MALDI Biotyping for Microorganism Identification in Clinical Microbiology

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Abstract In 1996, independent publications demonstrated the application of MALDI-TOF MS for microorganism identification using whole-cell profile mass spectra. As these were characteristic for distinct bacterial and fungal species, they could be used as a molecular fingerprint. Subsequently, sample preparation, data evaluation with bioinformatics and further aspects of the technology were further improved. Ease-of-use, rapidity and accuracy of this technology finally led to the implementation of MALDI-TOF MS into routine analytical work, particularly in clinical microbiology.

Today, microorganism identification by MALDI-TOF MS is performed in many clinical microbiology laboratories and is increasingly replacing the conventional methods utilized for decades. In this chapter, the impact of this technology on clinical diagnostics is described. Furthermore, exemplary protocols for microorganism identification are presented. Specialized protocols even allow identification of more demanding microorganisms like mycobacteria and filamentous fungi as well as identification from liquid culture media inoculated with patient specimen. Some analytical systems allow the extension of reference databases with own entries and an insight into evaluation algorithms, interpretation of results and creation of own references is given.

Further, MALDI-TOF MS has proven its utility in identification of food-borne and veterinary microorganisms. These topics as well as regulatory aspects together with the necessary steps for qualification and validation are also covered for the interested reader.

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1 Introduction

Early in the development of mass spectrometry (MS) technologies their applicability to microorganism analysis, in particular the identification of microbes, was already discussed (Anhalt and Fenselau 1975; Sinha et al. 1985; Heller et al. 1987). The real breakthrough was achieved with the appearance of matrix-assisted laser desorption/ionization (MALDI)-time-of-flight (TOF) MS, a technology which combines soft ionization of large biomolecules with short analysis time. In 1996, three independent scientific publications showed ground-breaking results in the application of MALDI-TOF MS for microorganism identification using whole-cell profile mass spectra (Claydon et al. 1996; Holland et al. 1996; Krishnamurthy and Ross 1996).

The idea was to use the microbial MALDI-TOF profile mass spectra of whole cells as a molecular fingerprint, being characteristic for bacteria and fungi.

During the first years, several scientists worked on the improvement of sample preparation (Welham et al. 1998; Gantt et al. 1999; Smole et al. 2002; Vaidyanathan et al. 2002), reproducibility (Wang et al. 1998; Demirev et al. 1999; Saenz et al. 1999; Williams et al. 2003), bioinformatic data evaluation (Arnold and Reilly 1998; Jarman et al. 1999; Jarman et al. 2000; Pineda et al. 2000), and applicability of the technique to different microbial groups (Haag et al. 1998; Nilsson 1999; Amiri-Eliasi and Fenselau 2001; Conway et al. 2001; Bernardo et al. 2002; Hettick et al. 2004; Krader and Emerson 2004; Mandrell et al. 2005). Ryzhov and Fenselau (2001) found that most of the peaks which can be observed in typical bacterial profile spectra are derived from ribosomal and other high-abundant housekeeping proteins, which explains the robustness and reproducibility of approaches analysing profiles in the mass range of 2–20 kDa.

A first commercial system, launched by the company Micromass (Manchester, UK), analyzed fingerprints in the lower mass region (approximately up to m/z 4000) but did not succeed in routine laboratories. Therefore, MALDI-TOF MS profiling for microorganisms stayed a scientific topic in the area of mass spectrometry for quite some time but did not enter the area of routine (clinical) microbiology.

This situation started to change in 2007 with the introduction of a new generation of commercially available systems in clinical microbiology, equipped with easy-to-use software solutions and broadly applicable reference databases, supported with standard operating procedures (SOPs) for sample preparation. All these systems analyzed biomolecules in the mass range of approximately m/z 2000–20,000. Mellmann and co-workers compared the performance of the MALDI Biotyper system (Bruker Daltonik GmbH, Bremen, Germany) for the identification of Gram-negative non-fermenting bacteria with sequence analysis of 16S ribosomal DNA which is currently the ‘gold standard’ for identification of bacterial species (Mellmann et al. 2008). They found that for this bacterial group, which is very difficult to characterize by biochemical tests, 85.9% of strains were correctly identified. This performance was superior to the established biochemical routine analysis sys-

tems. Subsequently, the same group performed the first large multi-centre study for this technology (Mellmann et al. 2009). In this international study, eight laboratories analyzed 60 blind-coded non-fermenting bacteria samples each and achieved an inter-laboratory reproducibility of 98.75%. Only six of the 480 samples were misidentified due to sample interchanges (four samples) or contamination (one sample) or could not be identified because of insufficient signal intensity (one sample). The authors concluded that this level of reproducibility is usually only achievable with DNA sequence-based methods and that MALDI-TOF MS for microorganism identification is therefore a highly reproducible technology, a prerequisite for any routine utilization.

2 Applications

2.1 MALDI Biotyping in Medical Microbiology: An Overview

Medical microbiology plays an important role in the diagnosis and treatment of infectious diseases today. In case of an infection, patient specimens are collected and examined with laboratory methods to identify the infection-causing microorganisms like bacteria, fungi and viruses.

While viruses are usually identified using molecular methods, bacteria and fungi are generally identified from cultures grown on solid or in liquid media. Fast growing microorganisms form small colonies on solid media after 24–48 h. Shape and color of individual colonies as well as their microscopic features can be used for a first categorization of the microorganism. Gram staining is generally used for a further quick characterization step. Final identification is normally performed using biochemical reactions and tests that indicate the metabolic characteristics of the species of interest during growth for about another 6 h for fast growing bacteria (like enterobacteria, enterococci or staphylococci) in automated systems and up to 48 h for slower growing organisms or with fewer characteristic metabolic reactions.

MALDI biotyping, a modern, fast and accurate method suitable for routine analysis allows the identification of microorganisms in a few minutes from a colony. This has led to ‘a fundamental shift in routine practice of clinical microbiology’ as described by Clark et al. (Clark et al. 2013), while other authors call it an ‘ongoing revolution’ (Seng et al. 2009). The latter publication and work from Eigner and colleagues (Eigner et al. 2009) were the first reports of studies investigating the broad utilization of MALDI-TOF MS biotyping in a clinical microbiology laboratory. These studies revealed excellent results for the identification of microorganisms occurring in clinical routine. Subsequently, a number of studies have proven the superiority of the technology in comparison to traditional biochemical test systems (Marklein et al. 2009; Nagy et al. 2009; Bader et al. 2011; Bizzini et al. 2010; van Veen et al. 2010; Bille et al. 2012; Dhiman et al. 2011).

A particular strength of MALDI-TOF MS (with an appropriate database) is the potential to identify not only the frequently occurring but also rare and difficult-to-

identify organisms which normally have to be characterized by DNA sequencing if identification is at all possible at species level (Bizzini et al. 2011). Thereby, species name can now be assigned for some bacteria, which were previously classified by many laboratories only to the genus level or an even more general systematic group, e.g. 'Gram-positive, non-sporulating rods' or 'Gram-negative non-fermenting rods'. Thus, MALDI-TOF MS profiling improves the quality of diagnostic microorganism identification through both greater accuracy and higher taxonomic resolution.

For example, in the group of yeasts, the *Candida parapsilosis* complex and the *Candida haemulonis* complex can now be resolved as distinct species (Quiles-Melero et al. 2012; Cendejas-Bueno et al. 2012). For bacteria, important examples are the corynebacteria (Alatoom et al. 2012; Vila et al. 2012) and *Haemophilus influenzae/haemolyticus* (Zhu et al. 2013; Bruin et al. 2014). For some groups of microorganisms, which usually had to be referred to reference laboratories, identification can now be performed by routine laboratories. This applies in particular to mycobacteria (Lotz et al. 2010; Saleeb et al. 2011; Balada-Llasat et al. 2013; Buchan et al. 2014) and filamentous fungi (Cassagne et al. 2011; Iriart et al. 2012; Lau et al. 2013; Schulthess et al. 2014), where MALDI-TOF MS profiling can also be employed to get a first-line identifier.

2.2 *The Impact of MALDI-TOF MS on the Clinical Microbiology Laboratory*

The methods and procedures described in this chapter are suitable for the identification of microorganisms in the microbiology laboratory. With the introduction of this technology, it has also been possible to optimize established routine workflows that use conventional methods. Formerly, microbial identification and antimicrobial susceptibility testing (AST) were performed in parallel after an initial 'triage' by colony morphology to decide which test panels could be the most appropriate. Today, in many cases where morphological assessment is difficult, AST can be started based on the precise identification of the microorganism by MALDI biotyping, and corresponding AST panels can be applied. Rapid identification also allows earlier therapy adjustment. This is particularly true for slow growing organisms or when differentiation by conventional biochemical methods is difficult.

Another important benefit of MALDI-TOF MS biotyping is its precision and high discriminatory power. Reviews of laboratory statistics of the MVZ (Medical Center) Dr. Eberhard & Partners (Dortmund, Germany)—one of the major independent laboratories in Germany—show a significant enhancement in identification of microorganisms after the introduction of MALDI-TOF MS. Sited in Dortmund this laboratory is located in the heart of the Ruhr valley which is one of the largest urban agglomerations in Europe with about five million inhabitants. The Department of Microbiology of the MVZ Dr. Eberhard & Partners serves as a diagnostic partner for several hospitals and many doctors in private practices as well as for other laboratories. In the year 2009, before the introduction of a MALDI-

TOF system at the Department of Microbiology, a total of 299 different species from 115 genera were found. By the end of 2009 first tests with a MALDI biotyping system had started, and the new technology was introduced into routine analysis a few months later. In 2010, the first year with MALDI-TOF MS as an additional routine identification method, the reports showed a total of 438 different species (+46%) from 146 different genera (+27%). Especially in the group of anaerobic bacteria and non-fermenters, which are usually difficult to identify by conventional methods, a broad variety of species can now be differentiated. Also, the commonly consolidated 'coryneform bacteria' can now be broken down into distinct species.

