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Contribution of MALDI-TOF mass spectrometry in the microbiological diagnosis and clinical management of patients suffering from infectious diseases

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Doctor (PhD) in Biomedical and Pharmaceutical Sciences

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List of abbreviations

ASC	Advanced spectra classifiers
AST	Antimicrobial susceptibility testing
CCI	Composite correlation index
CF	Cystic fibrosis
CFU	Colony forming unit
CHCA	α-cyano-4-hydroxycinnamic acid
CL	Central laboratory
CLSI	Clinical and Laboratory Standards Institute
CoNS	Coagulase-negative staphylococci
CPE	Cytopathogenic effect
DHB	2,5-dihydroxybenzoic acid
DL	Distant laboratory
DNA	Deoxyribonucleic acid
EHEC	Enterohaemorrhagic *Escherichia coli*
ESI	Electrospray ionisation
FDA	Food and Drug Administration
HA-MRSA	Hospital-associated MRSA
ICU	Intensive care unit
IDS	Infectious diseases specialist
IgM	Immunoglobulin M
IVD	In vitro diagnosis
kDa	Kilodalton
LIS	Laboratory information system
m/z	Mass-to-charge ratio
MALDI	Matrix-assisted laser desorption/ionisation
MIC	Minimal inhibitory concentration
MLSA	Multi-locus sequence analysis
MLST	Multi-locus sequence typing
MPCC	Minimal profile change concentration
MS	Mass spectrometry
MSP	Mass spectra projection
MSSA	Methicillin susceptible *Staphylococcus aureus*
MRSA	Methicillin resistant *S. aureus*
NRC	National reference centre
PCR	Polymerase chain reaction
PFGE	Pulsed field gel electrophoresis
PVL	Panton-Valentine leukocidin
RFMP	Restriction fragment mass polymorphism
RMI	Rapid microbial identification
RNA	Ribonucleic acid
RUO	Research use only
SAS	Statistical analysis software
ST	Subtype
TAT	Turn-around-time
TFA	Trifluoroacetic acid
TOF	Time-of-flight
TTI	Time-to-identification
UTI	Urinary tract infection

Abstract

In infected patients, the rapid identification of pathogens is critical. After a long period of slow technological improvement, the microbiology laboratory is now undergoing significant evolution. This work evaluated the contribution of recent MALDI-TOF MS technology in terms of the diagnosis and clinical management of patients and its implementation in the laboratory of tomorrow. The studies were conducted over a 3.5-year period, mostly in the iris public hospital network of Brussels.

First, we confirmed the accurate performance of MALDI-TOF MS in the identification of routine isolates, regardless of whether the Biotyper (92.7% correct species identification) or VITEK MS (93.2%) (n=986) commercial system was used, and demonstrated the supremacy of this technology over conventional identification techniques for fastidious bacteria, including *Campylobacter* and related organisms (98.3%, 72.2% and 79.9% correct species identification by Biotyper, Vitek NH Card and API Campy, respectively; n=234).

Second, we showed that the direct MALDI-TOF MS identification of bacteria from positive blood cultures was not only feasible but also led to an 24-h reduction in the time-to-identification. In an adult population, more than 13% of the direct identifications from positive blood cultures resulted in the faster adaptation of the antimicrobial treatment.

Third, we demonstrated that MALDI-TOF MS could easily be implemented in a network, which was associated with significant cost savings and reduction in the time-to-identification. Finally, our promising *Blastocystis* subtyping results suggest that the number of MALDI-TOF MS applications may be increased.

In the future, automation of the technique will make its use in clinical laboratories even easier, eliminating the use of conventional identification techniques. Improvement of the preanalytical procedures is also important to make MALDI-TOF MS a suitable instrument for resistance and toxicity mechanism detection and subtyping.

1. Introduction

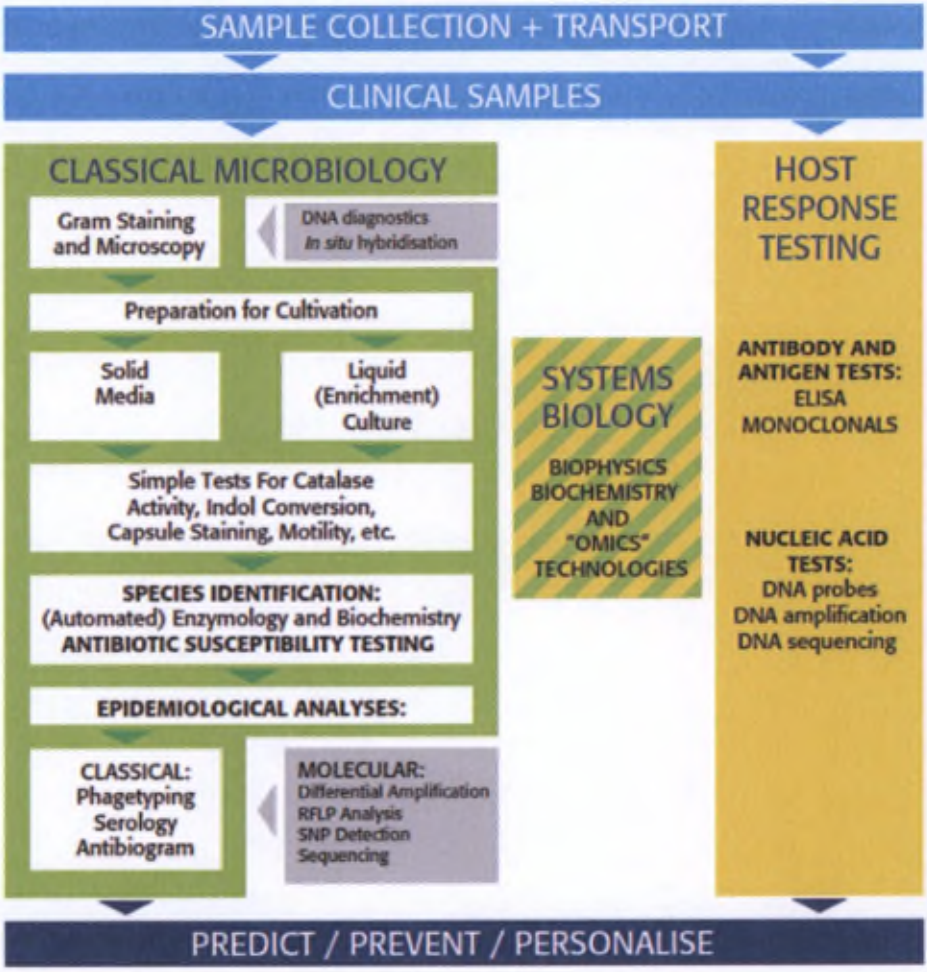


Figure 1. Schematic review of activities and procedures in the laboratory of microbiology (from van Belkum et al. 2013).

1.1. The current microbiology laboratory

1.1.1. Background

Rapid identification of the agents (bacteria, viruses or fungi) responsible for human infectious diseases is critical for patient management in terms of diagnosis and efficiency in the choice of the empirical treatment (Ho et al. 2011). However, clinical microbiology - and bacteriology in particular - has evolved slowly and in many cases is still using the numerous technologies developed in the 19th century (van Belkum et al. 2013). As the identification of a microorganism may take days or weeks and occasionally requires as many as 20 biochemical tests, the need for rapid and reliable alternative procedures is increasing (Ho et al. 2011). A schematic review of the activities and procedures in the microbiology laboratory is presented in Figure 1 (van Belkum et al. 2013).

1.1.2. Bacteriology

After isolation on differential growth media, the identification of bacteria in the clinical laboratory is mainly based on phenotypic tests, including Gram staining, colony shape and size determination, microscopy, motility, growth requirements, oxidase and catalase tests and others. These tests lead to preliminary species assessment, followed by manual or semi-automated biochemical and extended enzymology tests to provide final species identification. This second set of tests often relies on metabolic processes of the microorganism and therefore requires growth and subsequent long incubation times (Bizzini et al. 2010a, van Belkum 2013). Such conventional procedures are laborious, complex and time-consuming (approximately 18 h on average), and species assignment may be inaccurate (Lartigue et al. 2013, Sogawa et al. 2011). Molecular biology techniques such as real-time polymerase chain reactions (PCR) or 16S RNA sequencing are gradually being developed and used as an alternative. However, these analyses are still expensive, time-consuming, technically demanding and not suited for use with the majority of clinical samples commonly analysed in a clinical laboratory (Bizzini et al. 2010a, Lartigue et al. 2013). New biophysical technologies, including matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS), are now appearing in the clinical laboratory (van Belkum et al. 2013).

1.1.3. Mycology

The rapid and accurate identification of fungi as the causal agent of an infection in immunocompromised patients is of particular importance (Nye et al. 2006, Klutts et al. 2011). The cultivation of samples is usually performed on Sabouraud dextrose medium with or without antibiotic supplementation; samples are ideally incubated for four weeks at 30°C (Hazen 1996). A combination of morphological observations and assimilation/fermentation results (germ-tube test, trehalose assimilation test, etc.) are collected to identify yeasts. *Candida* sp. not identified by such rapid methods should nevertheless be investigated further, as accurate identification is required to provide the correct antifungal therapy. Several commercial systems, including chromogenic agar media and biochemical or enzymatic panels (e.g., API ID 20C, API ID 32C and Vitek ID YST systems (bioMérieux, Marcy l'Etoile, France)), are widely used; however, they are occasionally disadvantageous due to limited databases and potential misidentifications (Nye et al. 2006, Marklein et al. 2009).

For the diagnosis of invasive fungal infections, the results from mycological cultures are often obtained too late and are not sufficiently specific, as fungi are a part of the human saprophytic flora and common laboratory contaminants. The serological determination of galactomannan has therefore been introduced as a non-invasive diagnostic tool in the management of haematological patients in whom aspergillosis is suspected (Cuenca-Estrella et al. 2008). Molecular biology tests are still being developed, but there are several limitations to their implementation in the clinical laboratory, including the high cost per analysis, high level of technical expertise required and need for special laboratory facilities (Hazen 1996).

1.1.4. Parasitology

In clinical laboratories, the diagnosis of parasitic infections is mainly based on microscopic examination and involves recognition of the parasite according to its size and morphology. Other techniques, including fluorescence staining, antigen detection by enzyme immunoassay or immunochromatography and molecular biology, are also available.

1.1.5. Virology

Cell culture is the traditional diagnostic tool in virology and is still performed in specialised laboratories. The technique is difficult to standardise and is expensive in terms of skilled staff time, reagents and space (Madeley 1999). However, it is often necessary because it is the only technique that provides biological material for further isolation and susceptibility testing and allows the detection of multiple unsuspected viruses. Every 1-2 days, cell lines are microscopically observed to detect characteristic cytopathogenic effects (CPE), allowing the technologist to identify the virus responsible for the infection. These CPE may appear after only 1 or 2 days or may take as long as 3 weeks, depending on the causal agent. Several methods of antigen detection, mainly fluorescent antibody staining, are widely used and of particular interest for slow growing or labile viruses. These methods are rapid and do not depend on virus viability in the sample, which simplifies sample handling and transport (Storch 2000). More recently, molecular biology methods have been developed for routine purposes. These methods allow the simultaneous detection of multiple viruses in a sample or the quantitation of viral load (HIV, HBV, etc.) through the sensitive detection of specific viral nucleic acids. Finally, serological methods may allow a diagnosis from a single sample when IgM antibodies are present. These methods help define the specific antiviral immunity of the patient and are useful for chronic infections in which the presence of any antiviral antibodies is always indicative of infection (Storch 2000).

