JCM
Journals.ASM.org

Performances of the Vitek MS Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry System for Rapid Identification of Bacteria in Routine Clinical Microbiology

Damien Dubois,[a,b] Marion Grare,[a,b] Marie-Françoise Prere,[a,b] Christine Segonds,[a,b] Nicole Marty,[a,b] and Eric Oswald[a,b]

Laboratoire de Bactériologie-Hygiène, Institut Fédératif de Biologie, Centre Hospitalo-Universitaire, Toulouse, France,[a] and Université Paul Sabatier-Toulouse III, Toulouse, France[b]

Rapid and cost-effective matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS)-based systems will replace conventional phenotypic methods for routine identification of bacteria. We report here the first evaluation of the new MALDI-TOF MS-based Vitek MS system in a large clinical microbiology laboratory. This system uses an original spectrum classifier algorithm and a specific database designed for the identification of clinically relevant species. We have tested 767 routine clinical isolates representative of 50 genera and 124 species. Vitek MS-based identifications were performed by means of a single deposit on a MALDI disposable target without any prior extraction step and compared with reference identifications obtained mainly with the VITEK2 phenotypic system; if the identifications were discordant, molecular techniques provided reference identifications. The Vitek MS system provided 96.2% correct identifications to the species level (86.7%), to the genus level (8.2%), or within a range of species belonging to different genera (1.3%). Conversely, 1.3% of isolates were misidentified and 2.5% were unidentified, partly because the species was not included in the database; a second deposit provided a successful identification for 0.8% of isolates unidentified with the first deposit. The Vitek MS system is a simple, convenient, and accurate method for routine bacterial identification with a single deposit, considering the high bacterial diversity studied and as evidenced by the low prevalence of species without correct identification. In addition to a second deposit in uncommon cases, expanding the spectral database is expected to further enhance performances.

Due to the dramatic increase of bacterial resistance and to the ecological cost of broad-spectrum antimicrobial therapies, rapid and accurate identification (ID) of bacteria is essential for the appropriate management of infections. Conventional identification methods require at least 4 to 12 h, and molecular methods are not suitable for large-scale routine identification.

Nearly 40 years ago, chemists proposed to identify bacterial cultures via the detection of small organic molecules using mass spectrometry (2). More than 10 years later, matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) allowed the detection of intact larger biomolecules, such as proteins, and was further developed for microbial ID in routine clinical laboratories (13). During the analysis process, proteins are ionized without fragmentation by the coordinated action of the laser and the small organic acids of the matrix and separated on the basis of their mass-to-charge ratios, a process which results in a characteristic mass spectral profile. Microbial ID is based on the comparison of the protein spectrum generated from intact whole bacterial cells to a database of species-specific reference protein profiles using a particular algorithm.

In the mid-1990s, different groups developed their own libraries of bacterial reference mass spectra and software for bacterial identification and taxonomic classification. After a decade of optimization of the method parameters, like the reproducibility of mass spectral profiles at different locations, the robustness to account for variations and variability in culture conditions, the application to the majority of clinically relevant bacteria, and an automated mass spectral analysis, the MALDI-TOF MS-based bacterial ID became suitable for routine use in applied laboratories (11). Commercial, user-friendly devices containing different algorithms for the classification of bacterial protein mass patterns and associated with databases including several thousand bacterial reference entries for bacterial ID, represented mainly by the Biotyper (Bruker Daltonics, Germany), Vitek MS RUO (formerly Saramis) and Vitek MS (bioMérieux, France), and Andromas (Andromas SAS, France) systems, were available (8, 19). Numerous studies reported the fast, easy-to-use, cost-effective, and, thus, high-throughput performances of these MALDI-TOF MS systems for bacterial ID in clinical laboratories using duplicate deposits on a MALDI target (4, 5, 7, 9, 15, 20, 24).

The objective of the present study was to evaluate the performances and technical practicability of the Vitek MS system (bioMérieux), a recently commercialized MALDI-TOF-based method using an original spectrum classifier algorithm (i.e., comparison of the presence and the absence of specific peaks between the obtained spectrum and the typical spectrum of each claimed species, previously determined with 10 different reference strains, using an analysis mass range from 3,000 to 17,000 Da) and a database of 586 species (including 508 bacterial and 78 fungal taxa). In contrast to most previous studies that analyzed the ID performances of other MALDI-TOF-based systems with two deposits or even protein extraction (5, 7, 15, 20, 24), the Vitek MS was assessed here

Received 6 February 2012 **Returned for modification** 29 February 2012
Accepted 4 May 2012
Published ahead of print 16 May 2012
Address correspondence to Damien Dubois, dubois.d@chu-toulouse.fr.

doi:10.1128/JCM.00343-12

using a single deposit without any prior extraction step from bacterial colonies.

(This work was presented in part at the 51st Interscience Conference on Antimicrobial Agents and Chemotherapy [ICAAC], Chicago, IL, 2011.)

MATERIALS AND METHODS

Bacterial isolates. In order to capture the clinically significant broad bacterial diversity that was encountered in our large medical laboratory's routine, bacterial isolates, including no more than 30 consecutive isolates per species (any surplus isolates tested for a given species were kept for performance analysis), were prospectively recovered over a 6-week period from various clinical specimens (such as blood, urine, stool, pus, biopsy specimens, cerebrospinal fluid, respiratory tract, wound specimens, and swabs from any site of the body) and different medical departments. Isolate duplicates (i.e., from the same patient) were discarded.

The isolates were recovered after the laboratory's routine ID and purity control on an appropriate agar plate (5% sheep blood agar, chocolate agar, or buffered charcoal-yeast extract [BCYE] agar medium; bioMérieux) and under appropriate atmosphere (aerobic, microaerophilic, or anaerobic incubation) after 24 h to 72 h of incubation at 35°C.