Arguably, this new accuracy in identification will also lead to an increase of knowledge about individual microorganisms and their infection potential. The possibility of extending the database with your own entries offers the potential for easy detection of uncommon microorganisms. To date, nucleic acid sequencing is the 'gold standard' in microorganism identification and remains the last option in selected cases where other identification methods are insufficient. With the subsequent creation of an own MSP ('main spectrum', i.e. reference database entry), uncommon microbes can easily be re-identified without the need for laborious and costly biomolecular sequencing. Even for yet unnamed species, recognition of recurrence of these microorganisms is now possible. Therefore, in addition to the manufacturers' databases major laboratories often also employ an increasing number of their own reference spectra for routine diagnostics. As the manufacturers' reference databases have grown over the years a broad range of identifications is now possible. A further extension of these reference libraries can be expected. Statistics from MVZ Dr. Eberhard & Partners, where the standard reference libraries are extended by about 200 self-acquired MSPs, show that in the year 2014 more than 700 different species from 190 genera were recorded. Compared to species identification before the implementation of MALDI-TOF MS, this is an increase of about 150%. In addition, MSPs of 21 different yet unnamed bacterial species detected these microorganisms in about 130 cases. This clearly stresses the great potential of MALDI-TOF MS in routine diagnostics of microbiology laboratories and its future prospect for a better knowledge about microorganisms in infections.

2.3 Regulatory Aspects

While in the beginning only research-use-only (RUO) systems were available for the identification of microorganisms by MALDI-TOF MS, this has changed in the following years. The pioneers who have introduced the technology into the diagnostic field validated the instrumentation, software and databases in thorough studies with reference strains and clinical isolates.

Aspects like the performance for specific microorganism groups as well as the robustness of the method and repeatability from day to day and user to user have to be proven and documented. If the system is labeled and distributed as RUO by the manufacturer and not intended for diagnostic purpose, the user takes full responsi-

bility for the functionality and results of the system. On the other hand, users have the flexibility to define and control their own diagnostic device. For the improvement of results of a self-validated RUO system, several specialists have reported extensions of the manufacturer's database, modifications of preparation techniques or adaptations of acceptance criteria (Christensen et al. 2012; Khot et al. 2012; Theel et al. 2012; Ford and Burnham 2013; McElvania TeKippe et al. 2013; Murugaiyan et al. 2014).

In 2009, the first IVD-CE labeled system became available in Europe, for diagnostic identification of bacteria and yeast in clinical microbiology laboratories, the IVD MALDI Biotyper (Bruker Daltonik GmbH). Later, with the VITEK® MS IVD (bioMérieux, Marcy l'Etoile, France) a further system became available. With such a system, the manufacturer takes over the validation for clinical usage and later responsibility for the function of the system—provided that the system is utilized in the area of intended use and according to the instructions of the manufacturer.

In the USA, a clearance of such a device for in vitro diagnostic usage by the US Food and Drug Administration (FDA) is necessary and was obtained for both the MALDI Biotyper—Clinical Application (Bruker Daltonics, Billerica, MA, USA) and the VITEK® MS IVD (bioMérieux) in 2013. In particular, the FDA process involved detailed and expensive studies including investigations of robustness and repeatability, media compatibility and influence of all possible environmental conditions to the analyses. Furthermore, several thousands of samples had to be analyzed in clinical studies, and the results were compared to DNA sequencing as the current 'gold standard'. All studies resulted in the proof of excellent performance for the investigated MALDI-TOF MS systems which may therefore become a new 'gold standard' for microbial identification in the near future.

In Germany, laboratories offering diagnostic services to others need an accreditation according to DIN EN ISO 15189. Since 2009 accreditation of new methods using MALDI-TOF MS for microorganism differentiation has become possible, with sufficient familiarization as well as specific quality assurance being mandatory.

2.4 MALDI-TOF MS Profiling of Veterinary and Food-Borne Organisms

In parallel to the field of medical microbiology, MALDI-TOF MS has also conquered the field of microbial identification and characterization in veterinary and food microbiology. The reasons for success are the same as before: speed, accuracy and cost-effectiveness. As regulatory hurdles are lower in the veterinary field, in particular for university veterinary institutes, which are used to perform their own validation of methods, MALDI-TOF MS could even spread faster in veterinary microbiology, e.g. in the USA.

Besides studies to investigate the broad applicability of MALDI-TOF MS in these areas (Boehme et al. 2013; Wragg et al. 2014), studies for microorganisms

from particular diseases and certain microorganisms, which are difficult to identify by traditional methods, have been performed (Alispahic et al. 2010; Taniguchi et al. 2014; Frey and Kuhnert 2015). It has to be mentioned that less sophisticated biochemical ready-to-use kits are available in veterinary microbiology because of the smaller commercial interest. Further, as not only pathogens but also hosts are very variable the diversity of microorganisms is significantly higher than in human microbiology. Both are very good reasons to use a universal technology like MALDI-TOF MS profiling.

Barreiro and colleagues found MALDI-TOF MS profiling very suitable for pathogens isolated from milk of cows with mastitis (Barreiro et al. 2010). Excellent identification of group D Streptococci could be demonstrated (Werner et al. 2012). Grosse-Herrenthey and co-workers investigated the performance of MALDI-TOF MS for the identification and differentiation of clostridia, a group of anaerobic, Gram-positive sporulating bacteria which comprises a number of pathogens with veterinary but also medical relevance (Grosse-Herrenthey et al. 2008). They analyzed a total of 64 clostridial strains of 31 different species and found an excellent differentiation of these. It was even possible to identify species, which are normally difficult to differentiate by traditional methods, such as *C. chauvoei* and *C. septicum*.

The group of Campylobacter species and the related genera Helicobacter and Arcobacter were also found to be well identified and differentiated by MALDI-TOF MS profiling. Alispahic and colleagues analyzed 144 clinical isolates of these genera using whole spectral profiles (Alispahic et al. 2010). It was found that correct identification could even be obtained from bacteria stored at room temperature or at 4 °C up to 9 days prior to being tested. Other groups also found MALDI-TOF MS an excellent tool for the identification of these species, which partially are also important human pathogens (Bessède et al. 2011; Martiny et al. 2011; Taniguchi et al. 2014). For *Campylobacter jejuni*, effective distinction of subgroups has recently been reported. It was possible to group specific *C. jejuni* subgroups of phylogenetically related isolates in distinct clusters by principle component analysis (PCA) and hierarchical clustering (Zautner et al. 2013).

Another study demonstrated that MALDI-TOF MS profiling represents a fast and reliable method for the identification and differentiation of Gallibacterium species, with applications in clinical diagnostics (Alispahic et al. 2011). Even the very difficult-to-identify pathogenic algae from the genus Prototheca, occurring in mastitis and sometimes infecting milkers, could be reliably identified (von Bergen et al. 2009; Murugaiyan et al. 2012). This is one of the examples where MALDI-TOF MS profiling can be of particular diagnostic power as the algae might initially be misidentified as yeast which could lead to an ineffective treatment with antimycotic drugs.

Another case for the importance of accurate technology can be made in the field of food-borne pathogens. The power of identifying accurately Campylobacter species has already been described. However, the most well-known bacteria causing food-borne diseases are Salmonella species. Reliable identification of the genus

Salmonella has been described by several researchers (Dieckmann et al. 2008; He et al. 2010) but a serovar differentiation which would be highly desirable for Salmonella species is currently questionable. There have been reports that the highly pathogenic serovar *Salmonella typhi* might be differentiated from the other residual members of the genus (Kuhns et al. 2012; Martiny et al. 2012; Schaumann et al. 2013), but this still has to be validated. A method described for the rapid detection of Salmonella species in stool samples of diseased people, which comprises the enrichment of the bacteria in a liquid selective broth (Sparbier et al. 2012), may also be adapted to food products but will need further development and validation work.

Identification of further food-borne pathogens as described for Listeria species (Barbuddhe et al. 2008; Hsueh et al. 2014), Bacillus species (Farfour et al. 2012) and Yersinia species (Ayyadurai et al. 2010; He et al. 2010; Lasch et al. 2010; Stephan et al. 2011) may be mainly applied for confirmation of clinical diagnostics and/or further developed towards fast detection tests as shown in a study with spiked samples for Listeria (Jadhav et al. 2014).

2.5 *Instrument Qualification and Method Validation* (for the Interested Reader: Can Be Skipped by the MALDI Novice)

2.5.1 Qualification as an Analytical Instrument: DQ-IQ-OQ-PQ

Instrument qualification of automated, computerized systems is a critical step and has to follow accepted official 'good manufacturing practices' (GMP) guidelines. All initial activities are usually summarized as design qualification (DQ) steps. This includes system documentation of hardware and software, intended use considerations/statements, user and/or functional requirement declarations and vendor assessments in coordination with a dedicated risk management defined by GMP guidelines for analytical procedures (e.g. USP, EP, ICH, PDA Tech Report33; cf. http://www.gmp-navigator.com/nav_guidelines.html; http://www.ich.org/home.html; http://www.usp.org/; http://www.pda.org/).

Next, installation qualification (IQ) documents as well as operational qualification (OQ) procedures are fully documented references to ensure that the system is installed and operational according to dedicated specifications to guarantee the intended use. It typically includes a system suitability test in a final performance qualification step (PQ) with several different freshly grown microorganisms.

In addition, a set of operational and administrative SOPs, preventive maintenance plans/activities and complete method validation scenarios are a crucial part of initial considerations. Once these steps are initiated any changes or amendments to software, hardware, methods, SOPs, etc. are not allowed or have to follow a strin-

gent risk management-based approach usually handled by change control under well-defined, mandatory quality management (QM) rules and followed by a final approval through the responsible qualified person (QP). These QM rules are based on the aforementioned established GMP guidelines and conventions and are meant to show (objectively) whether any change in the complete process will influence the outcome/intended use of the system negatively. A small change in the process may require a complete new validation procedure.

2.5.2 Validation

The in-house validation part requires a setup of independent experiments generally starting with an equivalence study showing the sensitivity and specificity of the new method compared to an established standard method. Discrepancies between both methods have to be resolved by utilizing established DNA sequencing techniques (the current 'gold standard') or additional methods if required. Several strains of each relevant group (e.g. Gram-positive and Gram-negative bacteria, spore-formers and non-spore-formers, fungal species if relevant and additional relevant groups if existing) have to be freshly prepared and identified. Further, a system performance check or system suitability check needs to be run before every experiment to document correct system operation and demonstrate a fit-for-purpose state.

A system suitability test shall include at least one well characterized and officially certified strain. In addition, a negative test with a strain delivering a true detectable MALDI-TOF MS profile but resulting in 'no identification' after data processing (because it is not contained in the respective database) has to be successfully employed throughout all experiments.

