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Performance of Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry for Identification of Bacterial Strains Routinely Isolated in a Clinical Microbiology Laboratory[▽]

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Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) has recently been introduced in diagnostic microbiology laboratories for the identification of bacterial and yeast strains isolated from clinical samples. In the present study, we prospectively compared MALDI-TOF MS to the conventional phenotypic method for the identification of routine isolates. Colonies were analyzed by MALDI-TOF MS either by direct deposition on the target plate or after a formic acid-acetonitrile extraction step if no valid result was initially obtained. Among 1,371 isolates identified by conventional methods, 1,278 (93.2%) were putatively identified to the species level by MALDI-TOF MS and 73 (5.3%) were identified to the genus level, but no reliable identification was obtained for 20 (1.5%). Among the 1,278 isolates identified to the species level by MALDI-TOF MS, 63 (4.9%) discordant results were initially identified. Most discordant results (42/63) were due to systematic database-related taxonomical differences, 14 were explained by poor discrimination of the MALDI-TOF MS spectra obtained, and 7 were due to errors in the initial conventional identification. An extraction step was required to obtain a valid MALDI-TOF MS identification for 25.6% of the 1,278 valid isolates. In conclusion, our results show that MALDI-TOF MS is a fast and reliable technique which has the potential to replace conventional phenotypic identification for most bacterial strains routinely isolated in clinical microbiology laboratories.

In the clinical diagnostic microbiology laboratory, the identification of bacterial or yeast isolates is currently mainly based on phenotypic characteristics, such as growth on different media, colony morphology, Gram stain, and various biochemical reactions. Altogether, these techniques allow the identification of most bacterial isolates with great accuracy, but they are costly and time-consuming.

The matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) technique can be used to generate protein fingerprint signatures from whole bacterial cells (9). By comparing these fingerprints to a database of reference spectra by the use of various algorithms, bacteria can be rapidly identified (9). Even though the first study of the use of MS for the identification of bacteria dates back to 1975 and was performed by Anhalt and Fenselau (1), MALDI-TOF MS devices designed for use under routine conditions have only recently been commercially introduced.

Over the past few years, this technique has been used in specific studies that have essentially assessed its ability to identify different bacterial genera among Gram-negative rods, such as *Escherichia coli* and other members of the *Enterobacteriaceae* family (5, 6); Gram-positive cocci, such as *Staphylococcus aureus* and streptococci (8, 12); and some Gram-positive rods, such as *Bacillus cereus* and *Listeria* species (3, 18). Very recently, the first study to have assessed the performance of MALDI-TOF MS for the identification of bacterial strains isolated from clinical samples has been published (20). That study showed that MALDI-TOF MS enables the accurate identification to the species level of 84.1% of the 1,660 strains tested (20).

In our own clinical microbiology laboratory, we prospectively assessed the performance of MALDI-TOF MS for the identification of bacterial and yeast strains routinely isolated from clinical samples.

MATERIALS AND METHODS

Clinical isolates collection. Over a 4-week period, all bacteria and yeast isolates retrieved from clinical samples and identified in our laboratory to the species level by using conventional methods were tested in parallel by MALDI-TOF MS. For each isolate, the growth medium used for the identification was recorded.

Conventional identification. Identification of each isolate to the species level was performed by various phenotypic tests, such as the catalase test (bioMérieux, Marcy l'Etoile, France), tests with the Slidex Staph plus system (bioMérieux), the oxidase test (Becton Dickinson), the pyrrolidonylarylamidase test (Remel Inc.), and tests with either the Vitek2 or the API system (bioMérieux). Quality control of commercial systems was performed by periodically testing a range of ATCC strains. The criterion used for the acceptance of the identifications obtained with the Vitek2 system was that the isolate was identified as the only choice with a >93% probability. The criteria used for the acceptance of the identifications obtained with the API system were that the isolate was identified with >90% certainty and a typicity index of >0.75. When phenotypic identification failed or was not absolutely certain (n = 11), sequenced-based molecular identification was performed, as described previously (21).

