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Biomedical Mass Spectrometry in Today's and Tomorrow's Clinical Microbiology Laboratories

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Clinical microbiology is a conservative laboratory exercise where base technologies introduced in the 19th century remained essentially unaltered. High-tech mass spectrometry (MS) has changed that. Within a few years following its adaptation to microbiological diagnostics, MS has been introduced, embraced, and broadly accepted by clinical microbiology laboratories throughout the world as an innovative tool for definitive bacterial species identification. Herein, we review the current state of the art with respect to this exciting new technology and discuss potential future applications.

In a minireview published in this journal in 2006, Alvin Fox concluded that biomedical mass spectrometry was still "daunting to many in the clinical microbiology community." Fox hoped that his review would support the acceptance of biophysical diagnostics in general (13). Now, only 6 years later, matrix-assisted laser desorption/ionization–time of flight mass spectrometry (MALDI-TOF MS) has been embraced by the clinical microbiology community as the next-generation tool for definitive species identification of a variety of microorganisms (6, 23, 28).

MALDI-TOF MS is an analytical method suited for the fast and precise assessment of the mass of molecules in a range of 100 Da to 100 kDa. Conceptually, the method is simple: biomolecules such as proteins are embedded in crystals of a so-called matrix (low-molecular-weight organic acids, usually including an aromatic group). By absorbing the energy of a short laser pulse, the molecules are desorbed and ionized by charge transfer. The ionized molecules are then accelerated in an electrical field and eventually collide with a detector at the end of the flight tube. The ions are separated according to their mass-to-charge ratio (m/z), with a shorter TOF for the smaller ions. This cycle is repeated at a frequency of 50 or more hertz, which leads to series of molecular detections that are accumulated into a mass spectrum. MALDI-TOF MS allows for simultaneous detection of multiple ion species without a preceding separation step (e.g., liquid chromatography), rendering it suitable for whole-cell MS. For the identification of microorganisms by MALDI-TOF MS, 10^5 to 10^6 fresh cells, representing a fraction of a colony on an agar plate, are generally sufficient. The current article will be limited to a description and discussion of the use of the technology, not its intrinsic capacities.

Mass spectrometers are continuously being refined: their sensitivity is said to increase significantly with each succeeding generation. The current MALDI-TOF MS equipment is suited for the detection of essentially all proteins and (amplified [sections of]) DNA molecules. However, for future analytical applications, more sensitive and specific systems will be required. Such systems include surface-enhanced laser desorption/ionization-TOF (SELDI-TOF) MS, liquid chromatography coupled to electrospray ionization triple quadrupole (LC-ESI-QqQ) MS, or LC coupled to ESI-Q-TOF (LC-ESI-Q-TOF MS). LC-ESI-QqQ in selected or multiple-reaction mode (SRM or MRM) has been used for routine detection of small molecules, including metabolites and drugs. More recently, LC-ESI-QqQ has been suggested as a replacement for classical enzyme-linked immunosorbent assays (ELISAs) for the quantitation of proteins in complex matrices (12). These MS technologies are already used extensively in research settings. It will only be a matter of time before they are introduced in routine clinical microbiology laboratories. It is noteworthy that the most popular routine diagnostic procedure to date, MALDI-TOF MS, was developed 2 decades ago to facilitate the analysis of solid compounds next to the customary volatile compounds (29). Table 1 compares some of the characteristics of LC-ESI-QqQ with those of MALDI-TOF MS.

The availability of novel MS platforms will further revolutionize diagnostic microbiology; although significant hurdles still need to be overcome, it will become a tool for microbial subtyping, antimicrobial susceptibility testing (AST), and the detection of virulence factors. These applications will be supported by the ability of these platforms to analyze molecules other than peptides and proteins (e.g., fatty acids, low-molecular-weight metabolites, and oligo- and polysaccharides). To date, clinically validated diagnostic MS systems in microbiology are based solely on the MALDI-TOF principle and are commercially available from a limited number of manufacturers.