To show method robustness, small but deliberate changes during the analytical process have to be demonstrated. These can be shown by experiments at different temperatures and humidity, using different pipettes if exact volumes are required, different time frames between sample handling and processing steps and/or utilizing different lots of matrix and solvents.

For the precision of the method, the degree of agreement of multiple runs of the same sample or different suspensions of the same bacterial material (usually in the same lab within a short time) will be recorded. If different people on different days (ideally in different labs) using different preparations of the same bacterial sample are included, the intra- and inter-assay repeatability (intermediate precision) can be shown and documented.

3 Materials and Protocols

The protocols usually applied for routine microbial identification by MALDI-TOF MS profiling are quite similar among the different commercially available systems. In the following, protocols are described for the MALDI Biotyper (Bruker Daltonics), one of the leading systems with about 1500 installed instruments at the end of 2014. Generally, these protocols can also be applied or transferred to other MALDI-TOF mass spectrometers.

3.1 Chemicals for Microorganism Identification by MALDI-TOF MS Biotyping

- HPLC water, ethanol, acetonitrile (ACN), trifluoroacetic acid and formic acid
- Matrix: α-Cyano-4-hydroxycinnamic acid (CHCA)
- Standard solvent composition: ACN:water:trifluoroacetic acid (50%:47.5%:2.5% (v:v:v))

All chemicals used should be of highest purity (intended for use in mass spectrometry or HPLC).

The matrix solution is typically prepared by adding 250 μL of the standard solvent to 2.5 mg of CHCA and vortexing the mixture until all matrix crystals are completely dissolved. The manufacturers usually offer small tubes with preportioned amounts of matrix to which just the adequate volume of solvent has to be added. The standard solvent is also available as a ready-to-use solution (cat. no. 19182; Sigma-Aldrich).

The matrix solution can be stored in the dark at room temperature for up to 2 weeks. As the organic solvents are volatile and ACN is strongly hygroscopic, it is recommended to review storage conditions. Storing small volumes in big tubes or containers as well as working for long time with opened containers for reagents can result in a shift in concentrations that render MALDI-TOF MS profiling measurements impossible.

3.2 Spectrum Acquisition

The MALDI-TOF mass spectrometer is typically operated in linear mode. The spectrum is acquired in an m/z range of 2000–20,000 targeting ribosomal proteins, which are highly conserved and in general independent on culture conditions. It is recommended to perform regular calibrations with a standard, covering the m/z range of interest. A total of up to 240 laser shots from different positions of the sample is sufficient to obtain a representative spectrum with many characteristic

peaks. Latest software versions allow an early termination of the spectral acquisition as soon as enough peaks with a satisfactory resolution or amplitude have been acquired. Statistics from routine diagnostics in medical microbiology show that about 25% of the measurements are finished after 40–120 shots, while 50% of the measurements need less than 200 shots (Data source: MVZ Dr. Eberhard & Partners). This saves time and extends the laser life time as well as intervals between servicing for source cleaning.

3.3 Direct Transfer (DT) Procedure

Besides the speed of identification, the ease-of-use in daily routine analysis has been one of the main reasons for the success of MALDI-TOF MS in medical microbiology. This is especially true for the direct transfer (DT) procedure, which can be applied to most cases of routine analysis in medical microbiology. From the colony to be identified some biological material is directly transferred to the steel target and smeared as a very thin film on the sample spot area. Best results can be obtained from cultures that are still growing. So in case of fast growing bacteria, fresh overnight cultures are suitable and identifications from slow growing organisms can be performed from cultures over several days. As proteins can quickly degrade from organisms stored in a refrigerator where they enter the lag phase, spectrum quality for such organisms can also quickly degrade. However, measurements from colonies stored at room temperature and kept in a metabolic active state show acceptable spectra even after days. For smearing the colony material onto the steel target, inoculation loops, pipette tips or wooden toothpicks are used in most laboratories. Sometimes, it needs some training to establish the right technique and amount of biological material that has to be applied to the sample spot. Usually, very thin films that can sometimes hardly be seen by the naked eye provide better results. Some users also prefer to employ ground steel targets instead of polished steel targets.[1] The sample spot is then directly overlaid with 1 µL of matrix solution, which is prepared according to the description in Sect. 3.1. After drying at room temperature, spectral acquisition (see Sect. 3.2) can be started. Figure 1 shows the steps of the DT method for a freshly grown colony picked from a blood agar plate.[2]

[1] The slightly rougher structure of ground steel targets can facilitate the smearing to the right thickness.

[2] Most culture media are blood-based. The haemoglobin in these media can produce artificial peaks disturbing the spectral acquisition or deteriorating the identification result. Thus, accidental transfers of parts of the culture media to the target should be avoided.

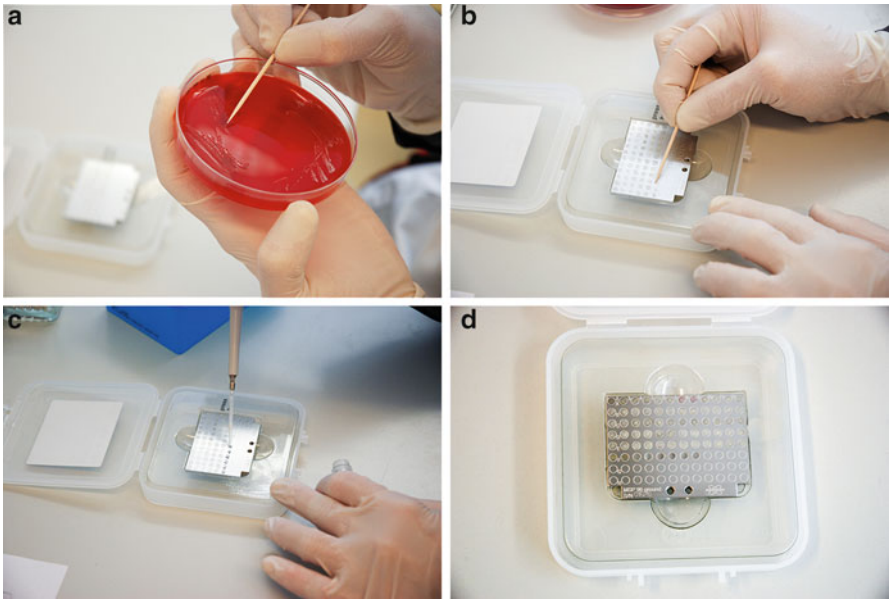

Fig. 1 Direct transfer (DT) procedure for microorganism identification by MALDI-TOF MS biotyping: (**a**) pick small amount of biomass from a bacterial colony of a freshly (overnight) grown culture, (**b**) transfer the biomass onto a sample spot area on a steel MALDI target plate and smear it out, producing a very thin film, (**c**) overlay with matrix, and (**d**) dry at room temperature before MALDI-TOF MS measurement

3.4 Extended Direct Transfer (EDT) Procedure

The DT procedure provides good-quality spectra for the majority of microorganisms in routine work. For some analytes like yeasts that have a more rigid cell wall, the extended direct transfer (EDT) procedure might be more advantageous. After smearing the colony material onto the MALDI target, the thin analyte film is overlaid with 1 μL of 70% formic acid. Then, after drying, 1 μL of the matrix solution is added and dried at room temperature. The formic acid can help to penetrate the cell walls and liberate the proteins of interest. Before applying the EDT method, it is advisable to check whether optimization of the standard DT procedure (cf. Sect. 3.3) could also improve results. In many cases, the amount of microorganism biomass transferred to the MALDI target is the more critical parameter.

3.5 Ethanol/Formic Acid Extraction (EFEx)

The best spectrum quality can be expected with the ethanol/formic acid extraction (EFEx) method. Compared to the DT and EDT procedure, it is more laborious and can be performed when the simpler methods give no satisfactory spectra. This elaborate extraction method is also appropriate for applications like strain sub-typing and the acquisition of reference spectra for extending the database when maximum spectrum quality is critical.

EFEx is performed in an Eppendorf® or similar tube with a volume of 1.5 mL by pipetting 300 µL of water into the tube and suspending a small amount of biological material (e.g. one single colony from a freshly grown culture of up to 5–10 mg) into it. Repeated pipetting (dispensing/aspirating) and/or vortexing facilitates the mixing process. Then, 900 µL of ethanol (absolute) are added and the solution is thoroughly mixed. The tube is then centrifuged at maximum speed for 2 min. The supernatant can be decanted and after another centrifugation step the remaining ethanol should be carefully removed without touching the pellet. Subsequently, the pellet is dried for some minutes at room temperature, followed by the addition of 1–80 µL of 70% formic acid. The exact amount depends on the amount of biological material entered into the extraction process: About 1–5 µL are sufficient for a small colony, while for bigger colonies 5–15 µL should be used. If a full 1-µL inoculation loop with colony material is used 10–40 µL are appropriate and for a 10-µL loop about 30–80 µL of 70% formic acid should be used and mixed well by pipetting (dispensing/aspirating) and/or vortexing. Then, pure ACN is added in the same amount as the formic acid and mixed. After centrifugation for 2 min, one should obtain a small pellet with the residual material and a supernatant with the extracted proteins. Then, 1 µL of the supernatant can be pipetted onto one sample spot of the steel target. After drying at room temperature, 1 µL of the matrix solution can be added and again dried at room temperature. To prevent chemical reactions like oxidation and methylation of the samples that lead to peak shifts in the spectrum, the supernatant with the extracted proteins should be used immediately and overlaid with the matrix solution as soon as possible (in general within 10 min).

Typical MALDI MS profile spectra using the EFEx procedure for the preparation of a variety of microorganisms from different phylogenetic branches are depicted in Fig. 2.