Figure 2. Mr. Koichi Tanaka japanese engineer and chemist who jointly won the 2002 Nobel Prize in Chemistry for his part in developing a new a mass spectrometric method for analyses of biological macromodules (from http://wbmsdg.org/wordpress/?s=tanaka; http://superstarsofscience.com/scientist/koichi-tanaka).

Figure 3. Prof. Franz Hillenkamp and Prof. Michael Karas (from http://www.uni-muenster.de/Rektorat/exec/upm.php?rubrik=Alle&neu=1&monat=200312&nummer=04827)

1.2. Matrix-Assisted Laser Desorption Ionisation Time-of-Flight Mass spectrometry (MALDI-TOF MS)

1.2.1. Background

Mass spectrometry is an analytical technique used to detect and determine the amount of a given analyte and its elemental composition through the measurement of the mass of gas-phase ions produced from this analyte (Watson et al. 2007).

Before 1970, nonvolatile and labile molecules were not amenable to mass spectrometry because the technique was only adapted to analytes with significant vapour pressure and able to produce gas-phase ions using electron ionisation or chemical ionisation techniques. After 1970, the development of desorption/ionisation techniques allowed the production of gas-phase ions from a sample in a condensed phase (Watson et al. 2007). The first bacterial characterisation provided by mass spectrometry was reported by Anhalt and Fenselau in 1975 and showed that unique mass spectra were produced from bacterial extracts of different genera and species (Anhalt et al. 1975).

Despite these first promising results, the improvement of bioinformatics and the development of sample preparation and mass spectrometry techniques, it has taken time for the technology to be adopted by clinical laboratories (Murray 2012). In early experiments, only molecules of low molecular masses were analysed because the ionisation process of biomolecules was too energy rich to avoid unpredictable analyte fragmentation. Fast atom bombardment, plasma desorption and laser desorption techniques have therefore been eclipsed by soft ionisation techniques, such as electrospray ionisation (ESI) and MALDI, developed in the late 1980s. Tanaka (Figure 2) was awarded part of the 2002 Nobel Prize in chemistry for his thesis on MALDI, which was first published in 1988. Hillenkamp and Karas (Figure 3) contributed to the development of routine techniques (Tanaka et al. 1988).

The introduction of MALDI-TOF MS has fundamentally revolutionised conventional diagnostic testing in Europe, and the recent 510(k) Food and Drug Administration (FDA) clearance to market MALDI-TOF MS will certainly lead to the complete rethinking of microbiology practices in the United States of America (Murray 2012, Dekker et al. 2011, Mitsuma et al. 2013, Kok et al. 2013).

Other MS techniques do exist that fall outside the scope of the present work (e.g., headspace gas chromatography MS, desorption electrospray ionisation MS).

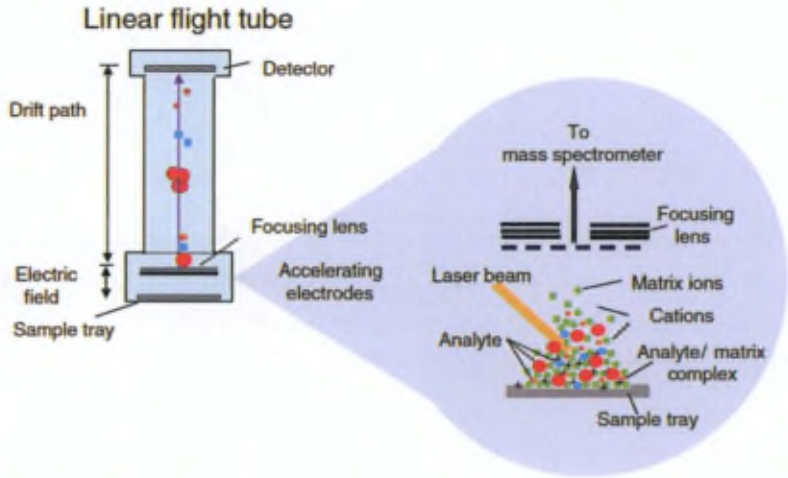


Figure 4. Principle of MALDI-TOF MS identification of microorganisms in schematic diagram. Laser impact causes thermal desorption of proteins of bacteria/yeasts embedded in matrix material and applied to the target plate (analytes shown as red, light blue, and orange spheres, the matrix is given as green spheres). In an electric field, ions are accelerated according to their mass and electric charge. The drift path allows further separation and leads to measurable differences in time-of-flight of the desorbed particles that are detected on top of the vacuum tube (from Wieser et al. 2012).

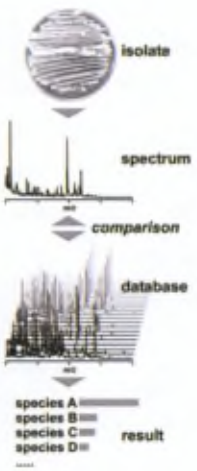


Figure 5. General scheme of MALDI-TOF MS based identification of microorganisms using microbial biomass, generating whole-cell mass spectra to derive a composite strain-specific mass spectrum and comparing the mass spectrum to a reference database (from Welker et al. 2011)

1.2.2. MALDI-TOF MS operating principle

The principles of microorganism identification by MALDI-TOF MS are presented in Figure 4.

MALDI-TOF MS allows the soft ionisation and vapourisation of large, nonvolatile biological molecules (Hillenkamp et al. 2007). The sample for analysis, which is usually taken from a culture plate that provides pure bacteria in sufficient quantities, is smeared onto a target plate and covered by a matrix solution (Lay 2000). When dried, the target plate is loaded in the ionisation chamber of the mass spectrometer. The crystal composed of the sample and matrix is usually exposed to nitrogen laser pulses, resulting in energy transfer from the matrix to nonvolatile analyte molecules with removal (desorption) of positively charged analyte cations in the gas phase (Croxatto et al. 2011). There is no subfragmentation, which makes the technique suitable for whole cell analysis (Dekker et al. 2011). Ionised particles are then accelerated through an electrostatic field within the flight tube under a vacuum and separated based on their mass-to-charge ratio (m/z), with smaller ions travelling faster than larger ions. The time of flight is measured precisely by the arrival of ionised particles at a detector. MALDI-TOF MS usually produces singly charged (z=1) ions, and the m/z therefore corresponds with the value of its mass (Croxatto et al. 2011). The spectral profile is generated and compared with a database of reference spectra, as illustrated in Figure 5. The identification is acquired based on the principle that spectral profiles vary between microorganisms and that some peaks are specific to genus, species and occasionally subspecies. Different strategies were followed to construct the databases. The first strategy consists in including a large number of peaks for each reference strain without focusing on species-specific peaks. In contrast, the second strategy consists in selecting a limited number of conserved species-specific and high-intensity peaks after performing assays on a panel of strains representative of the species (cf. 1.2.6; Carbonnelle et al. 2011).

1.2.2.1. Mass spectrometer

The mass spectrometer is composed of three functional units: an ionisation chamber, a time-of-flight mass analyser, where ions are separated according to their m/z, and a detector to monitor the separated ions (Croxatto et al. 2011).

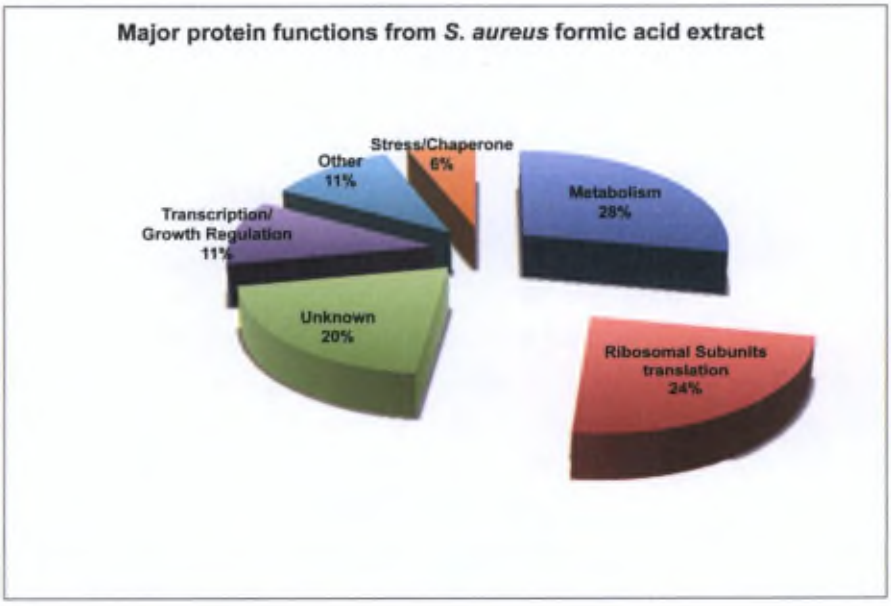


Figure 6. A summary of major functional protein groupings identified by LC-MS/MS in formic acid extracts of MRSA and MSSA strains. Ten strains of MRSA and MSSA were extracted in 35% formic acid/acetonitrile, protease digested and analysed by LC-MS/MS. Protein functional groupings include high abundance proteins detected in at least seven out of ten strains examined (adapted from Drake et al. 2011).

1.2.2.2. Matrix solution

The purpose of the matrix solution is to dilute the analyte molecules to avoid analyte-analyte interactions and to protect the analyte by absorbing the radiation. It is a means to couple the analyte to the energy of the laser by gently transferring charge to the analyte and producing intact protonated molecules (Lay 2000).

The matrix compound is composed of small acidic molecules that have a strong absorbance at the wavelength employed, typically 337 nm, and is usually dissolved in a mixture of solvents, including water, acetonitrile, ethanol and a strong acid, such as trifluoroacetic acid (TFA) (Croxatto et al. 2011). The sample is mixed with the matrix solution, and the matrix solvents penetrate the cell, making the intracellular proteins accessible for analysis. Co-crystallisation results from evaporation of the solvents; the matrix forms a crystal, and the proteins and other cellular compounds are embedded in the matrix. The composition of the matrix solvent mixture may influence the quality of the spectral profile via direct interaction with the microorganism and how that microorganism is incorporated into the matrix during the crystallisation process. Acetonitrile and a 2.5% TFA solution appeared to be optimal choices, leading to better protein profiles in the 3-14 kDa mass range (Domin et al. 1999, Williams et al. 2003).

The acidic nature of the matrix allows the protonation of basic analytes, which explains why most peaks in the MALDI-TOF MS spectrum correspond with ribosomal proteins because such proteins are particularly abundant (20% of total cellular proteins) and also very basic (Suh et al. 2004). Moreover, there is better protein solubility and more efficient extraction from bacterial cells at lower pH values (Sedo et al. 2011). Other highly conserved proteins with housekeeping functions are also detected (Murray 2012). The partition of the proteins detected in *Staphylococcus aureus* is presented in Figure 6.