BACTERIAL IDENTIFICATION: MARKET PERSPECTIVE AND MANUFACTURERS

Currently, the major commercial MALDI-TOF MS systems with validated microbial identification capacity are the Bruker Biotyper and the bioMérieux Vitek MS system (the latter developed by AnagnosTec [Germany] and Shimadzu Corporation [Japan]) (5, 25). Andromas (Paris, France) provides an additional database that can be used in combination with the Bruker platform (2). Other companies, including Waters (Manchester, United Kingdom), using MicrobeLynx software, also offer diagnostic MS solutions, albeit with lower market penetration. In general, to date, MS applications in clinical microbiology have been adopted more

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TABLE 1 Comparison of MALDI-TOF and LC-ESI-QqQ mass spectrometry

Characteristic	Description[a]	
	MALDI-TOF	LC-ESI-QqQ-MS
Ionization	Soft ionization with matrix	Soft ionization with solvents and electronebulization
Fragmentation	No (intact molecules)	Yes
Sample	Solid form (or liquid allowed to dry on target)	Liquid form (downstream of a liquid chromatography step)
Molecules	Mainly proteins, large glycopeptides, oligonucleotides, carbohydrates	Different molecules, especially peptides
Turnaround time	20-30 s per sample at laser frequency of 50 Hz to generate a spectrum Minutes from sample preparation to result	Minutes or hours depending on liquid chromatography adsorption/elution times
Throughput	Disposable target with multiple spots (48 to 96)	No batch mode at the moment due to LC step
Reagent	Chemical matrix	Chemical reagents for chromatographic separation and elution
	External calibrant	Internal calibrant
Material	Disposable target	Chromatographic column and precolumn Vials for LC injection
Quantification	Not well suited	Fully adapted
Specificity	Depending on MS specificity and proteins tested	Usually higher than MALDI when selected reaction monitoring (MS^2) is used
Sensitivity	Bacterial ID: 10^5 CFU using fingerprint approach Urine sample after purification without culture: 10^5 CFU/ml	To be explored
Integration into microbiology lab workflow	Yes (IVD-compliant systems)	No (research applications)
Today's clinical microbiology applications	Microbiology: identification of bacteria, yeast, and molds	None Quantitative assays for small molecules, such as vitamin D (outside microbiology field)

[a] ID, identification; MS^2, two subsequent mass assessments; IVD, *in vitro* diagnostic.

extensively in European countries than in North America, Asia, or the Pacific Rim.

All currently available diagnostic microbiology MS systems come with a spectral database and identification algorithms for the detection of conserved and microbe-specific peak patterns in whole-cell mass spectra. When the different systems and approaches are compared, limited differences in analytical performance are observed. Another MALDI-TOF MS system, focused on the analysis of nucleic acids, including PCR products and their derivatives, is offered by Sequenom (San Diego, CA) and uses the proprietary MassArray technology (27). The MassArray Analyzer systems combine molecular biology with MS. This system characterizes fragmented transcripts of PCR products to the primary sequence level, is fast, and has the ability to cost-effectively and in near-real time process tens to thousands of samples. The Abbott Ibis PLEX ID system (Abbott) employs similar principles.

Some microbial species remain more difficult to identify than others using MALDI-TOF; however, this is true for most diagnostic technologies (24). That said, microbiological and technological studies have shown that the various MALDI-TOF MS systems have comparable sensitivities and specificities. They facilitate identification of hundreds of different bacterial species and many clinically relevant fungi. The choice of one specific commercial system should be based on factors such as the integrity and extent of the database, cost, throughput, mechanical reliability, and ergonomics.

DATA MANAGEMENT

Data acquisition and management and the quality of the final experimental output (be it a spectrum needed for research activities or a clinical bacterial identification result) strongly depend on the algorithm used for interpretation of crude data. Various methods for adequate data interpretation have been described (23, 31). Of course, communication of results to "customers," who frequently are clinicians awaiting results for modification or initiation of antibiotic treatment, requires integration in laboratory or hospital information systems (LIS or HIS). Essentially, the basic experimental output is represented by a limited number of parameters: *m*/*z* peak position, intensity, and frequency or reproducibility. The "biggest" mathematical systems are the Bruker Main Spectrum analysis (MSP) and the bioMérieux SuperSpectrum and Advanced Spectra Classifier (ASC) approaches. The MSP technology involves the comparison of an individual, newly recorded spectrum to the entries of a database consisting of a collection of reference spectra from single reference strains (computed from multiple replicate spectra). SuperSpectra are constructed on the basis of an accumulation of spectra obtained in replicate for large numbers of random clinical and reference strains grown under different conditions. Newly recorded spectra are compared to the SuperSpectra, and hence microbial species identification is facilitated. The ASC approach is an extension of the SuperSpectrum approach, looking at the presence or absence of peaks through the entire mass range in the target species compared to all other species in the database. An important difference between the MSP and SuperSpectrum/ASC methods is how microbial biodiversity is (or is not) broadly included in the analysis. This has led to discussions of the adequacy of restricted numbers of individual isolates (e.g., type strains) versus larger numbers but maybe less-well-characterized clinical isolates to represent species in the respective databases. Currently, these are unresolved issues. However, both technologies seem to be performing well in practice. Of note, the Bruker Biotyper database contains more bacte-

rial species than does the bioMérieux Vitek MS; however, both systems address the large majority of clinically relevant species found in routine clinical practice, including the 20 bacteria that represent > 80% of isolates recovered from human clinical specimens.