The 767 isolates included in the study encompassed 282 *Enterobacteriaceae*, 94 nonfermentative Gram-negative rods, 47 other Gram-negative bacteria, 127 staphylococci and related species, 177 streptococci and related species, 30 anaerobes, and 10 other Gram-positive rods (Table 1).

Reference identification and results management. Isolates were simultaneously identified by the Vitek MS system and, as reference methods, by the conventional Vitek2 system using the GP, GN, NH, or ANC card (bioMérieux) if applicable or otherwise by genomic methods. Vitek2 IDs were performed according to the recommendations of the manufacturer, including complementary tests if required.

When the Vitek MS system proposed as a single choice or in a multiple choice the Vitek2 IDs to the species level, no further investigation was performed. In the case of discordant results between the Vitek MS and Vitek2 methods, low-discrimination results with Vitek2, or "no ID" results obtained with the Vitek2 or Vitek MS methods, genomic IDs were performed and then considered to be the reference ID.

Genomic IDs were performed using the sequencing-based Mastermix 16S Complete kit (Molzym GmbH) targeting the 5′ partial 16S rRNA gene as the first line. If the ID remained inconclusive, sequencing-based IDs using the *sodA* gene for coagulase-negative staphylococci, streptococci, and enterococci, the *recA* gene for the *Burkholderia cepacia* complex, and the 3′ partial 16S rRNA gene for other taxa were implemented (16, 17, 21). Moreover, the PCR- and hybridization-based system GenoType EHEC (Hain LifeScience), detecting the *ipaH* gene, and a serotyping method were used to confirm *Shigella* isolates. An optochin susceptibility test for *Streptococcus pneumoniae* isolates and a species-specific PCR targeting the *crgA* gene for *Neisseria meningitidis* isolates were also used (23).

MALDI-TOF MS. (i)Technical training. Prior to the assessment initiation, the four operators involved were trained for sample and slide preparation by performing three slides of 48 deposits with duplicate deposits per isolate during three independent days (one slide per day). Mucoid and rough isolates were included only in the third slide performed by each operator. A proficiency test using 16 strains with single deposits was passed by each operator.

(ii) Plate preparation. The disposable plate preparation was performed with the Vitek MS Preparation Station software to link sample information to the Vitek MS spectrophotometer using the single-use FlexiMass MALDI target plates, supplied in a 48-well microscope slide format, divided into three acquisition groups of 16 spots, and inoculated by picking an overnight culture with a 1-μl disposable loop and by smearing the specimen directly onto the plate (mostly one colony/deposit). The preparations were overlaid with 1 μl of matrix solution (saturated solution of α-cyano-4-hydroxycinnamic acid in 50% acetonitrile and 2.5% trifluoroacetic acid) and air dehydrated for 1 to 2 min at room temperature. As recommended by the manufacturer, the *Escherichia coli* ATCC 8739 strain, used as a calibrator and internal ID control, was inoculated on the calibration spots of each acquisition group (small spot in the middle of each acquisition group). Each bacterial isolate had been tested with a unique deposit.

(iii) Generation of mass spectra. Mass spectra were generated with a Vitek MS Axima Assurance mass spectrometer (bioMérieux) in positive linear mode at a laser frequency of 50 Hz with an acceleration voltage of 20 kV and an extraction delay time of 200 ns. For each spectrum, 500 shots in 5-shot steps from different positions of the target spot (automatic mode) were collected by the mass spectrometer operating in conjunction with the Acquisition Station software (Vitek MS version 1.0.0). Measured mass spectra ranged from 2,000 to 20,000 Da.

(iv) MS identification. For each bacterial sample, mass fingerprints were processed by the compute engine and the advanced spectrum classifier (ASC) algorithm associated with the Vitek MS system, which then automatically identifies the organism by comparing the characteristics of the spectrum obtained (presence and absence of specific peaks) with those of the typical spectrum of each claimed species.

The ASC algorithm is a supervised learning method that analyzes spectral data and recognizes patterns used to build the knowledge base. It can be classified in the "nonprobabilistic linear classifier" family. In most cases, one pattern in the knowledge base corresponds to one species. Sometimes, when two (or more) species cannot be separated efficiently with MALDI-TOF technology, only one pattern is created by using all the spectra collected for both species. On the other hand, when spectra collected for one species are so variable due to strain variability or culture conditions like incubation time and culture medium, several patterns are created for one species. For each species integrated in the knowledge base, the variability of the spectra is evaluated with clustering application and interspectra distance calculation.

The spectral database was built by the manufacturer as follows. Ten isolates belonging to the same species were carefully selected to take into account diversity in clinical specimen origins, geographic origins (different countries), and year of isolation. All isolates were previously characterized by phenotypic and/or molecular methods. The spectral database was built through an experimental plan design, including several culture media, several media suppliers, different incubation times ranging from 18 h to 24 h to up to 72 h, and several mass spectrometers. Masses from 2 to 20 kDa were collected, and the analysis focused on the 3- to 17-kDa mass range.

The ASC algorithm compared the generated spectra to the expected spectrum of each organism or organism group of the database to provide identification. A percent probability, or confidence value, which represents the similarity in terms of presence/absence of specific peaks between the generated spectrum and the database spectra, was calculated by the algorithm. A perfect match between the spectrum and the unique spectrum of a single organism or organism group provided a confidence value of 99.9% ("good ID"). When a perfect match was not obtained, it was still possible for the spectrum to be sufficiently close to that of a reference spectrum such that a clear decision was provided about the organism ID ("good ID," confidence value of >60 to 99.8%). If a unique ID pattern was not recognized, a list of possible organisms was given ("low discrimination" [LD], confidence value of >60%) or the strain was determined to be outside the scope of the database ("no ID"). The range of percent probabilities in the single-choice case was 60 to 99%. Values closer to 99.9% indicate a closer match to the typical pattern for the given organism. When the confidence value obtained was below 60, the organism was considered nonidentified.

