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Comparison of the New VITEK MS PRIME System with the Matrix-Assisted Laser Desorption Ionization Biotyper Microflex LT for the Identification of Microorganisms

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Background: Matrix-assisted laser desorption ionization (MALDI) is the cornerstone of bacterial identification. The performance of a new MALDI time-of-flight mass spectrometry VITEK MS PRIME (VMS-P) system was compared with that of the MALDI Biotyper Microflex LT (MBT) system, which is routinely used in our laboratory.

Methods: Sixteen bacterial and yeast reference strains cultured in 20 different media were analyzed over 10 consecutive rounds using both systems. Bacterial and yeast isolates from the routine workflow were processed using both systems. Microcolonies were identified after a 4-hour agar subculture from positive blood culture bottles, without extraction.

Results: To determine the repeatability based on the reference strains, 1,190 spots were processed using each system. Correct identification was achieved for 94.0% (MBT) and 98.4% (VMS-P; $P<0.01$) of spots. Among these, 83.0% (MBT) and 100.0% (VMS-P) were identified with a high degree of confidence. For 1,214 spots from routine isolates, species identification was achieved for 90.0% (MBT) and 91.4% (VMS-P; $P=0.26$) of spots. For 69.8% (MBT) and 87.4% (VMS-P) of the spots, identification was achieved with a high degree-of-confidence score. When identification was performed using both systems, the agreement between them was 97.9%. The identification of microcolonies from positive blood culture bottles was achieved for 55.5% (MBT) and 70.2% (VMS-P; $P=0.01$) of spots.

Conclusions: The MBT and VMS-P systems perform similarly in routine daily practice. The new VMS-P system shows high repeatability, better confidence scores for identification, and promising ability to identify microcolonies.

Key Words: Blood culture, Microbiology, Spectrometry, Mass, Matrix-assisted laser desorption ionization

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INTRODUCTION

The effectiveness of matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) for identifying microorganisms encountered in routine clinical practice has been demonstrated for bacteria and yeast [1-3]. Since the early 2010s, this technology has been successfully implemented in several European laboratories [4]. For the management of infected patients, early identification of the causative species is crucial. MALDI-TOF MS is essential for the rapid identification of pathogens, particularly those causing blood infections [4-6]. VITEK MS (VMS) (bioMérieux SA, Marcy l'Etoile, France) and MALDI

Biotyper Microflex LT (MBT) (Bruker Corporation, Leipzig, Germany) have been the two main MALDI-TOF MS systems available over the last 10 years. They were approved by the United States Food and Drug Administration in August and November 2013, respectively. MS profile analysis is performed using MYLA for VITEK MS 3.0 (bioMérieux SA) for the VMS system and MBT Compass IVD software for the MBT system. The performance of these two MALDI-TOF MS systems has been comparatively evaluated in three other studies, which showed similar identification efficiency and workflow robustness [7-9]. Both systems correctly identified most isolates at the genus (98.0% and 98.0%, 98.8% and 98.1%, and 99.0% and 99.2% for the VMS and MBT systems, respectively) and species (97.2% and 96.6%, 87.0% and 93.0%, and 93.7% and 98.1%, respectively) levels [7-9]. Recently, a new MALDI-TOF MS identification system, VITEK MS PRIME (VMS-P), which includes a new spectrometer, hardware and software for data acquisition and analysis, and an updated user interface, was designed by bioMérieux SA. The objective of this study was to compare the performance of the new VMS-P system with that of the routinely used MBT system.

METHODS

Study design

This prospective study was conducted in the Microbiology Department of Georges Pompidou European Hospital, Paris, France. Between October 2021 and January 2022, we assessed the performances of the MBT and VMS-P systems using two sets of microorganisms: reference strains and routine clinical isolates. Four technicians, who used the MBT system and were trained on the new VMS-P system, performed the experiments. Except for several non-specific recommended media analyzed in repeatability experiments, all technical recommendations of both manufacturers, including the use of their respective reagents, were applied. Recommendations for the VMS-P system are available online in the VITEK MS PRIME IVD User Manual 161150-1143A, as of February 2021 [10]. For the VMS-P system, the experimental protocol was followed according to documents provided by the Bruker Corporation technician during the last system update (based on the MBT Compass IVD User Manual, REF 1832771, Revision D, November 2019) that took place in our laboratory in February 2020.