Reference databases require continual updating as new information is accumulated. Database expansion with the addition of more diverse and biodiverse strains leads to a more robust identification tool. The ever-evolving microbial taxonomy, changes in the regional prevalences of infectious diseases, emerging infections, the discovery of new bacterial species, and local or pandemic changes in microbial epidemiology underscore the essential importance of continuous database revision with resultant diagnostic optimization. In clinical microbiology, no one system "fits all." This is also true of MS technology. For example, adequate MS-based identification of certain bacterial species is still problematic irrespective of the type of data analysis. Finally, adequate quality control methods and measures (which are essential for acquiring CE marking or FDA approval) are still at the developmental stage. Repetitive inclusion of a certain microbial cell preparation in most or many assay pools provides a good start for quality assurance/quality control (QA/QC) of MS-mediated identification, but more sophisticated tools may be required once the number of different MS tests expands.

CURRENT CLINICAL MICROBIOLOGY APPLICATIONS

When applying MS technologies, the amino acid sequences of specific target proteins can be determined (15). However, the diagnostic MALDI-TOF MS systems as marketed today are focused primarily on bacterial species identification. MALDI-TOF MS will replace some biochemical tests, since in terms of biological performance, MALDI-TOF MS provides identification results equal to or better than those obtained with these conventional methods. Comparing MALDI-TOF MS, conventional biochemical tests, and 16S rRNA gene sequencing for more than 1,000 common bacterial isolates, Benagli et al. (1) reported that for 94.7% of the isolates, MALDI-TOF MS results were identical to those obtained with conventional systems and that 16S sequencing confirmed MALDI-TOF MS identification in 63% of the discordant results.

The reference databases employed in *in vitro* diagnostics applications of MS are closed and cannot be manipulated because of regulatory mandates. So within these clinical-legal confines, the sample processing step is of pivotal importance. Protocols for direct MS-mediated testing of clinical materials or for the identification of precultured microorganisms are still debated and are of varying quality. Comparative studies are many, but real gold standard technology has not yet been made available to the diagnostic community (19, 20, 22). Some methods for the processing of positive blood culture samples have been published, but especially in the case of direct testing of other materials (e.g., urine specimens), consensus still has not been reached (11, 22). The generation of spectra is next. To date, all different systems allow for the reliable identification of hundreds of species, including, for instance, the mycobacteria (10) and their subspecies (30). Novel applications of MS are being developed, and this will further strengthen the clinical laboratory position of MS in the future.

INNOVATIVE APPLICATIONS

The clinical microbiology MS field is developing rapidly, and innovative applications that will add diagnostic value continue to emerge. Some specific examples will be highlighted below.

Microbial typing. DNA microheterogeneity currently forms the basis of the most rigorous epidemiological typing tools. It has been demonstrated for certain species that strains can also be distinguished by MALDI-TOF MS, thereby rendering this technology suitable for epidemiological investigations. Comparison of MALDI-TOF MS and multilocus sequence typing (MLST), the current gold standard portable typing methodology for many bacterial species, showed a nearly complete overlap between clonal clusters as identified by MLST and MS-based strain grouping. Specific *Salmonella* serovars can be equally well grouped on the basis of MS (8). MS-based strain clustering for staphylococci can be performed on the basis of the selective absence or presence of 10 to 15 specific *m*/*z* peaks in the spectra (32). For pneumococci, essentially the same phenomenon was observed: again a 99% overlap between MLST and MS-derived strain clusters was documented (9). Still, there is no uniform MALDI-TOF MS "strain-typing" protocol available today. Currently, individual assays need to be developed specifically for each species of interest. This could involve exploration of other mass ranges, other matrices, and other extraction methods. However, bacterial typing using MS is an emerging field, and many innovative approaches will be forthcoming during the coming years.