The overall correct ID was defined as including the following levels: (i) correct ID to the species level, when the system proposed the reference species ID as a single choice or with low discrimination to the subspecies level (with any level of confidence), (ii) correct ID to the genus level, when the system proposed the reference species ID among a set of low-discrimination results including species of the same genera, and (iii) correct ID

TABLE 1 Valid Vitek MS results of 767 bacterial isolates using a single deposit and no protein extraction step

Reference identification	No. of isolates	No. (%) of isolates with the indicated result[a]				
		Correct ID to the level of:				
		Species	Genus	Above genus	No ID	Mis-ID
Enterobacteriaceae	282	231 (82.2)	45 (16.0)	2 (0.7)	2 (0.7)	2 (0.7)
Citrobacter braakii	1	1 (100)				
Citrobacter freundii	12	9 (75)	2 (17)	1 (8)		
Citrobacter koseri	14	1 (100)				
Citrobacter youngae	2	1 (50)	1 (50)			
Enterobacter aerogenes	13	13 (100)				
Enterobacter asburiae	1		1 (100)			
Enterobacter cloacae	31		30 (97)		1 (3)	
Escherichia coli	31	30 (100)	1 (3)			
Escherichia fergusonii	2	1 (50)	1 (50)			
Escherichia vulneris	1	1 (100)				
Hafnia alvei	13	13 (100)				
Klebsiella oxytoca	27	27 (100)				
Klebsiella pneumoniae	33	33 (100)				
Morganella morganii	23	23 (100)				
Pantoea agglomerans	1	1 (100)				
Proteus mirabilis	30	28 (93)		1 (3)	1 (3)	
Proteus vulgaris	8		8 (100)			
Providencia rettgeri	1	1 (100)				
Providencia stuartii	4	4 (100)				
Raoultella ornithinolytica	2	2 (100)				
Raoultella planticola	1		1 (100)			
Serratia liquefaciens	1	1 (100)				
Salmonella group[d]	7	7 (100)				
Serratia marcescens	21	21 (100)				
Shigella flexneri	2					2 (100)
Nonfermentative Gram-negative rods	94	81 (86.2)	8 (8.5)	2 (2.1)	2 (2.1)	1 (1.1)
Achromobacter denitrificans	1		1 (100)			
Achromobacter xylosoxidans	5		5 (100)			
Acinetobacter baumannii complex[d]	5	5 (100)				
Acinetobacter lwoffii	2	1 (50)		1 (50)		
Acinetobacter radioresistens	1	1 (100)				
Acinetobacter ursingii	2	2 (100)				
Acinetobacter sp.[c]	1				1 (100)	
Aeromonas caviae[e]	1	1 (100)				
Aeromonas sobria	1		1 (100)			
Alcaligenes faecalis subsp. *faecalis*	4	3 (75)			1 (25)	
Burkholderia cepacia	1	1 (100)				
Burkholderia vietnamiensis	1		1 (100)			
Burkholderia stabilis	1	1 (100)				
Chryseobacterium indologenes	2	2 (100)				
Elizabethkingia meningoseptica	1	1 (100)				
Pseudomonas aeruginosa	36	35 (97)		1 (3)		
Pseudomonas putida	6	6 (100)				
Psychrobacter sp.[g]	1	1 (100)				
Ralstonia pickettii	1					1[b] (100)
Stenotrophomonas maltophilia	21	21 (100)				
Other Gram-negative bacteria	47	38 (80.9)	1 (2.1)	0	4 (8.5)	4 (8.5)
Aggregatibacter segnis	1					1 (100)
Haemophilus influenzae	21	19 (90)	1 (5)		1 (5)	
Haemophilus parainfluenzae	3	2 (67)				1[b] (33)
Pasteurella canis	1	1 (100)				
Pasteurella multocida	3	3 (100)				
Eikenella corrodens	1	1 (100)				
Moraxella catarrhalis	3	3 (100)				
Neisseria gonorrhoeae	1	1 (100)				
Neisseria meningitidis	2	2 (100)				

(Continued on following page)

TABLE 1 (Continued)