MALDI-TOF MS identification. The identification of the isolates by MALDI-TOF MS was performed on a Microflex LT instrument (Bruker Daltonics GmbH, Leipzig, Germany) with FlexControl (version 3.0) software (Bruker Daltonics) for the automatic acquisition of mass spectra in the linear positive mode within a range of 2 to 20 kDa, according to the instructions of the manufacturer. Of note, the Microflex LT instrument was periodically calibrated

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by using the Bruker Daltonics bacterial test standard, according to the instructions of the manufacturer. Automated analysis of the raw spectral data was performed by the MALDI BioTyper automation (version 2.0) software (Bruker Daltonics) and by use of a library of 3,290 spectra (database update of 2 September 2008) and the default settings.

The identification was first performed by touching the surface of the investigated colony with a sterile pipette tip and directly applying the small amount of sample on polished or ground steel MSP 96 target plates (Bruker Daltonics). Of note, this was done by the technician in charge of the sample and not by a specifically trained technician. The deposited bacteria or yeasts were overlaid with 1 μl of HCCA matrix (a saturated solution of α-cyano-4-hydroxycinnamic acid in 50% acetonitrile–2.5% trifluoroacetic acid) and air dried at room temperature to allow cocrystallization with the experimental sample. The spectra were then acquired by the mass spectrometer and were compared by using the BioTyper software. According to the criteria proposed by the manufacturer, a result was considered valid (accurate identification to the species level) whenever the score value was ≥2.0. When the scores obtained were <2.0, the samples were retested after a protein extraction step. Briefly, colony samples were resuspended in a 1.5-ml polypropylene tube (Eppendorf, Hamburg, Germany) containing 1 ml of a water-ethanol (1:2) solution. The cell suspension was centrifuged at 12,500 × *g* for 2 min, and the supernatant was discarded. The pellet was suspended in 25 μl of 70% formic acid in water and 25 μl of 100% acetonitrile. A final centrifugation was performed at 12,500 × *g* for 2 min. Then, 1 μl of supernatant was spotted on the MSP 96 target plate and allowed to dry at room temperature before it was overlaid with 1 μl of the HCCA matrix and analyzed as described above.

RESULTS

Comparison of conventional and MALDI-TOF MS-based identifications. During the study period, a total of 1,371 isolates were identified to the species level by conventional methods. MALDI-TOF MS yielded a valid score (score $x \geq 2.0$; accurate identification to the species level) for 1,278 (93.2%) isolates, an intermediate score ($1.7 \leq$ score $x < 2$; accurate identification to the genus level) for 73 (5.3%) isolates, and no reliable identification (score $x < 1.7$) for 20 (1.5%) isolates.

Among all 1,371 isolates tested, a valid result could be obtained for 964 strains (70.3%) after direct deposition and for 314 additional strains after protein extraction (22.9%). Among the 1,278 valid results, the identification matched at the species level in 95.1% (1,215/1,278) of the instances, matched at the genus level in 3% (39/1,278), and was discordant at the genus and species levels in 1.9% (24/1,278). MALDI-TOF MS identification to the species level was correct for 92.2% (671/728) of all Gram-negative bacilli tested, whereas it was correct for 99.2% (508/512) of Gram-positive cocci and 84.6% (11/13) of Gram-positive bacilli. Finally, it was 100% correct for the only Gram-negative coccus and the 24 yeasts tested. Table 1 provides detailed results for each species and the identities obtained by the conventional method and MALDI-TOF MS.

Overall, a valid and correct result at the species level was obtained for 88.6% (1,215) of all 1,371 strains tested in our study. Interestingly, of 73 nonvalid results with an intermediary score ($1.7 \leq$ score $x < 2$) even after the extraction step, 54 nonetheless yielded an identification at the species level congruent with the phenotypic identification. Hence, the overall rate of matching of the results between MALDI-TOF MS (valid and nonvalid results) and conventional techniques at the species level was 92.6% (1,269/1,371).

