Comparison of the Microflex LT and Vitek MS Systems for Routine Identification of Bacteria by Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry

Delphine Martiny,^a Laurent Busson,^a Ingrid Wybo,^b Rachid Ait El Haj,^a Anne Dediste,^a and Olivier Vandenberg^{a,c}

Department of Microbiology, Saint-Pierre University Hospital & Jules Bordet Institute, Brussels, Belgium^a; Department of Microbiology and Infection Control, Universitair Ziekenhuis Brussel, Vrije Universiteit Brussel, Brussels, Belgium^b; and Infectious Diseases Epidemiological Unit, Public Health School, Université Libre de Bruxelles, Brussels, Belgium^c

This study compared the performance of three matrix-assisted laser desorption ionization–time of flight mass spectrometry systems: Microflex LT (Bruker Daltonics, Bremen, Germany), Vitek MS RUO (Axima Assurance–Saramis database; bioMérieux, Marcy l’Etoile, France), and Vitek MS IVD (bioMérieux). A total of 1,129 isolates, including 1,003 routine isolates, 73 anaerobes, and 53 bacterial enteropathogens, were tested on the Microflex LT and Axima Assurance devices. The spectra were analyzed using three databases: Biotyper (Bruker Daltonics), Saramis, and Vitek MS (bioMérieux). Among the routine isolates requiring identification to the species level ($n = 986$), 92.7% and 93.2% were correctly identified by the Biotyper and Vitek MS databases, respectively. The Vitek MS database is more specific for the identification of *Streptococcus viridans*. For the anaerobes, the Biotyper database often identified *Fusobacterium* isolates to only the genus level, which is of low clinical significance, whereas 20% of the *Bacteroides* species were not identified or were misidentified by the Vitek MS database. For the enteropathogens, the poor discrimination between *Escherichia coli* and *Shigella* explains the high proportion of unidentified organisms. In contrast to the Biotyper database, the Vitek MS database properly discriminated all of the *Salmonella enterica* serovar Typhi isolates ($n = 5$). The performance of the Saramis database was globally poorer. In conclusion, for routine procedures, the Microflex LT and Vitek-MS systems are equally good choices in terms of analytical efficiency. Other factors, including price, work flow, and lab activity, will affect the choice of a system.

For decades, potentially pathogenic bacteria have routinely been identified in clinical laboratories using biochemical and phenotypic analyses, which usually require a time commitment ranging from a few hours to several days. Manual analyses are time-consuming, and semiautomated methods require large amounts of biological material, which can be a major disadvantage for the identification of fastidious microorganisms. Molecular methods have been demonstrated to have complementary value, but they are not practical for routine use due to their high cost.

The first studies regarding the identification of bacteria by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) were conducted toward the end of the 1990s (13). However, many years passed before the first commercial applications of this technology became available; this delay was due primarily to the lack of robust informatic tools and efficient databases. The technology was first made available as a research tool (20, 21), and it was commercialized for use in private and public laboratories in 2008.

An abundant collection of literature now highlights the many benefits that result from the use of this technology. Using only a small portion of a colony and a drop of matrix solution, MALDI-TOF MS can accurately identify bacteria within a few minutes at a moderate cost (25, 30, 31). Although the technique has proven to be valuable for the identification of common bacteria, numerous studies have recently shown that it is a promising tool that can also be used for species that are usually difficult to identify, such as yeasts, anaerobes, and fastidious microorganisms (2, 3, 12, 16, 17, 22, 32). The ability of MALDI-TOF MS to directly identify bacte-

ria in positive blood cultures also enhances the quality of patient management (11, 15, 19, 33, 34).

Therefore, MALDI-TOF MS is an important new technology and medical microbiologists are aware that this tool will revolutionize their practice and will soon replace most of the traditional identification methods (4, 6, 27).

In our laboratory, the Microflex LT system was first implemented as a diagnostic method for our *Campylobacter* National Reference Center activity (22). Since December 2010, it has been used as our main diagnostic method and has allowed us to routinely identify more than 90% of the bacterial isolates in our clinical samples.

Until April 2011, only Bruker Daltonics had commercialized a Conformité Européenne (CE)-marked MALDI-TOF MS system. In Europe, two manufacturers are now commercializing research use only (RUO) and *in vitro* diagnostic (IVD) MALDI-TOF MS systems.

The main aim of this study was to evaluate and compare the performance of these systems in terms of their analytical accuracy

Received 4 October 2011 Returned for modification 4 November 2011

Accepted 28 January 2012

Published ahead of print 8 February 2012

Address correspondence to Delphine Martiny, delphine_martiny@stpierre-bru.be.

Supplemental material for this article may be found at <http://jcm.asm.org/>.

Copyright © 2012, American Society for Microbiology. All Rights Reserved.

doi:10.1128/JCM.05971-11

FIG 1 Distribution of the origins of the 1,003 routine isolates tested in this study. RTI, respiratory tract infections; SSI, skin and soft tissue infections; LIQ, normally sterile body fluids; UTI, urinary tract infections; EPID, epidemiological samples (methicillin-resistant *S. aureus* and extended-spectrum β -lactamase screening); GEN, genital tract infections; BC, blood cultures.

and practicality to determine which system is the best option for use in a routine bacteriology laboratory.

MATERIALS AND METHODS

Population. The specimens tested were collected from hospitalized and ambulatory patients at the Jules Bordet Institute and Saint-Pierre University Hospital in Brussels, Belgium.

Bacterial isolates. From January to March 2011, a random selection of bacterial isolates that were recovered from multiple clinical sources, including wounds, urine, blood, tissue, biopsy specimens, and the respiratory tract, were prospectively included in this study. The distribution of the origins of the isolates is summarized in Fig. 1.

The samples were cultured using a variety of medium types that are traditionally used in our practice, including cystine lactose electrolyte-deficient, methicillin-resistant *Staphylococcus aureus*, Columbia, MacConkey, mannitol, and chocolate agars. For the two most frequently isolated species, the percentage of correct species identification was evaluated for each culture medium and is presented in Fig. 2.

All of the specimens were incubated overnight at $36 \pm 1^\circ\text{C}$.

In addition to this random selection, collection strains were added to ensure the coverage of anaerobes and enteric pathogens. The isolates of both panels were stored frozen prior to their identification.

The anaerobe panel, previously collected in a university hospital for a susceptibility survey, included 75 strains covering 28 species of 13 different genera (8). Although the anaerobes were of various origins, they were derived primarily from blood cultures and abdominal samples. After thawing, the isolates were subcultured twice on Schaedler 5% sheep blood agar (BD, Franklin Lakes, NJ) and incubated for 48 h at $36 \pm 1^\circ\text{C}$ under anaerobic conditions with an Anoxomat WS 80 device (Mart BV, Lichtenvoorde, The Netherlands). Two strains (one of *Campylobacter rectus* and one of *Clostridium septicum*) were not recovered from this panel (dead strains).

The enteropathogen panel included 53 strains that were received in the context of routine activities or as quality controls. This panel covered 20 species of 10 different genera. Most of the isolates were recovered from stool samples, with the exception of one *Aeromonas veronii* strain that was isolated from a rectal biopsy specimen. The strains were subcultured twice onto selective medium or Columbia agar, depending on the species.

Routine identification. Following Gram staining, the first identification method that was used was MALDI-TOF MS performed on an RUO Microflex LT (Bruker Daltonics). If the resulting identification was not included in the validation file (see the supplemental material) or the identification score was poor (<2), traditional methods such as biochemical tests, API, or the Vitek system (bioMérieux) were used. In cases of uncertain identification, molecular methods were also used (see the supplemental material). Anaerobes were previously identified by gas-liquid chromatographic analysis of their cellular fatty-acid composition using the microbial identification system; if needed, this procedure was complemented with biochemical and enzymatic tests. Enteropathogenic species were identified using biochemical and serological tests or commercial methods (API system, Vitek card). Moreover, most of the strains were submitted to national reference laboratories (see the supplemental material).