Several matrix solutions have been evaluated, with α-cyano-4-hydroxycinnamic acid (CHCA) being one of the most commonly used for microbiology (Williams et al. 2003). CHCA and 2,5-dihydroxybenzoic acid (DHB) allow optimal detection of lower mass ions, whereas sinapinic acid was better for detecting ions in the higher mass range. Sinapinic acid demonstrated lower sensitivity when compared with CHCA.

Table 1. List of the matrix commonly used for MALDI-TOF MS methods (adapted from Clark et al. 2013).

Matrix	Sample type(s) analyzed
Picolinic acid, 3-hydroxypicolinic acid, 3-aminopicolinic acid	Oligonucleotides, DNA, and biopolymers
2,5-dihydroxybenzoic acid	Oligosaccharides
α-cyano-4-hydroxycinnamic acid	Peptides and triacylglycerol
3,5-dimethoxy-hydroxycinnamic acid	Proteins
2-(4-hydroxyphenylazo)benzoic acid	Peptides, proteins, glycoproteins
2-mercaptobenzothiazole	Peptides, proteins, synthetic polymers
2,6-dihydroxyacetophenone	Glycopeptides, phosphopeptides
2,4,6-trihydroxyacetophenone	Oligonucleotides

Despite numerous studies on MALDI-TOF MS, the physical chemistry involved in the ionisation process is not sufficiently understood to make predictions about the best choice for a given application, and the choice of one matrix over another still relies on experience rather than science (van Baar 2010). A list of commonly used matrices for MALDI-TOF MS methods is presented in Table 1.

The development of new matrix solutions will expand the number of MALDI-TOF MS applications in the future.

1.2.2.3. Target plates

The analyte signal increases with decreasing surface-binding affinity as a consequence of unbound analyte being free for incorporation into the matrix solution (Peng et al. 2010). Though stainless steel target plates are widely used, numerous surface modifications have been tested to improve the signal resolution (Murray 2012). It is important to not create a liquid smear between the deposits that may result in cross-contamination. This phenomenon seems to be more frequent when using ground steel target plates instead of polished steel target plates, which are ideal for routine purposes (Bizzini et al. 2010b). Coating the target plates with Teflon also improves the concentration of the analyte/matrix during the crystallisation process, resulting in deposits that are technically easier to prepare and a reduction in cross-contamination between deposits (Murray 2012).

1.2.2.4. Deposits

1.2.2.4.1. Extraction

The first description of MALDI-TOF MS analysis of disrupted bacterial cells was reported in 1994 (Cain et al. 1994).

In the extraction procedure, chemicals or enzymes are used to lyse the colonies and release proteins in an extract that is deposed onto the MALDI-TOF MS target plate (Alatoom et al. 2011). This pretreatment inactivates the microorganisms and improves both sample stability and the detection of biomarkers above 15 kDa (Murray 2012). Background artefacts that impede the identification of typical peaks are removed (Fournier et al. 2012).

The extraction procedure is typically performed as follows. The colonies to be identified are mixed in 300 µl of deionised water, and 900 µl of absolute ethanol is then added. After the first centrifugation step (2 min at 16,600 × *g*), the pellet is washed with deionised water and dissolved in 50 µl of 70% formic acid and 50 µl of pure acetonitrile. After the second centrifugation step (2 min at 16,600 × *g*), the supernatant is deposited onto a target plate.

1.2.2.4.2. Whole cell

The first description of MALDI-TOF MS analysis of intact bacterial cells was reported in 1996 (Holland et al. 1996). Using this procedure, the authors showed that five bacterial species (*Enterobacter cloacae*, *Proteus mirabilis*, *Shigella flexneri*, *Escherichia coli* and *Serratia marcescens*) were correctly identified from a larger group by comparison of the spectra with reference spectra.

In the whole cell procedure, which will be called the "direct deposit" procedure throughout this work, colonies taken from growth plates are directly smeared onto the MALDI-TOF MS target plate (Alatoom et al. 2011).

The term «whole cell» is not completely accurate because the MALDI-TOF MS signals are more likely from proteins extracted from the cells and co-crystallised with the matrix (Williams et al. 2003).

1.2.2.4.3. What is the best option?

Conflicting observations have been reported regarding whether an extraction step is required before MALDI-TOF MS analysis.

Numerous authors have highlighted the benefits of using the extraction procedure, especially for Gram-positive bacteria, indicating that this procedure leads to significantly higher scores and a higher proportion of isolates correctly identified at the species level with a concomitant decrease in the proportion of unidentified organisms (Khot et al. 2012, Alatoom et al. 2011). The direct deposit procedure may result in poor score values because of the thick peptidoglycan cell wall of Gram-positive bacteria or due to metabolites, pigments and/or agar material on the surface of the cell that may interfere with the crystalisation process (McElvania TeKippe et al. 2013, Alatoom et al. 2011).

Nevertheless, most microorganisms can be processed by this fast and easy procedure because they are already lysed by exposure to the solvent mixture of the matrix solution (Croxatto 2011). A recent study even suggested that the time-consuming extraction procedure was useless and that direct deposit could lead to accurate results even when performing a single deposit instead of duplicate deposits (Szabados et al. 2012).

Several authors have also evaluated simplified extraction procedures by adding formic acid solutions directly to the deposit on the MALDI-TOF MS target plates with or without adopting adapted cutoff values. Such strategies appear to be efficient for the MALDI-TOF MS identification of yeasts, *Streptococcus* sp., *Aerococcus* sp. and nonfastidious, nonfermenting Gram-negative bacilli (van Herendael et al. 2012, Schulthess et al. 2013, McElvania TeKippe et al. 2013, Ford et al. 2013, Matsuda et al. 2012).

The fast direct deposit procedure remains easier to implement in clinical laboratories.

Figure 7.
Microflex LT marketed by
Bruker Daltonics
(Bremen, Germany)

Figure 8.
VITEK MS marketed by
bioMérieux
(Marcy l'Etoile, France)

Figure 9.
Andromas marketed by
Andromas
(Paris, France)

Table 2. Interpretative identification cutoff values for both the VITEK MS and Biotyper systems

		Indicator	Interpretation	Remark
VITEK MS	Numerical treshold	< 60%	Low confidence level	
		≥ 60%	High confidence level	
	Additionally (if ≥ 60%)		Medium confidence level High confidence level	low discrimination or conflicting results
Biotyper	Numerical treshold	≤1.7	No reliable identification	
		1.7-2	Probable genus identification	
		2-2.3	Secure genus identification, probable species identification	
		≥ 2.3	Highly probable species identification	
	10 matches-based indicator	A	Species consistency	
		B	Genus consistency	score <2 or different species with score >2
		C	No consistency	score <1.7 or different genus with score >1.7
	Identification-based indicator	Matching hint	Known limitation of the system	e.g. *E.coli* vs *Shigella* sp.

1.2.3. Commercial systems

To our knowledge, three commercial in-vitro diagnosis (IVD) systems are currently available in Europe: MALDI Biotyper (Bruker Daltonics, Bremen, Germany), VITEK MS (bioMérieux, Marcy l'Etoile, France) and Andromas (Andromas, Paris, France). The systems are presented in Figures 7, 8 and 9, respectively.

Each MALDI-TOF MS result is associated with a score value that is interpreted based on arbitrary defined cutoff criteria and provides information on the reliability of the identification result. The cutoff criteria recommended by the manufacturers for the two most marketed systems in Europe are presented in Table 2.

The MALDI Biotyper has been developed for use on instruments from Bruker Daltonics (Microflex LT in our laboratory). The Biotyper database includes a large number of main spectra projections (MSP), or "reference spectra", to which the acquired spectra are compared (Lartigue et al. 2013). The identification is provided with a score value based on the similarities between peaks of the acquired and reference full spectra.

The VITEK MS database has recently been developed by bioMérieux to be used on the VITEK MS system. The software uses an identification matrix calculated with the advanced spectrum classifier (ASC). For sample identification, the test spectrum is matched against the identification matrix, and the sum of bin weights is calculated for each of the species represented and transformed into a probability, which is then reported (Welker et al. 2011).

The specifications of the Microflex LT and VITEK MS are presented in the appendix section (appendix 9.1).

Andromas is the third IVD system in Europe and uses the SuperSpectrum algorithm (Carbonnelle et al. 2007). A SuperSpectrum contains a set of relevant species-specific peaks to which the unknown spectrum is compared.

SARAMIS is research use only (RUO) software developed by AnagnosTec (Postdam, Germany) for use on the Axima (Shimadzu, Kyoto, Japan) instrument. It has since been bought by bioMérieux. With this system, identifications are provided with a score value also using the SuperSpectrum algorithm (Lartigue et al. 2013).

The MSP and ASC/SuperSpectra algorithms are different in the manner in which they incorporate microbial biodiversity into the analysis, and the major issues concern the number of isolates introduced into the respective databases to represent species (restricted numbers of individual isolates versus more clinical isolates that may be less well-characterised). The best algorithm has yet to be determined, but both systems appear to be operational (van Belkum et al. 2012).

It should be noted that because of the different algorithms, score values are not comparable between the systems (Patel et al. 2013). As with any identification system, limitations in the databases and identification algorithms should be determined and taken into account when interpreting MALDI-TOF MS results (Patel et al. 2013, de Bel et al. 2010).

1.2.4. Preanalytical parameters affecting MALDI-TOF MS identification

1.2.4.1. Growth conditions

There are conflicting views regarding the influence of growth conditions on subsequent MALDI-TOF MS results. The growth media have been reported to affect the spectra produced by MALDI-TOF MS in several specific cases.

In two different studies, the growth of *Pseudomonas aeruginosa* on McConkey agar growth medium and subsequent use of the direct deposit procedure generated poorer identification results than when other growth media were used (Anderson et al. 2012, Bizzini et al. 2010b). The reasons for such observations remain unclear and could involve the presence of pigments in the McConkey agar growth medium or the induction of the mucoid phenotype of *P. aeruginosa* when grown on this medium (Anderson et al. 2012).

The spectral profile of *S. aureus* also appears to be influenced by cultivation conditions. For example, the addition of blood to the growth medium created peak variations throughout the spectral profile as a consequence of the detection of blood degradation products (Walker et al. 2002).

Additionally, growth media contain sodium chloride, and efforts should be made to suppress salt-containing ions during the MALDI-TOF MS process. The use of formic acid can improve spectrum resolution by increasing the proportion of ions formed by protonation relative to the number formed by sodium ion adduction (Lay 2000).

Other studies have reported no influence of the growth medium on MALDI-TOF MS identification results (Williams et al. 2003, Valentine et al. 2005).

Finally, the growth and storage times and number of subcultures could also alter the protein profiles and should be reduced whenever possible (Williams et al. 2003, Lay 2000, McElvania TeKippe 2013).

1.2.4.2. Technologist expertise

Preparation of the deposits is a crucial parameter, and the trend toward better quality deposits with increased experience has been reported many times (van Herendael et al. 2012, Seng et al. 2009).