Analysis of discordant results. Among the 1,278 identifications given as valid (score ≥ 2.0) by MALDI-TOF MS, we initially identified 63 (4.9%) discordant results by MALDI-TOF MS (39 at the species level and 24 at the genus level) in comparison to the identity indicated by the conventional method.

Whereas results with intermediate or nonreliable scores would not be deemed applicable, identifications with a score of ≥2.0 are considered valid, according to the BioTyper scoring system, and should therefore be trusted by the microbiologist. Thus, it is of the uttermost importance that the cause of the discrepant results in this category be analyzed.

For seven discordant results, repetition of the conventional method of identification showed that the initial identity obtained by the conventional method was incorrect and that the identity obtained by MALDI-TOF MS was correct. The remaining 56 discordant results can be classified into four different categories (Table 2). Category S consists of discordances due to the inability of the MALDI-TOF spectra to discriminate closely related species. It consisted of two *Shigella sonnei* isolates and one *Shigella flexneri* isolate which were identified as *E. coli* by MALDI-TOF MS. Because *Shigella* spp. and *E. coli* exhibit great similarity at the genomic level (11), it was expected that their similar proteomes prevented their differentiation by MALDI-TOF MS (a limitation also present with phenotypic tests, such as the Vitek2 system). Category M consisted of discordances due to the MALDI-TOF MS database, such as a likely error in the MALDI-TOF reference spectra for two *Propionibacterium acnes* wrongly identified as *Eubacterium brachy* by use of the BioTyper software. The category M discordances may also be due to the closeness of the spectra present in the database and related to two different bacterial species. Category V represents the absence of the isolate in the Vitek2 system database. Finally, category T is related to taxonomical discordances.

Yield of the acetonitrile-formic acid extraction step. The yield of the extraction was assessed for results that achieved a valid score at the end of the process. The valid score for the 1,278 samples was obtained by direct application for 964 (75.4%) and after an extraction step for 314 (25.6%).

It could be hypothesized that Gram-positive bacteria or yeasts, because of their cell wall structure, might more frequently need an extraction step to yield a valid score. This was indeed the case for yeast samples, for which only 4% (1/24) of the valid results were obtained by direct application of the colony. However, for Gram-positive and Gram-negative bacteria, the yields from direct application were almost the same, being about 75%. As an example, the yields of valid scores before the extraction step were 79% for *S. aureus*, 82% for *Enterococcus faecalis*, 92% for *Pseudomonas aeruginosa*, 74% for *E. coli*, 58% for *Klebsiella pneumoniae*, and 58% for *Staphylococcus epidermidis*. Of note, for *E. coli*, the growth medium from which isolates are recovered must be taken into account (see below).

Influence of growth conditions. The culture conditions and the growth time are factors which influence the protein expression pattern of bacteria and might therefore alter the results of MALDI-TOF MS identification (22). In the present study, we observed a statistically significant difference in the identification yield for Gram-negative bacteria grown on MacConkey agar in comparison to that for the same species grown on rich medium, such as sheep blood or chocolate agar. Thus, by using the direct deposition technique, only 56% of 108 *E. coli* isolates from MacConkey medium were identified correctly with a valid

TABLE 1. Comparison between conventional and MALDI-TOF MS-based identification techniques for 1,278 valid MALDI-TOF MS results