Repeatability experiments based on reference strains

Sixteen reference strains, mostly comprising aerobic and anaerobic bacteria and yeast encountered in clinical practice, were selected (Table 1). No ATCC species known to be difficult to identify using MALDI-TOF MS were included. Frozen strains were plated on Columbia agar supplemented with c (v/v) sheep blood (COS; bioMérieux SA) and incubated for 24–48 hours at 35°C under suitable conditions. Isolated colonies grown on COS were plated for a second subculture on different agar media (Table 1) provided by four manufacturers: (i) bioMérieux SA, (ii) Oxoid (distributed by Thermo Fisher Diagnostics SAS, Dardilly, France), (iii) Becton Dickinson (Le Pont-de-Claix, France), and (iv) Bio-Rad (Marnes-la-Coquette, France). After incubation for 24 hours at 35°C, 10 duplicates of each colony were spotted, by a single technician, onto 96-well MBT target plates, in addition to 48-well VMS-P disposable target slides. In-tube protein extraction was not performed before spotting the bacterial and yeast isolates on either system. For yeast, on-target spots were covered with 0.5 µL of 70% formic acid before matrix application. When the isolates had grown into small colonies, several colonies (sufficient for 20 spots) were used. Each spot was covered with 1 µL of alpha-cyano-4-hydroxycinnamic acid matrix from either bioMérieux SA or Bruker Corporation. Targets were processed using their respective MS instruments.

Comparison of bioMérieux SA and Bruker Corporation MALDI-TOF MS systems using clinical strains

Isolated strains (in the routine workflow) were prospectively included without selection. When the optimal number of technicians was on duty, bacterial and yeast isolates from the previous day's cultures were identified in parallel using both MALDI-TOF MS systems, regardless of the agar medium used. Only one spot per colony was processed by a single technician using the VMS-P and MBT systems. The treatment of the clinical isolates before matrix application was the same as that used for the aforementioned reference strains. Small colonies (mainly of *Streptococcus* and anaerobic bacteria) were pooled.

Specific case of identification from blood culture bottles

Bacterial identification of microcolonies was performed after blood culture followed by subculture. Blood culture specimens were collected in BacT/ALERT FA/FN Plus bottles (bioMérieux SA) and incubated at 35°C in the BacT/ALERT VIRTUO automated system (bioMérieux SA). Bacteria-positive cultures were plated on COS under suitable conditions. Microcolonies were identified using the MBT system after 4 hours of growth at 35°C [11]. Extraction was not performed before spotting. Microcolonies were analyzed in parallel, after routine MBT and VMS-P protocols.

Table 1. Strains and agar media used for repeatability experiments, including abbreviations (in parentheses)