1.2.5. Reproducibility

Over time, minor variations were observed in the spectral profiles of *Bacillus atrophaeus* and *E. coli*, likely resulting from variations in the growth procedures and efficiency of solvent extraction prior to analysis. These variations emphasised the need for standardised sample preparation procedures (Saenz et al. 1999). The same conclusion was highlighted by Walker et al., who suggested that the differences in intensities observed between peaks produced by the same organisms are inherent to the technique and should be considered when determining baseline levels (Walker et al. 2002). These authors also showed good intra- and interlaboratory reproducibility when studying *S. aureus* strains.

A large international multicentre study involving eight laboratories from five countries on two continents (Europe n=6, USA n=2) was conducted by Mellmann et al. and also showed a high level of interlaboratory reproducibility of MALDI-TOF MS results, usually achieved only with DNA sequence-based methods. Briefly, 60 blind-coded, nonfermenting bacterial samples were shipped to the eigth laboratories. Of the 480 total samples, 467 (97.3%) were correctly identified at the species level (Mellmann et al. 2009).

Several studies have evaluated the reproducibility of MALDI-TOF MS results using different mass spectrometers. Though spectra acquired from different instruments look different in terms of relative peaks intensities and general profiles, the m/z of many ions are similar and a direct comparison of spectral profiles between laboratories could be performed if a standardised protocol, including interlab fingerprints from combined spectra, is used (Wunschel et al. 2005). When comparing the results obtained from three MALDI-TOF MS mass spectrometers that have different mass resolving power, namely the AutoFlex II, the AutoFlex Speed and the UltrafleXtreme (Bruker Daltonics, Bremen, Germany), this parameter was shown to have no influence on the quality of the MALDI-TOF MS results (Sogawa et al. 2011).

1.2.6. Postanalytical parameters affecting MALDI-TOF MS identification

1.2.6.1. Adaptation of cutoff criteria

The adaptation of the cutoff criteria may improve the rate of correct species identification of the numerous species that are frequently correctly identified but with low score values (e.g., *Bacteroides sp.*, *Staphylococcus lugdunensis*, *Streptococcus dysgalactiae*), therefore limiting the need for additional unnecessary testing (Szabados et al. 2012).

A reduced threshold (1.7 vs. 2 to consider an identification reliable at the species level) has recently been reported to improve the identification of Gram-positive bacteria and to enhance species identification by 14.3% (McElvania TeKippe et al. 2013). Similar observations have been reported for Gram-negative bacteria (Ford et al. 2013).

Khot et al. used a computational approach to determine the optimal cutoff criteria for species and genus level identification. They suggested that 1.9 could be an optimal species cutoff criterion because it increased the rate of correct species identification by 6.9% without significantly influencing the rate of misidentification. They also demonstrated that 1.7 was the optimal genus cutoff criterion (Khot et al. 2012).

Another study suggested that cutoff criteria could be adapted for each bacterial species but that such adaptations would be difficult to manage without efficient interpretation algorithms and bioinformatic tools (Schulthess et al. 2013); the expertise of the microbiologist in this field is also of particular importance (Szabados et al. 2012).

1.2.6.2. Database optimisation

Numerous authors have established a link between the quality of the identification and the number of corresponding spectra in the database (Khot et al. 2012, Schulthess et al. 2013, Seng et al. 2009).

For example, Alatoom et al. used the Biotyper database to demonstrate that when the database contained ≥ 9 spectra from a given species, 75-100% of the isolates from that species were correctly identified. This result may be explained by the fact that an increased number of spectra for the same species in the database will likely better reflect the intraspecies diversity due to variations in protein expression between strains and potentially under different conditions (Alatoom et al. 2011).

It is important to note that regular updates of the database are required (van Belkum et al. 2013). In most studies, the complementation of commercial databases with additional in-house spectra yielded significant improvements in the rate of correct identification at the species level (Sogawa et al. 2012, Schulthess et al. 2013, Verroken et al. 2010, Benagli et al. 2012).

The impact of such updates is illustrated throughout the present work, with concrete examples for several types of microorganisms.

1.2.7. Performance in a routine practice

1.2.7.1. Pros and cons

1.2.7.1.1. Pros

1.2.7.1.1.1. Ease of use

Despite its high-end technology, MALDI-TOF MS is easy to use. The mass spectrometer usually shows a single button to press to open a small trap where the target plate is introduced. The associated software is also user-friendly (Bizzini et al. 2010a).

1.2.7.1.1.2. Improved identification capacity and efficiency

The clinical relevance and pathogenic potential of previously unappreciated taxa are highlighted by the increased species resolution provided by MALDI-TOF MS (McElvania TeKippe et al. 2013). Seng et al. reported an increase in the number of bacterial species identified in their laboratory from 160 in 2002 to 278 in 2012 (Seng et al. 2013). This result may be explained by the fact that a larger number of isolates are identified from each clinical sample by technologists and that the MALDI-TOF MS databases, which are easily updated, are 10-fold broader than those of conventional identification systems (Seng et al. 2013, Sogawa et al. 2012).
Additionally, these authors also reported an increase in their annual analysis capacity from 46,079 analyses in 2002 to 66,989 analyses in 2012.

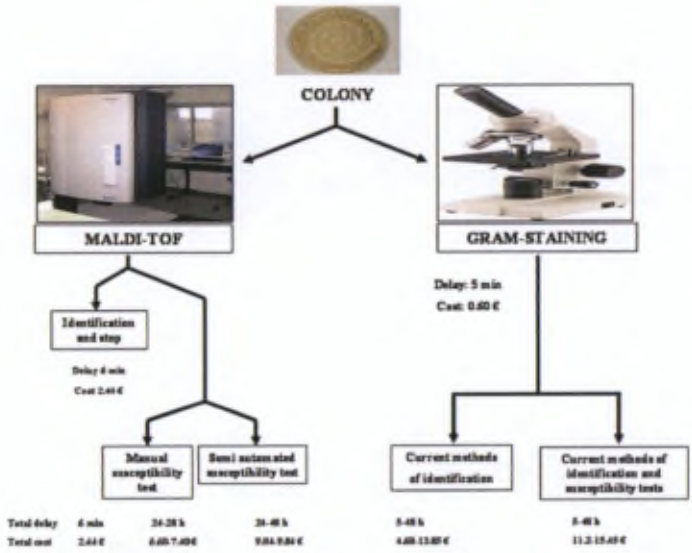


Figure 10. Identification costs, workflow and delay for the MALDI-TOF MS identification of bacteria (from Seng et al. 2009).

1.2.7.1.1.3. Cost

Numerous studies have compared the cost of MALDI-TOF MS identification with conventional identification techniques and showed variable results depending on the design of the financial analysis (Bizzini et al. 2010b, Seng et al. 2009 and 2013, Gaillot et al. 2011, Neville et al. 2011, van Belkum et al. 2012, Tan et al. 2012, Ford et al. 2013, Dhiman et al. 2011).

Early studies that have included consumables, reagents and salaries and considered the depreciation of the instrument over a 5-year period determined the cost of MALDI-TOF MS identification to be approximately one-quarter of that of conventional identification techniques (Seng et al. 2009, Cherkaoui et al. 2010). The identification costs, workflow and analysis times evaluated by Seng et al. in 2009 are presented in Figure 10.

However, other parameters not taken into account in these studies also favourably influence the cost of MALDI-TOF MS analysis compared with conventional identification techniques and include the decreased need for waste disposal, secondary phenotypic analysis and DNA/RNA sequencing and for subcultures and inoculation of multiple growth media (Bizzini et al. 2010b, Gaillot et al. 2011, van Belkum 2012).

The financial analysis should also consider the laboratory's characteristics, including the prevalence of the isolated microorganisms and the identification procedures used, and should take into account the performance of the identification systems (Tan et al. 2012).

Recent studies have reported a cost reduction ranging from 2- to 96-fold with the use of MALDI-TOF MS identification compared with conventional identification techniques and gene sequencing (Seng et al. 2013, Tan et al. 2012, Gaillot et al. 2011).

Finally, Ford et al. reported that the cost of 16S gene sequencing of isolates showing unreliable MALDI-TOF MS identification results (determined to be 6% of all tested isolates) might be balanced by the low cost of MALDI-TOF MS analysis and the reduced need for additional biochemical testing (Ford et al. 2013).

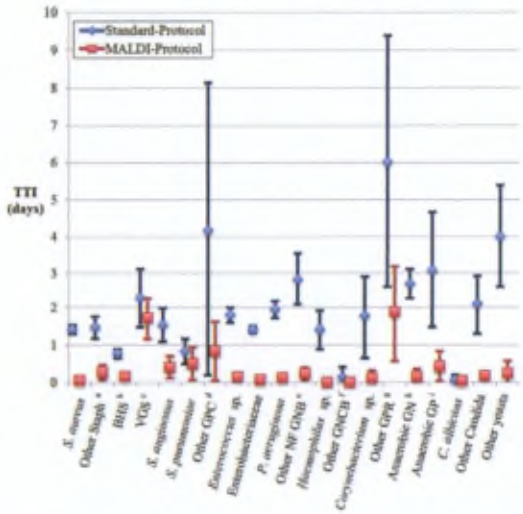


Figure 11. TTI and 95% confidence interval by MALDI-TOF MS and standard protocols.
The blue and red symbols represent the mean TTI by the standard and MALDI-TOF MS protocols and the error bars represent the 95% confidence interval.
The organisms identified were as follow: other Staph[a], staphylococci other than *S. aureus*; BHS[b], beta-hemolytic *Streptococcus*; VGS[c], viridans group *Streptococcus*; GPC[d], Gram-positive cocci members of the Enterobacteriaceae family, NF GNB[e], glucose-nonfermenting Gram-negative bacilli; GNCB[f], fastidious Gram-negative coccobacilli; GPR[g], Gram-positive rod; Anaerobic GN[h], anaerobic Gram-negative bacteria; Anaerobic GP[i], anaerobic Gram-positive bacteria (from Tan et al. 2012).

1.2.7.1.1.4. Rapidity

The MALDI-TOF MS analysis time was much shorter than the analysis time of most conventional and gene sequencing identification techniques, ranging from 1 min 46 sec to 8 min 30 sec depending on the instrument used (Seng et al. 2013).

The entire identification process is thus considerably shortened, and MALDI-TOF MS identification results may be available to clinicians one day earlier than when using other identification techniques for most isolates; the differences are even more pronounced with biochemically inert or slow growing organisms (Neville et al. 2010, Seng et al. 2013, Tan et al. 2012). The time-to-identification (TTI) by MALDI-TOF MS and conventional identification techniques evaluated in a study by Tan et al. is presented in Figure 11 (Tan et al. 2012). Rapid identification by MALDI-TOF MS may be used in combination with local antimicrobial stewardship programs to efficiently optimise early empirical antimicrobial treatments (Seng et al. 2009).

1.2.7.1.1.5. Other advantages

Misinterpretation in the early identification process has no impact on the quality and accuracy of MALDI-TOF MS identification because it does not rely on any upstream information, unlike most conventional identification techniques (Gram stain and/or examination of the colonies to determine which panel of tests to choose) (McElvania TeKippe et al. 2013).