Species	No. of isolates	No. (%) of isolates with results:			
		Matching at the species level	Matching at the genus level	Nonmatching	Matching at the species level by direct application[a]
Gram positive					
Staphylococcus aureus	270	270 (100)			214 (79)
Enterococcus faecalis	79	79 (100)			65 (82)
Staphylococcus epidermidis	48	48 (100)			28 (58)
Streptococcus agalactiae	45	45 (100)			26 (58)
Enterococcus faecium	15	15 (100)			13 (87)
Streptococcus pneumoniae	11	11 (100)			8 (73)
Streptococcus pyogenes	9	8 (89)	1 (11)		7 (88)
Enterococcus gallinarum	7	7 (100)			5 (71)
Clostridium difficile	6	6 (100)			5 (83)
Staphylococcus haemolyticus	5	5 (100)			5 (100)
Streptococcus dysgalactiae	5	4 (80)	1 (20)		3 (75)
Bacillus cereus	3	3 (100)			(0)
Staphylococcus lugdunensis	3	3 (100)			1 (33)
Streptococcus anginosus	3	3 (100)			1 (33)
Streptococcus constellatus	3	2 (67)	1 (33)		(0)
Enterococcus casseliflavus	2	2 (100)			(0)
Propionibacterium acnes	2			2 (100)	(0)
Staphylococcus capitis	2	2 (100)			(0)
Staphylococcus hominis	2	1 (50)	1 (50)		1 (100)
Brevibacterium casei	1	1 (100)			1 (100)
Clostridium perfringens	1	1 (100)			1 (100)
Staphylococcus saprophyticus	1	1 (100)			1 (100)
Staphylococcus simulans	1	1 (100)			1 (100)
Staphylococcus warneri	1	1 (100)			1 (100)
Gram negative					
Escherichia coli	257	254 (99)	1	2 (1)	189 (74)
Pseudomonas aeruginosa	99	98 (99)	1 (1)		90 (92)
Klebsiella pneumoniae	59	57 (97)	1 (2)	1 (2)	33 (58)
Enterobacter cloacae	48	29 (60)	19 (40)		21 (72)
Proteus mirabilis	42	42 (100)			36 (86)
Haemophilus influenzae	35	35 (100)			32 (91)
Klebsiella oxytoca	31	28 (90)	1 (3)	2 (6)	15 (54)
Stenotrophomonas maltophilia	30	17 (57)		13 (43)	9 (53)
Morganella morganii	22	22 (100)			22 (100)
Citrobacter koseri	17	17 (100)			16 (94)
Serratia marcescens	14	14 (100)			10 (71)
Citrobacter freundii	10	8 (80)	2 (20)		6 (75)
Moraxella catarrhalis	9	9 (100)			9 (100)
Proteus vulgaris	9	9 (100)			7 (78)
Pseudomonas stutzeri	7	7 (100)			6 (86)
Bacteroides fragilis	5	4 (80)	1 (20)		4 (100)
Enterobacter aerogenes	5	5 (100)			1 (20)
Agrobacterium tumefaciens	4	2 (50)	1 (25)	1 (25)	2 (100)
Salmonella spp.	4	4 (100)			4 (100)
Campylobacter jejuni	3	2 (67)	1 (33)		2 (100)
Achromobacter xylosoxidans	2		2 (100)		(0)
Burkholderia cepacia	2	1 (50)	1 (50)		1 (100)
Citrobacter braakii	2	2 (100)			1 (50)
Haemophilus parainfluenzae	2	1 (50)	1 (50)		1 (100)
Providencia rettgeri	2	2 (100)			2 (100)
Pseudomonas putida	2		2 (100)		(0)
Shigella sonnei	2			2 (100)	(0)
Aeromonas hydrophila	1	1 (100)			1 (100)
Campylobacter coli	1		1 (100)		(0)
Fusobacterium necrophorum	1	1 (100)			1 (100)
Neisseria meningitidis	1	1 (100)			1 (100)
Shigella flexneri	1			1 (100)	(0)
Yeasts					
Candida albicans	16	16 (100)			(0)
Candida glabrata	5	5 (100)			(0)
Candida tropicalis	2	2 (100)			1 (50)
Candida norvegensis	1	1 (100)			

[a] Direct application on the MALDI-TOF MS target plate, without the protein extraction step.