Antimicrobial susceptibility testing (AST). In some cases, the detection of a single microbial gene product can potentially be correlated with resistance to one or more antimicrobial agents. This was investigated in the case of the unique *Bacteroides fragilis cfiA* carbapenemase. Investigators noted a good correlation of MS types with *cifA* types based on the evolutionary history of the species. Apparently, the *cif*A gene has been acquired by one lineage and has not been further spread by horizontal gene transfer (25, 33). Other resistance mechanisms that rely on the activity of single resistance determinants may be amenable to the same approach. MecA-dependent methicillin resistance is another example.

Recently, several studies have described MS-mediated detection of degradation of beta-lactam antimicrobials (3, 16, 18). Here, the enzymatic hydrolysis of the antimicrobial agent itself was monitored. This hydrolysis results in a mass shift of 18 Da which can be detected by MALDI-TOF MS analysis provided that multiple repeat measurements are performed. It was demonstrated that this method has a sensitivity of 97% and a specificity of 98% (18). The method can provide results within 1 to 2.5 h (3). Of note, it is likely that this time frame can be further shortened (16). Direct measurement of hydrolysis allows calculation of reaction speed and the affinity between the enzyme and the substrate. This may facilitate further characterization of the resistance mechanisms involved (16).

Microbial virulence. Differences in virulence profiles for bacterial isolates can be based on the selective determination of the presence or absence of certain *m*/*z* peaks in an MS spectrum. For example, once the molecular weight of a toxin or a hemolysin is known, specific searches for peaks at the corresponding *m*/*z* ratio can be made. We have recently detected the staphylococcal delta-toxin by MALDI-TOF MS and have shown that the presence or absence of this protein is associated with acute versus persistent infection (J. Gagnaire, O. Dauwalder, S. Boisset, S. Khau, A. Frey-

dière, M. Bes, G. Lina, M. Reverdy, A. Marchand, T. Geissmann, Y. Benito, G. Durand, J. Charrier, A. van Belkum, M. Welker, and F. Vandenesch, submitted for publication). Detection and identification of quorum-sensing signals, immune-modulatory proteins, and the binding of host factors, including antibodies, are targets for future research in this field.

Glycans. All current diagnostic MS applications in microbiology focus on the visualization of protein profiles. This is because proteins can be more easily ionized than, for instance, the strongly hydrophilic oligo- or polysaccharides. Still, these other biomolecules may have important diagnostic implications, and MS technologies for glycan detection have been developed. Chemical modification with aminoquinolines promoted the "ionizability" of saccharides (21). This significantly improved the detectability of such compounds to the attomolar level. Applications involving bacterial capsule or lipopoly- or oligonucleotide saccharides (LPS or LOS) have also been described. It is possible that endotoxin detection by MS could have an important clinical impact on the early detection of Gram-negative bacteremias and sepsis.

Nonbacteriological application. As was initially piloted with various insect species, MALDI-TOF MS can also be used for human tissue imaging (4) and the detection of pathogenic viruses (26). In addition, the technology is also finding its way into immunology. The use of high-performance liquid chromatography (HPLC) in tandem with MS facilitates the detection of a variety of antibody species and even antibody-antigen complexes (17).

SYSTEM AND WORKFLOW ENHANCEMENTS

Sample preparation for identification of microbes by MALDI-TOF MS remains laborious today. Some sample preparation robots are under development to ease the deposit of the sample and the matrix on the MALDI target. Miniaturization of mass spectrometers, improvements in laser pulse frequency, and the development of preprepared disposable target slides with prespotted chemical matrices are all areas currently being investigated. In an attempt to facilitate the integration of MALDI-TOF MS or other MS systems into the global workflow of clinical microbiology laboratories, so called "middleware" (software designed to enhance communication between various types of laboratory equipment and laboratory information systems) is being developed. The possibility of interfacing MS instrumentation directly to "smart phones" and other personal electronic information devices is also being explored.