MALDI-TOF MS analysis. For the purposes of this study, all of the isolates were smeared in double deposit by the same operator for both the Microflex LT and Axima Assurance analyses. No extraction with formic acid was performed. The spectra acquired using the Microflex LT system were analyzed with the Biotyper spectral database. The spectra acquired with the Axima Assurance system were first analyzed with the Saramis database (Vitek MS RUO system) and thereafter with the Vitek MS database (Vitek MS IVD system).

Bruker Daltonics MALDI-TOF MS analysis. The “direct transfer” procedure recommended by the manufacturer was used to identify strains with the Microflex LT. A portion of a colony in the exponential growth phase was smeared onto a 96-well target plate, and after drying, it was covered using $1 \mu\text{l}$ of α -cyano-4-hydroxycinnamic acid (CHCA) matrix solution. When it was dry, the target plate was loaded into the machine,

FIG 2 Influence of the growth medium on the percentage of correct species identifications for the two most frequently isolated species. CAP, colistin aztreonam blood agar plate; CLED, cystine lactose electrolyte-deficient agar; COL, Columbia agar; ESBL, chromogenic screening plate for the detection of extended-spectrum β -lactamase-producing organisms; MAC, MacConkey agar; MAN, mannitol agar; MRSA, chromogenic screening plate for detection of methicillin-resistant *S. aureus*.

TABLE 1 MALDI-TOF MS results for 17 strains requiring identification only to genus level

ID ^a (no. [%] of isolates)	Saramis			Biotyper			Vitek MS		
	No. of correct genus IDs	No. without ID	No. misidentified	No. of correct genus IDs	No. without ID	No. misidentified	No. of correct genus IDs	No. without ID	No. misidentified
<i>Micrococcus</i> sp. (2 [11.8])	2	0	0	2	0	0	2	0	0
<i>Corynebacterium</i> sp. (5 [29.4])	1	4	0	3	2	0	2	3	0
<i>Actinomyces</i> sp. (1 [5.9])	0	1	0	1	0	0	0	1	0
<i>Pseudomonas</i> sp. (1 [5.9])	0	1	0	1	0	0	1	0	0
<i>Neisseria</i> sp. (1 [5.9])	0	1	0	1	0	0	1	0	0
<i>Chryseobacterium</i> sp. (1 [5.9])	1	0	0	1	0	0	1	0	0
Coagulase-negative <i>Staphylococcus</i> sp. (1 [5.9])	1	0	0	1	0	0	1	0	0
Beta-hemolytic group C, <i>G</i> <i>Streptococcus</i> sp. (5 [29.4])	3	2	0	5	0	0	5	0	0
Total (17) [100.0]	8 (47.1)	9 (52.9)	0 (0.0)	15 (88.2)	2 (11.8)	0 (0.0)	13 (76.5)	4 (23.5)	0 (0.0)

^a ID, identification.

which was equipped with a 337-nm nitrogen laser. The spectra were recorded in the linear mode in a mass range of 2 to 20 kDa and subsequently analyzed using MALDI Biotyper Automation Control and Biotyper 2.0 software. At the time, the database included 3,740 spectra from 319 genera and 1,946 species. This database exists as both an IVD and an RUO tool; however, only the latter system was used in this study. Because the database (Biotyper) is the same in both configurations, the results would have been the same if they had been derived from the IVD version. Both the anaerobic and enteropathogenic isolates were analyzed using the MALDI Biotyper Realtime Classification and Biotyper 3.0 software. Compared with the previous version, the Biotyper 3.0 software provides additional information on the isolate identification via the “matching hints” function. Some identification results are accompanied by a comment informing the user of the limitations of the technique (e.g., species that are difficult to discriminate, species included in the same bacterial complex or group, species for which additional tests are needed. . .). The complete list of matching hints is provided by the manufacturer upon request. At that time, the database included 3,995 entries. For some enteropathogens (*Vibrio*, *Yersinia*, *Salmonella*), the spectra were also compared with the security-relevant (SR) database (data not shown).

bioMérieux MALDI-TOF MS analyses. bioMérieux MALDI-TOF MS analyses were performed according to the manufacturer's instructions. The methodology was the same as for the Microflex LT system. Briefly, a portion of a fresh colony was smeared onto a FlexiMass disposable target plate and then immediately covered with 1 μ l of ready-to-use CHCA matrix solution. After drying, the target plate was loaded into the Axima Assurance mass spectrometer (Shimadzu Corporation, Kyoto, Japan). Spectra were generated using the Launchpad v2.8 software program and compared to the Saramis database (originally developed by Anagnos-Tec, Gölme, Germany), which contained reference spectra for 1,288 bacteria, 2 algae, and 258 fungi; it also contained SuperSpectra for 878 species. SuperSpectra are computed from typical strains covering more than 90% of the intraspecific diversity in most species. Reference spectra correspond to all of the spectra obtained with the different isolates included in Saramis. Each spectrum of a tested sample is matched against the SuperSpectra database. Peak matches that yield identification results with confidence values exceeding 80% are considered significant and displayed. The software also tests for homology to reference spectra using a compare mode in the case of a confidence value below 80%.

This Axima Assurance system with Saramis is now called Vitek MS RUO. In a second step, spectra were exported and sent to the manufacturer on a USB key device for blind analysis using the Vitek MS IVD v1 database. At the time, this database included more than 25,000 spectra covering 586 species.

Data analysis. The identification criteria were chosen according to the cutoffs proposed by the manufacturers. For Biotyper, identifications with scores above 2 and between 1.7 and 2 were considered to be reliable at the species and genus levels, respectively. Identification scores below 1.7 were considered unacceptable.

Saramis database results were evaluated according to a colored index: green for percentages equal to or above 90%, yellow for those between 85 and 89.9%, and white for those below 85%. All of the identifications to the genus or species level that fell into the green zone, with a score above 90%, were considered reliable. Scores between 80 and 90% were also considered for acceptable identifications. A cutoff of 90% was chosen for Vitek MS. However, in cases of “low discrimination,” two results are provided for the same isolate, sometimes with a score above 90%. In these cases, the identification was often considered reliable only at the genus level (if both results showed the same genus identification) or was included in a complex because an accurate species identification was not clinically needed, e.g., the *Enterobacter cloacae* complex (see the supplemental material). Any identification of bacteria belonging to this complex was considered correct according to the respective cutoff values of the systems.

Classification. According to the confidence levels of the three software programs and the previously determined identification, results obtained from the three databases were classified into the following categories: “correct identification of genus and species,” “correct identification of genus,” “no identification,” “misidentification at the species level,” and “misidentification at the genus level.” “Not identified” organisms included organisms that could not be identified by the technique. The organisms that were unreliably identified (either with an unacceptable score value or with a comment suggesting low genus discrimination, i.e., *Escherichia coli* versus *Shigella*) were also classified in this category, even if the identification was correct. For each isolate, the top score of the two separate spots was taken into account. The classification of the results obtained for routine isolates, anaerobes, and enteric pathogens are presented in summary Tables 1 to 4.

Discrepancies. The first response to a discrepancy was to repeat the analysis using both the Microflex LT and Vitek MS systems to eliminate the possibility of contamination. The remaining discrepancies were resolved by performing additional biochemical and molecular tests (see the supplemental material).

Statistical methods. McNemar's chi square or exact binomial test was used to compare the results obtained by both methods with the same samples. These results are presented in summary Table 5. A Fisher test was used to evaluate the impact of the medium used on the quality of the *E. coli* and *S. aureus* identifications, respectively.