There is no specific equipment required for the implementation of such technology in the clinical laboratory except a chemical hood in which to perform the extraction procedure in case the direct deposit does not yield a valid result (see 1.2.2.4. "Deposits"; Bizzini et al. 2010a).

Finally, as it requires little biological material and few consumables and reagents compared with biochemical-based instruments (Keys et al. 2004), MALDI-TOF MS may be considered a "green" technology (Patel et al. 2013).

1.2.7.1.2. Cons

The major disadvantages of MALDI-TOF MS identification frequently reported by users are the poor discrimination between closely related taxa and the need for organism cultivation (Patel et al. 2013, van Belkum et al. 2013).
Several genera, species and/or pathovars require additional biochemical and/or serological testing to be well-characterised or discriminated. Examples include *E. coli* and *Shigella* sp., *Streptococcus pneumoniae* and *Streptococcus mitis/oralis* and *Salmonella* Typhi (Neville et al. 2011, Wojewoda 2013).

The identification of tiny or mucoid colonies may yield a small amount of protein and may generate poor quality spectra; in such cases, phenotypic identification methods may supplant the MALDI-TOF MS technique, as recently demonstrated in the discrimination between *Pantoea agglomerans* and mucoid *Pseudomonas* spp. (Ford et al. 2013, Alatoom et al. 2011).

The identification of microorganisms from polymicrobial cultures also remains problematic (Mitsuma et al. 2013, Gray et al. 2013).

Finally, the instrument and its maintenance are expensive (van Belkum et al. 2013, Pinto et al. 2011). However, this extra cost is balanced by the low cost of reagents and consumables.

Table 3. This table summarises seven major studies evaluating the global performance of MALDI-TOF MS for routine identification of bacteria and/or yeasts.

Study	Database	Entries or version	Cutoff	n	Genus ID %	Species ID %	No ID %	Error %	Period or origin of strains
Seng et al. CID 2009	Biotyper	2881	1.9/1.7	1660	95.5	84.2	2.8	1.7	16 weeks
van Veen et al. JCM 2010	Biotyper	2881	2/1.7	327	95.1	85.6	1.8	3.1	COLL
				980	97.1	92.0	1.1	1.7	5 weeks
Cherkaoui et al. JCM 2010	Biotyper	NR	1.7	720	94.4		NR	0.8	21 weeks
	Saramis	NR	70%	720	88.8		NR	0.4	
Bizzini et al. JCM 2010	Biotyper	3290	2/1.7	1371	91.5	88.6	6.8	1.8	4 weeks
Carbonnelle et al. JMM 2012	Biotyper	2	1.7	317	98.4	95.3	NR	NR	4 weeks
	Saramis	2008	70%	317	97.2	93.4	NR	NR	
	Biotyper	2	1.7	296	94.9	83.4	NR	NR	Collection
	Saramis	2008	70%	296	83.8	65.9	NR	NR	
Bille et al. CMI 2012	Andromas	NR	65%	2665	97.9	97.9	2.1	0	2 months
Dubois et al. JCM 2012	VITEK MS	1	60%	767	94.9	86.7	3.8	1.3	6 weeks

NR, not reported; COLL, collection strains

1.2.7.2. Bacteriology

Numerous studies are published every year that evaluate the performance of MALDI-TOF MS in the identification of bacterial isolates (Seng et al. 2009, Stults 1995, Dingle et al. 2013). Major works evaluating the potential of MALDI-TOF MS for bacterial identification and comparing its performance with conventional or molecular identification techniques are summarised in this section.

1.2.7.2.1. Global performance

Seven major studies evaluating the global performance of the different available MALDI-TOF MS systems are presented in Table 3.

The rate of correct species identification in these studies ranged from 84.1% to 97.9% when considering only routine isolates.

In the first study, most of the unidentified isolates were anaerobes, and most of the misidentified isolates were species for which there are known limitations of MALDI-TOF MS (e.g., *S. pneumoniae*, *E. cloacae*, *Stenotrophomonas maltophilia*) (Seng et al. 2009).

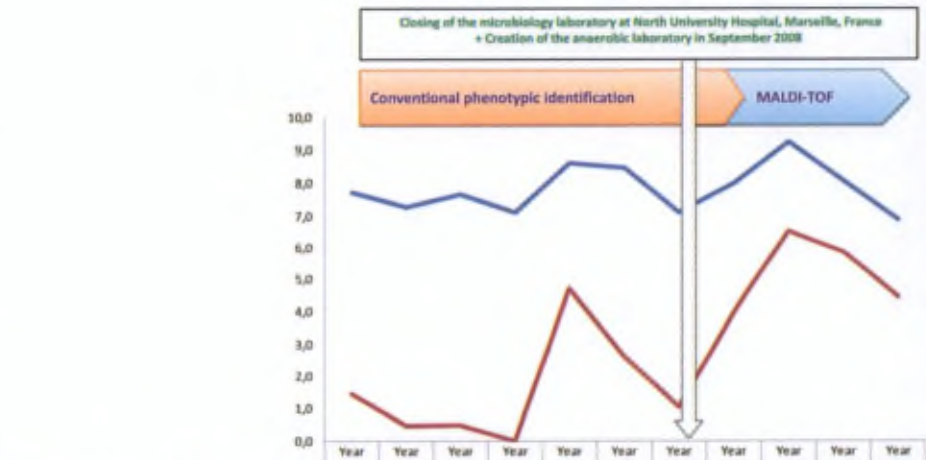
When analysing routine isolates, van Veen et al. observed that MALDI-TOF MS was particularly helpful in identifying staphylococci and HACEK isolates compared with conventional identification techniques. Based on the isolate collection results, the same authors concluded that better results were obtained from Enterobacteriaceae than from nonfermenting Gram-negative bacteria (van Veen et al. 2010).

The identification rates obtained using the SARAMIS database were usually lower than those obtained using the Biotyper database (Cherkaoui et al. 2010, Carbonnelle et al. 2012). Carbonnelle et al. highlighted a major issue with the poor identification of *Neisseria* sp. using SARAMIS (Carbonnelle et al. 2012), and Cherkaoui et al. concluded that the main differences in the identification results observed between systems could be due to database composition (Cherkaoui et al. 2010).

As expected, the rate of correct species identification obtained with updated databases was better than the rates observed in previous studies with older databases (Seng et al. 2009, Bizzini et al. 2010a).

A large study of Bille et al. illustrated excellent identification results obtained using the Andromas database (Bille et al. 2012).

The VITEK MS system also provided satisfactory results (Dubois et al. 2012).



	Year 2002	Year 2003	Year 2004	Year 2005	Year 2006	Year 2007	Year 2008	Year 2009	Year 2010	Year 2011	Year 2012
Well-identified species (n=) i.e. #species per 10,000 isolates	7,7	7,3	7,7	7,1	8,6	8,5	7,1	8,0	9,2	8,1	6,9
Rare species (n species =) i.e. #species per 10,000 isolates	1,4	0,5	0,5	0,0	4,7	2,6	1,1	4,0	6,5	5,9	4,5

Figure 12. Biodiversity of rare species identified in the routine identification of all clinical isolates tested during the last 11 years in a University Hospital in Marseille (from Seng et al. 2013).

1.2.7.2.2. Rare and fastidious growth bacteria

When encountered in the clinical laboratory, infrequently isolated microorganisms usually require numerous biochemical tests and/or expensive gene sequencing analysis that increase both the TAT and identification cost.
The performance of MALDI-TOF MS technology in identifying such microorganisms is less satisfactory than that observed for routine isolates (van Veen et al. 2010, Carbonnelle et al. 2012).

Recently, Khot et al. reported a rate of correct MALDI-TOF MS species identification of 88.3% for infrequently isolated bacteria versus more than 95.8% for frequently isolated bacteria (Khot et al. 2012). However, it has been demonstrated that MALDI-TOF MS can identify infrequent organisms that could not be identified using conventional identification techniques in more than 45% of cases, therefore avoiding the extra costs associated with 16S RNA sequencing. In the remaining 55% of cases, failure to provide accurate MALDI-TOF MS identification was due to the lack of adequate reference spectra in the database or poor quality spectra related to the structural properties of the cell wall of some bacteria or fastidious growth organisms (Bizzini et al. 2010b). This result again emphasises the need for frequent database updates (van Belkum et al. 2013).

A recent study focused on species for which there are fewer than 10 reports designating them as human pathogens in the Pubmed database. The authors discussed the influence of the implementation of MALDI-TOF MS technology on the total number of isolates analysed over time, the rate of rare species identification before and after the introduction of this new technology and the importance of regular database improvements (Seng et al. 2013). The biodiversity of the rare species identified in this laboratory over the last 11 years is presented in Figure 12.

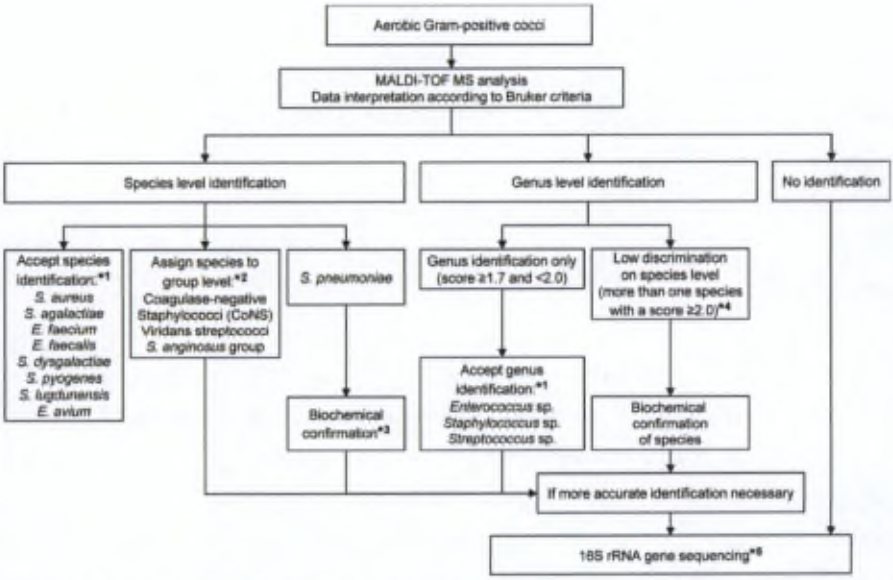


Figure 13. Suggestion of a practical algorithm for the identification of Gram-positive cocci in routine diagnotics complementing MALDI-TOF MS with phenotypic and molecular methods (from Schulthess et al. 2013).

1.2.7.2.3. Major concerns regarding Gram-positive bacteria

For years, Gram-positive bacteria recovered from clinical specimens have not been identified at the species level as often as Gram-negative bacteria in clinical laboratories. In the future, the number of species-level identifications of such organisms will likely increase due to the implementation of MALDI-TOF MS on a daily basis, resulting in increased knowledge about the clinical significance of numerous previously unappreciated organisms (McElvania TeKippe et al. 2013). As previously discussed, numerous strategies have been evaluated to enhance the quality of Gram-positive organism identification by MALDI-TOF MS, including the adaptation of cutoff criteria and evaluation of optimal extraction procedures (McElvania TeKippe et al. 2013, Schulthess et al. 2013). Nevertheless, several issues remain and are presented in this section. Recently, an algorithm has been proposed for the interpretation of Gram-positive MALDI-TOF MS identifications (Figure 13).