Medium	*Escherichia coli* ATCC 25922 (ESCCOL)	*Klebsiella pneumoniae* ATCC 700603 (KLEPNE)	*Pseudomonas aeruginosa* ATCC 27853 (PSEAER)	*Haemophilus influenzae* ATCC 49766 (HAEINF)	*Pasteurella multocida* ATCC 12945 (PASMUL)	*Bacteroides fragilis* ATCC 25585 (BACFRA)	*Bacteroides thetaiotaomicron* ATCC 29741 (BACTHE)	*Campylobacter jejuni* ATCC 33560 (CAMJEJ)	*Staphylococcus aureus* ATCC 29213 (STAAUR)	*Enterococcus faecalis* ATCC 29212 (ENTFAE)	*Bacillus subtilis* ATCC6633 (BACSUB)	*Streptococcus pneumoniae* ATCC 49619 (STRPNE)	*Streptococcus uberis* ATCC 700407 (STRUBE)	*Streptococcus pyogenes* ATCC 5641T (STRPYO)	*Candida albicans* ATCC 90028 (CANALB)	*Candida parapsilosis* ATCC 22019 (CANPAR)
Chocolate+PolyViteX, bioMérieux SA (PVX-BM)	•	•	•	•	•	•	•	•	•	•	•	•	•	•		
Chocolate+PolyViteX, Oxoid (PVX-OX)	•	•	•	•	•	•	•	•	•	•	•	•	•	•		
Columbia+5% horse blood, bioMérieux SA (COH-BM)	•	•	•		•	•	•	•	•	•	•	•	•	•		
Columbia+5% sheep blood, Oxoid (COH-OX)	•	•	•		•	•	•	•	•	•	•	•	•	•		
ChromID CPS Elite, bioMérieux SA (CPSE-BM)	•	•	•						•	•	•					
ChromID CPS Opaque, bioMérieux SA (CPSO-BM)	•	•	•						•	•	•					
UriSelect 4, Bio-Rad (URI4-BR)	•	•	•						•	•	•					
Brilliance UTI clarity, Oxoid (UTI-OX)	•	•	•						•	•	•					
CLED agar, bioMérieux SA (CLED-BM)	•	•	•						•	•	•					
Drigalski, bioMérieux SA (DRIG-BM)	•	•	•													
Drigalski, Oxoid (DRIG-OX)	•	•	•													
ChromID ESBL, bioMérieux SA (ESBL-BM)		•														
Columbia ANC+5% horse blood, bioMérieux SA (ANC-BM)						•	•		•	•	•	•	•	•		
Columbia CAP+5% sheep blood, Oxoid (CAP-OX)						•	•		•	•	•	•	•	•		
Schaedler+5% sheep blood, Becton Dickinson (SCH-BD)						•	•									
ChromID Candida, bioMérieux SA (CID-BM)															•	•
Sabouraud Chloramphenicol Gentamicin, bioMérieux SA (SCG-BM)															•	•
CHROMagar Candida medium, Becton Dickinson (CHR-BD)															•	•
Brilliance Candida agar, Oxoid (BRC-OX)															•	•
Sabouraud Chloramphenicol, Oxoid (SCH-OX)															•	•

Solid circles indicate that the identification of the reference strain was performed on this agar medium.
Abbreviations: ANC, nalidixic acid+colistin; CAP, Oxoid antibiotic mix (including colistin) (an alternative to the ANC mix).

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Table 2. Repeatability experiment using different correct reference strain identifications classified by culture media and the new MALDI-TOF MS system, VMS-P, and the routinely used MBT system

Medium		N of correct reference strain identifications																
		ESC COL	KLE PNE	PSE AER	HAE INF	PAS MUL	BAC FRA	BAC THE	CAM JEJ	STA AUR	ENT FAE	BAC SUB	STR PNE	STR UBE	STR PYO	CAN ALB	CAN PAR	ALL
PVX-BM	MBT	10	10	10	10	**9**	10	10	10	10	**7**	10	10	**8**	**8**			132
	VMS-P	10	10	10	10	10	10	**6**	10	10	10	10	10	**9**	10			135
PVX-OX	MBT	10	10	10	10	10	10	10	10	10	10	10	**6**	**9**	10			135
	VMS-P	10	10	10	10	10	10	10	10	10	10	10	10	10	10			140
COH-BM	MBT	10	10	10		8	10	10	10	10	**9**	10	10	**8**	**6**			121
	VMS-P	10	10	10		10	10	10	10	10	10	10	10	10	10			130
COH-OX	MBT	10	10	10		10	10	10	10	10	10	10	10	10	10			130
	VMS-P	10	10	10		**9**	10	10	10	10	10	10	10	10	10			129
CPSE-BM	MBT	10	10	10						10	10	**6**						56
	VMS-P	10	10	10						10	**9**	10						59
CPSO-BM	MBT	10	10	**7**						10	10	**9**						56
	VMS-P	10	10	**9**						10	10	10						59
URI4-BR	MBT	10	10	**8**						10	**7**	10						55
	VMS-P	10	10	10						10	10	10						60
UTI-OX	MBT	10	10	10						10	**9**	10						59
	VMS-P	10	10	10						10	10	10						60
CLED-BM	MBT	10	10	**9**						10	10	10						59
	VMS-P	10	10	10						10	10	10						60
DRIG-BM	MBT	**3**	**9**	**9**														21
	VMS-P	**9**	**6**	10														25
DRIG-OX	MBT	**6**	**9**	10														25
	VMS-P	10	10	10														30
ESBL-BM	MBT		10															10
	VMS-P		10															10
ANC-BM	MBT						10	10		10	**8**	**9**	10	**6**	**8**			71
	VMS-P						10	10		10	10	10	10	10	10			80
CAP-OX	MBT						10	10		10	10	**9**	10	**8**	**9**			76
	VMS-P						10	10		10	10	10	10	10	10			80
SCH-BD	MBT						10	10										20
	VMS-P						10	10										20
CID-BM	MBT															10	10	20
	VMS-P															10	10	20
SGC-BM	MBT															10	10	20
	VMS-P															**9**	**9**	18
CHR-BD	MBT															10	**8**	18
	VMS-P															10	10	20
BRC-OX	MBT															10	**8**	18
	VMS-P															10	**6**	16