TABLE 2 MALDI-TOF MS results for 986 strains requiring identification to species level

Genus and species/serovar	No. of isolates	% of isolates	Saramis					Biotyper
			No. with correct genus and species IDs	No. with correct genus ID	No. with no ID	No. misidentified to species level	No. misidentified to genus level	No. with correct genus and species IDs
<i>Achromobacter xylosoxidans</i>	1	0.1	0	0	1	0	0	0
<i>Acinetobacter baumannii</i>	5	0.5	3	0	2	0	0	5
<i>Citrobacter lwoffii</i>	1	0.1	0	0	1	0	0	0
<i>Citrobacter freundii</i>	2	0.2	0	2	0	0	0	2
<i>Citrobacter koseri</i>	9	0.9	0	7	2	0	0	9
<i>Corynebacterium pseudodiphtheriticum</i>	1	0.1	1	0	0	0	0	0
<i>Delftia acidovorans</i>	1	0.1	0	0	1	0	0	1
<i>Enterobacter aerogenes</i>	10	1.0	8	0	2	0	0	10
<i>Enterobacter cloacae</i>	38	3.9	15	1	22	0	0	38
<i>Enterobacter avium</i>	1	0.1	1	0	0	0	0	0
<i>Enterobacter faecalis</i>	48	4.9	44	0	4	0	0	46
<i>Enterobacter faecium</i>	16	1.6	15	0	1	0	0	15
<i>Escherichia coli</i>	338	34.5	302	0	36	0	0	329
<i>Gardnerella vaginalis</i>	1	0.1	1	0	0	0	0	0
<i>Haemophilus influenzae</i>	30	3.1	25	0	5	0	0	30
<i>Haemophilus parainfluenzae</i>	2	0.2	1	0	0	1	0	0
<i>Haemophilus parahaemolyticus</i>	1	0.1	1	0	0	0	0	1
<i>Hafnia alvei</i>	2	0.2	2	0	0	0	0	2
<i>Klebsiella oxytoca</i>	6	0.6	4	0	2	0	0	6
<i>Klebsiella pneumoniae</i>	37	3.8	27	0	10	0	0	32
<i>Moraxella catarrhalis</i>	9	0.9	4	0	5	0	0	9
<i>Morganella morganii</i>	4	0.4	4	0	0	0	0	4
<i>Proteus mirabilis</i>	27	2.8	27	0	0	0	0	27
<i>Proteus vulgaris</i>	1	0.1	0	1	0	0	0	1
<i>Providencia stuartii</i>	1	0.1	1	0	0	0	0	1
<i>Pseudomonas aeruginosa</i>	60	6.1	57	0	3	0	0	59
<i>Pseudomonas fluorescens</i>	1	0.1	0	0	1	0	0	0
<i>Roultella ornitholytica</i>	1	0.1	0	0	1	0	0	1
<i>Roultella planticola</i>	1	0.1	0	0	1	0	0	0
<i>Salmonella Typhi</i>	1	0.1	0	1	0	0	0	0
<i>Serratia liquefaciens</i>	1	0.1	1	0	0	0	0	1
<i>Serratia marcescens</i>	7	0.7	5	0	2	0	0	4
<i>Staphylococcus aureus</i>	183	18.7	183	0	0	0	0	180
<i>Staphylococcus capitis</i>	4	0.4	1	0	3	0	0	4
<i>Staphylococcus chromogenes</i>	1	0.1	0	0	1	0	0	0
<i>Staphylococcus cohnii</i>	2	0.2	0	0	2	0	0	0
<i>Staphylococcus epidermidis</i>	38	3.9	38	0	0	0	0	35
<i>Staphylococcus haemolyticus</i>	7	0.7	5	0	2	0	0	4
<i>Staphylococcus hominis</i>	7	0.7	7	0	0	0	0	5
<i>Staphylococcus lugdunensis</i>	2	0.2	2	0	0	0	0	2
<i>Staphylococcus saprophyticus</i>	1	0.1	1	0	0	0	0	0
<i>Staphylococcus schleiferi</i>	1	0.1	0	0	0	1	0	1
<i>Staphylococcus simulans</i>	1	0.1	0	0	1	0	0	1
<i>Stenotrophomonas maltophilia</i>	4	0.4	3	0	1	0	0	3
<i>Streptococcus agalactiae</i>	29	3.0	27	0	2	0	0	25
<i>Streptococcus anginosus</i>	7	0.7	0	6	1	0	0	6
<i>Streptococcus constellatus</i>	2	0.2	0	2	0	0	0	0
<i>Streptococcus gordonii</i>	1	0.1	1	0	0	0	0	1
<i>Streptococcus pyogenes</i>	8	0.8	5	0	3	0	0	8
<i>Streptococcus pneumoniae</i>	15	1.5	2	7	6	0	0	6
<i>Streptococcus porcinus</i>	1	0.1	0	0	1	0	0	0
<i>Streptococcus viridans</i> ^a	8	0.8	2	4	1	1	0	0
Total no. (%)	986	100.0	826 (83.8)	31 (3.1)	126 (12.8)	3 (0.3)	0 (0.0)	914 (92.7)

(Continued on next page)

TABLE 2 (Continued)

Biotyper				Vitek MS				
No. with correct genus ID	No. with no ID	No. misidentified to species level	No. misidentified to genus level	No. with correct genus and species IDs	No. with correct genus ID	No. with no ID	No. misidentified to species level	No. misidentified to genus level
1	0	0	0	0	1	0	0	0
0	0	0	0	5	0	0	0	0
0	1	0	0	0	0	1	0	0
0	0	0	0	1	0	1	0	0
0	0	0	0	7	0	2	0	0
0	0	1	0	1	0	0	0	0
0	0	0	0	0	0	1	0	0
0	0	0	0	9	0	1	0	0
0	0	0	0	34	0	4	0	0
0	1	0	0	1	0	0	0	0
1	1	0	0	45	0	3	0	0
1	0	0	0	16	0	0	0	0
4	5	0	0	333	0	4	0	1
0	1	0	0	1	0	0	0	0
0	0	0	0	30	0	0	0	0
0	0	2	0	0	0	1	1	0
0	0	0	0	1	0	0	0	0
0	0	0	0	2	0	0	0	0
0	0	0	0	5	0	1	0	0
1	4	0	0	24	0	13	0	0
0	0	0	0	9	0	0	0	0
0	0	0	0	4	0	0	0	0
0	0	0	0	27	0	0	0	0
0	0	0	0	0	1	0	0	0
0	0	0	0	1	0	0	0	0
0	1	0	0	58	0	1	0	1
0	0	1	0	1	0	0	0	0
0	0	0	0	0	0	1	0	0
0	0	1	0	0	0	1	0	0
1	0	0	0	0	1	0	0	0
0	0	0	0	1	0	0	0	0
0	0	3	0	7	0	0	0	0
2	1	0	0	177	0	6	0	0
0	0	0	0	3	0	1	0	0
0	1	0	0	0	0	1	0	0
0	2	0	0	2	0	0	0	0
3	0	0	0	34	0	4	0	0
1	2	0	0	6	0	1	0	0
2	0	0	0	6	0	1	0	0
0	0	0	0	2	0	0	0	0
1	0	0	0	1	0	0	0	0
0	0	0	0	0	0	0	1	0
0	0	0	0	1	0	0	0	0
1	0	0	0	2	0	2	0	0
3	1	0	0	27	0	2	0	0
1	0	0	0	6	0	1	0	0
1	1	0	0	2	0	0	0	0
0	0	0	0	1	0	0	0	0
0	0	0	0	7	1	0	0	0
1	8	0	0	12	0	2	1	0
1	0	0	0	1	0	0	0	0
2	2	4	0	6	0	1	1	0
28 (2.8)	32 (3.2)	12 (1.2)	0 (0.0)	919 (93.2)	4 (0.4)	57 (5.8)	4 (0.4)	2 (0.2)

^a Non-beta-hemolytic streptococci showing a resistance to optochin (no additional test was needed from a clinical perspective).