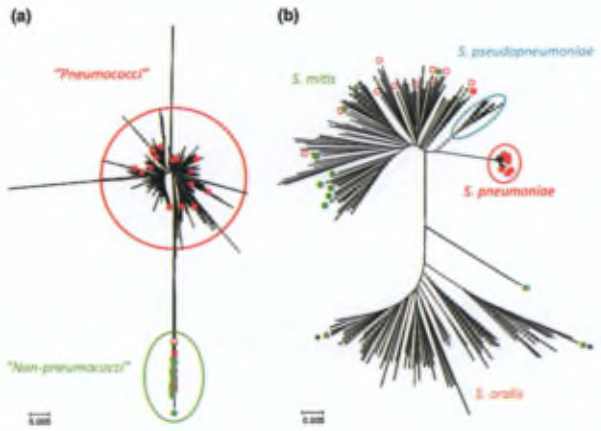


Figure 14. (a) phylogenetic trees constructed on the concatenated sequences of six housekeeping gene fragments displaying the clonal relationship between the strains under study and 6497 *S. pneumoniae* strains stored in the *S. pneumoniae* / MLST database. (b) phylogenetic trees constructed on the concatenated sequences of seven housekeeping gene fragments and displaying the clonal relationship between the strains under study and the 244 strains of the *S. mitis* group stored in the eMLSA database. In both figures, optochin-susceptible strains are marked by red filled circles, while optochin-resistant strains are marked by green filled circles. Red unfilled circles indicate the strains that were primarily characterised as optochin-susceptible, but optochin susceptibility has not been confirmed for repeated tests (from Ikryannikova et al. 2012).

1.2.7.2.3.1. Streptococci

Among α-haemolytic streptococci, *S. pneumoniae* is a major human pathogen responsible for severe infections, including bloodstream infections, severe pneumonia and meningitis. Its discrimination from other members of this group is therefore of clinical importance, as streptococci usually occur commensally in the human body, except in immunocompromised patients or if there is access to the blood. Conventional identification techniques are not always accurate, and the reliable multilocus sequence analysis (MLSA), which provides reliable results, is expensive and time-consuming (Ikryanikova et al. 2012). Despite its better performance than conventional identification techniques for the identification of α-haemolytic streptococci (Lopez Roa et al. 2013, Davies et al. 2012), the high degree of similarity between the mass spectra of *S. mitis*, *S. oralis* and *S. pneumoniae* makes it difficult to discriminate between these species using MALDI-TOF MS. Figure 14 illustrates this close relationship (Ikryanikova et al. 2012). Therefore, the identification of all isolates suspected to be *S. pneumoniae* is often confirmed by additional methods, such as the optochin test (McElvania TeKippe et al. 2013).

However, several authors have recently suggested that specific peaks could contribute to the discrimination of *S. pneumoniae* from *S. mitis/oralis* (m/z 6949, 9876, 9975) and the discrimination of species within the *S. mitis* group (Ikryanikova et al. 2012, Werno et al. 2012). Several recent studies evaluated the performance of the VITEK MS, 1.0 and 2.0, for the discrimination of *S. pneumoniae* from nonpneumococcal species of the *S. mitis* group and reported that this system is able to accurately discriminate between these related organisms (Dubois et al. 2013, Branda et al. 2013, Rychert et al. 2013, Moon et al. 2013). There are also conflicting results regarding the MALDI-TOF MS discrimination of the new species *Streptococcus pseudopneumoniae*, the clinical significance of which is still unknown (Wessels et al. 2012, Dubois et al. 2013).

For most other streptococci, MALDI-TOF MS outperformed conventional identification techniques, showing 100% concordance with the Lancefield classification for beta-haemolytic streptococci, perfect identification of enterococci and complete agreement with 16S RNA sequencing for the identification of *Aerococcus* sp. (Lartigue et al. 2009, Cherkaoui et al. 2011, Fang et al. 2012, Senneby et al. 2013). A recent study even suggested that MALDI-TOF MS was able to detect the highly virulent group B *Streptococcus* subtype (ST)-17 (Lartigue et al. 2011).

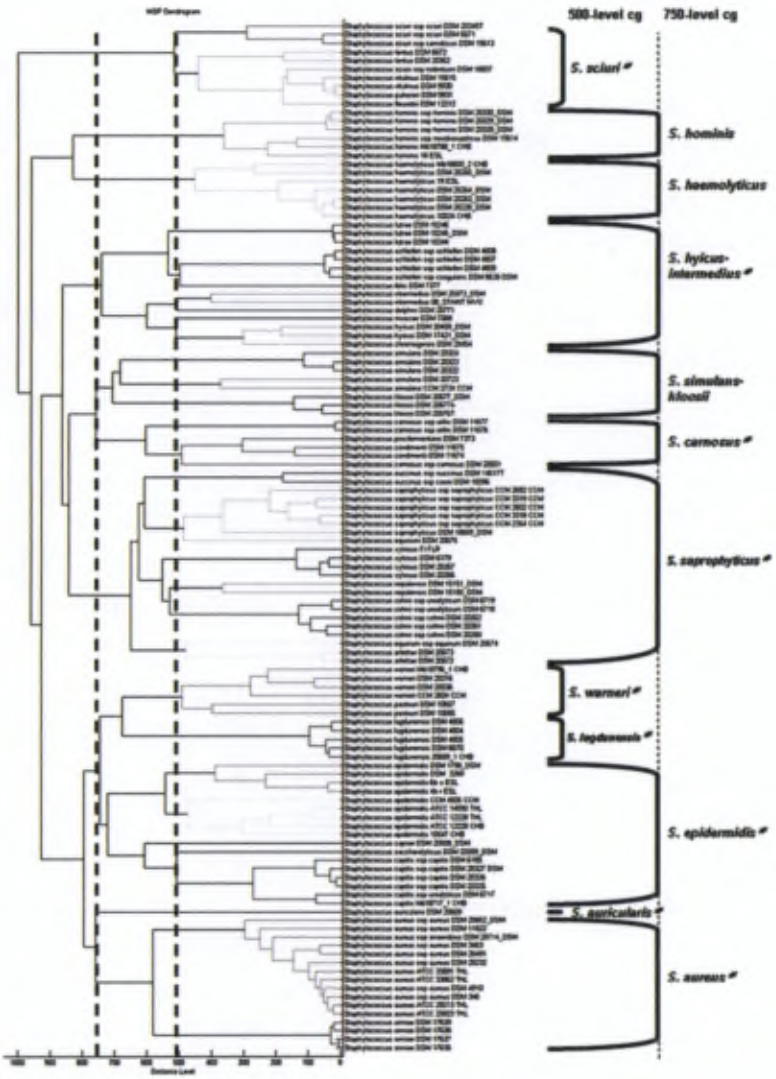


Figure 15. Classification of staphylococcal reference strains shown in score-oriented dendrogram of staphylococcal reference strains included in the database. The "terms 500-level cg" and "750-level cg" define cluster groups (cg) based on the branching pattern using critical distance levels of 500 and 750, respectively. ♯, cluster groups as defined by the phylogenetic analysis of 16s rRNA genes. (from Dubois et al. 2010).

1.2.7.2.3.2. Staphylococci

The accurate identification of staphylococci is of particular importance because *S. aureus* is one of the most frequently isolated nosocomial pathogens responsible for a wide range of infections and because coagulase-negative staphylococci (CoNS) can show pathogenic potential, particularly in immunocompromised patients that have implanted devices (Heikens et al. 2005).

Several authors have demonstrated the benefits of MALDI-TOF MS identification of CoNS compared with conventional biochemical identification techniques (Carbonelle et al. 2007, Dupont et al. 2010). Dupont et al. reported correct CoNS species identification rates of 97.4%, 79% and 78.6% using the MALDI-TOF MS, Phoenix (BD, Erembodegem, Belgium) and Vitek 2 systems (bioMérieux, Marcy l'Etoile, France), respectively (Dupont et al. 2010).

MALDI-TOF MS is easier to perform than conventional identification techniques, rarely results in misidentification, includes large databases that are easily updated and could even have sufficient discriminatory power to group isolates below the species level (Dupont et al. 2010, Dubois et al. 2010). A MALDI-TOF MS-based phylogenetic tree of CoNS isolates is presented in Figure 15.

In studies comparing it to the accurate *tuf* gene sequencing, Carpaji et al. demonstrated that MALDI-TOF MS (Biotyper) was an accurate, easy, cheap and fast method for the identification of clinically important CoNS, whereas Bergeron et al. concluded that the molecular technique was better than MALDI-TOF MS when using the SARAMIS system (Carpaji et al. 2011, Bergeron et al. 2011).

To our knowledge, there is no particular problem related to the use of MALDI-TOF MS in the identification of *S. aureus* strains, and the rates of correct *S. aureus* identification in the different studies were satisfactory (Bizzini et al. 2010b, van Veen et al. 2010). The potentials for MALDI-TOF MS typing for *S. aureus* lineage determination and toxin detection are discussed later in this work (sections 1.2.9.5 and 1.2.9.6).

1.2.7.2.3.3. Other Gram-positive bacteria

The identification of Gram-positive rods is often challenging in clinical laboratories, and MALDI-TOF MS may offer an alternative to inaccurate conventional identification techniques.

For example, the laboratory skills necessary for the diagnosis of diphtheria have declined in many parts of the world, both in routine microbiological laboratories and in diphtheria reference centres. Identification of the non-diphtheriae *Corynebacterium* sp. is also particularly challenging, and conventional identification techniques can yield inaccurate results. After updating the commercial databases, several authors have demonstrated that MALDI-TOF MS is a useful approach for the identification of most *Corynebacterium* sp. (Konrad et al. 2010, Alatoom et al. 2012). MALDI-TOF MS analysis of *Corynebacterium* sp. requires an extraction step because of the peptidoglycan layer and mycolic acids present in the cell wall.
The current system is unable to detect diphtheria toxin, which is larger than the detected ribosomal proteins. Additionally, closely related species, such as *Corynebacterium aurimucosum* and *Corynebacterium minutissimum*, cannot be discriminated.

The establishment of a specific database in combination with a boiling step and formic acid extraction procedure have been particularly helpful in the identification of *Nocardia* sp., for which conventional identification techniques require skilled technologists and are usually difficult to interpret (Verroken et al. 2010, Leli et al. 2013a).

Finally, MALDI-TOF MS shows promising results for the discrimination of *Bacillus* sp. (Fernandez-No et al. 2013) and outperforms conventional techniques in the identification of *Actinomyces* sp., yielding 99% correct species identification versus only 33% using the API Coryne system (bioMérieux, Marcy l'Etoile, France), a system that also leads to a high rate of misidentification (Ng et al. 2012).