TABLE 2. Analysis of the 56 remaining discordant results between conventional and MALDI-TOF MS-based identification techniques

Conventional identification (no. of isolates)	MALDI-TOF MS identification (no. of isolates)	Type of discordance[a]	Interpretation
Enterobacter cloacae (19)	*Enterobacter hormaechei* (12), *Enterobacter kobei* (3), *Enterobacter asburiae* (3), *Enterobacter* sp. (1)[c]	V/T/M	*E. hormaechei*, *E. kobei*, and *E. asburiae* all belong to the *E. cloacae* complex group (16). For 11/19 of these isolates, the 2nd-best MALDI identification choice was *E. cloacae*. The Vitek2 system GN card should identify *E. asburiae* but does not specifically identify *E. hormaechei* and *E. kobei* species.
Stenotrophomonas maltophilia (13)	*Pseudomonas hibiscicola* (11), *Pseudomonas beteli* (2)	T/M	*P. hibiscicola* and *P. beteli* are heterotypic synonyms of *S. maltophilia* which should not be used according to the recent taxonomy (2). For 8/13 of these isolates, the 2nd-best MALDI identification choice was *S. maltophilia*.
Pseudomonas putida (2)	*Pseudomonas monteilii* (1), *Pseudomonas* sp. (1)[c]	V/T/M	*P. monteilii* belongs to the *P. putida* group (2). The Vitek2 system GN card does not specifically identify *P. monteilii*.
Pseudomonas aeruginosa (1)	*Pseudomonas corrugata* (1)	V	The Vitek2 system GN card does not specifically identify *P. corrugata*.
Shigella sonnei (2)	*Escherichia coli* (2)	S	*Shigella* and *Escherichia* are very closely related species (11).
Shigella flexneri (1)	*Escherichia coli* (1)	S	*Shigella* and *Escherichia* are very closely related species (11).
Agrobacterium tumefaciens (2)	*Agrobacterium rhizogenes* (1), *Rhizobium radiobacter* (1)	T	The current taxonomy of the *Rhizobiaceae* family is debated (4). *A. tumefaciens* and *A. rhizogenes* are synonymous with *Rhizobium radiobacter* and *Rhizobium rhizogenes*, respectively.
Klebsiella oxytoca (2)	*Raoultella ornithinolytica* (2)	V	The 16S rRNA sequencing identification was *R. ornithinolytica*. *Raoultella* is a genus which has recently been separated from the *Klebsiella* genus (7). However, the two should have been identified by the Vitek2 system.
Citrobacter freundii (2)	*Citrobacter braakii* (2)	V/T	*C. freundii* and *C. braakii* are closely related *Citrobacter* species which were previously reported to be members of the *C. freundii* complex (10).
Propionibacterium acnes (2)	*Eubacterium brachy* (2)	M	The 16S rRNA sequencing identification was *P. acnes*. *P. acnes* and *E. brachy* are unrelated anaerobic Gram-positive rods.
Bacteroides fragilis (1)	*Bacteroides thetaiotaomicron* (1)	T	*B. thetaiotaomicron* belongs to the *B. fragilis* group (15).
Burkholderia cepacia (1)	*Burkholderia dolosa* (1)	T	*B. dolosa* belongs to the *B. cepacia* complex (23).
Streptococcus constellatus (1)	*Streptococcus anginosus* (1)	M[b]	The 16S rRNA sequencing identification was *S. constellatus*. Repetition of five independent direct deposits and three protein extractions subsequently consistently yielded a MALDI identification of *S. constellatus*.
Streptococcus pyogenes (1)	*Streptococcus agalactiae* (1)	M[b]	The 16S rRNA sequencing identification was *S. pyogenes*. Repetition of five independent direct deposits and three protein extractions subsequently consistently yielded a MALDI identification of *S. pyogenes*.
Streptococcus dysgalactiae (1)	*Streptococcus pyogenes* (1)	M[b]	The 16S rRNA sequencing identification was *S. dysgalactiae*. Repetition of five independent direct deposits yielded two MALDI identifications of *S. dysgalactiae* (score < 2) and three nonreliable results. Repetition of three independent protein extractions yielded a MALDI identification of *S. pyogenes* (score < 2).
Haemophilus parainfluenzae (1)	*Haemophilus influenzae* (1)	M[b]	The 16S rRNA sequencing identification was *H. parainfluenzae*. A 2nd MALDI analysis provided a new valid result that matched the conventional identification (no further repeats could be performed because of a lack of growth of the initial strain).
Achromobacter xylosoxidans (1)	*Achromobacter ruhlandii* (1)	M[b]	The 16S rRNA sequencing identification was *A. xylosoxidans*. Repetition of five independent direct deposits yielded four MALDI identifications of *A. ruhlandii* and one *A. xylosoxidans*. Repetition of three protein extractions yielded two MALDI identifications of *A. xylosoxidans* and one *A. ruhlandii*.
Campylobacter coli (1)	*Campylobacter jejuni* (1)	M[b]	16S rRNA sequencing cannot differentiate *C. coli* from *C. jejuni*. Repetition of five independent direct deposits and three protein extractions subsequently consistently yielded a MALDI identification of *C. coli*.
Escherichia coli (1)	*Escherichia albertii* (1)	V	The 16S rRNA sequencing identification was *E. albertii*. Repetition of five independent direct deposits yielded three MALDI identifications of *E. albertii* and two *E. coli*. Repetition of three protein extractions yielded three MALDI identifications of *E. albertii*. The Vitek2 system GN card does not specifically identify E. *albertii*.
Klebsiella pneumoniae (1)	*Klebsiella oxytoca* (1)	M[b]	The 16S rRNA sequencing identification was *K. pneumoniae*. Repetition of five independent direct deposits yielded two MALDI identifications of *K. pneumoniae*, two *K. variicola*, and one nonreliable result. Repetition of three protein extractions yielded two MALDI identifications of *K. pneumoniae* and one *K. variicola*.