3.6 Extraction from Blood Cultures

3.6.1 Sepsityper Protocol

Blood cultures play an important role in medical microbiology. They allow the detection of pathogens in blood stream infections. Although these infections are severe and can lead to serious complications, there is usually only a small number

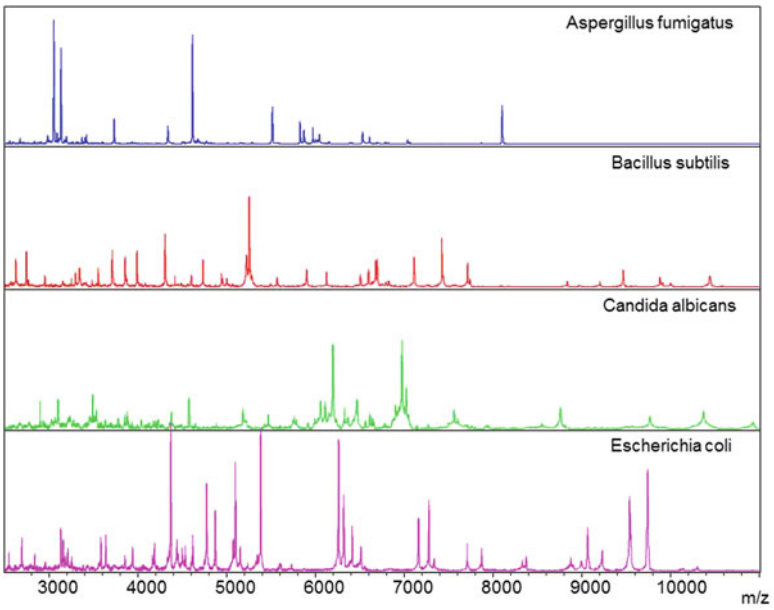

Fig. 2 MALDI-TOF MS profiles acquired using the ethanol/formic acid extraction (EFEx) method for four microorganisms belonging to different phylogenetic branches: the mold *Aspergillus fumigatus*, the Gram-positive bacterium *Bacillus subtilis*, the yeast *Candida albicans*, and the Gram-negative bacterium *Escherichia coli*

of 1–10 microorganisms per millilitre that can be detected. Therefore, liquid media in bottles are used as enrichment culture. A volume of 10–15 mL of blood is collected from the patient, inoculated into a bottle and incubated in an automated system that regularly checks for growth. As soon as the system detects growth (usually after several hours to days depending on the microorganism concentration and speed of growth), the liquid medium with the enriched microorganisms can be used for inoculation of usual solid media for further culture for at least another 24–48 h and subsequent identification and susceptibility testing. As blood stream infections can show a quite severe clinical picture an early initiation of antimicrobial therapy is essential. This is the reason why for an initial analysis direct microscopy with Gram staining is performed as soon as the blood culture has turned positive in order to estimate the group of organisms and a potentially effective therapy. MALDI-TOF MS now allows the identification directly from a positive blood culture bottle without subculture and identification results can be available about 12–24 h earlier.

There are several protocols published for the extraction of proteins for microorganism identification directly from positive blood cultures (La Scola and Raoult 2009; Christner et al. 2010; Moussaoui et al. 2010; Stevenson et al. 2010; Ferreira et al. 2011; Schubert et al. 2011). The main objective of all procedures is to enrich the cultured microorganisms and to separate them from blood cells and other blood

components. Especially, the haemoglobin of the erythrocytes can perturb the MALDI-TOF measurement and need to be excluded.

For the MALDI Biotyper system, the manufacturer offers a ready-to-use kit (MBT Sepsityper IVD Kit) which has been investigated in more than 20 published studies (see e.g. Kok et al. 2011; Schubert et al. 2011; Yan et al. 2011; Buchan et al. 2012; Juiz et al. 2012; Klein et al. 2012; Loonen et al. 2012; Schieffer et al. 2014) and is currently the only IVD-CE kit for this purpose. The kit comprises a lysis buffer, a washing buffer and 1.5-mL plastic reaction/microcentrifugation tubes. As the procedure is based on the EFEx method, the same reagents as for EFEx should also be used.

The extraction is started by transferring about 1 mL of the blood culture fluid to the 1.5-mL plastic reaction tube. In the next step, 200 µL of the lysis buffer are added and mixed by vortexing for about 10 s.[3] The tube is then centrifugated at 13,000 rpm for about 2 min and the supernatant is removed. The remaining pellet is suspended in 1 mL of the washing buffer and mixed by pipetting several times up and down (i.e. dispensing/aspirating). After another centrifugation step for 1 min at 13,000 rpm, the supernatant is again removed. The EFEx procedure can now be performed using the resulting pellet by suspending it in 300 µL of HPLC-grade water, adding 900 µL of ethanol and mixing the solution. After finishing these extraction steps, 1 µL of the extract can be transferred to a spot on the target, dried and then overlaid with 1 µL of matrix solution (cf. Sect. 3.5). The MALDI-TOF MS profiling measurement can then be performed with standard methods.[4]

As haemoglobin will produce large artificial peaks in MALDI-TOF MS measurements, which render the MS spectra useless, several additional washing steps after lysis with the supplied washing buffer can help reducing such problems.

3.6.2 Alternative Protocols

There have been other methods published for the extraction of proteins from blood culture. For instance, saponin (Ferroni et al. 2010) or ammonium chloride (Prod'hom et al. 2010) can be used for lysis, while the separation of microorganisms from blood cells can be performed with differential centrifugation and gel separator tubes (Stevenson et al. 2010; Moussaoui et al. 2010) or just with simple sedimentation (La Scola and Raoult 2009; Christner et al. 2010; Ferreira et al. 2011).

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[3] For blood culture bottles that contain charcoal for adsorbing antimicrobial substances, filtering the blood culture/lysis buffer mixture in the first steps with a spin column like SigmaPrep (cat. no. SC1000-1KT, Sigma-Aldrich; centrifugation for 2 min at 2000 rpm) can remove the distracting particles. For blood cultures with resin particles this procedure is not needed.

[4] Sometimes it is advisable to accept slightly lower score values. In the rare cases when more than one pathogenic microorganism is suspected in the blood culture or when Gram staining already shows multiple morphologies in microscopy, mixture algorithms can be applied for identification. However, in general, blood cultures only contain one microorganism.

3.7 Limitations of Standard Extraction Methods

The described procedures for microorganism profiling, especially the effortless DT method (cf. Sect. 3.3), have disburdened the routine microorganism identification process in medical microbiology. Furthermore, new workflows have been made possible as subsequent steps can now be based on precise identification results. Nevertheless, there are microorganisms that are resistant to standard extraction procedures and give low-quality spectra with no or only a low number of peaks.

As some of these pathogen groups can play an important role in infectious diseases adapted protocols have been developed or are still under review.

3.7.1 Filamentous Fungi

One of the above mentioned groups of microorganisms are the filamentous fungi. Usually, culture on solid media is performed with subsequent microscopy of the grown fungi. Identification is then based on the micromorphological structures observed in the different growth states of the fungus. Since these different growth states and also the sporulation of the fungi provide problems for MS spectrum acquisition, special culture conditions and extraction procedures can produce analytically more valuable spectra.

Different protocols have been published with several starting from mycel grown on solid media (Cassagne et al. 2011; Iriart et al. 2012; Lau et al. 2013; Ranque et al. 2014), one using a short incubation of mycel in liquid medium to unify the fungal material before extraction (Schulthess et al. 2014).

Figure 3 shows the identification of a fungus by conventional microscopy (a) and mass spectral analysis (b).

3.7.2 Mycobacteria

For mycobacteria, special extraction methods are currently under review. These bacteria have only a low number of ribosomes, and standard extraction methods cannot usually overcome their rigid cell walls. Generally, the currently adapted methods apply silica or zirkonia microbeads to disrupt cell aggregates and make the cell surface more accessible to the extraction solution (Lotz et al. 2010; Saleeb et al. 2011; El Khéchine et al. 2011; Balada-Llasat et al. 2013; Buchan et al. 2014). Reported identification rates are in the range of 80–90% of strains occurring in clinical routine work. The results show some limitations in distinguishing very closely related species, similar to the limitations of 16S rDNA sequencing but in some cases somewhat superior. As an example, the *Mycobacterium tuberculosis* complex members cannot be differentiated from each other but there are reports that *Mycobacterium abscessus* and *Mycobacterium massiliense*, both sometimes

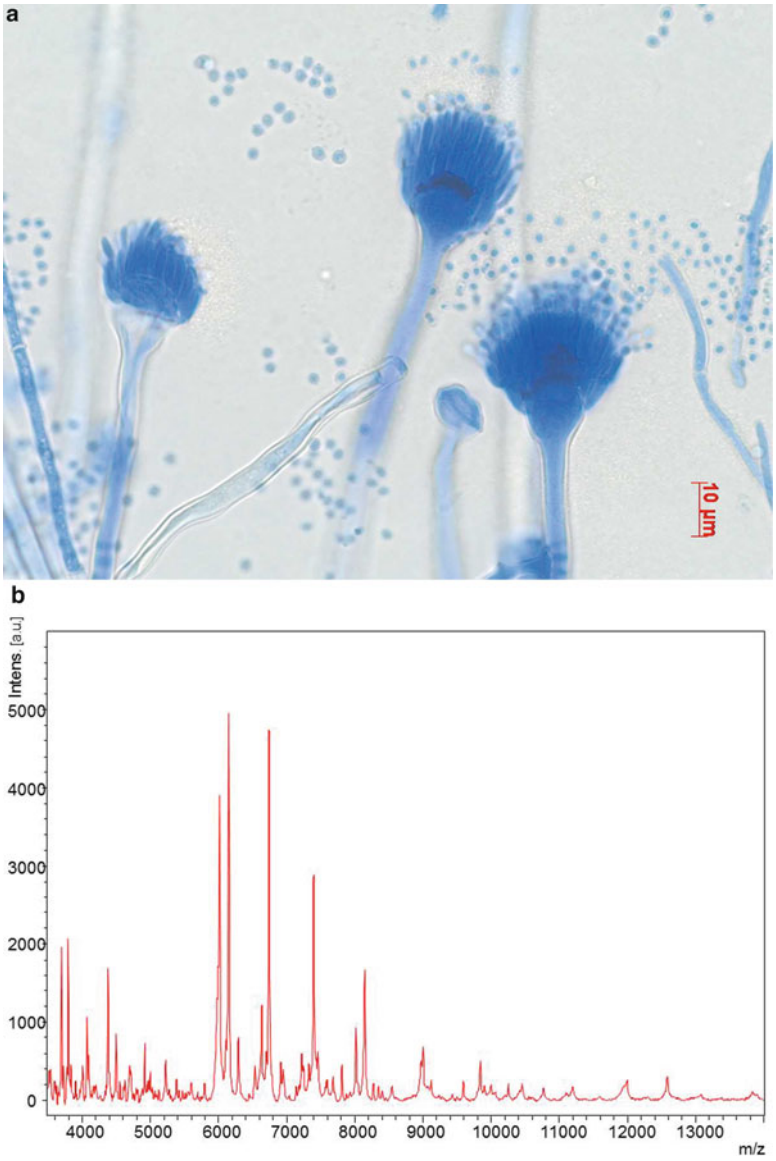

Fig. 3 Identification of the filamentous fungus *Aspergillus terreus*: (*top*) by microscopy using the characteristic size and shape of cells and micromorphological structures (e.g. from conidia (spores), which are formed on long cells sitting on round vesicles); (*bottom*) by MALDI-TOF MS profile analysis, allowing identification 2 days before characteristic morphological structures are observed by microscopy