(*Continued to the next page*)

Table 2. Continued

Medium		N of correct reference strain identifications																
		ESC COL	KLE PNE	PSE AER	HAE INF	PAS MUL	BAC FRA	BAC THE	CAM JEJ	STA AUR	ENT FAE	BAC SUB	STR PNE	STR UBE	STR PYO	CAN ALB	CAN PAR	ALL
SCH-OX	MBT															**9**	**8**	17
	VMS-P															10	10	20
ALL (N of spots)	MBT	110	120	110	20	40	70	70	40	110	110	110	60	60	60	50	50	1,190
	VMS-P	110	120	110	20	40	70	70	40	110	110	110	60	60	60	50	50	1,190
ALL (N of identification)	MBT	99	118	103	20	37	70	70	40	110	100	103	56	49	51	49	44	1,119
	VMS-P	109	116	109	20	39	70	66	40	110	109	110	60	59	60	49	45	1,171
ALL (% identification)	MBT	90.0	98.3	93.6	100.0	92.5	100.0	100.0	100.0	100.0	90.9	93.6	93.3	81.7	85.0	98.0	88.0	94.0
	VMS-P	99.1	96.7	99.1	100.0	97.5	100.0	94.3	100.0	100.0	99.1	100.0	100.0	98.3	100.0	98.0	90.0	98.4

Species and media abbreviations are listed in Table 1.
Numbers in bold indicate <10/10 correct identifications.
Abbreviations: MALDI-TOF MS, matrix-assisted laser desorption ionization time-of-flight mass spectrometry; MBT, MALDI Biotyper Microflex LT; VMS-P, VITEK MS PRIME.

The identification of microcolonies from blood cultures is not recommended by the manufacturers. Bruker Corporation recommends extracting bacteria present in blood culture bottles using the MALDI Sepsityper kit, whereas bioMérieux SA recommends using colonies grown for 18–24 hours and preferably to a size of approximately 3 mm. The bioMérieux SA User Manual explains that it is possible to use colonies that are considerably smaller or larger without providing further details. The identification results obtained using microcolonies are presented separately.

Analysis of MBT data

The first analysis of the spectral data was performed using MBT Compass IVD (software version: 4.2.90; database version: 9). The spectra obtained were compared with 8,326 reference spectra included in the MBT database, covering 2,887 species and 512 genera. The built-in algorithm computes a logarithmic value (score) ranging from 0 to 3 for identification accuracy. A score <1.69 was considered an unacceptable identification and was recorded as "no identification." A score between 1.70 and 1.99 was considered a low-confidence identification to the species level. Scores ≥2.00 indicated high-confidence identification to the species level. Only the highest scores provided by the MBT Compass IVD software were used for species identification and comparison with VMS-P data. When no peak was detected, the result was recorded as a "technical failure."

Analysis of VMS-P data

Spectral data were analyzed using VITEK MS software and the VMS-P CE-IVD-certified reference database (version 3.2), comprising 47,204 spectra corresponding to 15,556 strains covering 1,316 species (1,095 bacteria and 221 fungi). Identification was performed with confidence values ranging from <25.0% to 99.9% [10]. A percentage ≥60% indicated good species identification. Percentages of approximately 25%, 33%, and 50% indicated low discrimination among four, three, and two species, respectively [10]. Percentages <25% were interpreted as "no identification" and not reported. When no peak was detected, the result was recorded as a "technical failure."