[a] T, taxonomical; S, MALDI-TOF MS technique related (spectra not discriminative enough); V, Vitek2 database related; M, MALDI-TOF MS database related. A discordant isolate might belong to multiple categories.

[b] The MALDI-TOF MS identification yielded inconstant results due to either difficult-to-differentiate strains or a lack of sufficient reference spectra in the BioTyper database.

[c] The presence of reference spectra of strains identified only to the genus level is misleading and precludes definitive identification to the species level; such spectra should be discarded from the database.

score (≥2.0), whereas 87% of 148 *E. coli* isolates from other growth media were identified correctly with a valid score ($P < 0.001$). A subanalysis indicated that *E. coli* colonies taken from the MacConkey medium of urine culture devices, i.e., Uricult (Orion Diagnostica, Finland) and Diaslide (Novamed Ltd., Israel), yielded an even lower percentage of isolates with a valid score before extraction, that is, 35% of 34 cases. This low percentage may be due to poorer growth conditions in the device (thin medium, lower oxygen content). After the extraction step, there was no difference in the correct identifications between *E. coli* isolates taken from any media, since 100% of the *E. coli* isolates were correctly identified. Therefore, bacterial samples taken from MacConkey medium might directly be processed through the extraction protocol, especially when they are isolated from urine culture devices.

Technician-related discordant results. During the study period, we unequivocally identified 39 (3%) cases in which an inversion between two isolates present in the same sample had occurred. The MALDI-TOF MS target plates containing the 96 spots for sample deposition are relatively small (53 mm by 41 mm), and the interspot distance is ca. 4 mm. Therefore, even though the act of spotting bacterial samples is simple, it must nonetheless be carefully performed to avoid such inversions. The data for inverted isolates were corrected prior to all statistical analyses.

DISCUSSION

MALDI-TOF MS has successfully been used for the identification of a wide array of bacterial and fungal species (6, 9, 12, 14, 19). However, apart from our own work, we are aware of only one study that has assessed the performance of MALDI-TOF MS-based identification under routine laboratory conditions; that study showed a rate of accurate identification to the species level of 84% (20).