TABLE 3 MALDI-TOF MS results for 73 anaerobic strains^a

Genus and species	Saramis				Biotyper				Vitek MS			
	No. (%) of isolates	No. with correct genus and species IDs	No. with correct genus ID	No. with no ID	No. misidentified to species level	No. with correct genus and species IDs	No. with correct genus ID	No. with no ID	No. misidentified to species level	No. with correct genus and species IDs	No. with correct genus ID	No. with no ID
<i>Bacteroides caccae</i>	2 (2.7)	0	2	0	0	2	0	0	0	2	0	0
<i>Bacteroides fragilis</i>	14 (19.2)	3	0	11	0	14	0	0	0	14	0	0
<i>Bacteroides ovatus</i>	4 (5.5)	0	0	4	0	4	0	0	0	3	0	1
<i>Bacteroides thetaiotaomicron</i>	8 (11.0)	0	0	8	0	8	0	0	0	6	1	0
<i>Bacteroides vulgatus</i>	3 (4.1)	0	0	3	0	3	0	0	0	1	0	0
<i>Bacteroides xylanisolvens</i>	1 (1.4)	0	0	1	0	0	0	0	1	0	0	1
<i>Bifidobacterium wadsworthia</i>	1 (1.4)	0	0	1	0	0	1	0	0	0	0	0
<i>Campylobacter rectus</i>	1 (1.4)	0	0	1	0	0	1	0	0	0	0	1
<i>Clostridium clostridioforme</i>	2 (2.7)	0	0	2	0	1	1	0	0	0	0	1
<i>Clostridium perfringens</i>	2 (2.7)	2	0	0	0	2	0	0	0	2	0	0
<i>Clostridium ramosum</i>	1 (1.4)	1	0	0	0	0	0	1	0	1	0	0
<i>Eggerthella lenta</i>	4 (5.5)	0	0	4	0	2	0	2	0	4	0	0
<i>Fusobacterium necrophorum</i>	5 (6.8)	1	0	4	0	2	3	0	0	4	0	1
<i>Fusobacterium nucleatum</i>	5 (6.8)	0	0	5	0	0	3	2	0	5	0	0
<i>Fusobacterium varium</i>	2 (2.7)	0	0	2	0	0	2	0	0	2	0	0
<i>Parabacteroides distasonis</i>	2 (2.7)	0	0	2	0	2	0	0	0	0	0	2
<i>Parvimonas micra</i>	4 (5.5)	0	0	4	0	1	3	0	0	4	0	0
<i>Peptoniphilus harei</i>	1 (1.4)	0	0	1	0	1	0	0	0	0	0	1
<i>Peptoniphilus ivorii</i>	1 (1.4)	0	0	1	0	0	0	1	0	0	0	1
<i>Peptostreptococcus species</i>	1 (1.4)	0	0	1	0	0	0	1	0	0	0	1
<i>Prevotella bivia</i>	1 (1.4)	0	0	1	0	1	0	0	0	0	0	1
<i>Prevotella buccae</i>	2 (2.7)	0	0	2	0	2	0	0	0	2	0	0
<i>Prevotella denticola</i>	1 (1.4)	0	0	1	0	0	0	1	0	1	0	0
<i>Prevotella intermedia</i>	2 (2.7)	0	0	2	0	0	1	1	0	1	1	0
<i>Propionibacterium acnes</i>	1 (1.4)	0	0	1	0	0	0	1	0	1	0	0
<i>Veillonella parvula</i>	2 (2.7)	0	0	2	0	0	1	0	1	2	0	0
Total no. (%)	73 (100.0)	7 (9.6)	0 (0.0)	66 (90.4)	0 (0.0)	45 (61.6)	16 (21.9)	10 (13.7)	2 (2.7)	55 (75.3)	2 (2.7)	13 (17.8)
												1 (1.4)

^a Two dead strains (one *C. rectus*, one *C. septicum*).

TABLE 4 MALDI-TOF MS results for 53 enteropathogenic strains

Genus and species/serovar isolates	Saramis				Biotyper				Vitek MS			
	No. (%) of correct IDs	No. correct genus and species	No. correct ID	No. with no ID	No. misidentified to species level	No. misidentified to genus level	No. correct IDs	No. with correct genus ID	No. misidentified to species level	No. misidentified to genus level	No. correct IDs	No. with correct genus ID
<i>Aeromonas veronii</i>	1 (1.9)	0	0	1	0	0	0	1	0	0	1	0
<i>Acrobacter butzleri</i>	1 (1.9)	0	0	1	0	0	0	1	0	0	0	0
<i>Campylobacter coli</i>	3 (5.7)	0	0	3	0	0	3	0	0	0	0	0
<i>Campylobacter fetus</i>	1 (1.9)	1	0	0	0	0	1	0	0	0	0	0
<i>Campylobacter jejuni</i>	5 (0.4)	3	0	2	0	0	5	0	0	0	5	0
<i>Campylobacter lari</i>	2 (3.8)	0	0	2	0	0	2	0	0	0	2	0
<i>Clostridium difficile</i>	11 (20.8)	6	0	5	0	0	11	0	0	0	0	0
<i>Escherichia coli</i> O157 (nontoxigenic)	2 (3.8)	0	0	2	0	0	2	0	0	0	2	0
<i>Plesiomonas shigelloides</i>	1 (1.9)	1	0	0	0	0	0	1	0	0	0	0
<i>Salmonella</i> Darban	2 (3.8)	0	1	1	0	0	0	1	0	1	0	0
<i>Salmonella</i> Enteritidis	1 (1.9)	0	0	1	0	0	0	1	0	0	1	0
<i>Salmonella</i> Newport	1 (1.9)	0	0	1	0	0	0	0	0	0	0	0
<i>Salmonella</i> Saint-Paul	1 (1.9)	0	1	0	0	0	0	1	0	0	1	0
<i>Salmonella</i> Typhimurium	5 (9.4)	0	0	5	0	0	0	4	0	1	0	0
<i>Shigella boydii</i>	1 (1.9)	0	0	1	0	0	0	0	1 ^a	0	0	0
<i>Shigella flexneri</i>	4 (7.5)	0	0	3	0	1	0	0	4 ^a	0	0	0
<i>Shigella sonnei</i>	2 (3.8)	0	0	0	0	2	0	0	2 ^a	0	0	0
<i>Vibrio cholerae</i>	2 (3.8)	0	0	2	0	0	0	2	0	0	2	0
<i>Yersinia enterocolitica</i>	2 (3.8)	0	0	2	0	0	2	0	0	0	0	0
Total no. (%)	53 (100)	11 (20.8)	2 (3.8)	37 (70.0)	0 (0.0)	3 (5.7)	26 (49.1)	17 (32.1)	7 (13.2)	3 (5.7)	0 (0.0)	35 (66.0)

^a All *Shigella* species were misidentified as *E. coli* by both Vitek MS and Biotyper. However, a comment denouncing the poor discrimination between these organisms was provided. These isolates were thus classified as unidentified instead of misidentified.