Discrepancies between the results obtained using both MALDI-TOF MS systems

Differences observed between the two MALDI-TOF MS systems were classified into four categories. The first was minor species differences: (i) the same identification was performed using the two MALDI-TOF MS systems (the VMS-P system offers the choice of identification between taxa [50%/50%]; the MBT system gives one taxon along with a comment explaining the difficulty in discriminating between taxa. For example, *Bacteroides dorei/Bacteroides vulgatus* was identified by the VMS-P system, whereas with the MBT system, *Bacteroides vulgatus* was associated with the comment "*The species* vulgatus/dorei *from* Bacteroides *genus display a very similar profile, so it is difficult to distinguish between these species*. Bacteroides dorei *is not included in the MBT database*"); (ii) species belonging to the same species group (particularly the *Enterobacter cloacae* complex); and (iii) taxonomic changes (synonymy). The other three categories were (i) the same genus, in which different species belonged to the same

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genus; (ii) contamination, in which two species were present in the specimen, but a lack of precision probably occurred in the selection process before spotting; and (iii) wrongly picked colonies from a likely technical error (wrong plate or colony) or those picked from a mix of non-isolated colonies (polymorphic bacterial flora).

Statistical methods and ergonomic comparison of both MALDI-TOF MS systems

The results obtained using the two MALDI-TOF MS systems were compared using Fisher's exact analysis. $P<0.05$ was considered significant. Several characteristics were assessed for ergonomic comparison. The objective criterion was technical differences between the protocols, and the subjective criterion was ease of instrument handling and use. The latter was assessed through oral interviews with four technicians who operated the spectrometer.

RESULTS

Repeatability experiments based on reference strains

Depending on their growth on each type of medium, 16 reference strains cultured on several different agar media were spotted 10 times; 1,190 spots were processed on both MALDI-TOF MS systems (Table 1). Correct identification was achieved for 1,119/1,190 (94.0%) and 1,171/1,190 (98.4%) spots ($P<0.01$) using the MBT and VMS-P systems, respectively (Table 2). The lowest rate of correct identification was achieved after culture on Drigalski agar with only 46/60 (76.7%) and 55/60 (91.7%) spots correctly identified using the MBT and VMS-P systems, respectively (DRIG-BM+DRIG-OX) (Table 2). Culture on chocolate agar supplemented with PolyViteX (bioMérieux SA) achieved the highest efficiency, with 267/280 (95.4%) and 275/280 (98.2%) correct identifications using the MBT and VMS-P systems, respectively (PVX-BM+PVX-OX) (Table 2). With the MBT system, the percentages for bacterial and yeast identification were not significantly different at 94.7% and 93.0%, respectively ($P=0.49$), compared with those for the VMS-P system at 98.7% and 94.0%, respectively ($P<0.01$).

Among the 1,119/1,190 (94.0%) spots identified using the MBT system, 988/1,190 (83.0%) and 131/1,190 (11.0%) spots were identified with high and low confidence, respectively (Table 3). With the VMS P system, 1,171/1,190 (98.4%) identifications were classified as "good," and 1,163/1,171 (99.3%) were identified with a confidence of 99.9% (Table 3).

Table 3. Scores and percentage confidence values for spots processed using the new MALDI-TOF MS system, VMS-P, and the routinely used MBT system

Scores and % confidence values	N of spots (%)	Reference strains																
		ESC COL	KLE PNE	PSE AER	HAEINF	PAS MUL	BAC FRA	BAC THE	CAM JEJ	STA AUR	ENT FAE	BAC SUB	STR PNE	STR UBE	STR PYO	CAN ALB	CAN PAR	ALL
MBT system																		
No identification	<1.7	11	2	7	0	3	0	0	0	0	10	7	4	11	9	1	6	71 (6.0)
Identification with low confidence	1.7–1.9	1	0	0	0	3	0	0	2	17	23	53	0	14	4	9	5	131 (11.0)
Identification with high confidence	>2	98	118	103	20	34	70	70	38	93	77	50	56	35	47	40	39	988 (83.0)
Total		110	120	110	20	40	70	70	40	110	110	110	60	60	60	50	50	1,190 (100.0)
VMS-P system																		
No identification	<25.0%	1	4	1		1		4			1			1		5	1	19 (1.6)
Low discrimination	25.0–50.0%																	
Good identification	60.0–99.8%		5								2							8 (0.7)
Good identification	99.9%	109	111	109	20	39	70	66	40	110	107	59	60	59	110	45	49	1,163 (97.7)
Total		110	120	110	20	40	70	70	40	110	110	59	60	60	110	50	50	1,190 (100.0)

Species and media abbreviations are listed in Table 1.
Values in bold correspond to the total number of identifications performed for each reference strain. For instance, 10 duplicates of each *Escherichia coli* colony isolated from 11 agar media were spotted on target plates conducting to 110 identifications.
Abbreviations: MALDI-TOF MS, matrix-assisted laser desorption ionization time-of-flight mass spectrometry; MBT, MALDI Biotyper Microflex LT; VMS-P, VITEK MS PRIME.