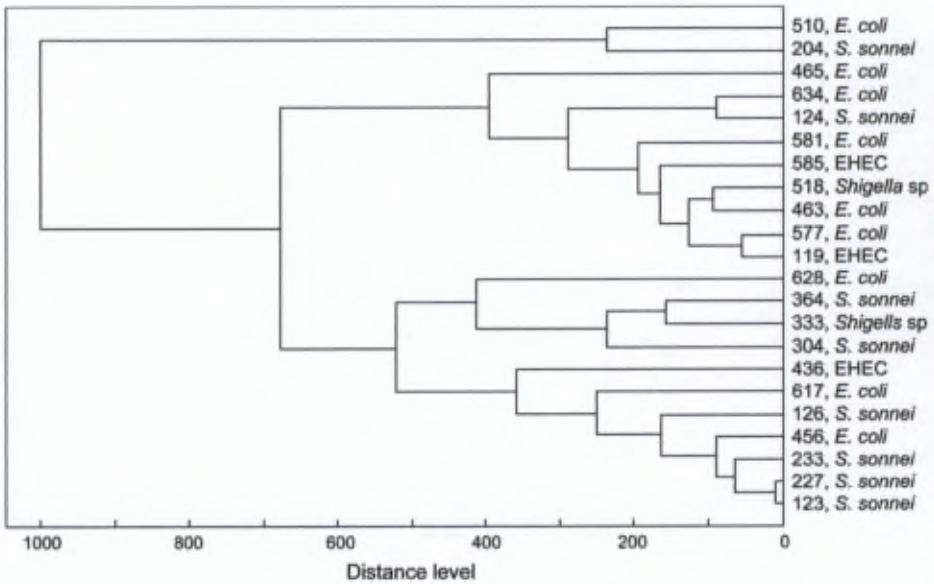


Figure 16. Log (score)-derived MSP dendrogram from *Shigella*, enterohaemorrhagic *E. coli* (EHEC), and *E. coli* using the MALDI-TOF MS-based Biotyper system. The dendrogram was generated with the distance set at Euclidian and linkage set at completion (from He et al. 2010).

1.2.7.2.4. Major concerns regarding Gram-negative bacteria

In most studies evaluating its global performance from a clinical perspective, MALDI-TOF MS appears to yield better identification results for Gram-negative bacteria than Gram-positive bacteria (Cherkaoui et al. 2010, Bizzini et al. 2010b, van Veen et al. 2010) and outperforms conventional biochemical identification techniques, especially for less frequently encountered species (Saffert et al. 2011). However, there are several limitations of MALDI-TOF MS, which are presented in this section. These limitations should be taken into account when interpreting the Gram-negative bacteria identification results.

1.2.7.2.4.1. Enterobacteriaceae

Enterobacteriaceae are usually better identified using MALDI-TOF MS compared with all other bacterial groups (van Veen et al. 2010). However, for several genera or complexes, such as *Citrobacter* sp. or the *E. cloacae* complex, the MALDI-TOF MS identification is occasionally limited to the genus level (Richter et al. 2013, Bizzini et al. 2010b). In these particular situations, laboratories should consider reporting at the genus/complex level, and the database should provide a warning (Saffert et al. 2011).

As illustrated in Figure 16, the MALDI-TOF MS-based spectra of *E. coli* and *Shigella* sp. are highly similar and equally distributed (Hé et al. 2010, Saffert et al. 2011). *Shigella* is absent from the Biotyper database and should be systematically discriminated from *E. coli* using both biochemical and serological tests when identifying non- or slow lactose-fermenting isolates from stool samples, intestinal biopsies and other intestinal samples. (Hé et al. 2010, Neville et al. 2011). Recently Khot et al. suggested that this limitation could be due to lack of sufficient analysis tools rather than inherent shortcomings of the method (Khot et al. 2013).

Similarly, isolates correctly identified as *Salmonella* require additional biochemical and serological tests to exclude/confirm *S.* Typhi and *Salmonella* Paratyphi (Neville et al. 2011). While some serovar-specific peaks have been identified by Dieckmann et al., MALDI-TOF MS can only be used as a rapid screening tool, and serotyping methods are still required (Dieckmann et al. 2011). Several biomarkers have also recently been identified that could discriminate *S.* Typhi from non-Typhi strains (Kuhns et al. 2012).

The identification of other enteric bacterial pathogens, such as *Clostridium difficile*, *Campylobacter* sp. and *Yersinia* sp., is easily provided by MALDI-TOF MS (Bessède et al. 2011, Stephan et al. 2011).

Finally, the discrimination of *Raoultella* sp. from *Klebsiella oxytoca* by both MALDI-TOF MS and conventional identification techniques is unreliable. Using Biotyper, the application of an additional 10% cutoff criterion (a difference of 10% in the identification score of the first MALDI-TOF MS match and the score of the first discrepant match) for isolates dispaying the better score value for *Raoultella ornithinolytica* could increase *Raoultella* genus identification accuracy (de Jong et al. 2013).

1.2.7.2.4.2. Nonfermenting Gram-negative bacteria

The correct species identification rate of nonfermenting Gram-negative bacteria is usually lower than that of enteric Gram-negative bacteria (Ford et al. 2013). Rapid and reliable identification are nevertheless required because nonfermenting Gram-negative bacteria can cause severe opportunistic infections in immunocompromised patients.

Several studies focused on the nonfermenting bacteria responsible for infections in patients with cystic fibrosis (CF) evaluated the use of MALDI-TOF MS for the identification of such organisms. The systems (VITEK MS and Biotyper) performed better than conventional identification techniques at providing rapid and reliable identification of *P. aeruginosa*, *Achromobacter* sp. and isolates of the *Burkholderia cepacia* complex (Marko et al. 2012, Fernandez-Olmos et al. 2012). This result is of clinical importance because a delay in the identification of *B. cepacia* complex members can result in unnecessary isolation measures and because CF patients infected with such organisms are not eligible for lung transplantation (Alby et al. 2013).

Studies evaluating MALDI-TOF MS performance in the identification of *Acinetobacter* sp. have reported variable results. Alvarez-Buylla et al. noted the limited database for *Acinetobacter* sp. other than *A. baumannii* and emphasised the need for its expansion (Alvarez-Buylla et al. 2012). In contrast, the system appeared to be adequate for the identification of isolates from the *Acinetobacter baumannii* group, yielding correct species identification of 98% of all tested isolates after updating the database to include *Acinetobacter pittii* and *Acinetobacter nosocomialis* (Espinal et al. 2012).

Recently, a study evaluating the performance of the VITEK MS 2.0 in the identification of non-*Enterobacteriaceae* Gram-negative bacilli reported a correct identification rate of 78.2% at the species level (Manji et al. 2013).

1.2.7.2.4.3. Other Gram-negative bacteria

Haemophilus parainfluenzae, *Aggregatibacter aphrophilus*, *Cardiobacterium hominis*, *Eikenella corrodens* and *Kingella kingae* are Gram-negative bacteria members of the HACEK group. These organisms are responsible for endocarditis and a wide range of other serious infections involving bones, joints, head and neck, lungs and other tissues. MALDI-TOF MS is a promising tool for the identification of these fastidious organisms but requires an update of the database, as demonstrated by Couturier et al., who reported an improvement in the species identification rate from 66% to 79% after the addition of in-house reference spectra (Couturier et al. 2011). A recent study evaluating the SARAMIS 4.09 database compared with conventional identification techniques of 140 HACEK isolates reported a correct species identification rate of 86.4% using the MALDI-TOF MS technique versus only 60.7% and 77.4% using the Remel RapidID NH system (Thermo Scientific, Zellik, Belgium) and Vitek 2 NH Card system (bioMérieux, Marcy l'Etoile, France), respectively (Powell et al. JCM 2013).

Discrimination between *Haemophilus* sp. is particularly important due to the different clinical presentations related to each species; *Haemophilus influenzae* is responsible for respiratory tract infections, whereas *H. parainfluenzae* is a saprophyte of the upper respiratory tract that may occasionally causes endocarditis. The etiologic role of *Haemophilus haemolyticus* remains controversial. To date, cultures and biochemical tests remain the gold standard, although misidentifications can occur. Several authors have evaluated the use of MALDI-TOF MS for the discrimination between these species compared with conventional and/or molecular identification techniques (Frickmann et al. 2013, Zhu et al. 2013). Frickmann et al. reported poor performance of both the SARAMIS system and conventional identification techniques for the identification of *Haemophilus* sp. and obtained better results using the Biotyper system after the database was completed with additional *H. haemolyticus* reference strains that were previously lacking (Frickmann et al. 2013). Zhu et al. presented MALDI-TOF MS as a reliable and rapid tool for the discrimination between nontypable *H. influenzae* and *H. haemolyticus* (Zhu et al. 2013). However, the great spectral similarity between *H. influenzae* and *H. haemolyticus* can lead to questionable results because both species may appear in the top of the match list with reliable and acceptable score values.

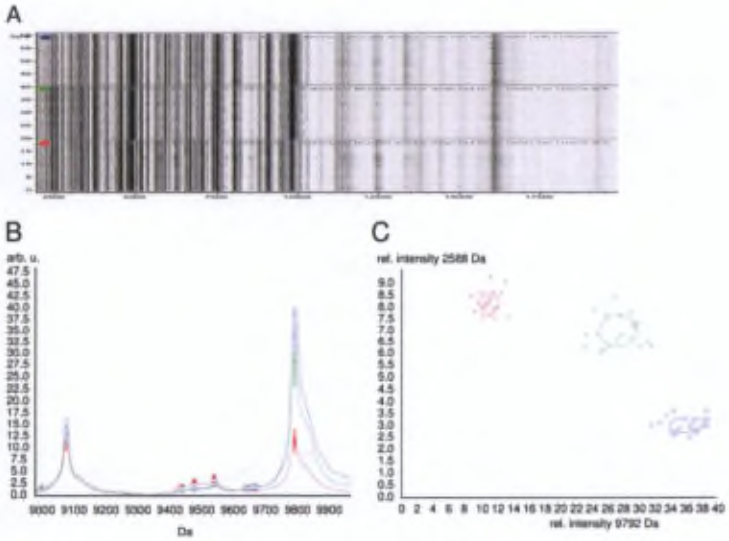


Figure 17. Comparison of spectra originating from *Brucella abortus* bv 1 grown for 24, 72 and 144h. (A) gel-view of spectra obtained from *B. abortus* bv 1 grown for 24 (red), 72 (green) and 144 (blue) hours of cultivation. Although the qualitative mass pattern remained stable over time, marked quantitative changes were observed which are shown in (B) and (C). Intensities of various peaks significantly changed over time (e.g. 9,792 Da), while others, such as 9,008 Da, did not (B). A scatter plot representing peak intensities at 2,558 Da and 9,792 Da (C) indicates that spectra generated at different points in time can be well separated by relative peak intensities (from Karger et al. 2013).

There are conflicting views regarding the potential discrimination between species inside the genus *Brucella* using MALDI-TOF MS (Ferreira et al. 2010a, Karger et al. 2013). Particular attention should be paid to the age of the culture when analysing these organisms by MALDI-TOF MS, as considerable peak changes are observed according to the growth phase, as presented in Figure 17 (Karger et al. 2013).