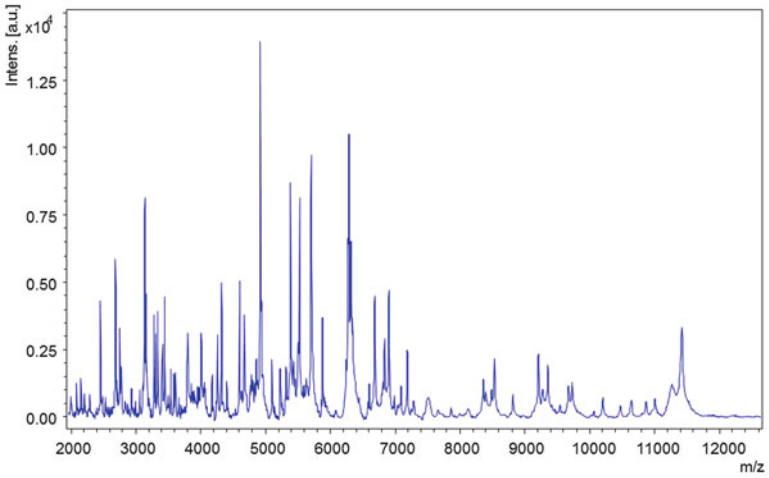

Fig. 4 Profile mass spectrum of the mycobacterium *Mycobacterium fortuitum* after bead-supported extraction of proteins. The microorganism was cultivated on solid Löwenstein–Jensen medium

regarded as subspecies *M. abscessus*, can be differentiated (Tseng et al. 2013; Fangous et al. 2014).

A further field of improvement is the identification of mycobacteria directly from liquid media which have been directly inoculated with patient sample material (generally sputum or aspirates from the patient's respiratory tracts). This might be the most significant contribution of MALDI-TOF MS towards mycobacteria identification as it will further accelerate microbiological routine analysis. However, problems with low cell count in the samples and interference from the patient's sample matrix may have to be overcome to achieve this.

A MALDI mass spectrum collected based on the microbead preparation method published by Buchan and co-workers (Buchan et al. 2014) is shown in Fig. 4.

3.8 Interpretation of Identification Results

Identification through the dedicated software of the different manufacturers is often based on proprietary algorithms. These algorithms differ significantly from each other. The calculation and interpretation of values for identification purposes is defined by the corresponding manufacturer.

With the MALDI Biotyper system a log(score) value is calculated to indicate reliability of the identification result. For the match of a spectrum with each database reference entry (MSP), a score is initially calculated by multiplication of

three independent values. These values are (a) the proportion of peaks from the newly acquired spectrum, which match with peaks from the reference peak lists of the database entries (MSPs), (b) the proportion of the MSP peaks, which match with peaks from the unknown spectrum, and (c) the overall correlation of peak height of both the unknown spectrum and the MSPs. The result is a list of scores between 0 and 1000. The most similar MSP has the highest score value. By taking the logarithm the respective log(scores) are calculated and then ranked by value. The highest log(score) value (maximum of 3) is used for identification, while the following matches can be used for a reliability/quality check.

Initially, the manufacturer defined thresholds for identifications at the genus (≥1.7) and species (≥2.0) level. These were also the acceptance criteria applied in the respective IVD-CE study by the manufacturer in 2008/2009. Subsequent studies of users have shown that the thresholds might be too stringent and sensitivity (proportion of identifications) of the system can be increased without significantly losing specificity, i.e. inducing a higher rate of misidentifications (Theel et al. 2011; Alatoom et al. 2012; Fedorko et al. 2012). Szabados and co-workers even proposed the utilization of species-specific thresholds (Szabados et al. 2012). As the database has evolved and expanded over the years, the manufacturer removed the genus-specific limit and redefined the log(score) values of 1.7 and 2.0 as thresholds for low-confidence and high-confidence identification at the species level, respectively. Similarly, the threshold for identification of microorganisms directly isolated from positive blood cultures was adjusted.

In diagnostics, it is sometimes advisable to know which score level can be reached for different species under routine conditions. This can help with the interpretation of the results. For example, enterobacteria usually yield high log(score) levels up to 2.3 or more. In cases where identifications show lower log(score) levels than usual the identification result should be reviewed as the lower score can be a sign of impure or mixed cultures or other problems in spectra acquisition that can lead to a lower discriminatory power. Statistics of scores from prior identifications for particular species can be utilized to find additional species-specific criteria for acceptance of the identification results.

3.9 Databases

Acquired spectra are typically compared to a database of reference spectra (cf. Sect. 3.8). Current database versions have grown over time to several thousand references and are suitable for routine use in medical microbiology.

Nevertheless, due to the broad range of microorganisms that can occur in routine clinical microbiology analysis there are cases when good-quality spectra show no matches against the database. The software then shows implausible results with scores indicating low reliability of identification. Contrary to conventional biochemical identification systems, the software of MALDI-TOF MS-based systems in principle allows the extension of the database. As the databases of different manu-

facturers are generated differently, based on different mathematical algorithms and identification principles, there are also differences between the systems regarding their possibility to extend the database with own reference spectra.

The Vitek MS system (bioMérieux) is a pure IVD system, which allows no expansion of the database by the user. This is not only due to the restrictions imposed by the principles of an *in vitro* diagnostic system but also to a limitation of the algorithm used for the generation of the database. In short, the advanced spectra classifier algorithm uses a network of biomarkers to characterize a reference spectrum of a given species. For this, a spectrum is divided into thousands of bins. For each bin, the value for identification of the species as well as its value for identification of all other species in the database is calculated. The result is not a database of reference spectra but a kind of a 'biomarker network'. This network has to be calculated anew for each database extension which is only done by the manufacturer for database updates.

However, the VITEK MS plus system has a second, RUO, database which is based on SARAMIS (Spectral ARchive And Microbial Identification System) formerly developed by the company ANAGNOSTEC in Germany. SARAMIS stores raw peak lists from measured strains as well as the so-called 'Super Spectra' in its database. 'Super Spectra' are the main tool for identification. They consist of curated peak lists from multiple measurements with a manually introduced weighting of characteristic peaks for genus and species. The user can also introduce own 'Super Spectra' to extend the database. This is performed based on own measurements and a manual weighting process of the characteristic peaks.

The MALDI Biotyper database is available in two IVD versions, which do not support any extensions by the user. The one called MBT-CA (MALDI Biotyper—Clinical Application) is dedicated to the US diagnostic market, which is regulated by the FDA (US Food and Drug Administration). The other system is the IVD-CE MALDI Biotyper, which is intended for the European market and other countries where European certifications are applied. In addition, a research-use-only system (MALDI Biotyper RUO) is available. It uses the same algorithms as both diagnostic systems and contains the same general database as the IVD-CE MALDI Biotyper. The MALDI Biotyper RUO database can be extended by the user with own reference spectra (MSP).

3.9.1 Database Extension and Creation of Own MSPs for the MALDI Biotyper System

The creation of own MSPs in the MALDI Biotyper software is quite simple and compatible with the usual workflow in microorganism identification. For this, a spectrum with the best possible quality should be acquired. Thus, the optimum protein extraction method should be applied for the given microorganism. In general, the EFEx method is used for bacteria. For compensation of a potentially high measurement variance, the spectrum is acquired multiple times. Therefore, the extract is

transferred to eight sample spots on the target. These eight spots are measured at least three times resulting in 24 spectra of the pure microorganism. An accurate calibration of the MALDI-TOF MS instrument before measurement is mandatory.

After alignment of the 24 microorganism spectra in the flexAnalysis spectra evaluation software (Bruker Daltonik GmbH), they can be reviewed visually and selected in a manual process. Spectra that show conspicuous deviations from the other spectra can then be excluded. In general, it is advisable to use about 20 spectra for the calculation and creation of the MSP in the MALDI Biotyper software for best database entry creation. This ensures an adequate weighting of frequent and less stable peaks. The resulting MSP should be checked by running an identification of itself against the existing database entries. If there are no implausible results and the new strain can be clearly differentiated from other MSPs, it can be used in routine identification. In case of database updates by the manufacturer, own database entries should be reviewed and checked for conflicts with new entries.

