al. reported that only one of 99 *P. aeruginosa* isolates analyzed by the Bruker system was

limited to genus-level agreement with Vitek 2; 8% of the isolates required extraction (1). A third Bruker system study showed 100% concordance with the BD Phoenix identification for 69 *P. aeruginosa* isolates (23).

B. cepacia complex organisms, present in respiratory cultures from 2.5% of CF patients (6), can have serious consequences for CF patients. Infection control measures to prevent transmission are essential due to epidemic strains of *Burkholderia cenocepacia*, *B. dolosa*, and *B. multivorans* associated with the acute respiratory decline of “cepacia syndrome” (11, 14). Commercial phenotypic systems have difficulty distinguishing members of the *B. cepacia* complex from one another and from *B. gladioli*, *Ralstonia*, *Pandora*, and *Cupriavidus* species (15). CF patient care centers send isolates suspected of belonging to the *B. cepacia* complex to the *B. cepacia* Research Laboratory and Repository for identification by molecular assays (20).

All 16 *B. cepacia* complex isolates in the current study were identified to a species or complex level that agreed with molecular methods. The Vitek MS provided more species-level identifications that agreed with the reference method than with the Bruker system (56% versus 31%). One *B. multivorans* Bruker system result required extraction. An earlier study of 52 *B. cepacia* complex isolates analyzed with the Bruker system reported higher species-level agreement (83%) than the current study (7).

Although *S. maltophilia* is present in respiratory cultures from 14% of CF patients (6), the impact of this organism on respiratory function is unclear (10, 26). All 55 *S. maltophilia* isolates were identified to the species level by Vitek MS in the current study. Bruker system identification was limited to the genus level for 25% of the isolates and 53% of isolates required an extraction step. The Bruker system species identification was a heterotypic synonym (*Pseudomonas beteli*, *P. geniculata*, *P. hibiscicola*) for 7 of 41 isolates that were interpreted as *S. maltophilia*. Degand et al. reported 100% species-level agreement for Bruker system identification of 32 *S. maltophilia* isolates from 12 CF patients (7). Two other Bruker system studies found 100% species-level agreement with phenotypic methods for *S. maltophilia* (1, 23); in one of the studies, 27% of isolates needed extraction (1).

A. xylosoxidans was recovered from the respiratory cultures of 6% of CF patients in 2010 (6). Like *S. maltophilia*, the effect of *A. xylosoxidans* on the pulmonary function of CF patients is uncertain (9, 11). Distinguishing *A. xylosoxidans* from other NFGNB using phenotypic methods can be difficult (25). The identification of 12 isolates in the current study with 16S rRNA gene sequencing was limited to the genus level and one of those isolates could not be identified by either MS system. The Vitek MS provided “Family III Alcaligenaceae” identifications for 7 of the 12 *Achromobacter* spp. isolates. The Bruker system gave the same identification as API for 3 *A. xylosoxidans* isolates that Vitek MS designated *Achromobacter* spp. Ten of the 15 *Achromobacter* isolates required extraction for Bruker system identification, while only one isolate required extraction with Vitek MS. Degand et al. reported Bruker system species identifications that agreed with API 20 NE for all 54 *A. xylosoxidans* isolates recovered from 12 CF patients (7).

The identification of organisms by MS offers several advantages in comparison to currently used methods. Shortening the time period required to identify an organism from days to minutes should improve clinical outcomes. The lower cost in comparison to current identification systems is another advantage of this technology (2, 8, 19). The manufacturer of each MS system is conduct-

ing multicenter clinical trials with comparison to 16S rRNA gene sequencing as the reference method for FDA clearance. For clinical trials, the duplicate spot testing performed for this study is not being used and a more simplified extraction method may be performed directly on the target plate. The database for each system is continuing to be developed, and the components are being optimized for routine clinical laboratory use. Therefore, the accuracy of identification provided by the updated software is likely better than that reported in the current study.

An extraction step was employed more often to generate a valid identification from the Bruker system (20 of 52 isolates) in comparison to Vitek MS (2 of 23 isolates). Optimization of the Bruker system database and testing procedures for direct bacterial isolate testing may eventually eliminate the need for off-plate extraction.

In conclusion, the identification of NFGNB by both MALDI-TOF MS systems was superior to conventional biochemical methods. The Bruker Biotyper identified more isolates to genus or species level but required an extraction step more often than the Vitek MS. The percentage of correct species-level identifications was similar for the two MS systems. The accuracy of both MS systems should improve further with ongoing database development. The accuracy, ease of use, low reagent cost, and speed of MALDI-TOF MS support the implementation of this technology for identification of NFGNB. Routine identification of NFGNB from CF patients by MALDI-TOF MS systems should provide a better understanding of the role microbes play in progression of this disease.