In the present study, identification by MALDI-TOF MS yielded a valid score for 1,278 (93.2%) of 1,371 isolates identified by conventional methods. Among these valid results, the rate of matching at the species level was 95.1% (1,215/1,278), and 63 discordant results were identified.

Of these 63 discordant identifications, 7 were due to an error in the initial conventional identification. The 56 remaining discordant results were mainly due to inaccurate taxonomic assignment of a given spectra in the MALDI-TOF MS database, a recent change in the taxonomy of a given species, or different levels of precision in which a strain was identified by the conventional method versus the MALDI-TOF MS approach (Table 2). Discordant results were encountered only three times (three *Shigella* isolates misidentified as *E. coli*) due to the limit of resolution of the MALDI-TOF MS method. The taxonomical discordances might easily be corrected by an update of the database, by automatic correction by the integrated interpretative software, or by the clinical microbiologist. Thus, for all MALDI-TOF MS identifications yielding either *Enterobacter hormaechei*, *Enterobacter kobei*, or *Enterobacter asburiae*, it is sound to provide meaningful information to the clinician, i.e., *E. cloacae* in the present example (pending new data which might render a more precise identification relevant).

Sample preparation (i.e., direct application of the colony on the target plate) is simple and the teaching of laboratory technicians is straightforward, but the application must nonetheless be carefully performed. Indeed, we observed that the application of too much material results in a reduction in the quality of the results. Apart from the risk of isolate inversion due to the small interspot distance on the target plate, we also observed that when the sample is overlaid with the matrix solution, care must be taken not to induce a liquid smear between spots, which may result in cross contamination. This smearing phenomenon occurred more frequently when ground steel target plates were used than when polished steel target plates were used, which may therefore indicate that use of the latter may be more suitable for routine identification purposes.

The extraction technique itself required more hands-on time than direct deposition but always yielded excellent results. Typically, the extraction procedure for a single sample takes ca. 6 min, but the per sample processing time is reduced by batch processing. The quality of the spectra obtained by this technique is superior to the quality of the spectra obtained by direct deposition and therefore resulted in better scores. Our results show that protein extraction increases the overall yield of valid results by 25%. As an example, for the most common Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) pathogens encountered in our study, protein extraction was required in 21% and 26% of the cases, respectively. However, it should be performed only as a systematic first step for yeasts and colonies isolated from urine culture devices and/or MacConkey agar.

In addition to the identification of colonies grown on agar plates, MALDI-TOF MS also has the potential to directly identify pathogens in biological fluids, such as urine samples (13). In these cases, however, further studies are warranted to assess the sensitivity of the method as well as the ability of the method to identify mixed cultures. Indeed, in our experience, when mixed bacterial samples were analyzed by MALDI-TOF MS, either no reliable results (score < 1.7) were obtained or only one of the pathogens present in the mixture was detected (data not shown). Improvements in the sample preparation and detection algorithms used by the manufacturer might increase the sensitivity and specificity of detection. As an example, the identification of filamentous fungi is another important application, but it requires both method and database improvements that shall undoubtedly be addressed in further studies (for a review, see reference 19). As well, further work must be performed in order to standardize the MALDI-TOF MS protocols for identification under routine laboratory conditions, especially considering factors such as interlaboratory variability related to the use of instruments from different manufacturers (17, 24).

Since MALDI-TOF MS is a new technique that will undoubtedly change the way of functioning of microbiology laboratories, further prospective studies aimed at assessing its cost-effectiveness and time to results in comparison to those for conventional techniques will be required.

In conclusion, our study indicates that MALDI-TOF MS has the potential to replace conventional identification techniques for the majority of routine isolates in the clinical microbiology laboratory. The technique itself is simple to use, fast, and reliable. Further improvements in sample preparation and the availability of databases specifically designed for the identification of clinically significant strains will certainly improve

both the efficiency and the accuracy of MALDI-TOF MS in this setting.

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