References

Alatoom AA, Cazanave CJ, Cunningham SA, Ihde SM, Patel R (2012) Identification of non-diphtheriae corynebacterium by use of matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 50:160–163. doi:10.1128/JCM.05889-11

Alispahic M, Hummel K, Jandreski-Cvetkovic D, Nöbauer K, Razzazi-Fazeli E, Hess M, Hess C (2010) Species-specific identification and differentiation of Arcobacter, Helicobacter and Campylobacter by full-spectral matrix-associated laser desorption/ionization time of flight mass spectrometry analysis. J Med Microbiol 59:295–301. doi:10.1099/jmm.0.016576-0

Alispahic M, Christensen H, Hess C, Razzazi-Fazeli E, Bisgaard M, Hess M (2011) Identification of Gallibacterium species by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry evaluated by multilocus sequence analysis. Int J Med Microbiol 301:513–522. doi:10.1016/j.ijmm.2011.03.001

Amiri-Eliasi B, Fenselau C (2001) Characterization of protein biomarkers desorbed by MALDI from whole fungal cells. Anal Chem 73:5228–5231

Anhalt JP, Fenselau C (1975) Identification of bacteria using mass spectrometry. Anal Chem 47:219–225. doi:10.1021/ac60352a007

Arnold RJ, Reilly JP (1998) Fingerprint matching of E. coli strains with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry of whole cells using a modified correlation approach. Rapid Commun Mass Spectrom 12:630–636. doi:10.1002/(SICI)1097-0231(19980529)12:10<630::AID-RCM206>3.0.CO;2-0

Ayyadurai S, Flaudrops C, Raoult D, Drancourt M (2010) Rapid identification and typing of Yersinia pestis and other Yersinia species by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. BMC Microbiol 10:285. doi:10.1186/1471-2180-10-285

Bader O, Weig M, Taverne-Ghadwal L, Lugert R, Gross U, Kuhns M (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. doi:10.1111/j.1469-0691.2010.03398.x

Balada-Llasat JM, Kamboj K, Pancholi P (2013) Identification of mycobacteria from solid and liquid media by matrix-assisted laser desorption ionization-time of flight mass spectrometry in the clinical laboratory. J Clin Microbiol 51:2875–2879. doi:10.1128/JCM.00819-13

Barbuddhe SB, Maier T, Schwarz G, Kostrzewa M, Hof H, Domann E, Chakraborty T, Hain T (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. doi:10.1128/AEM.02689-07

Barreiro JR, Ferreira CR, Sanvido GB, Kostrzewa M, Maier T, Wegemann B, Böttcher V, Eberlin MN, dos Santos MV (2010) Short communication: Identification of subclinical cow mastitis pathogens in milk by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. J Dairy Sci 93:5661–5667. doi:10.3168/jds.2010-3614

Bernardo K, Pakulat N, Macht M, Krut O, Seifert H, Fleer S, Hünger F, Krönke M (2002) Identification and discrimination of Staphylococcus aureus strains using matrix-assisted laser desorption/ionization-time of flight mass spectrometry. Proteomics 2:747–753. doi:10.1002/1615-9861(200206)2:6<747::AID-PROT747>3.0.CO;2-V

Bessède E, Solecki O, Sifré E, Labadi L, Mégraud F (2011) Identification of Campylobacter species and related organisms by matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry. Clin Microbiol Infect 17:1735–1739. doi:10.1111/j.1469-0691.2011.03468.x

Bille E, Dauphin B, Leto J, Bougnoux M-E, Beretti J-L, Lotz A, Suarez S, Meyer J, Join-Lambert O, Descamps P, Grall N, Mory F, Dubreuil L, Berche P, Nassif X, Ferroni A (2012) MALDI-TOF MS Andromas strategy for the routine identification of bacteria, mycobacteria, yeasts, Aspergillus spp. and positive blood cultures. Clin Microbiol Infect 18:1117–1125. doi:10.1111/j.1469-0691.2011.03688.x

Bizzini A, Durussel C, Bille J, Greub G, Prod'hom G (2010) Performance of matrix-assisted laser desorption ionization-time of flight mass spectrometry for identification of bacterial strains routinely isolated in a clinical microbiology laboratory. J Clin Microbiol 48:1549–1554. doi:10.1128/JCM.01794-09

Bizzini A, Jaton K, Romo D, Bille J, Prod'hom G, Greub G (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. doi:10.1128/JCM.01463-10

Boehme K, Fernández-No IC, Pazos M, Gallardo JM, Barros-Velázquez J, Cañas B, Calo-Mata P (2013) Identification and classification of seafood-borne pathogenic and spoilage bacteria: 16S rRNA sequencing versus MALDI-TOF MS fingerprinting. Electrophoresis 34:877–887. doi:10.1002/elps.201200532

Bruin JP, Kostrzewa M, van der Ende A, Badoux P, Jansen R, Boers SA, Diederen BMW (2014) Identification of Haemophilus influenzae and Haemophilus haemolyticus by matrix-assisted laser desorption ionization-time of flight mass spectrometry. Eur J Clin Microbiol Infect Dis 33:279–284. doi:10.1007/s10096-013-1958-x

Buchan BW, Riebe KM, Ledeboer NA (2012) Comparison of the MALDI Biotyper system using Sepsityper specimen processing to routine microbiological methods for identification of bacteria from positive blood culture bottles. J Clin Microbiol 50:346–352. doi:10.1128/JCM.05021-11

Buchan BW, Riebe KM, Timke M, Kostrzewa M, Ledeboer NA (2014) Comparison of MALDI-TOF MS with HPLC and nucleic acid sequencing for the identification of Mycobacterium species in cultures using solid medium and broth. Am J Clin Pathol 141:25–34. doi:10.1309/AJCPBPUBUDEW2OAG

Cassagne C, Ranque S, Normand A-C, Fourquet P, Thiebault S, Planard C, Hendrickx M, Piarroux R (2011) Mould routine identification in the clinical laboratory by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. PLoS One 6, e28425. doi:10.1371/journal.pone.0028425

Cendejas-Bueno E, Kolecka A, Alastruey-Izquierdo A, Theelen B, Groenewald M, Kostrzewa M, Cuenca-Estrella M, Gómez-López A, Boekhout T (2012) Reclassification of the Candida haemulonii complex as Candida haemulonii (C. haemulonii group I), C. duobushaemulonii sp.

nov. (C. haemulonii group II), and C. haemulonii var. vulnera var. nov.: three multiresistant human pathogenic yeasts. J Clin Microbiol 50:3641–3651. doi:10.1128/JCM.02248-12

Christensen JJ, Dargis R, Hammer M, Justesen US, Nielsen XC, Kemp M, Danish MALDI-TOF MS Study Group (2012) Matrix-assisted laser desorption ionization-time of flight mass spectrometry analysis of Gram-positive, catalase-negative cocci not belonging to the Streptococcus or Enterococcus genus and benefits of database extension. J Clin Microbiol 50:1787–1791. doi:10.1128/JCM.06339-11

Christner M, Rohde H, Wolters M, Sobottka I, Wegscheider K, Aepfelbacher M (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. doi:10.1128/JCM.01831-09

Clark AE, Kaleta EJ, Arora A, Wolk DM (2013) Matrix-assisted laser desorption ionization-time of flight mass spectrometry: a fundamental shift in the routine practice of clinical microbiology. Clin Microbiol Rev 26:547–603. doi:10.1128/CMR.00072-12

Claydon MA, Davey SN, Edwards-Jones V, Gordon DB (1996) The rapid identification of intact microorganisms using mass spectrometry. Nat Biotechnol 14:1584–1586. doi:10.1038/nbt1196-1584

Conway GC, Smole SC, Sarracino DA, Arbeit RD, Leopold PE (2001) Phyloproteomics: species identification of Enterobacteriaceae using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. J Mol Microbiol Biotechnol 3:103–112

Demirev PA, Ho YP, Ryzhov V, Fenselau C (1999) Microorganism identification by mass spectrometry and protein database searches. Anal Chem 71:2732–2738

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. doi:10.1128/JCM.02381-10

Dieckmann R, Helmuth R, Erhard M, Malorny B (2008) Rapid classification and identification of salmonellae at the species and subspecies levels by whole-cell matrix-assisted laser desorption ionization-time of flight mass spectrometry. Appl Environ Microbiol 74:7767–7778. doi:10.1128/AEM.01402-08

Eigner U, Holfelder M, Oberdorfer K, Betz-Wild U, Bertsch D, Fahr A-M (2009) Performance of a matrix-assisted laser desorption ionization-time-of-flight mass spectrometry system for the identification of bacterial isolates in the clinical routine laboratory. Clin Lab 55:289–296

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. doi:10.1371/journal.pone.0024720

Fangous M-S, Mougari F, Gouriou S, Calvez E, Raskine L, Cambau E, Payan C, Héry-Arnaud G (2014) Classification algorithm for subspecies identification within the Mycobacterium abscessus species, based on matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 52:3362–3369. doi:10.1128/JCM.00788-14

Farfour E, Leto J, Barritault M, Barberis C, Meyer J, Dauphin B, Le Guern A-S, Leflèche A, Badell E, Guiso N, Leclercq A, Le Monnier A, Lecuit M, Rodriguez-Nava V, Bergeron E, Raymond J, Vimont S, Bille E, Carbonnelle E, Guet-Revillet H, Lécuyer H, Beretti J-L, Vay C, Berche P, Ferroni A, Nassif X, Join-Lambert O (2012) Evaluation of the Andromas matrix-assisted laser desorption ionization-time of flight mass spectrometry system for identification of aerobically growing Gram-positive bacilli. J Clin Microbiol 50:2702–2707. doi:10.1128/JCM.00368-12

Fedorko DP, Drake SK, Stock F, Murray PR (2012) Identification of clinical isolates of anaerobic bacteria using matrix-assisted laser desorption ionization-time of flight mass spectrometry. Eur J Clin Microbiol Infect Dis 31:2257–2262. doi:10.1007/s10096-012-1563-4

Ferreira L, Sánchez-Juanes F, Porras-Guerra I, García-García MI, García-Sánchez JE, González-Buitrago JM, Muñoz-Bellido JL (2011) Microorganisms direct identification from blood culture by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Clin Microbiol Infect 17:546–551. doi:10.1111/j.1469-0691.2010.03257.x

Ferroni A, Suarez S, Beretti J-L, Dauphin B, Bille E, Meyer J, Bougnoux M-E, Alanio A, Berche P, Nassif X (2010) Real-time identification of bacteria and Candida species in positive blood culture broths by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 48:1542–1548. doi:10.1128/JCM.02485-09

Ford BA, Burnham C-AD (2013) Optimization of routine identification of clinically relevant Gram-negative bacteria by use of matrix-assisted laser desorption ionization-time of flight mass spectrometry and the Bruker Biotyper. J Clin Microbiol 51:1412–1420. doi:10.1128/JCM.01803-12

Frey J, Kuhnert P (2015) Identification of animal Pasteurellaceae by MALDI-TOF mass spectrometry. Methods Mol Biol 1247:235–243. doi:10.1007/978-1-4939-2004-4_18

Gantt SL, Valentine NB, Saenz AJ, Kingsley MT, Wahl KL (1999) Use of an internal control for matrix-assisted laser desorption/ionization time-of-flight mass spectrometry analysis of bacteria. J Am Soc Mass Spectrom 10:1131–1137. doi:10.1016/S1044-0305(99)00086-0

Grosse-Herrenthey A, Maier T, Gessler F, Schaumann R, Böhnel H, Kostrzewa M, Krüger M (2008) Challenging the problem of clostridial identification with matrix-assisted laser desorption and ionization-time-of-flight mass spectrometry (MALDI-TOF MS). Anaerobe 14:242–249. doi:10.1016/j.anaerobe.2008.06.002