TABLE 5 Comparison of the results obtained using the three systems

System and application (no. of isolates tested)	ID ^b	Saramis		Biotyper	
		% Correctly identified	P value	% Correctly identified	P value
Biotyper					
Routine (986) ^a	1	83.8 vs 92.7	<0.001	NA ^d	NA
	1 + 2	86.9 vs 95.5	<0.001	NA	NA
	3	12.8 vs 3.2	<0.001	NA	NA
	4	0.3 vs 1.2	<0.01	NA	NA
Enteric pathogens (53 [all])	1	20.8 vs 49.1	<0.001	NA	NA
Anaerobes (73 [all])	1	9.6 vs 61.6	<0.001	NA	NA
Vitek MS					
Routine (986) ^a	1	83.8 vs 93.2	<0.001	92.7 vs 93.2	0.608
	1 + 2	86.9 vs 93.6	<0.001	95.5 vs 93.6	<0.05
	3	12.8 vs 5.8	<0.001	3.2 vs 5.8	<0.01
	4	0.3 vs 0.4	1	1.2 vs 0.4	<0.05
<i>S. pneumoniae</i> (15)	1	ND ^c	ND	40.0 vs 80.0	0.070
<i>S. viridans</i> (8)	1	ND	ND	0 vs 75	<0.05
<i>S. pneumoniae</i> + <i>S. viridans</i> (23)	1	ND+	ND	26.1 vs 78.3	<0.01
All enteric pathogens (53)	1	20.8 vs 66.0	<0.001	49.1 vs 66.0	<0.01
	1 + 2	ND	ND	81.1 vs 84.9	0.625
	3	ND	ND	13.2 vs 15.1	1
	4	ND	ND	5.7 vs 0	0.25
<i>S. Typhi</i> (5)	1	ND	ND	0 vs 100	0.0625
All <i>Salmonella</i> spp. (15)	1	ND	ND	0 vs 33.3	0.0625
All anaerobes (73)	1	9.6 vs 75.3	<0.001	61.6 vs 75.3	0.068
	1 + 2	ND	ND	83.6 vs 78.1	0.522
	3	ND	ND	13.7 vs 17.8	0.491
	4	ND	ND	2.7 vs 2.7	1
<i>Bacteroides</i> species (32)	1	ND	ND	96.9 vs 78.1	<0.05
<i>Fusobacterium</i> species (12)	1	ND	ND	16.7 vs 91.7	<0.01
	1 + 2	ND	ND	58.3 vs 91.7	0.125

^a All isolates that needed to be identified to the species level.^b ID, identification; 1, correct ID of genus and species; 2, correct ID of genus; 3, no ID; 4, misidentification to species level.^c ND, not done.^d NA, no application.

Practical points. To compare the practical strengths and weaknesses of the three systems, we also considered the time required for the analysis. The time needed to identify 96 bacterial strains was evaluated for both the Microflex LT and Vitek MS RUO systems. This evaluation was conducted when the systems were working concurrently on the same strains.

Other practical considerations, including price, the difficulty of the method, the ease of use of the software, and the ability to use the device in a standard laboratory, were included in our comparison and are presented in summary Table 6. All of the practical considerations related to the use of the Vitek MS IVD system were assessed thanks to training organized in the research and development laboratory of the manufacturer.

RESULTS

Among the 1,003 routine isolates included in the study, which included 52 bacterial species of 27 different genera, 986 were identified to the species level for clinical purposes. For the 17 remaining organisms, identification to the genus level was sufficiently informative for our routine practice. Most of the latter organisms were considered to be contaminants (e.g., corynebacteria, micrococci, and *Pseudomonas* spp. other than *Pseudomonas aeruginosa*).

The results obtained from the three databases (Biotyper, Saramis, and Vitek MS) for the strains requiring identification to the genus or species level are presented in Tables 1 and 2, respectively.

For a comparison of the results obtained by the three systems, see Table 5.

For the bacterial strains that are routinely identified to the genus level ($n = 17$), 88.2%, 76.5% and 47.1% of the isolates were correctly identified by the Biotyper, Vitek MS, and Saramis databases, respectively. Most of the corynebacteria were not identified by the Saramis and Vitek MS systems; this was also the case for one *Actinomyces* species, which was recognized by the Biotyper system. The percentages of strains remaining unidentified by the Biotyper, Vitek MS, and Saramis systems were 11.8%, 23.5%, and 52.9%, respectively. No invalid identifications were observed.

Among the 986 isolates that are routinely identified to the species level, 92.7%, 93.2%, and 83.8% (cutoff at 90%) were correctly identified to the species level by the Biotyper, Vitek MS, and Saramis systems, respectively. The rates of correct species identification by the Biotyper and Vitek MS databases were similar ($P = 0.608$), whereas that of the Saramis database was significantly poorer ($P < 0.001$). Only two false identifications at the genus level were observed, and both occurred with the Vitek MS system; one *E. coli* isolate was identified as *Klebsiella oxytoca*, and *P. aeruginosa* from a respiratory sample was identified as *Neisseria subflava*.

TABLE 6 Comparison of practical parameters related to the use of the three systems

Property	Microflex LT	Vitek MS RUO	Vitek MS IVD	Remarks
User friendliness				
Ready-to use Matrix solution	No	Yes	Yes	For Vitek-MS systems, matrix solution must be deposited each two spots
Facility of preparing smear	Very easy	Easy	Easy	
Disposable targets	Yes	Yes	Yes	
Reusable targets	Yes	No	No	
Software	Easy to use	Not easy to use	Very easy to use	
Time for 96 identifications				
Time to prepare work list (min)	<5	5–10	ND ^a	
Time to load target and make vacuum	2	5		
Time for analysis (min)	40	55		
Time for 16 identifications (min)	ND	ND	15	No ID before success of QC at end of run (each 16 IDs)
Quality				
IVD	Yes	No	Yes	Need for validation before clinical reporting
RUO	Yes	Yes	No	
Quality management	Easy	Easy	Very easy	
Cost ^c				
Device	+	NA ^b	++	Based on catalog prices
Reactants	+++	NA	+	
Maintenance	++	NA	+++	
Implementation				
Noise	Silent	Noisy	Noisy	
Size	Smaller	Bulkier	Bulkier	
Connectivity	Via LIS	NA	Via Myla	
Capacity	1 × 96	4 × 48	4 × 48	

^a ND, not done.^b NA, data not available.^c Prices increasing with the number of crosses.

Both errors likely resulted from contamination with other strains that were present in the same sample. The rate of false identification to the species level was 1.2% using the Biotyper system and lower than 1% for the other systems ($P < 0.05$).

Most of the errors that were observed with the Biotyper system resulted from poor discrimination between species inside the *Serratia* and *Haemophilus* genera and from Bruker Daltonics policy of avoiding false negatives for *Streptococcus pneumoniae*. As described by the manufacturer, *S. pneumoniae* strains are not misidentified by the Biotyper system, but some *Streptococcus mitis/oralis* species are erroneously identified as *S. pneumoniae* due to the poor discrimination between these related species. Considering the identification of *Streptococcus viridans*, the Vitek MS database led to better results ($n = 8$, $P < 0.05$). The identification of *S. pneumoniae* was not significantly different using both databases ($n = 15$, $P = 0.07$) and one of these isolates was erroneously identified as *S. mitis/oralis* by the Vitek MS database.

At a cutoff of 90% (Vitek MS, Saramis) or a score above 1.7 (Biotyper), the percentage of unidentified organisms was 12.8% using the Saramis system and 5.8% using the Vitek MS system. With only 3.2% of the organisms unidentified, the Biotyper database was significantly better ($P < 0.01$). An additional 65/986 isolates (6.6%) were correctly identified by the Saramis database with scores between 80 and 90%, which left the percentage of unidentified organisms at 6.16% when the lower cutoff (80%) was used.