Table 4. Comparative performance of the VMS-P and MBT systems for the identification of clinical isolates to the species level

	Clinical isolates grown for 24–48 hours																					Bacterial isolates from blood cultures (microcolonies grown for 3–4 hours)						
	Bacteria							Yeast							All microorganisms													
	MBT			VMS-P			N of spots	MBT			VMS-P			N of spots	MBT			VMS-P			N of spots	MBT			VMS-P			N of spots
	ID +	ID -	TF	ID+	ID -	TF		ID+	ID -	TF	ID+	ID -	TF		ID +	ID -	TF	ID +	ID -	TF		ID+	ID -	TF	ID+	ID -	TF	
	•			•			780	•			•			247	•			•			1,138	•			•			111
	•				•		36	•				•		2	•				•		47	•				•		9
	•					•	20	•					•	7	•					•	28	•					•	1
		•		•			44		•		•			3		•		•			85		•		•			38
		•			•		17		•			•		1		•			•		63		•			•		45
		•				•	7		•				•	1		•				•	14		•				•	6
			•	•			23			•	•			12			•	•			39			•	•			4
			•		•		9			•		•		0			•		•		13			•		•		4
			•			•	5			•			•	0			•			•	5			•			•	0
N of spots	836	68	37	847	62	32	941	256	5	12	262	3	8	273	1,092	73	49	1,109	65	40	1,214	121	89	8	153	58	7	218
% of spots leading to species identification	88.8			90.0				93.8			96.0				90.0			91.4				55.5			70.2			
P	0.45							0.33							0.26							<0.01						
% of spectra without identification	7.2			6.6				1.8			1.1				6.0			5.4				40.8			26.6			
P	0.65							0.72							0.54							<0.01						
% of technical failures	3.9			3.4				4.4			2.9				4.0			3.3				3.7			3.2			
P	0.62							0.5							0.43							1.00						

Abbreviations: ID+, identification by a matrix-assisted laser desorption ionization time-of-flight mass spectrometry system; ID-, spectrum acquisition without associated identification; MBT, MALDI Biotyper Microflex LT; TF, technical failure causing an absence of spectra; VMS-P, VITEK MS PRIME.

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Table 5. Ergonomic characteristics of the two MALDI-TOF MS systems

	VMS-P	MBT
Multi-target loading	Present (16 targets)	Absent
Automatic data extraction for analysis	Possible	Not possible
Dedicated device for spotting	Present	Absent
Trackability of the matrix lot number based on scanning and recording of QR code on interface directly from the packaging	Present	Absent
Autonomy of users for MALDI-TOF MS calibration	Present	Hotline only
Deposition of an HCCA matrix	After spotting of all four isolates	After spotting of all isolates
Standard preparation	Standard *Escherichia coli* fresh culture (18–24 hours)	Mix ready to use
Standard failure	All positions used in the 16-position square must be re-spotted	Target can be re-read using a new fresh standard
Availability of non-used positions for a new run	Only non-used positions in a virgin 16-position square	All non-used positions are available
Protocol(s) for analysis	Bacteria or yeast	Only one protocol
Second run for a target previously read	Performed automatically after an acquisition failure, but not possible manually (new spots mandatory)	Possible

Abbreviations: HCCA, alpha-cyano-4-hydroxycinnamic acid; MALDI-TOF MS, matrix-assisted laser desorption ionization time-of-flight mass spectrometry; MBT, MALDI Biotyper Microflex LT; VMS-P, VITEK MS PRIME.