Reference identification	No. of isolates	No. (%) of isolates with the indicated result[a]				
		Correct ID to the level of:				
		Species	Genus	Above genus	No ID	Mis-ID
Neisseria subflava/flavescens/perflava	1	1 (100)				
Neisseria mucosa	1					1 (100)
Campylobacter fetus subsp. *fetus*[f]	1	1 (100)				
Campylobacter jejuni subsp. *jejuni*	3	2 (67)				1 (33)
Helicobacter pylori	3				3 (100)	
Legionella pneumophila	2	2 (100)				
Staphylococci and related species	127	117 (92.1)	3 (2.4)	3 (2.4)	4 (3.1)	0
Staphylococcus aureus	36	35 (97)			1 (3)	
Staphylococcus capitis	9	9 (100)				
Staphylococcus caprae	2	2 (100)				
Staphylococcus carnosus subsp. *carnosus*	1	1 (100)				
Staphylococcus cohnii ssp. *cohnii*[f]	1	1 (100)				
Staphylococcus epidermidis	36	35 (97)		1 (3)		
Staphylococcus haemolyticus	3	2 (67)			1 (33)	
Staphylococcus hominis subsp. *hominis*	5	5 (100)				
Staphylococcus intermedius	3		3 (100)			
Staphylococcus lugdunensis	9	9 (100)				
Staphylococcus pseudintermedius	1	1 (100)				
Staphylococcus saprophyticus	5	5 (100)				
Staphylococcus schleiferi	1	1 (100)				
Staphylococcus sciuri	1	1 (100)				
Staphylococcus simulans	4	4 (100)				
Staphylococcus warneri	6	4 (67)		2 (33)		
Staphylococcus xylosus	1	1 (100)				
Staphylococcus condimenti[c]	1				1 (100)	
Staphylococcus pasteuri[c]	1				1 (100)	
Micrococcus luteus/lylae	1	1 (100)				
Enterococcus avium	8	5 (63)	1 (13)	2 (25)		
Enterococcus casseliflavus	1	1 (100)				
Enterococcus durans	1	1 (100)				
Enterococcus faecalis	38	3 (100)				
Enterococcus faecium	17	16 (94)			1 (6)	
Enterococcus gallinarum	1	1 (100)				
Streptococcus agalactiae	32	32 (100)				
Streptococcus pyogenes	8	8 (100)				
Streptococcus dysgalactiae subsp. *equisimilis*[f]	8	8 (100)				
Streptococcus dysgalactiae subsp. *dysgalactiae*[f]	1	1 (100)				
Streptococcus canis[c]	1					1[b] (100)
Streptococcus anginosus	14	14 (100)				
Streptococcus constellatus	9	8 (89)	1 (11)			
Streptococcus intermedius	2	2 (100)				
Streptococcus pneumoniae	19	19 (100)				
Streptococcus mitis/oralis	9	9 (100)				
Streptococcus gallolyticus subsp. *pasteurianus*[f]	2	2 (100)				
Streptococcus parasanguinis	2	2 (100)				
Streptococcus vestibularis	1		1 (100)			
Streptococcus australis	1					1 (100)
Granulicatella adiacens	1	1 (100)				
Vagococcus fluvialis	1	1 (100)				
Anaerobes	30	25 (83)	0	1 (3)	4 (13)	0
Anaerococcus hydrogenalis[c]	1				1 (100)	
Bacteroides vulgatus	1	1 (100)				
Bacteroides fragilis	8	8 (100)				
Bacteroides ovatus	1	1 (100)				
Bacteroides uniformis	2	2 (100)				
Clostridium difficile	5	5 (100)				
Clostridium perfringens[h]	2	2 (100)				

(Continued on following page)

TABLE 1 (Continued)

Reference identification	No. of isolates	No. (%) of isolates with the indicated result[a]				
		Correct ID to the level of:				
		Species	Genus	Above genus	No ID	Mis-ID
Clostridium celerecrescens[c]	1				1 (100)	
Finegoldia magna	1	1 (100)				
Prevotella intermedia	2	2 (100)				
Prevotella nanceiensis[c]	1				1 (100)	
Prevotella nigrescens[c]	1				1 (100)	
Propionibacterium acnes	2	2 (100)				
Propionibacterium avidum	1			1 (100)		
Veillonella parvula	1	1 (100)				
Other Gram-positive rods	10	4 (40)	3 (30)	0	2 (20)	1 (10)
Corynebacterium amycolatum	1		1 (100)			
Corynebacterium striatum	4	4 (100)				
Corynebacterium fastidiosum/segmentosum[c]	1				1 (100)	
Corynebacterium macginleyi[c]	1				1 (100)	
Lactobacillus rhamnosus	1					1 (100)
Bacillus cereus/thuringiensis/mycoides[d]	2		2 (100)			
Total	767	665 (86.7)	63 (8.2)	10 (1.3)	19 (2.5)	10 (1.3)

[a] ID, identification; mis-ID, misidentification; species, correct identification at the species level (single choice or low discrimination at the subspecies level); genus, correct identification at the genus level (low discrimination at the species level); above genus, correct identification proposed among a set of low-discrimination results including species of different genera.
[b] LD discrepancies (sets of low-discrimination results not including the reference species).
[c] Species absent from the Vitek MS database. Using sequencing-based genomic methods and according to nucleotide public databases, the *Acinetobacter* sp. isolate corresponded to an unnamed *Acinetobacter* species.
[d] The species group is the final Vitek MS identification. The subspecies or species included in each species group are as follows: for the *Salmonella* group, *S. enterica* subsp. *enterica*, *S. enterica* serovar Enteritidis, *S. enterica* serovar Paratyphi B, *S. enterica* serovar Paratyphi C, *S. enterica* serovar Typhimurium, and *Salmonella* spp.; for the *Acinetobacter baumannii* complex, *A. baumannii*, *A. calcoaceticus*, *Acinetobacter* genomospecies 3, and *Acinetobacter* genomospecies TU13.
[e] The *Aeromonas hydrophila/caviae* species group is displayed as a species group result by the Vitek MS.
[f] The Vitek MS did not differentiate between the subspecies *S. cohnii* subsp. *cohnii* and *S. cohnii* subsp. *urealyticum*, *S. dysgalactiae* subsp. *dysgalactiae* and *S. dysgalactiae* subsp. *equisimilis*, *S. gallolyticus* subsp. *pasteurianus* and *S. gallolyticus* subsp. *gallolyticus*, and *C. fetus* subsp. *fetus* and *C. fetus* subsp. *venerealis*.
[g] This isolate was identified as *Psychrobacter phenylpyruvicus* by the Vitek MS system, but the *Psychrobacter* species was undeterminable by sequencing.
[h] These isolates do not possess the epsilon toxin gene.

above the genus level, when the system proposed the reference species ID among a set of low-discrimination results including species of different genera.

When a human error or a poor-quality deposit occurred (including the warning messages "bad spectrum," "not enough peaks," "too many peaks," and "too much background noise" or in the case of calibration/control failure), the incriminated isolates or all the isolates of the incriminated acquisition group had been retested with a single deposit and the second result had been taken into account for the analysis. For informative purposes, samples with "no ID" or "mis-ID" first-spot results were secondarily retested with a single spot.