E. coli ($n = 338$) and *S. aureus* ($n = 183$) were the most fre-

quently isolated species. The rate of correct species identification ranged from 94.4% (Columbia agar, Biotyper database) to 100% and from 95.5% (Columbia agar, Vitek MS database) to 100% for *E. coli* and *S. aureus*, respectively (Fig. 2). The Fisher test indicated that the quality of the identification was not related to the choice of the culture medium.

The anaerobes identified by the three databases are presented in Table 3. The Biotyper and Vitek MS databases correctly identified 61.6% and 75.3% of the isolates, respectively, to the species level ($P = 0.068$). Most of the *Fusobacterium* and *Parvimonas* isolates were only identified to the genus level using the Biotyper database. Considering the species identification of *Fusobacterium* isolates, the Vitek MS was significantly better than the Biotyper database ($P < 0.01$) but this difference disappeared when the isolates correctly identified to the genus level were included ($P = 0.125$). In contrast, *Bacteroides* species were identified more accurately by the Biotyper database than by the Vitek MS database ($P < 0.05$). Only one genus error was observed (Vitek MS), and it was probably due to a contaminant. Among the 73 cultured isolates, more than 90% remained unidentified at the SuperSpectra level using the Saramis database. This proportion was slightly improved by using a lower cutoff of 80% (84.9% versus 90.4%). The other two databases yielded similar proportions of unidentified isolates (13.7% and 17.8% for the Biotyper and Vitek MS databases, respectively; $P = 0.491$).

The enteric pathogens identified by the three databases are described in Table 4. As expected, all of the *Shigella* strains were

identified as *E. coli*. Both the Biotyper and Vitek MS databases misidentified the *Shigella* isolates; however, the identification results were accompanied by a comment explaining the inability to discriminate between these two genera. This poor discrimination resulted in high percentages of isolates unidentified by those databases (13.2 and 15.1% by the Biotyper and Vitek MS databases, respectively; $P = 1$). Similar comments were also provided by the Biotyper system for the identification of several *Salmonella* isolates. For these pathogens, the Vitek MS database allowed the correct discrimination of *Salmonella enterica* serovar Typhi ($n = 5$). This difference was not statistically significant, but additional studies including a larger number of isolates are needed. This difference resulted in a 5.7% false serotype identification rate by the Biotyper database versus no false identifications by the Vitek MS database ($P = 0.25$). No error was observed at the genus level with either the Biotyper or the Vitek MS system. Again, the Saramis database yielded poorer results; nearly 70% of the isolates were unidentified at the SuperSpectra level ($P < 0.001$). This database allowed the correct identification of only 20% of the isolates. An additional 15% were correctly identified when the cutoff was lowered to 80%.

The use of the Bruker complementary SR database, which includes *Vibrio cholerae*, *S. Typhi*, and *Yersinia pestis*, did not improve the discrimination of the *Salmonella* and *Vibrio* species. No confusion between *Y. enterocolitica* and *Y. pestis* was observed with this database. Actually, using only the SR database, *Y. enterocolitica* was identified as *Y. pestis* but with an unreliable score value (data not shown).

The Microflex LT, Vitek MS RUO (Saramis), and Vitek MS IVD systems were also compared in terms of practicality. The main observations are summarized in Table 6.

The preparation of the deposits and the analyses were similar for all of the systems. With the Microflex LT, the matrix solution is not immediately ready to use; a few minutes is required to rehydrate the lyophilized CHCA. In cases of bad preparation, losses may occur. However, the matrix solution is deposited on dried bacterial smears, which allows the technologist to prepare the entire batch of isolates and then add the matrix solution at the end. With both of the other systems, the matrix solution must be applied immediately after the bacterial smear, which is more time-consuming. Bruker Daltonics offers both single-use and reusable targets, whereas bioMérieux offers only single-use targets.

The Vitek MS IVD system seemed to have more-user-friendly software. With several windows needing to be open on a single screen, the AXIMA Launch Pad software for the Vitek MS RUO system was probably the most difficult to use.

The Microflex LT identifies an entire target plate (96 isolates) in approximately 45 min, whereas the Vitek MS RUO system requires more than 1 h. With the Vitek MS IVD system, which uses the same AXIMA Assurance mass spectrometer, the results are displayed by acquisition group (containing up to 16 samples) and no identification is available before the end of the run (including the quality controls). In the case of poor quality control, the run must be repeated with new deposits and no identification is provided.

In terms of the quality management parameters, both the Vitek MS IVD and Biotyper databases are CE marked. Both manufacturers offer RUO versions that require validation by the laboratory before the data can be reported to clinicians. The Vitek MS RUO (Saramis database) is provided by bioMérieux, whereas Bruker

Daltonics offers a research tool that includes the same database as the IVD system (Biotyper database). This manufacturer also offers the possibility of combining both the IVD and RUO versions on the same device. The Vitek MS IVD system has a more highly developed quality management system because it contains dedicated positions for quality controls and a well-defined traceability system.

Because prices and reimbursement conditions may differ between countries and laboratories, an accurate cost analysis has not been done. However, according to the prevailing catalog prices and the information that was obtained from the sales organizations in Belgium, a global evaluation was conducted. The Vitek MS IVD system, including Myla middleware, is more expensive than the Microflex LT system, but the reagents are cheaper. This factor may be offset by the availability of the reusable Biotyper targets. Both manufacturers offer several maintenance packages. In Belgium, the Vitek MS “omnium” is more expensive than the Bruker Daltonics “all in” package, but it includes a backup machine in case of system breakdown.

Both the Vitek MS IVD and IVD Microflex LT systems permit connections to other technologies via either a laboratory information system (Bruker Daltonics) or a proprietary middleware solution called Myla (bioMérieux). Therefore, identifications may be transmitted to other devices for the management of susceptibility testing.

Finally, the Vitek-MS system is bulkier and noisier than the Microflex LT system, which can be installed on a table. However, the Vitek-MS system allows the analysis of four target plates of 48 spots each in a single run versus one plate of 96 spots for the Microflex LT system.

DISCUSSION

MALDI-TOF MS represents a major revolution in the practice of bacteriology in clinical microbiology laboratories (5, 7, 9, 24, 30).

Currently, two companies offer such devices in Europe: Bruker Daltonics and bioMérieux. The aim of the present study was to identify the best option for implementing MALDI-TOF MS in a routine laboratory. To our knowledge, this is the first study to evaluate both IVD systems under routine conditions. Additional research tools were also evaluated in this study.

For isolates requiring identification to the species level ($n = 986$), the analytical sensitivities of the two IVD systems were similar (92.7% and 93.2% correct species identifications by the Biotyper and Vitek MS systems, respectively [$P = 0.608$]). A lower percentage of correct identifications to the species level was obtained using the Saramis database (83.8% [$P < 0.001$]).

Similarly, Cherkaoui et al. obtained better performance using the Biotyper database (88.8% versus 94.4% for the Saramis and Biotyper databases, respectively, with a cutoff of 70% for Saramis) (10). The lower performance of the Saramis database observed in our study than that previously reported may be partially explained by the presence of numerous unidentified isolates and the defined identification criteria that were used. Indeed, in the present study, the colored index was considered to be a cutoff but the manufacturer also suggests that a score of over 80%, rather than 90%, is consistent with highly accurate identification. If this new cutoff had been used, half of the unidentified organisms would have been added to the “correctly identified to the species level” category.

A total of 30% of the isolates unidentified by the Vitek MS system and the Biotyper database were *E. coli* or *Klebsiella*. This result may be explained by the morphology of the colonies be-

cause mucus can lead to thicker smears and generate incorrect identification results.

Most of the discrepancies were resolved by reanalyzing the strains, and they were found to be caused by contamination from the concomitant growth of other organisms in the sample (see the supplemental material). However, even when analyses are performed by the same operator, errors may occur. This suggests that a major disadvantage of the technology results from one of its primary assets: a small amount of biological material is required for identification. This fact must be considered carefully when susceptibility testing is performed as a second step.