GENERAL COMMENTS

No single diagnostic technology is universally applicable. This is certainly true of MS. Intracellular pathogens pose a problem, beta-hemolytic streptococci are difficult to identify to the species level, and an important pathogen, such as *Streptococcus pneumoniae*, is still hard to identify by MS. Further it is currently impossible to distinguish strains of *Escherichia coli* from those of *Shigella* spp. In addition, current MS applications depend on cultivation of microbes. Direct identification in clinical material remains problematic. However, in comparison to selected other novel technologies, e.g., PCR, sequencing, probe-mediated identification and detection tools, and even some selective culture-based diagnostics, MS technology offers several distinct advantages (7). The utility of MS was convincingly demonstrated by Gaillot et al. (14). These authors reported that the introduction of MS and the associated reduction in conventional bacterial species identification procedures led to a cost savings of 90%, a reduction in the amount of laboratory waste from >1,400 kg to <50 kg per year, a significant shortening in the length of time to bacterial identification, and a reduced need for DNA/RNA sequencing. While MS did not lead to a reduction in staffing requirements, nor was there a reduction in the time required for determining antimicrobial susceptibility profiles, MS was clearly found to have had a positive impact on laboratory function. As MS technology is more broadly applied in clinical microbiology laboratories, it will be of central importance to investigate its impact on disease outcomes. As with all technologies in clinical microbiology, their true value is best measured in the context of outcomes among patients with infection.

CONCLUDING REMARKS AND FUTURE PERSPECTIVE

In the future, MS technology in clinical microbiology will be extended to include characterization of host and microbial proteins, sugars, fatty acids, nucleic acids, and metabolites in a single clinical sample (25). This will require greater integration into laboratory workflow and further technical improvement, especially with respect to analyte detection sensitivity. Refinement of diagnostic databases and the development of methods compatible with the direct detection of (enriched or labeled) pathogens in clinical specimens will also be important. Where the use of MS is now limited to profiling of microbial proteins with high intracellular concentrations, future applications will involve complete "omics" and probably also "meta-omics" approaches based on even more sophisticated MS technologies, hopefully with application directly to clinical specimens.

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REFERENCES

1. **Benagli C, Rossi V, Dolina M, Tonolla M, Petrini O.** 2011. Matrix-assisted laser desorption ionization-time of flight mass spectrometry for the identification of clinically relevant bacteria. PLoS One. **6**:e16424.
2. **Bille E, et al.** 2011. MALDI-TOF MS Andromas strategy for the routine identification of bacteria, mycobacteria, yeasts, Aspergillus spp. and positive blood cultures. Clin. Microbiol. Infect. [Epub ahead of print.] doi: 10.1111/j.1469-0691.2011.03688.x.
3. **Burckhardt I, Zimmermann S.** 2011. Using matrix-assisted laser desorption ionization–time of flight mass spectrometry to detect carbapenem resistance within 1 to 2.5 hours. J. Clin. Microbiol. **49**:3321–3324.
4. **Cazares LH, Troyer DA, Wang B, Drake RR, Semmes OJ.** 2011. MALDI tissue imaging: from biomarker discovery to clinical applications. Anal. Bioanal. Chem. **401**:17–27.
5. **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.
6. **Croxatto A, Prod'hom G, Greub G.** 22 August 2011. Applications of MALDI-TOF mass spectrometry in clinical diagnostic microbiology. Microbiol. FEMS Rev. [Epub ahead of print.] doi:10.1111/j.1574-6976.2011.00298.x.
7. **Dhiman N, Hall L, Wohlfiel SL, Buckwalter SP, Wengenack NL.** 2011. Performance and cost analysis of matrix-assisted laser desorption ionization–time of flight mass spectrometry for routine identification of yeast. J. Clin. Microbiol. **49**:1614–1616.
8. **Dieckmann R, Malorny B.** 2011. Rapid screening of epidemiologically important Salmonella enterica subsp. enterica serovars by whole-cell matrix-assisted laser desorption ionization-time of flight mass spectrometry. Appl. Environ. Microbiol. **77**:4136–4146.