Calculation of global assessment indices. For the MALDI-TOF-based identification method, positive predictive values to the genus level and to the species level, considering isolates with correct IDs to the genus level true positives and isolates with correct IDs to the species level true positives, respectively, were calculated. Misidentified isolates were considered false positives. Negative predictive value, considering isolates with an absence of ID and belonging to species not included in the database true negatives and isolates with an absence of ID and belonging to species included in the database false negatives, was calculated.

RESULTS

Technical practicability. During the study, one out of 48 spot calibrations performed failed and 2.2% of all generated spectra were uninterpretable. These latter errors corresponded to seven "too many/not enough peaks" and 10 "bad spectrum" warning messages and were not associated with any particular taxonomic group or colonial characteristics. Fifteen out of these 17 isolates were correctly identified to the species level with a second deposit. One *Staphylococcus epidermidis* isolate and one *Klebsiella oxytoca* isolate again generated a bad spectrum and then were discarded from the analysis.

Global identification performances. During the study period, 767 isolates were analyzed by the Vitek MS system and, in parallel, by the conventional Vitek2 system (760 isolates, 99.1%) or directly by genotypic methods (7 isolates, 0.9%, for *Legionella pneumophila*, *Helicobacter pylori*, and *Bacillus cereus/thuringiensis/mycoides* species). Implementation of DNA-based ID methods to manage discrepancies or to obtain a more accurate reference ID (to the species or subspecies level) was performed for 79 (10.3%) isolates. Reference IDs proposed by the Vitek MS as a single choice (SC), whatever the confidence value, or included in a multiple-choice result (LD with up to four proposed species) were considered overall correct IDs. Among the 767 isolates, including 124 species and 50 genera, 738 (96.2%) isolates were correctly identified by the MALDI-TOF MS system as defined previously (Table 1). No IDs and discordant results (mis-IDs) were obtained for 2.5% and 1.3% of the isolates, respectively.

(i) Correct identifications. A correct ID to the species or subspecies level was obtained for 86.7% ($n = 665$) of the isolates, with confidence values of 99.9%, 90.0% to 99.8%, and 80.0% to 89.9% for 97.6%, 2.0%, and 0.3% of these isolates, respectively. A correct

TABLE 2 Correct identifications proposed by the Vitek MS among multiple-choice results including species of different genera[a]

Reference ID	Proposed ID results (confidence value [%])			
	1st choice	2nd choice	3rd choice	4th choice
Acinetobacter lwoffii	*Acinetobacter lwoffii* (99.9)	*Mycobacterium tuberculosis* (99.9)	*Mycobacterium bovis* (99.9)	
Citrobacter freundii	*Citrobacter freundii* (99.0)	*Citrobacter braakii* (99.9)	*Citrobacter koseri* (92.4)	*Haemophilus influenzae* (71.5)
Enterococcus avium	*Enterococcus avium* (99.9)	*Clostridium butyricum* (96.6)		
Enterococcus avium	*Enterococcus avium* (99.9)	*Clostridium butyricum* (85.9)	*Bacillus atrophaeus* (78.8)	
Micrococcus luteus	*Micrococcus luteus/lylae* (99.9)	*Bacillus thuringiensis* (99.9)	*Bacillus mycoides* (79.1)	*Bacillus cereus* (79.1)
Propionibacterium avidum	*Propionibacterium avidum* (96.8)	*Clostridium butyricum* (87.7)		
Proteus mirabilis	*Proteus mirabilis* (99.9)	*Streptococcus constellatus* (71.3)		
Pseudomonas aeruginosa	*Pseudomonas oryzihabitans* (99.4)	*Peptoniphilus indolicus* (99.2)	*Pseudomonas aeruginosa* (82.0)	
Staphylococcus epidermidis	*Staphylococcus epidermidis* (99.9)	*Leuconostoc pseudomesenteroides* (99.7)		
Staphylococcus warneri[b]	*Staphylococcus warneri* (99.9)	*Prevotella buccalis* (99.9)		

[a] ID, identification.
[b] 2 isolates.

ID to the genus level only, that is, the correct species ID was included in a multiple-choice result of species from the same genus, was obtained for 8.2% ($n = 63$) of the isolates. These LD results to the species level proved to be recurrent in 79.3% of cases, including species complexes such as *Enterobacter cloacae/asburiae* ($n = 31$), *Proteus vulgaris/penneri* ($n = 8$), *Achromobacter xylosoxidans/denitrificans* ($n = 6$), *Bacillus cereus/thuringiensis/mycoides* ($n = 2$), and *Staphylococcus intermedius/pseudintermedius* ($n = 3$). LD results above the genus level, that is, with the correct ID proposed among species of different genera, were obtained for 1.3% ($n = 10$) of the isolates (Table 2), some of which also seem to be recurrent, like the *Staphylococcus warneri*/*Prevotella buccalis* LD result for some *S. warneri* isolates. An identical and high confidence value was mostly obtained for each proposed species in the case of an LD result to the species level or above the genus level. In the few cases in which a confidence value difference occurred, it argued either for or against the correct species ID.

(ii) Incorrect identifications. Ten isolates were misidentified by the Vitek MS system, six of them with the correct or closed genus (Table 3). Two *Shigella* isolates were misidentified as *E. coli*, even after retest. One *Aggregatibacter segnis* isolate (a species formerly belonging to the *Haemophilus* genus), misidentified as *Haemophilus influenzae*, was correctly identified with a second deposit, whereas one *Neisseria mucosa* isolate was misidentified as its closely related species *Neisseria subflava* twice. Belonging to species absent from the database, one *Streptococcus australis* isolate was misidentified as *Streptococcus parasanguinis*, even after one retest, and one *Streptococcus canis* isolate gave an LD result between other species of the "pyogenic" group and a correct "no ID" result with a second deposit.