In several cases, the traditional methods were responsible for the discrepancy, which suggests that MALDI-TOF MS may soon replace certain conventional and limited techniques (see the supplemental material). Similar conclusions have already been reported by several authors who have compared the performance of MALDI-TOF MS with that of conventional microbiological techniques (4, 6, 27).

The other discrepancies were related to the databases and/or known limitations of the MALDI-TOF MS technique.

The accuracy of the Biotyper database was found to be lower than that of the Saramis and Vitek MS databases. A third of the false identifications arose from Bruker Daltonics policy of avoiding false negatives for *S. pneumoniae*. Currently, MALDI-TOF MS cannot correctly discriminate between *S. mitis/oralis* and *S. pneumoniae*. The Saramis system has solved this problem by identifying strains as “*mitis/oralis/pneumoniae*” streptococci. This solution has led to fewer errors, but it is neither efficient nor satisfactory in routine practice because an optochin test is still needed to establish a definitive diagnosis (25). Our results suggest, however, that the Vitek MS IVD system is more specific than the other systems for the identification of *S. viridans* ($P < 0.05$).

Another source of errors for the Biotyper database was the discrimination of species within the genus *Serratia*. Species identification errors also occurred for *Staphylococcus schleiferi* when the spectrum was analyzed using the Vitek MS and Saramis databases, and all of the systems produced false results when identifying one or both of the included strains of *Haemophilus parainfluenzae*. Updates to the databases may solve the difficulties with such species.

In contrast to the lower accuracy of the Biotyper database, the Vitek MS system led to a higher rate of unidentified isolates ($n = 32$ and $n = 57$ for the Biotyper and Vitek MS databases, respectively; $P < 0.01$).

For *E. coli* and *S. aureus*, which represent more than 50% of the isolates in our routine practice (521/1003), the growth medium used does not influence the quality of the identification by mass spectrometry (Fig. 2). However, a broader study should be conducted in order to evaluate the culture agar impact on mass spectrometric identification of other bacterial species.

MALDI-TOF MS seems to be less effective for the identification of anaerobes than for aerobic organisms. In a recent comparison of the Saramis and Biotyper databases, Veloo et al. found that the Biotyper database performed better when identifying species of the *B. fragilis* group, but they also noted that the analysis of Gram-positive cocci with this technology required a time-consuming extraction step to obtain the same results as those obtained using the Saramis database and direct deposit (32). In the present study, the results obtained with the Saramis database were unsatisfactory. This major difference probably resulted from the

different correct-identification cutoffs adopted in the two studies. However, the Biotyper database performed similarly in the identification of anaerobic organisms in both studies. Comparing the Biotyper and Vitek MS systems, the Biotyper database allowed better identification of *Bacteroides* species ($P < 0.05$). The Vitek MS database showed better results for *Fusobacterium* species ($P < 0.01$); however, this difference should not impact clinical management. Indeed, the Gram coloration of these species is often sufficiently informative for their identification. Moreover, the identification of these organisms to the genus level was not statistically significantly different using both databases ($P = 0.125$).

Although mass spectrometry is not competitive as a routine identification technique for anaerobes, it could be helpful in conjunction with conventional tests. In several cases, an extraction procedure would also improve the quality of the identification (18). Future expansion of the databases will likely improve the performance of this technique for anaerobe identification.

MALDI-TOF MS technology is a powerful tool that can be used in routine laboratories for the diagnosis of enteric diseases. It is particularly useful for the rapid discrimination of normal flora from potential pathogens that are isolated from stool samples. For pathogen identification itself, the limitations of MALDI-TOF MS must be considered. Initially, the identification of *Shigella* or *E. coli* will still require additional tests according to the nature of the sample. Second, biochemical and serological tests will still be required to accurately identify *Salmonella* species. Additional studies should be conducted in order to evaluate the ability of the Vitek MS database to differentiate *S. Typhi* from other *Salmonella* serotypes; this is indeed of major interest from both the clinical management and public health perspectives. Even when the SR database is used in parallel, the Biotyper database cannot discriminate these serotypes. Compared to the Biotyper database, the Vitek MS system showed better identification of enteric pathogens to the species level ($P < 0.01$). However, the difference between the two databases did not remain when the rate of correct genus identification was taken into account ($P = 0.625$) and this panel included nonconsecutive clinical isolates. This explains the high number of *S. Typhi* and *V. cholerae*, species that are usually less frequently encountered in our routine practice.

These results suggest that the major factors that influence the quality of MALDI-TOF MS identifications are the purity of the strain, the amount of biological material smeared onto the target plate, and the experience of the technologist. Indeed, no major differences were observed in the analytical performance of the Biotyper and Vitek MS databases for the identification of most routine isolates. The distinction of *Bacteroides* species by the Biotyper database and that of *S. viridans*, *Fusobacterium*, and enteric pathogen species by the Vitek MS system are actually the only differences that were observed between the IVD databases. Clinically, the distinction of *Bacteroides* species is probably the only significant difference between the systems. Indeed, the identification of *Fusobacterium* to the genus level is usually informative enough and an additional optochin test must still be performed in order to discriminate *S. pneumoniae* and *S. viridans*. However, the potential of the Vitek MS database to differentiate *S. Typhi* from other *Salmonella* species requires further investigation.

Applied to the routine data set ($n = 986$), the matching hints included in the Biotyper 3.0 software would have led to a modified result classification. According to our routine algorithm (and considering the low incidence of *Shigella* in samples other than stool

samples), the matching hint related to the *E. coli-Shigella* discrimination is not taken into account when enteropathogenic bacteria are not suspected. The reclassification of *S. viridans* isolates would have affected our conclusion in terms of the accuracy of the systems (no longer any difference among the three databases), whereas other modifications (e.g., *Citrobacter* and *Acinetobacter* isolate classification) would not have had a significant impact in the present study.

The Saramis database was weaker than the others, particularly for anaerobes and enteric pathogens. The use of a lower cutoff and an updated database would improve this performance in the future.

The main limitation of this study was the absence of yeasts and mycobacterial isolates. The ability of MALDI-TOF MS to identify yeasts and fungi has been demonstrated many times (1, 14, 23, 26, 29). In a large comparison, Bader et al. found no significant difference between the Saramis and Biotyper databases when identifying yeasts (2). Additional studies are still needed to determine the efficacy of MALDI-TOF MS for the identification of mycobacteria, but a recent publication by Saleeb et al. has shown encouraging results (28). To our knowledge, no study has evaluated the performance of the Vitek MS system with these organisms or with routine isolates.

Because labor shortages and financial constraints have forced clinical microbiology laboratories to use their available resources more efficiently, we also analyzed the practical performance of the three systems.

The first question that must be answered regarding the acquisition of a MALDI-TOF spectrometer is whether it is intended for research or only for routine laboratory use. In the former case, an RUO system will be necessary, and the Microflex LT would be the preferred option because of its improved analytical performance and user-friendly software. Moreover, this system may be coupled with the IVD version for routine use. The ability to combine the IVD and RUO systems is certainly a major advantage for laboratories that want to develop their own database and also use their spectrometer as a research tool. Choosing an RUO-only system requires the creation of a larger, more robust validation file.

Because of their similar analytical performance, the choice between the IVD Microflex LT and Vitek MS IVD systems for routine use only depends primarily on practical issues such as price and integration into the lab.

As previously described, no major differences in the sample preparation requirements were observed and both of the systems have strengths and weaknesses.