Comparative performance of both MALDI-TOF MS systems in identifying clinical isolates

Although not recommended by the manufacturers, microcolonies of 1,214 clinical isolates comprising 941 bacteria and 273 yeast from blood cultures were analyzed using both MALDI-TOF MS systems (Table 4). When both MBT and VMS-P systems were used, identification results were available for 1,092/1,214 (90.0%) and 1,109/1,214 (91.4%) isolates (P=0.26; Table 4), revealing 124 and 123 taxa, respectively. A list of all species identified using both MALDI-TOF MS systems is provided in Supplemental Data Table S1. Overall, 1,091/1,092 (99.9%) and 1,105/1,109 (99.6%) species identifications were performed using the MBT and VMS-P systems, respectively. Good identifications were achieved for 847/1,214 (69.8%) and 1,062/1,214 (87.4%) isolates using the MBT and VMS-P systems, respectively.

Overall, 1,024/1,214 (84.3%) spots were subjected to identification, including at least one microorganism, using both MALDI-TOF MS systems. In total, 907/1,024 (88.6%) isolates were identical. Among the 117 results that differed, 69 showed minor differences, 22 comprised the same genus, 11 were contaminated, and 15 had erroneous colony selection (Supplemental Data Table S2). Excluding contamination (11) and colony selection errors (15) but including minor differences (69) from the identical results (907), the agreement between the species identification achieved using both MALDI-TOF MS systems was 97.8% ([907+69)/(1,024–11–15)]).

Microcolonies isolated from blood cultures

In total, 218 additional bacterial isolates from blood cultures (microcolonies) were analyzed using both MALDI-TOF MS systems. Species identification was achieved for 55.5% and 70.2% (P=0.01) of isolates identified by the MBT and VMS-P systems, respectively (Table 4). The MBT system did not identify 42 spots, whereas the VMS-P system did. Of the corresponding 42 isolates, 35 (83.3%) were gram-positive (27 belonged to the genus *Staphylococcus*). No identification of 10 spots was achieved with the VMS P system but was achieved with the MBT system, where six isolates were gram-positive.

Ergonomic characteristics

The ergonomic characteristics of both MALDI-TOF MS systems are summarized in Table 5.

DISCUSSION

We compared the performance of VMS-P, a new MALDI-TOF MS system, and the MBT system currently used in our laboratory. While the new VMS-P system had been recently compared with the first VITEK MS [12], to our knowledge, this is the first comparative evaluation between this new platform and the MBT system. In routine daily practice, both the VMS-P and MBT systems performed similarly, with 97.8% agreement in terms of microorganism identification at the species level, when used ac-

cording to the manufacturer's recommendations. The VMS-P system achieved the best results for microcolonies from blood cultures. The repeatability achieved for the VMS-P system was greater than that achieved for the MBT system.

Repeatability experiments were performed using several agar media that were neither specifically recommended nor forbidden by the manufacturers. Only Columbia supplemented with blood (with or without colistin and nalidixic acid) and chocolate agar were included in the MBT User Manual. The VMS-P User Manual is not explicit and only recommends the use of "frequently used" agar media with blood, such as Columbia. A more detailed document is available (certificate of compatibility with bioMérieux analyses, REF SYS224), indicating that several agar media are compatible with the VMS-P system [10]. In real-life settings, numerous media are used daily, including selective media for urine specimens. We did not consider culturing isolates on a non-validated medium and subsequently plated them for supplementary subculture on a validated agar medium. If some media, such as Drigalski, are known to yield poor identification results, at a time when second-generation MALDI-TOF MS systems are available, an extension of the agar media list recommended by the manufacturers is necessary, and studies using larger panels of agar media are desirable.

In routine daily practice, both MALDI-TOF MS systems showed similar identification performance as results were provided for 90.0% and 91.4% of colonies spotted using the MBT and VMS-P systems, respectively. The percentage of technical failures leading to the absence of spectra (low-quality spots, matrix crystallization, or technical errors) was similar for both MALDI-TOF MS systems, consistent with previous studies on the identification efficiency and workflow robustness between the MBT system and the first VMS system [7-9].

Concerning *Escherichia coli/Shigella* identification, both MALDI-TOF MS systems showed similar results. With the MBT system, regardless of the isolate, the identification result of "*Escherichia* coli" was always associated with the comment "*Escherichia* coli *is close to* Shigella *and* Escherichia fergusonii. *Cannot be clearly distinguished*." With the VMS-P system, the result of "*Escherichia coli*" was always associated with an orange warning that *Escherichia coli* has been identified, but it could be a *Shigella* or an *Escherichia coli O157* isolate.