One *Campylobacter jejuni* isolate and one *Lactobacillus rhamnosus* isolate were misidentified as the distant species *Citrobacter braakii* and *Propionibacterium avidum*, respectively, but were correctly identified with a second deposit. With an LD result between species of distant genera, the misidentified *Haemophilus parainfluenzae* and *Ralstonia pickettii* isolates were correctly identified to the species level with a second deposit.

(iii) No identifications. The Vitek MS system gave an absence

TABLE 3 Misidentifications of the Vitek MS system[a]

Reference ID	Proposed IDs (confidence value [%]) for first spot as single or multiple choice			ID result for second spot
	1st choice	2nd choice	3rd choice	
Aggregatibacter segnis	*Haemophilus influenzae* (99.4)			*Aggregatibacter segnis* (99.9)
Campylobacter jejuni	*Citrobacter braakii* (84.4)			*Campylobacter jejuni* (99.9)
Haemophilus parainfluenzae	*Haemophilus haemolyticus* (98.8)	*Enterobacter aerogenes* (93.5)		*Haemophilus parainfluenzae* (99.9)
Lactobacillus rhamnosus	*Propionibacterium avidum* (99.3)			*Lactobacillus rhamnosus* (99.9)
Neisseria mucosa	*Neisseria subflava* (99.9)			*Neisseria subflava* (99.9)
Ralstonia pickettii	*Prevotella melaninogenica* (99.9)	*Staphylococcus saprophyticus* (99.9)	*Chryseobacterium gleum* (96.2)	*Ralstonia pickettii* (99.9)
Shigella flexneri[b]	*Escherichia coli* (99.9)			*E. coli* (99.9)
Streptococcus australis	*Streptococcus parasanguinis* (99.9)			*Streptococcus parasanguinis* (99.9)
Streptococcus canis	*Streptococcus dysgalactiae* subsp. *dysgalactiae* (99.9)	*Streptococcus dysgalactiae* subsp. *equisimilis* (99.9)	*Streptococcus equi* subsp. *equi* (99.9)	No ID

[a] ID, identification.
[b] 2 isolates.

of ID for 19 (2.5%) isolates that were tested again using one deposit for informative purposes (Table 1). Nine isolates belonging to nine species were not included in the database of the MALDI-TOF MS system, including one *Acinetobacter* sp. (unnamed species in public nucleotide databases), two staphylococci, two corynebacteria, and four anaerobe isolates, for which the system gave the same "no ID" answer after reading a second deposit. With the analysis of an additional deposit, six out of 10 isolates with species included in the database were correctly identified. One *Staphylococcus haemolyticus* isolate among three tested in the study and three *Helicobacter pylori* isolates again gave a "no ID" result despite the additional retest.

(iv) Global assessment indices. According to the criteria detailed in Materials and Methods, the positive predictive values to the genus level and to the species level of the Vitek MS system were 98.6 and 98.5, respectively, and the negative predictive value was 47.4.

Analysis of the *Streptococcaceae* group. Considering the organism groups largely tested, the Vitek MS gave a good ID overall to the genus level with a single deposit for 98.2% of the *Enterobacteriaceae*, 94.7% of the nonfermentative Gram-negative rods, 94.5% of the staphylococci, and 97.2% of the streptococci and related isolates (Table 1), with some species of the last group reported to be difficult to discriminate using MALDI-TOF MS systems. Focusing on the 177 isolates belonging to the *Streptococcaceae* family that were tested, 93.9% of the enterococci (62 out of 66 isolates, including six species) and 98% of the pyogenic streptococci (49 out of 50 isolates, including four species) were correctly identified to the species level with a single deposit (Table 1). Of note, the LD results to the subspecies level seem to be recurrent for *Streptococcus dysgalactiae* subsp. *dysgalactiae* and *S. dysgalactiae* subsp. *equisimilis* ($n = 9$), probably due to the resolution limit of the settled system. Among the milleri group streptococci, 24 out of 25 isolates were correctly identified to the species level and one LD result was obtained for a *Streptococcus constellatus* isolate with *Streptococcus anginosus*, whereas five isolates were identified as other alpha- or nonhemolytic streptococci by the Vitek2 system. The Vitek MS system correctly identified to the species level all 19 *S. pneumoniae* isolates and 15 out of 17 alpha- or nonhemolytic *Streptococcaceae* isolates, including nine *Streptococcus mitis/oralis* isolates. One *S. australis* isolate, a species not included in the Vitek MS database, was misidentified as *S. parasanguinis*, and one *Streptococcus vestibularis* isolate was not discriminated from the closed *Streptococcus salivarius* subsp. *salivarius* species. In contrast, the Vitek2 system misidentified two alpha-hemolytic streptococci isolates as milleri group streptococci.

DISCUSSION

The introduction of the high-tech MALDI-TOF MS technology in clinical laboratories is reducing the time required while improving the accuracy of bacterial identification. Without an intensive training background of the operators, the technical ownership of the Vitek MS system is straightforward and fast, as previously mentioned (5). However, the operator must remain vigilant in routine practice during sample preparation because of reduced interspot distances (especially for spots near the *E. coli* calibrant spot) that can mix two bacterial deposits, particularly during the matrix application step, as happened during the training period.

Many authors evaluating other MALDI-TOF MS-based systems have previously reported the use of a formic acid-based protein extraction using a bacterial lysis step or directly on the bacterial smear before matrix application to be needed, mainly for Gram-positive bacteria (1, 5, 9, 15, 24). In this study, the use of the Vitek MS system generated a low frequency of unusable spectra without formic acid use that is compatible and convenient for routine practice. Only one *Staphylococcus epidermidis* isolate and one *Klebsiella oxytoca* isolate twice generated bad spectra; in routine practice, these two isolates should have then been managed using a formic acid extraction step. These good performances of Vitek MS spectral acquisition for both Gram-negative and Gram-positive isolates may be due to the efficient displacement raster of the laser on the deposit. The laser scans the entire sample, and the instrument acquires good-quality subspectra from each 5-shot step. When 100 good-quality subspectra are not reached during the first large screening of the deposit, the laser goes back onto sample areas giving good-quality subspectra to obtain sufficient data (30 is the minimum acceptable number of subspectra) before the average spectrum data are analyzed.