Because of its strict quality control, the Vitek MS IVD system will likely simplify laboratory quality management, which may constitute a major advantage of this system. However, this strict control will certainly delay the acquisition of identifications, and it may increase the final cost of the analysis by unnecessarily requiring a repeated series or, indirectly, through the loss of unused positions on the target.

The installation of a MALDI-TOF MS platform in a laboratory requires work flow management to avoid waiting for the identification work station. From this perspective, the larger load capacity of the Vitek MS IVD system would be an advantage. Finally, both systems can be easily implemented in a routine laboratory and the customer service that is provided by both manufacturers is satisfactory.

Conclusion. The design and maintenance of robust and effi-

cient MALDI-TOF MS systems are new challenges for manufacturers. Databases must be dynamic, software must be easy to use, and the entire system must meet quality standards. If the system is to be used for nonroutine research purposes, the combined IVD/RUO Microflex LT is certainly the ideal system. For routine purposes, however, the Microflex LT and Vitek MS IVD systems both offer good analytical performance. Both the specific sales conditions and the work flow of each lab will be decisive factors in the choice between the Bruker Daltonics and bioMérieux products.

ACKNOWLEDGMENTS

This work was partially sponsored by bioMérieux, Marcy l'Etoile, France.

We thank Sonia Chatellier, bioMérieux, for her assistance during this study. We also thank Michèle Dramaix, School of Public Health, Université Libre de Bruxelles, for her help in the statistical analysis process.

REFERENCES

1. Alanio A, et al. 2011. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry for fast and accurate identification of clinically relevant *Aspergillus* species. *Clin. Microbiol. Infect.* 17:750–755.
2. Bader O, et al. 2011. Improved clinical laboratory identification of human pathogenic yeasts by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. *Clin. Microbiol. Infect.* 17:1359–1365.
3. Barbuddhe SB, et al. 2008. Rapid identification and typing of *Listeria* species by matrix-assisted laser desorption ionization–time of flight mass spectrometry. *Appl. Environ. Microbiol.* 74:5402–5407.
4. Benagli C, Rossi V, Dolin 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.
5. Bizzini A, Durussel C, Bille J, Greub G, Prod'homme 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. Bizzini A, et al. 2011. Matrix-assisted laser desorption ionization-time of flight mass spectrometry as an alternative to 16S rRNA gene sequencing for identification of difficult-to-identify bacterial strains. *J. Clin. Microbiol.* 49:693–696.
7. Bizzini A, Greub G. 2010. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry, a revolution in clinical microbial identification. *Clin. Microbiol. Infect.* 16:1614–1619.
8. Blairon L, et al. 2010. Vitek 2 ANC Card versus BBL Crystal Anaerobe and RapID ANA II for identification of clinical anaerobic bacteria. *Anaerobe* 16:355–361.
9. Carbone E, et al. 2011. MALDI-TOF mass spectrometry tools for bacterial identification in clinical microbiology laboratory. *Clin. Biochem.* 44:104–109.
10. 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.
11. Christner M, et al. 2010. Rapid identification of bacteria from positive blood culture bottles by use of matrix-assisted laser desorption-ionization time of flight mass spectrometry fingerprinting. *J. Clin. Microbiol.* 48:1584–1591.
12. Couturier MR, Mehinovic E, Croft AC, Fisher MA. 2011. Identification of HACEK clinical isolates by matrix-assisted laser desorption ionization-time of flight mass spectrometry. *J. Clin. Microbiol.* 49:1104–1106.
13. Demirev PA, Ho Y-P, Ryzhov V, Fenselau C. 1999. Microorganisms identification by mass spectrometry and protein database searches. *Anal. Chem.* 71:2732–2738.
14. Dhiman N, Hall L, Wohlfel SL, Buckwalter SP, Wengenack NL. 2011. Performance and cost analysis of MALDI-TOF mass spectrometry for routine identification of yeast. *J. Clin. Microbiol.* 49:1614–1616.
15. Drancourt M. 2010. Detection of microorganisms 304 in blood specimens using matrix-assisted laser desorption ionization time-of-flight mass spectrometry: a review. *Clin. Microbiol. Infect.* 16:1620–1625.
16. Ferreira L, et al. 2010. Identification of *Brucella* by MALDI-TOF mass spectrometry. Fast and reliable identification from agar plates and blood cultures. *PLoS One* 5:e14235.
17. Gaia V, Casati S, Tonolla M. 2011. Rapid identification of *Legionella*

- spp. by MALDI-TOF MS based protein mass fingerprinting. *Syst. Appl. Microbiol.* 34:40–44.
18. 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.
19. Kaleta EJ, et al. 2011. Comparative analysis of PCR-electrospray ionization/mass spectrometry (MS) and MALDI-TOF/MS for the identification of bacteria and yeast from positive blood culture bottles. *Clin. Chem.* 57:1057–1067.
20. Keys CJ, et al. 2004. Compilation of a MALDI-TOF mass spectral database for the rapid screening and characterization of bacteria implicated in human infectious diseases. *Infect. Genet. Evol.* 4:221–242.
21. Leuschner RGK, Beresford-Jones N, Robinson C. 2004. Difference and consensus of whole cell *Salmonella enterica* subsp. *enterica* serovars matrix-assisted laser desorption/ionization time-of-flight mass spectrometry spectra. *Lett. Appl. Microbiol.* 38:24–31.
22. Martiny D, et al. 2011. Accuracy of the API Campy system, the Vitek 2 *Neisseria-Haemophilus* (NH) Card and the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) for the identification of *Campylobacter* and related organisms. *Clin. Microbiol. Infect.* 17:1001–1006.
23. McTaggart LR, et al. 2011. Rapid identification of *Cryptococcus neoformans* and *Cryptococcus gattii* by matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry. *J. Clin. Microbiol.* 49:3050–3053.
24. Mellmann A, et al. 2009. High interlaboratory reproducibility of matrix-assisted laser desorption/ionization–time of flight mass spectrometry-based species identification of nonfermenting bacteria. *J. Clin. Microbiol.* 47:3732–3734.
25. 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.
26. Putignani L, et al. 2011. MALDI-TOF mass spectrometry proteomic phenotyping of clinically relevant fungi. *Mol. Biosyst.* 7:620–629.
27. Saffert RT, et al. 2011. Comparison of Bruker Biotyper matrix-assisted laser desorption/ionization–time of flight mass spectrometer to BD Phoenix automated microbiology system for identification of Gram-negative bacilli. *J. Clin. Microbiol.* 49:887–892.
28. Saleeb PG, Drake SK, Murray PR, Zelazny AN. 2011. Identification of mycobacteria in solid-culture media by matrix-assisted laser desorption/ionization–time of flight mass spectrometry. *J. Clin. Microbiol.* 49:1790–1794.
29. Santos C, Paterson RMM, Venâncio A, Lima N. 2010. Filamentous fungal characterizations by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *J. Appl. Microbiol.* 108:375–385.
30. 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.
31. van Veen SQ, Claas ECJ, 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.
32. Veloo ACM, Knoester M, Degener JE, Kuijper EJ. 2011. Comparison of two matrix-assisted laser desorption/ionization–time of flight mass spectrometry methods for the identification of clinically relevant anaerobic bacteria. *Clin. Microbiol. Infect.* 17:1501–1506.
33. Wybo I, et al. 2011. Differentiation of *cfiA*-negative and *cfiA*-positive *Bacteroides fragilis* isolates by matrix-assisted laser desorption/ionization–time of flight mass spectrometry. *J. Clin. Microbiol.* 49:1961–1964.
34. Yan Y, et al. 2011. Improved identification of yeast species directly from positive blood culture medium by combining Sepsityper specimen processing and Microflex analysis with the matrix-assisted laser desorption/ionization Biotyper system. *J. Clin. Microbiol.* 49:2528–2532.