Haag AM, Taylor SN, Johnston KH, Cole RB (1998) Rapid identification and speciation of Haemophilus bacteria by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. J Mass Spectrom 33:750–756. doi:10.1002/(SICI)1096-9888(199808)33:8<750::AID-JMS680>3.0.CO;2-1

He Y, Li H, Lu X, Stratton CW, Tang Y-W (2010) Mass spectrometry biotyper system identifies enteric bacterial pathogens directly from colonies grown on selective stool culture media. J Clin Microbiol 48:3888–3892. doi:10.1128/JCM.01290-10

Heller DN, Cotter RJ, Fenselau C, Uy OM (1987) Profiling of bacteria by fast atom bombardment mass spectrometry. Anal Chem 59:2806–2809

Hettick JM, Kashon ML, Simpson JP, Siegel PD, Mazurek GH, Weissman DN (2004) Proteomic profiling of intact mycobacteria by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Anal Chem 76:5769–5776. doi:10.1021/ac049410m

Holland RD, Wilkes JG, Rafii F, Sutherland JB, Persons CC, Voorhees KJ, Lay JO (1996) Rapid identification of intact whole bacteria based on spectral patterns using matrix-assisted laser desorption/ionization with time-of-flight mass spectrometry. Rapid Commun Mass Spectrom 10:1227–1232. doi:10.1002/(SICI)1097-0231(19960731)10:10<1227::AID-RCM659>3.0.CO;2-6

Hsueh P-R, Lee T-F, Du S-H, Teng S-H, Liao C-H, Sheng W-H, Teng L-J (2014) Bruker biotyper matrix-assisted laser desorption ionization-time of flight mass spectrometry system for identification of Nocardia, Rhodococcus, Kocuria, Gordonia, Tsukamurella, and Listeria species. J Clin Microbiol 52:2371–2379. doi:10.1128/JCM.00456-14

Iriart X, Lavergne R-A, Fillaux J, Valentin A, Magnaval J-F, Berry A, Cassaing S (2012) Routine identification of medical fungi by the new Vitek MS matrix-assisted laser desorption ionization-time of flight system with a new time-effective strategy. J Clin Microbiol 50:2107–2110. doi:10.1128/JCM.06713-11

Jadhav S, Sevior D, Bhave M, Palombo EA (2014) Detection of Listeria monocytogenes from selective enrichment broth using MALDI-TOF Mass Spectrometry. J Proteomics 97:100–106. doi:10.1016/j.jprot.2013.09.014

Jarman KH, Daly DS, Petersen CE, Saenz AJ, Valentine NB, Wahl KL (1999) Extracting and visualizing matrix-assisted laser desorption/ionization time-of-flight mass spectral fingerprints. Rapid Commun Mass Spectrom 13:1586–1594. doi:10.1002/(SICI)1097-0231(19990815)13:15<1586::AID-RCM680>3.0.CO;2-2

Jarman KH, Cebula ST, Saenz AJ, Petersen CE, Valentine NB, Kingsley MT, Wahl KL (2000) An algorithm for automated bacterial identification using matrix-assisted laser desorption/ionization mass spectrometry. Anal Chem 72:1217–1223

Juiz PM, Almela M, Melción C, Campo I, Esteban C, Pitart C, Marco F, Vila J (2012) 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 31:1353–1358. doi:10.1007/s10096-011-1449-x

Khot PD, Couturier MR, Wilson A, Croft A, Fisher MA (2012) Optimization of matrix-assisted laser desorption ionization-time of flight mass spectrometry analysis for bacterial identification. J Clin Microbiol 50:3845–3852. doi:10.1128/JCM.00626-12

Klein S, Zimmermann S, Köhler C, Mischnik A, Alle W, Bode KA (2012) Integration of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry in blood culture diagnostics: a fast and effective approach. J Med Microbiol 61:323–331. doi:10.1099/jmm.0.035550-0

Kok J, Thomas LC, Olma T, Chen SCA, Iredell JR (2011) Identification of bacteria in blood culture broths using matrix-assisted laser desorption-ionization Sepsityper™ and time of flight mass spectrometry. PLoS One 6, e23285. doi:10.1371/journal.pone.0023285

Krader P, Emerson D (2004) Identification of archaea and some extremophilic bacteria using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. Extremophiles 8:259–268. doi:10.1007/s00792-004-0382-7

Krishnamurthy T, Ross PL (1996) Rapid identification of bacteria by direct matrix-assisted laser desorption/ionization mass spectrometric analysis of whole cells. Rapid Commun Mass Spectrom 10:1992–1996. doi:10.1002/(SICI)1097-0231(199612)10:15<1992::AID-RCM789>3.0.CO;2-V

Kuhns M, Zautner AE, Rabsch W, Zimmermann O, Weig M, Bader O, Groß U (2012) Rapid discrimination of Salmonella enterica serovar Typhi from other serovars by MALDI-TOF mass spectrometry. PLoS One 7, e40004. doi:10.1371/journal.pone.0040004

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. doi:10.1371/journal.pone.0008041

Lasch P, Drevinek M, Nattermann H, Grunow R, Stämmler M, Dieckmann R, Schwecke T, Naumann D (2010) Characterization of Yersinia using MALDI-TOF mass spectrometry and chemometrics. Anal Chem 82:8464–8475. doi:10.1021/ac101036s

Lau AF, Drake SK, Calhoun LB, Henderson CM, Zelazny AM (2013) Development of a clinically comprehensive database and a simple procedure for identification of molds from solid media by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 51:828–834. doi:10.1128/JCM.02852-12

Loonen AJM, Jansz AR, Stalpers J, Wolffs PFG, van den Brule AJC (2012) An evaluation of three processing methods and the effect of reduced culture times for faster direct identification of pathogens from BacT/ALERT blood cultures by MALDI-TOF MS. Eur J Clin Microbiol Infect Dis 31:1575–1583. doi:10.1007/s10096-011-1480-y

Lotz A, Ferroni A, Beretti J-L, Dauphin B, Carbonnelle E, Guet-Revillet H, Veziris N, Heym B, Jarlier V, Gaillard J-L, Pierre-Audigier C, Frapy E, Berche P, Nassif X, Bille E (2010) Rapid identification of mycobacterial whole cells in solid and liquid culture media by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 48:4481–4486. doi:10.1128/JCM.01397-10

Mandrell RE, Harden LA, Bates A, Miller WG, Haddon WF, Fagerquist CK (2005) Speciation of Campylobacter coli, C. jejuni, C. helveticus, C. lari, C. sputorum, and C. upsaliensis by matrix-assisted laser desorption ionization-time of flight mass spectrometry. Appl Environ Microbiol 71:6292–6307. doi:10.1128/AEM.71.10.6292-6307.2005

Marklein G, Josten M, Klanke U, Müller E, Horré R, Maier T, Wenzel T, Kostrzewa M, Bierbaum G, Hoerauf A, Sahl H-G (2009) Matrix-assisted laser desorption ionization-time of flight mass spectrometry for fast and reliable identification of clinical yeast isolates. J Clin Microbiol 47:2912–2917. doi:10.1128/JCM.00389-09

Martiny D, Dediste A, Debruyne L, Vlaes L, Haddou NB, Vandamme P, Vandenberg O (2011) Accuracy of the API Campy system, the Vitek 2 Neisseria-Haemophilus card and matrix-

assisted laser desorption ionization time-of-flight mass spectrometry for the identification of Campylobacter and related organisms. Clin Microbiol Infect 17:1001–1006. doi:10.1111/j.1469-0691.2010.03328.x

Martiny D, Busson L, Wybo I, El Haj RA, Dediste A, Vandenberg O (2012) 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. J Clin Microbiol 50:1313–1325. doi:10.1128/JCM.05971-11

McElvania Tekippe E, Shuey S, Winkler DW, Butler MA, Burnham C-AD (2013) Optimizing identification of clinically relevant Gram-positive organisms by use of the Bruker Biotyper matrix-assisted laser desorption ionization-time of flight mass spectrometry system. J Clin Microbiol 51:1421–1427. doi:10.1128/JCM.02680-12

Mellmann A, Cloud J, Maier T, Keckevoet U, Ramminger I, Iwen P, Dunn J, Hall G, Wilson D, Lasala P, Kostrzewa M, Harmsen D (2008) Evaluation of matrix-assisted laser desorption ionization-time-of-flight mass spectrometry in comparison to 16S rRNA gene sequencing for species identification of nonfermenting bacteria. J Clin Microbiol 46:1946–1954. doi:10.1128/JCM.00157-08

Mellmann A, Bimet F, Bizet C, Borovskaya AD, Drake RR, Eigner U, Fahr AM, He Y, Ilina EN, Kostrzewa M, Maier T, Mancinelli L, Moussaoui W, Prévost G, Putignani L, Seachord CL, Tang YW, Harmsen D (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. doi:10.1128/JCM.00921-09

Moussaoui W, Jaulhac B, Hoffmann A-M, Ludes B, Kostrzewa M, Riegel P, Prévost G (2010) Matrix-assisted laser desorption ionization time-of-flight mass spectrometry identifies 90% of bacteria directly from blood culture vials. Clin Microbiol Infect 16:1631–1638. doi:10.1111/j.1469-0691.2010.03356.x

Murugaiyan J, Ahrholdt J, Kowbel V, Roesler U (2012) Establishment of a matrix-assisted laser desorption ionization time-of-flight mass spectrometry database for rapid identification of infectious achlorophyllous green micro-algae of the genus Prototheca. Clin Microbiol Infect 18:461–467. doi:10.1111/j.1469-0691.2011.03593.x

Murugaiyan J, Walther B, Stamm I, Abou-Elnaga Y, Brueggemann-Schwarze S, Vincze S, Wieler LH, Lübke-Becker A, Semmler T, Roesler U (2014) Species differentiation within the Staphylococcus intermedius group using a refined MALDI-TOF MS database. Clin Microbiol Infect 20:1007–1015. doi:10.1111/1469-0691.12662

Nagy E, Maier T, Urban E, Terhes G, Kostrzewa M, ESCMID Study Group on Antimicrobial Resistance in Anaerobic Bacteria (2009) Species identification of clinical isolates of Bacteroides by matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry. Clin Microbiol Infect 15:796–802. doi:10.1111/j.1469-0691.2009.02788.x