LD results between species of different genera, accounting for 14 (1.8%) of the tested isolates, were not reported in the literature for other MALDI-TOF MS-based ID systems. The basis of this phenomenon may lie in the spectrum classifier algorithm that takes into account the absence and the presence of species-specific peaks. Although most of the correct species IDs included in such LD results can be found out with growth conditions or by simple and immediate tests (catalase, Gram staining, pigmentation), the correct species was proposed for only 11 out of 14 isolates with these LDs; a correct result (species ID or "no ID") was obtained for the three other isolates using a second spot. As a consequence and according to our subsequent experience, these few LD results should not be removed by complementary tests, but the isolates should be retested in order to obtain a single choice. Moreover, whatever the level of the LD results, confidence values do not appear to be reliable to determine the right species. Nevertheless, recurrent LD results to the species level have mostly no impact on isolate management and bioclinical interpretation, since species show similar pathogenicities and antibiotics susceptibility patterns (for example, *E. cloacae/asburiae*) and/or can be discriminated by simple and immediate tests (for example, indole test for *P. vulgaris/penneri*). For other LD results to the species level, an additional deposit should also be performed, as it mostly provided the correct species ID during our subsequent experience.

The mis-ID of *Shigella* isolates as *E. coli* was previously reported with other MALDI-TOF MS-based ID systems (4–6, 15, 20). These results are not surprising since the genus *Shigella* belonged genetically to the *E. coli* species and was kept in this way to differentiate these "specific *E. coli*" isolates with a particular virulence toward humans (12). This point reflects the resolution limits of the MALDI-TOF MS-based ID method currently used for routine bacterial IDs. These mis-IDs are a major drawback from a clinical point of view, particularly for stool sample analysis, that needs to be overcome by conventional phenotypic testing. Regarding the other major mis-IDs (*Campylobacter jejuni*, *Lactobacillus rhamnosus*, and *Aggregatibacter segnis* isolates), one cannot rule out a technical mistake or an undetected mixed culture generating two superimposed spectra that were wrongly interpreted as a unique spectrum and, thus, as a third species. These major incorrect results could have been amended in routine practice according to Gram staining, growth conditions, colonial features, and oxidase/catalase tests. These few but none-

theless critical mis-IDs confirmed that, like any identification system, experienced laboratory personnel have to manage Vitek MS results and take into account bacterial and clinical data, as highlighted by other authors using different MALDI-TOF MS-based systems (4, 15, 22).

Nine isolates belonging to nine species not included in the Vitek MS database twice obtained the same "no ID" answer, highlighting the specificity of the algorithm used in these bacterial groups. Two out of four *Corynebacterium* species and four out of 15 anaerobic species collected in our routine practice were not identified. The lack of species diversity of corynebacteria and anaerobes in the currently available database should be addressed for overall routine use. As noted by other authors, our results emphasize the widespread ignorance and failure to correctly identify anaerobic species by biochemical methods in medical bacteriology (10, 14, 25, 26). Moreover, from microbiologist views and for clinical purposes, the ID to the genus level of many anaerobes is usually sufficient, but that is unfortunately not achievable with the original algorithm of the tested system.

With the analysis of an additional deposit, six out of 10 isolates with species included in the database were correctly identified. As mentioned for another MALDI-TOF MS-based bacterial ID system, these results may indicate that deposition of an excessive amount of bacteria during sample spot preparation can lead to a loss of accuracy of the Vitek MS system, providing quality spectrum warning messages or "no ID" results (5). As a consequence, all isolates with a "no ID" result given by the Vitek MS using one deposit should be retested in routine practice. The absence of IDs for one *S. haemolyticus* isolate and all three *H. pylori* isolates tested twice may be due to a lack of species diversity among the 10 reference spectra embedded in the database or the need for a prior protein extraction.

The "good ID" performances for the largely tested *Enterobacteriaceae*, nonfermentative Gram-negative rod, staphylococci, and streptococci groups using only one deposit and no extraction step were reported previously in only one study (4). In addition to the spectral acquisition step, the good results obtained with the Vitek MS system may be due to the database building using 10 different reference strains for each species and generating different spectra for each strain under different culture conditions in order to set its typical spectrum. A previous report has noted that including at least 10 strains per species in the database with many replicates per strain is a prerequisite to obtain an accurate MALDI-TOF MS ID (20). By allowing quick and reliable IDs of *Streptococcaceae* isolates, the Vitek MS showed better ID performances for milleri group streptococci and other alpha- or nonhemolytic *Streptococcaceae* than did the Vitek2 system. In contrast to previous studies using other MALDI-TOF MS systems and although our results need to be confirmed on a larger number of isolates, the Vitek MS appears to particularly discriminate *S. pneumoniae*, an undeniable pathogenic species, from other alpha-hemolytic streptococci (3, 4, 6, 7, 15, 18, 20, 22, 24).