9. **Dunne EM, et al.** 2011. Multilocus sequence typing of Streptococcus pneumoniae by use of mass spectrometry. J. Clin. Microbiol. **49**:3756–3760.
10. **El Khéchine A, Couderc C, Flaudrops C, Raoult D, Drancourt M.** 2011. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry identification of mycobacteria in routine clinical practice. PLoS One **6**:e24720.
11. **Ferreira L, et al.** 2010. Direct identification of urinary tract pathogens from urine samples by matrix-assisted laser desorption ionization–time of flight mass spectrometry. J. Clin. Microbiol. **48**:2110–2115.
12. **Fortin T, et al.** 2009. Clinical quantitation of prostate-specific antigen biomarker in the low nanogram/milliliter range by conventional bore liquid chromatography-tandem mass spectrometry (multiple reaction monitoring). Mol. Cell Proteomics **8**:1006–1015.
13. **Fox A.** 2006. Mass spectrometry for species or strain identification after culture or without culture: past, present, and future. J. Clin. Microbiol. **44**:2677–2680.
14. **Gaillot O, et al.** 2011. Cost-effectiveness of switch to matrix-assisted laser desorption ionization-time of flight mass spectrometry for routine bacterial identification. J. Clin. Microbiol. **49**:4412.
15. **Glowalla E, Tosetti B, Krönke M, Krut O.** 2009. Proteomics-based identification of anchorless cell wall proteins as vaccine candidates against *Staphylococcus aureus.* Infect. Immun. **77**:2719–2729.
16. **Hooff GP, et al.** 11 November 2011. Characterization of β-lactamase enzyme activity in bacterial lysates using MALDI-mass spectrometry. J. Proteome Res. [Epub ahead of print.] doi:10.1021/pr200858r.
17. **Hoofnagle AN, Wener MH.** 2009. The fundamental flaws of immunoassays and potential solutions using tandem mass spectrometry. J. Immunol. Methods **347**:3–11.
18. **Hrabák J, Walková R, Studentová V, Chudácková E, Bergerová T.** 2011. Carbapenemase activity detection by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J. Clin. Microbiol. **49**:3222–3227.
19. **Juiz PM, et al.** 26 October 2011. A comparative study of two different methods of sample preparation for positive blood cultures for the rapid identification of bacteria using MALDI-TOF MS. Eur. J. Clin. Microbiol. Infect. Dis. [Epub ahead of print] doi:10.1007/s10096-011-1449-x.
20. **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.
21. **Kaneshiro K, Fukuyama Y, Iwamoto S, Sekiya S, Tanaka K.** 2011. Highly sensitive MALDI analyses of glycans by a new aminoquinoline-labeling method using 3-aminoquinoline/α-cyano-4-hydroxycinnamic acid liquid matrix. Anal. Chem. **83**:3663–3667.
22. **La Scola B, Raoult D.** 2009. Direct identification of bacteria in positive blood culture bottles by matrix-assisted laser desorption ionisation time-of-flight mass spectrometry. PLoS One **4**:e8041.
23. **La Scola B.** 2011. Intact cell MALDI-TOF mass spectrometry-based approaches for the diagnosis of bloodstream infections. Expert Rev. Mol. Diagn. **11**:287–298.
24. **Martiny D, Busson L, El Haj RA, Dediste A, Vandenberg O.** 2012. Comparison of Microflex LT and Vitek MS systems for routine identification of bacteria by matrix-assisted laser desorption ionization–time of flight mass spectrometry. J. Clin. Microbiol. **50**:1313–1325.
25. **Nagy E, Becker S, Sóki J, Urbán E, Kostrzewa M.** 2011. Differentiation of division I (cfiA-negative) and division II (cfiA-positive) Bacteroides fragilis strains by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. J. Med. Microbiol. **60**(Pt 11):1584–1590.
26. **Sjöholm MI, Dillner J, Carlson J.** 2008. Multiplex detection of human herpesviruses from archival specimens by using matrix-assisted laser desorption ionization–time of flight mass spectrometry. J. Clin. Microbiol. **46**:540–545.
27. **Syrmis MW, et al.** 2011. Comparison of a multiplexed MassARRAY system with real-time allele-specific PCR technology for genotyping of methicillin-resistant *Staphylococcus aureus.* Clin. Microbiol. Infect. **17**:1804–1810.
28. **Van Herendael BH, et al.** 23 August 2011. Validation of a modified algorithm for the identification of yeast isolates using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS). Eur. J. Clin. Microbiol. Infect. Dis. [Epub ahead of print.] doi:10.1007/s10096-011-1383-y.
29. **Vestal ML.** 2011. The future of biological mass spectrometry. J. Am. Soc. Mass Spectrom. **22**:953–959.
30. **Weigoldt M, et al.** 2011. Differential proteome analysis of Mycobacterium avium subsp. paratuberculosis grown in vitro and isolated from cases of clinical Johne's disease. Microbiology **157**(Pt 2):557–565.
31. **Welker, M.** 2011. Proteomics for routine identification of microorganisms. Proteomics **11**(15):3143–3153.
32. **Wolters M, et al.** 2011. MALDI-TOF MS fingerprinting allows for discrimination of major methicillin-resistant Staphylococcus aureus lineages. Int. J. Med. Microbiol. **301**:64–68.
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.