Nilsson CL (1999) Fingerprinting of Helicobacter pylori strains by matrix-assisted laser desorption/ionization mass spectrometric analysis. Rapid Commun Mass Spectrom 13:1067–1071. doi:10.1002/(SICI)1097-0231(19990615)13:11<1067::AID-RCM612>3.0.CO;2-N

Pineda FJ, Lin JS, Fenselau C, Demirev PA (2000) Testing the significance of microorganism identification by mass spectrometry and proteome database search. Anal Chem 72:3739–3744

Prod'hom G, Bizzini A, Durussel C, Bille J, Greub G (2010) Matrix-assisted laser desorption ionization-time of flight mass spectrometry for direct bacterial identification from positive blood culture pellets. J Clin Microbiol 48:1481–1483. doi:10.1128/JCM.01780-09

Quiles-Melero I, García-Rodríguez J, Gómez-López A, Mingorance J (2012) Evaluation of matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry for identification of Candida parapsilosis, C. orthopsilosis and C. metapsilosis. Eur J Clin Microbiol Infect Dis 31:67–71. doi:10.1007/s10096-011-1277-z

Ranque S, Normand A-C, Cassagne C, Murat J-B, Bourgeois N, Dalle F, Gari-Toussaint M, Fourquet P, Hendrickx M, Piarroux R (2014) MALDI-TOF mass spectrometry identification of filamentous fungi in the clinical laboratory. Mycoses 57:135–140. doi:10.1111/myc.12115

Ryzhov V, Fenselau C (2001) Characterization of the protein subset desorbed by MALDI from whole bacterial cells. Anal Chem 73:746–750

Saenz AJ, Petersen CE, Valentine NB, Gantt SL, Jarman KH, Kingsley MT, Wahl KL (1999) Reproducibility of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry for replicate bacterial culture analysis. Rapid Commun Mass Spectrom 13:1580–1585. doi:10.1002/(SICI)1097-0231(19990815)13:15<1580::AID-RCM679>3.0.CO;2-V

Saleeb PG, Drake SK, Murray PR, Zelazny AM (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. doi:10.1128/JCM.02135-10

Schaumann R, Knoop N, Genzel GH, Losensky K, Rosenkranz C, Stîngu CS, Schellenberger W, Rodloff AC, Eschrich K (2013) Discrimination of Enterobacteriaceae and non-fermenting gram negative bacilli by MALDI-TOF Mass Spectrometry. Open Microbiol J 7:118–122. doi:10.2174/1874285801307010118

Schieffer KM, Tan KE, Stamper PD, Somogyi A, Andrea SB, Wakefield T, Romagnoli M, Chapin KC, Wolk DM, Carroll KC (2014) Multicenter evaluation of the Sepsityper™ extraction kit and MALDI-TOF MS for direct identification of positive blood culture isolates using the BD BACTEC™ FX and VersaTREK(®) diagnostic blood culture systems. J Appl Microbiol 116:934–941. doi:10.1111/jam.12434

Schubert S, Weinert K, Wagner C, Gunzl B, Wieser A, Maier T, Kostrzewa M (2011) Novel, improved sample preparation for rapid, direct identification from positive blood cultures using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. J Mol Diagn 13:701–706. doi:10.1016/j.jmoldx.2011.07.004

Schulthess B, Ledermann R, Mouttet F, Zbinden A, Bloemberg GV, Böttger EC, Hombach M (2014) Use of the Bruker MALDI Biotyper for identification of molds in the clinical mycology laboratory. J Clin Microbiol 52:2797–2803. doi:10.1128/JCM.00049-14

Seng P, Drancourt M, Gouriet F, La Scola B, Fournier P-E, Rolain JM, Raoult D (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. doi:10.1086/600885

Sinha MP, Platz RM, Friedlander SK, Vilker VL (1985) Characterization of bacteria by particle beam mass spectrometry. Appl Environ Microbiol 49:1366–1373

Smole SC, King LA, Leopold PE, Arbeit RD (2002) Sample preparation of Gram-positive bacteria for identification by matrix assisted laser desorption/ionization time-of-flight. J Microbiol Methods 48:107–115

Sparbier K, Weller U, Boogen C, Kostrzewa M (2012) Rapid detection of Salmonella sp. by means of a combination of selective enrichment broth and MALDI-TOF MS. Eur J Clin Microbiol Infect Dis 31:767–773. doi: 10.1007/s10096-011-1373-0

Stephan R, Cernela N, Ziegler D, Pflüger V, Tonolla M, Ravasi D, Fredriksson-Ahomaa M, Hächler H (2011) Rapid species specific identification and subtyping of Yersinia enterocolitica by MALDI-TOF mass spectrometry. J Microbiol Methods 87:150–153. doi:10.1016/j.mimet.2011.08.016

Stevenson LG, Drake SK, Murray PR (2010) Rapid identification of bacteria in positive blood culture broths by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 48:444–447. doi:10.1128/JCM.01541-09

Szabados F, Tix H, Anders A, Kaase M, Gatermann SG, Geis G (2012) Evaluation of species-specific score cutoff values of routinely isolated clinically relevant bacteria using a direct smear preparation for matrix-assisted laser desorption/ionization time-of-flight mass spectrometry-based bacterial identification. Eur J Clin Microbiol Infect Dis 31:1109–1119. doi:10.1007/s10096-011-1415-7

Taniguchi T, Sekiya A, Higa M, Saeki Y, Umeki K, Okayama A, Hayashi T, Misawa N (2014) Rapid identification and subtyping of Helicobacter cinaedi strains by intact-cell mass spectrometry profiling with the use of matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 52:95–102. doi:10.1128/JCM.01798-13

Theel ES, Hall L, Mandrekar J, Wengenack NL (2011) Dermatophyte identification using matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 49:4067–4071. doi:10.1128/JCM.01280-11

Theel ES, Schmitt BH, Hall L, Cunningham SA, Walchak RC, Patel R, Wengenack NL (2012) Formic acid-based direct, on-plate testing of yeast and Corynebacterium species by Bruker Biotyper matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 50:3093–3095. doi:10.1128/JCM.01045-12

Tseng S-P, Teng S-H, Lee P-S, Wang C-F, Yu J-S, Lu P-L (2013) Rapid identification of M. abscessus and M. massiliense by MALDI-TOF mass spectrometry with a comparison to sequencing methods and antimicrobial susceptibility patterns. Future Microbiol 8:1381–1389. doi:10.2217/fmb.13.115

Vaidyanathan S, Winder CL, Wade SC, Kell DB, Goodacre R (2002) Sample preparation in matrix-assisted laser desorption/ionization mass spectrometry of whole bacterial cells and the detection of high mass (>20 kDa) proteins. Rapid Commun Mass Spectrom 16:1276–1286. doi:10.1002/rcm.713

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. doi:10.1128/JCM.02071-09

Vila J, Juiz P, Salas C, Almela M, de la Fuente CG, Zboromyrska Y, Navas J, Bosch J, Agüero J, de la Bellacasa JP, Martínez-Martínez L (2012) Identification of clinically relevant Corynebacterium spp., Arcanobacterium haemolyticum, and Rhodococcus equi by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 50:1745–1747. doi:10.1128/JCM.05821-11

Von Bergen M, Eidner A, Schmidt F, Murugaiyan J, Wirth H, Binder H, Maier T, Roesler U (2009) Identification of harmless and pathogenic algae of the genus Prototheca by MALDI-MS. Proteomics Clin Appl 3:774–784. doi:10.1002/prca.200780138

Wang Z, Russon L, Li L, Roser DC, Long SR (1998) Investigation of spectral reproducibility in direct analysis of bacteria proteins by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Rapid Commun Mass Spectrom 12:456–464. doi:10.1002/(SICI)1097-0231(19980430)12:8<456::AID-RCM177>3.0.CO;2-U

Welham KJ, Domin MA, Scannell DE, Cohen E, Ashton DS (1998) The characterization of micro-organisms by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Rapid Commun Mass Spectrom 12:176–180. doi:10.1002/(SICI)1097-0231(19980227)12:4<176::AID-RCM132>3.0.CO;2-T

Werner G, Fleige C, Fessler AT, Timke M, Kostrzewa M, Zischka M, Peters T, Kaspar H, Schwarz S (2012) Improved identification including MALDI-TOF mass spectrometry analysis of group D streptococci from bovine mastitis and subsequent molecular characterization of corresponding Enterococcus faecalis and Enterococcus faecium isolates. Vet Microbiol 160:162–169. doi:10.1016/j.vetmic.2012.05.019

Williams TL, Andrzejewski D, Lay JO, Musser SM (2003) Experimental factors affecting the quality and reproducibility of MALDI TOF mass spectra obtained from whole bacteria cells. J Am Soc Mass Spectrom 14:342–351. doi:10.1016/S1044-0305(03)00065-5

Wragg P, Randall L, Whatmore AM (2014) Comparison of Biolog GEN III MicroStation semi-automated bacterial identification system with matrix-assisted laser desorption ionization-time of flight mass spectrometry and 16S ribosomal RNA gene sequencing for the identification of bacteria of veterinary interest. Journal of Microbiological Methods 105:16–21. doi: 10.1016/j.mimet.2014.07.003

Yan Y, He Y, Maier T, Quinn C, Shi G, Li H, Stratton CW, Kostrzewa M, Tang Y-W (2011) Improved identification of yeast species directly from positive blood culture media by combining Sepsityper specimen processing and Microflex analysis with the matrix-assisted laser

desorption ionization Biotyper system. J Clin Microbiol 49:2528–2532. doi:10.1128/JCM.00339-11

Zautner AE, Masanta WO, Tareen AM, Weig M, Lugert R, Groß U, Bader O (2013) Discrimination of multilocus sequence typing-based Campylobacter jejuni subgroups by MALDI-TOF mass spectrometry. BMC Microbiol 13:247. doi:10.1186/1471-2180-13-247

Zhu B, Xiao D, Zhang H, Zhang Y, Gao Y, Xu L, Lv J, Wang Y, Zhang J, Shao Z (2013) MALDI-TOF MS distinctly differentiates nontypable Haemophilus influenzae from Haemophilus haemolyticus. PLoS One 8, e56139. doi:10.1371/journal.pone.0056139