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REFERENCES

- Bizzini A, Durussel C, Bille J, Greub G, Prod'hom G. 2010. Performance of matrix-assisted laser desorption ionization-time of flight mass spectrometry for identification of bacterial strains routinely isolated in a clinical microbiology laboratory. *J. Clin. Microbiol.* 48:1549–1554.
- Cherkaoui A, et al. 2010. Comparison of two matrix-assisted laser desorption ionization-time of flight mass spectrometry methods with conventional phenotypic identification for routine identification of bacteria to the species level. *J. Clin. Microbiol.* 48:1169–1175.
- Clarridge JE. 2004. Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. *Clin. Microbiol. Rev.* 17:840–862.
- Clinical and Laboratory Standards Institute. 2008. Interpretive criteria for identification of bacteria and fungi by DNA target sequencing; approved guideline. CLSI document MM18-A. Clinical and Laboratory Standards Institute, Wayne, PA.
- Cloud JL, et al. 2010. Comparison of traditional phenotypic identification methods with partial 5' 16S rRNA gene sequencing for species-level identification of nonfermenting gram-negative bacilli. *J. Clin. Microbiol.* 48:1442–1444.
- Cystic Fibrosis Foundation. 2011. Patient registry 2010. Annual data report. Cystic Fibrosis Foundation, Bethesda, MD.
- Degand N, et al. 2008. Matrix-assisted laser desorption ionization–time of flight mass spectrometry for identification of nonfermenting gram-negative bacilli isolated from cystic fibrosis patients. *J. Clin. Microbiol.* 46:3361–3367.
- Dhiman N, Hall L, Wohlfiel SL, Buckwalter SP, Wengenack NL. 2011. Performance and cost analysis of matrix-assisted laser desorption ionization-time of flight mass spectrometry for routine identification of yeast. *J. Clin. Microbiol.* 49:1614–1616.
- Gibson RL, Burns JL, Ramsey BW. 2003. Pathophysiology and manage-

- ment of pulmonary infections in cystic fibrosis. *Am. J. Respir. Crit. Care Med.* 168:918–951.
10. Goss CH, et al. 2004. Association between *Stenotrophomonas maltophilia* and lung function in cystic fibrosis. *Thorax* 59:955–959.
 11. Hauser AR, Jain M, Bar-Meir M, McColley SA. 2011. Clinical significance of microbial infection and adaptation in cystic fibrosis. *Clin. Microbiol. Rev.* 24:29–70.
 12. Justesen US, et al. 2011. Species identification of clinical isolates of anaerobic bacteria: a comparison of two matrix-assisted laser desorption ionization-time of flight mass spectrometry systems. *J. Clin. Microbiol.* 49:4314–4318.
 13. Lay JO. 2001. MALDI-TOF mass spectrometry of bacteria. *Mass Spectrom. Rev.* 20:172–194.
 14. LiPuma JJ. 2010. The changing microbial epidemiology in cystic fibrosis. *Clin. Microbiol. Rev.* 23:299–323.
 15. LiPuma JJ, Currie BJ, Peacock SJ, VanDamme PAR. 2011. *Burkholderia*, *Stenotrophomonas*, *Ralstonia*, *Cupriavidus*, *Pandoraea*, *Brevundimonas*, *Comamonas*, *Delftia*, and *Acidovorax*, p 692–713. In Versalovic J, et al (ed), *Manual of clinical microbiology*, 10th ed. ASM Press, Washington, DC.
 16. LiPuma JJ, et al. 1999. Development of rRNA-based PCR assays for identification of *Burkholderia cepacia* complex isolates recovered from cystic fibrosis patients. *J. Clin. Microbiol.* 37:3167–3170.
 17. Liu L, et al. 2002. Ribosomal DNA-directed PCR for identification of *Achromobacter (Alcaligenes) xylosoxidans* recovered from cystic fibrosis patients. *J. Clin. Microbiol.* 40:1210–1213.
 18. Mahenthiralingam E, et al. 2000. DNA-based diagnostic approaches for identification of *Burkholderia cepacia* complex, *Burkholderia vietnamiensis*, *Burkholderia multivorans*, *Burkholderia stabilis*, and *Burkholderia cepacia* genomovars I and III. *J. Clin. Microbiol.* 38:3165–3173.
 - 18a. Marko DC, et al. 2011. Abstr. 111th Gen. Meet. Am. Soc. Microbiol., abstr C-3352.
 19. Neville SA, et al. 2011. Utility of matrix-assisted laser desorption ionization-time of flight mass spectrometry following introduction for routine laboratory bacterial identification. *J. Clin. Microbiol.* 49:2980–2984.
 20. Reik R, Spilker T, LiPuma JJ. 2005. Distribution of *Burkholderia cepacia* complex species among isolates recovered from persons with or without cystic fibrosis. *J. Clin. Microbiol.* 43:2926–2928.
 21. Riordan JR, et al. 1989. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science* 245:1066–1073.
 22. Rowe SM, Miller S, Sorscher EJ. 2005. Cystic fibrosis. *N. Engl. J. Med.* 352:1992.
 23. Saffert RT, et al. 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.
 24. van Veen SQ, Claas EC, Kuijper EJ. 2010. High throughput identification of bacteria and yeast by matrix-assisted laser desorption ionization-time of flight mass spectrometry in conventional medical microbiology laboratories. *J. Clin. Microbiol.* 48:900–907.
 25. Von Konig CW, Riffelmann M, Coenye T. 2011. *Bordetella* and related genera, p 739–750. In Versalovic J, et al (ed), *Manual of clinical microbiology*, 10th ed. ASM Press, Washington, DC.
 26. Waters V, et al. 2011. *Stenotrophomonas maltophilia* in cystic fibrosis. *Am. J. Respir. Crit. Care Med.* 183:635–640.
 27. Welsh MJ, Smith AE. 1993. Molecular mechanisms of CFTR chloride channel dysfunction in cystic fibrosis. *Cell* 73:1251–1254.