In conclusion, the Vitek MS system allows a fast and reliable acquisition of bacterial ID for most bacterial species isolated routinely in a medical laboratory with only one deposit of crude bacteria and without any extraction step. The remarkable performance of the Vitek MS system may be due to its novel laser displacement mode, original algorithm, and quality of the database building. It is worth noting that the analysis of the presence and the absence of specific peaks by the spectrum classifier algorithm of the Vitek MS is double edged. When the tested species is missing from the database, the algorithm gives a clear absence of ID in most cases, a result which is overall what is expected for an ID system. However, due to the limited species diversity of the database for some taxa groups, as for some anaerobe taxa, the absence of ID results to the genus level is unfortunate and requires the use of a conventional identification system. For routine purposes, in addition to the isolates that generated a spectrum of poor quality, we suggested a retest with one to two deposits for isolates that give "no ID" and "no recurrent" LD results with a first deposit, as false "no ID" results can be obtained with deposits of poor quality and the confidence values are indicators with weak usefulness in cases of LD, respectively. Considering the higher bacterial diversity included in this study than in routine practice and the low prevalence of species without correct identification, the performances should be even better for routine activity in clinical laboratories. However, expanding the spectral database is warranted, particularly for anaerobic, coryneform, and some highly pathogenic bacteria, in order to use almost exclusively this system for isolate IDs in routine medical practice.

ACKNOWLEDGMENTS

Funding for this study was provided by bioMérieux.

We thank the clinical microbiology laboratory technologists for their help in collecting isolates for this study and Françoise Bertin, Brigitte Gras-Potier, Brigitte Rigonnet, Véronique Tudal, and Emmanuel Cartron for helpful technical assistance.

REFERENCES

1. **Alatoom AA, Cunningham SA, Ihde SM, Mandrekar J, Patel R.** 2011. Comparison of direct colony method versus extraction method for identification of Gram-positive cocci by use of Bruker Biotyper matrix-assisted laser desorption ionization–time of flight mass spectrometry. J. Clin. Microbiol. **49**:2868–2873.
2. **Anhalt JP, Fenselau C.** 1975. Identification of bacteria using mass spectrometry. Ann. Chem. **47**:219–225.
3. **Benagli C, Rossi V, Dolina M, Tonolla M, Petrini O.** 2011. Matrix-assisted laser desorption ionization-time of flight mass spectrometry for the identification of clinically relevant bacteria. PLoS One **6**:e16424. doi:10.1371/journal.pone.0016424.
4. **Bille E, et al.** 2011. MALDI-TOF MS Andromas strategy for the routine identification of bacteria, mycobacteria, yeasts, *Aspergillus* spp. and positive blood cultures. Clin. Microbiol. Infect. doi:10.1111/j.1469-0691.2011.03688.x.
5. **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.
6. **Blondiaux N, Gaillot O, Courcol RJ.** 2010. MALDI-TOF mass spectrometry to identify clinical bacterial isolates: evaluation in a teaching hospital in Lille. Pathol. Biol. (Paris) **58**:55–57. (In French.)
7. **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.
8. **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.
9. **Haigh J, Degun A, Eydmann M, Millar M, Wilks M.** 2011. Improved performance of bacterium and yeast identification by a commercial matrix-assisted laser desorption ionization–time of flight mass spectrometry system in the clinical microbiology laboratory. J. Clin. Microbiol. **49**:3441.
10. **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.
11. **Kallow W, Erhard M, Shah HN, Raptakis E, Welker M.** 2010. MALDI-

TOF MS for microbial identification: years of experimental development to an established protocol, p 255–276. *In* Shah HN, Gharbia SE (ed), Mass spectrometry for microbial proteomics. John Wiley & Sons, Ltd, Chichester, United Kingdom.
12. **Kaper JB, Nataro JP, Mobley HL.** 2004. Pathogenic *Escherichia coli*. Nat. Rev. Microbiol. **2**:123–140.
13. **Karas M, Hillenkamp F.** 1988. Laser desorption ionization of proteins with molecular masses exceeding 10,000 daltons. Anal. Chem. **60**:2299–2301.
14. **La Scola B, Fournier PE, Raoult D.** 2011. Burden of emerging anaerobes in the MALDI-TOF and 16S rRNA gene sequencing era. Anaerobe **17**:106–112.
15. **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.
16. **Poyart C, Quesne G, Boumaila C, Trieu-Cuot P.** 2001. Rapid and accurate species-level identification of coagulase-negative staphylococci by using the *sodA* gene as a target. J. Clin. Microbiol. **39**:4296–4301.
17. **Prere MF, Fayet OA.** 2011. A specific polymerase chain reaction test for the identification of Streptococcus pneumoniae. Diagn. Microbiol. Infect. Dis. **70**:45–53.
18. **Risch M, et al.** 2010. Comparison of MALDI TOF with conventional identification of clinically relevant bacteria. Swiss Med. Wkly. **140**:w13095. doi:10.4414/smw.2010.13095.
19. **Sauer S, Kliem M.** 2010. Mass spectrometry tools for the classification and identification of bacteria. Nat. Rev. Microbiol. **8**:74–82.
20. **Seng P, et al.** 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.
21. **Slinger R, et al.** 2007. Pyrosequencing of a *recA* gene variable region for *Burkholderia cepacia* complex genomovar identification. Diagn. Microbiol. Infect. Dis. **58**:379–384.
22. **Szabados F, et al.** 2012. Evaluation of species-specific score cutoff values of routinely isolated clinically relevant bacteria using a direct smear preparation for matrix-assisted laser desorption/ionization time-of-flight mass spectrometry-based bacterial identification. Eur. J. Clin. Microbiol. Infect. Dis. **31**:1109–1119.
23. **Taha MK.** 2000. Simultaneous approach for nonculture PCR-based identification and serogroup prediction of *Neisseria meningitidis*. J. Clin. Microbiol. **38**:855–857.
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. **Veloo AC, Erhard M, Welker M, Welling GW, Degener JE.** 2011. Identification of Gram-positive anaerobic cocci by MALDI-TOF mass spectrometry. Syst. Appl. Microbiol. **34**:58–62.
26. **Veloo AC, Welling GW, Degener JE.** 2011. The identification of anaerobic bacteria using MALDI-TOF MS. Anaerobe **17**:211–212.