The new VMS-P system is as or more reliable than the MBT system, which has been the reference in our laboratory for 10 years. Although no difference was observed between bacterial and yeast isolates after 24–48 hours of culture, a significant difference was observed in the identification of microcolonies isolated from blood cultures incubated for 4 hours without pretreatment before spotting (Table 4). The identification of young positive blood subcultures is correlated with the duration of agar medium incubation [11]. This parameter was the same for both MALDI-TOF MS systems (MALDI-TOF MS process after a 4-hour subculture at 35°C) and cannot explain the difference observed. In these instances, the performance of the VMS-P system was superior (70.2% vs. 55.5%), particularly for gram-positive isolates. This may allow laboratories to lower their requirements for expensive commercial blood culture extraction kits and could facilitate early blood culture identification; thus, improving the management of patients with bacteremia [13].

In 2014, Verroken, *et al.* [11] showed that the routine implementation of MBT identification based on young positive blood subcultures could provide accurate clinical results in >80% of bacteremia episodes. A previous comparative study of the identification rates of isolates from blood culture bottles using an extraction protocol before spotting showed similar results for the MBT system and the first VMS system (81.8% vs. 80.7%) [14]. The accuracy of the identification of microcolonies isolated from the subculture of positive blood culture bottles would benefit from optimization by the manufacturers. The VMS-P system identified almost all colonies to the species level, with a confidence of 99.9%, as compared with a significant proportion of colonies identified with a low confidence score (between 1.70 and 1.99) by the MBT system.

Compared with that of the MBT system, the main drawback of the VMS-P system is that the matrix must be added to all four spots simultaneously, which is problematic. The use of the MBT target plate allows the matrix to be added to all positions after placement of the last spot. The VMS-P target is divided into three zones, with 16 positions in each zone. Once reading begins, unused positions in a zone where at least one position has been spotted cannot be used, increasing costs for laboratories with only modest volumes. The two MALDI-TOF MS systems have different advantages and disadvantages (Table 5).

This study has some limitations, the main one being that the discrepancies were not resolved using the gold standard sequencing method. Some approximations are questionable, considering that differences in identification within the *Enterobacter cloacae* complex were not considered. Second, we included only routine isolates; no rare clinical isolates were identified in this study. An almost identical number of species (124 vs. 123) was identified using both MALDI-TOF MS systems; however, the numbers of species included in the respective MBT and VMS-P libraries were different (2,887 vs. 1,316). One may assume that

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rare species can be identified by the MBT system, but not by the VMS-P system. The design of this study did not allow us to address this question. Future assessments of both MALDI-TOF MS systems, focusing on the correct identification of exotic species, could be useful.

Finally, we compared the new system with a 10-year-old system, which could explain some of the differences between them. A comparison of the VMS-P system with another new MALDI-TOF MS system, namely, Biotyper Sirius (Bruker Corporation), would complement this study. Despite these limitations, we documented the ability of the VMS-P system to correctly identify microorganisms commonly isolated in a clinical microbiology laboratory. These results are consistent with those of Bardelli, *et al.* [12], who recently showed that VITEK MS PRIME performs similarly to VMS and reduces the time required for pathogen identification. In the future, it would be interesting to conduct a workflow study that defines the time necessary to obtain an identification, beginning at the spotting step until a result is obtained on the laboratory information system. The present study was not designed to address this question.

During routine daily practice, the performance of the VMS-P system was similar to that of the MBT system. The VMS-P system exhibited high repeatability and confidence scores for species identification. The VMS-P system showed promising ability to identify bacterial microcolonies isolated from blood cultures (particularly gram-positive bacteria) without pretreatment.

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AUTHOR CONTRIBUTIONS

Grohs P: Study Design, Data Analysis/Statistics, Writing–Original Draft, Writing–Review & Editing. Remaud E: Technical Investigations. Lath C: Technical Investigations. Vuong K: Technical Investigations. Parolini ML: Technical Investigations. Dannaoui E: Mycology Expertise, Writing—Review & Editing. Podglajen I: Study Design, Writing—Review & Editing.

CONFLICTS OF INTEREST

During the past 5 years, Dannaoui E has received grants from MSD and Gilead; travel grants from Gilead, MSD, Pfizer, and Astellas; and speakers' fees from Gilead, MSD, and Astellas. The other authors declare no conflicts of interest.

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