Finally, MALDI-TOF MS could replace sequencing techniques for the routine identification of *Legionella* sp. if the databases are updated; MALDI-TOF MS also appears to be an interesting approach for further in-depth studies of the epidemiology of *Pasteurella* species (Svarrer et al. 2012, Zangenah et al. 2013).

Table 4. This table summarises the major studies evaluating the performance of MALDI-TOF MS for the identification of anaerobic bacteria.

Study	db	Entries or version	E?	CO	n	Genus ID %	Species ID %	No ID %	Error %	Period or origin
Nagy et al.* CMI 2009	Biotyper	3260	Y	1.7/2	277	97.5	97.5	2.5	0.0	COLL
Veloo et al. CMI 2011	Biotyper	3746	N	1.7/2	79	51.9	35.4	41.8	6.3	CI
			Y			60.8	50.6	30.4	8.9	
	SARAMIS	2875 SSp 37804 RSp	N	50%		70.9	60.8	25.3	3.8	
Justesen et al. JCM 2011	Biotyper	3.1.1.0	P	2	290	67.2	67.2	24.8	7.9	CI 3 years + RS
	SARAMIS	update 2010		80%		60.7	49.0	37.9	1.4	
La Scola et al. Anaerobe 2011	EB	NR	N	1.9	544	NR	61	39	0	CI 1 year
Veloo et al. SAM 2011**	Sdb	89	N	50%	107	NR	89.7	10.3	0	CI
Wybo et al.* JCM 2012**	Biotyper	3.2.1.0	N	1.7/2	102	73.5	62.7	26.5	0	CI
	EB	3.2.1.0 + 23				89.2	83.3	10.8	0	
Culebras et al.* Anaerobe 2012	Biotyper	1.7/2.0	Y	1.9	193	99.5	87.0	0.5	6.7	CI 2 years
Fedorko et al. EJCMID 2012	Biotyper	2.0.4	Y	2	152	NR	78.9	17.8	3.3	RS + CI
Fournier et al. Anaerobe 2012	Biotyper	3995	N	1.7/2	238	87.0	66.4	13.0	0	CI
			Y			91.6	77.7	8.4	0	
Schmitt et al. JCM 2013	EB	4613 + 87	P	1.7/2	253	91.7	70.8	7.9	4.7	CI 6 months
Barreau et al. Anaerobe 2013	Biotyper	3.0	N	1.7/1.9	1325	99.0	92.5	1.0	0	CI 30 months
Garner et al. CMI 2013	VITEK MS	2.0	N	60%	651	92.5	91.2	7.5	0	CI + COLL

* Studies focusing on *Bacteroides* sp.
** Study focusing on Gram-positive anaerobic cocci
*** Study focusing on *Prevotella* sp.
db, database; EB, enriched Biotyper; Sdb, specific database;
E?, extraction?; Y, yes; N, no; P, on plate; CO, cutoff
RS, reference strain; COLL, collection strains; CI, clinical isolates; NR, not reported
Ssp, Superspectra; Rsp, reference spectra

1.2.7.2.5. Anaerobes

Because of its rapidity, MALDI-TOF MS is a promising tool for slow growing or biochemically inactive bacteria (Clark et al. 2013). Therefore, it is of particular interest for the identification of anaerobes, and numerous authors have evaluated MALDI-TOF MS performance in this particular field.

The major studies related to the use of MALDI-TOF MS for the identification of anaerobic isolates are summarised in Table 4.

While previously evaluated as an experimental tool, MALDI-TOF MS has quickly become a rapid identification tool with important benefits for clinical laboratories. The first experiments were conducted by Shah et al., who demonstrated that MALDI-TOF MS was able to discriminate between *Porphyromonas* species and showed value as an epidemiological tool in the study of *Fusobacterium nucleatum* subspecies (Shah et al. 2002).

From a clinical laboratory perspective, MALDI-TOF MS performance in the identification of anaerobic bacteria was first disappointing after the revolution it caused for aerobic bacteria. The rate of correct species identification ranged from 35.4% to 67.2% in different studies (Veloo et al. 2011a, Justesen et al. 2011). Not surprisingly, the major identification problems not only concerned species not included in the databases but also the discrimination of *Fusobacterium* sp. and *Propionibacterium* sp., which might be explained by their high intraspecies diversity (Justesen et al. 2011, Veloo et al. 2011b, Fedorko et al. 2012, Coltella et al. 2013). Incomplete databases may also be problematic for the user because there is no easy method to detect potential misidentifications if one of several closely related species is absent from the database, as was the case for *Bacteroides dorei* and *Bacteroides xylanisolvens* misidentification as *Bacteroides vulgatus* and *Bacteroides ovatus*, respectively, in a study by Culebras et al. (Schmitt et al. 2013, Coltella et al. 2013, Culebras et al. 2012). Most authors have therefore highlighted the need for database updates, such as adding reference spectra for unrepresented species and species present in the databases but for which poor results were observed (Schmitt et al. 2013, Wybo et al. 2012).

These adaptations were rapidly implemented and soon demonstrated the great potential of the technology for the identification of anaerobic bacteria. Veloo et al. reported a remarkable improvement in the rate of correct identification (more than 12% at both the species and genus levels) when retesting the isolates of their study against the updated version of the Biotyper database made available at the end of the study period (Veloo et al. 2011a). Nevertheless, the discrimination between *B. dorei* and *B. vulgatus* and between *B. ovatus* and *B. xylanisolvens* remains problematic (Pedersen et al. 2013).

More recently, Barreau et al. reported an improvement of nearly 30% in the rate of correct species identification (92.5% vs. 61%, n=1325) compared with a previous study also conducted on clinical isolates under the same analytical conditions but with an older database (Barreau et al. 2013, La Scola et al. 2011). With the updated database, the authors were also able to identify rare/recent species for the first time in their laboratory, and the number of "unknown" organisms was significantly decreased (Barreau et al. 2013). When studying *Prevotella* sp., Wybo et al. demonstrated that the addition of a second reference spectrum for *Prevotella bivia* allowed correct species identification of all isolates included in the study (Wybo et al. 2012).

While the importance of database quality has been recognised by all, there are conflicting views regarding the optimisation of spectral quality, the adaptation of cutoff criteria and the need for an extraction procedure when identifying anaerobes by MALDI-TOF MS. The extraction procedure for anaerobic organisms has been called into question, as a recent report suggests that the direct deposit procedure is not inferior to the extraction procedure and may even lead to the identification of previously unidentified strains (Fournier et al. 2012). Other authors have argued that it may avoid the need for additional tests (Fedorko et al. 2012). Veloo et al. suggested that the need for an extraction step could depend on the conditions used to create the reference spectra. This study highlighted the increased requirement for an extraction step when using the Biotyper database, which includes reference spectra acquired from strains subjected to the extraction procedure, compared with the SARAMIS database (Veloo et al. 2011a, Wybo et al. 2012).

However, recent studies that evaluated the performance of the Biotyper 3.0 and VITEK MS 2.0 databases without performing an extraction step showed correct species identification results in 92.5% and 91.2% of cases, respectively (Barreau et al. 2013, Garner et al. 2013). A compromise would be to perform an extraction on isolates that led to unreliable results using the direct deposit procedure (Coltella et al. 2013).

MALDI-TOF MS should soon replace conventional identification techniques such as API 20A or rapid ID 32C because of its improved reliability (Kierzkowska et al. 2013, Coltella et al. 2013, Culebras et al. 2012, Jamal et al. 2013a, Nagy et al. 2009 and 2012, Kliem et al. 2012).

Finally, the system appears to be a promising tool for both the identification and typing of anaerobic species such as *Propionibacterium* sp. and *Clostridium* sp. (Grosse-Herenthey et al. 2008, Nagy et al. 2013, Reil et al. 2011).

Table 5. This table summarises the major studies evaluating the performance of MALDI-TOF MS in medical mycology.

Study	Database	Entries or version	E?	CO	n	Genus ID %	Species ID %	No ID %	Error ID%	Period or origin
Marklein et al., 2009	Biotyper/EB	241	Y	1.7/2	267	92.5/100		NR	NR	CI 14 months
Stevenson et al., 2010	EB	2.0.4.0+ 109	Y	1.8/2	197	97.5	85.8	2.5	0	COLL
Bader et al., 2010	Biotyper	3.0	Y	2	1192	97.6	97.6	1.8	0.7	CI 6 months+ COLL
	SARAMIS	3.3.1	P	80%		96.1	96.1	3.7	0.2	
Pinto et al., 2011	Biotyper	3.1.2.0	Y	1.7/2	167	95.8	83.8	4.2	0	COLL
					67	94.0	79.1	6.0	0	CI 2 months
Dhiman et al., 2011	Biotyper	3740	Y	1.8/1.7	138 commun	97.0	96.3	2.9	0	CI 1 month
					103 uncommon	88.4	84.5	11.6	0	COLL
McTaggart et al., 2011	EB	2.0.1+ 26	Y	1.7/2	160 mainly *Cryptococcus* sp.	100	93.1	0	0	CI + COLL
Seyfarth et al., 2012	SARAMIS	NR	N	NR	83	100	94.0	3.6	2.4	CI 1 year (74) + COLL
Theel et al., 2012	Biotyper	3	P	1.5/1.7	90	95.6	81.1	4.4	1	NR
Westblade et al., 2013	VITEK MS	2.4	P	60%	852	96.6	96.1	2.8	0.6	COLL
Iriart et al., 2012	VITEK MS	V1	P	NR	192	95.8	95.8	3.7	0.5	CI 5 weeks
			N		44 *Aspergillus* sp.	81.8	81.8	18.2	0	CI 10 weeks
Bille et al., 2012	Andromas	NR	P	65%	162	98.8	98.8	1.2	0	CI 2 months
					64 *Aspergillus* sp.	95.2	98.4	1.6	0	
Rosenvinge et al., 2013	Biotyper	3.1.2.0	P	1.7	102	NR	90.2	9.8	0	COLL
	SARAMIS	4.09		80%			95.1	4.9	0	COLL
	Biotyper	3.1.2.0		1.7	98		85.0	15.0	0	CI
	SARAMIS	4.09		80%	99		100	0	0	CI
Lohmann et al., 2013	Biotyper	2.0.4.0	Y	SA	312	NR	87.2	12.2	0.6	CI 8 months
	SARAMIS	4.07	Y	SA			82.7	16.0	1.3	
Lacroix et al., 2013	Andromas	NR	P	70%	1383	98.3	98.3	1.5	0.2	CI 2 months
	Biotyper	3.3.106.0	Y	1.7/2	1383	98.3	98.3	1.3	0.4	
Won et al., 2013	VITEK MS	NR	P	60%	533	96.1	96.1	2.8	1.1	BC 1 year, MC
Mancini et al., 2013	VITEK MS	1.2.0.	P	SA	157	93.7	87.3	3.8	2.5	CI
	Biotyper	v3.0	Y	1.7/2		93.6	92.3	6.4	0	
	VITEK MS	1.2.0.	P	SA	40 non-Candida	85.0	72.5	2.5	12.5	
	Biotyper	3.0	Y	1.7/2		80.0	80.0	20	0	
Alanio et al., 2011	Andromas	28	P	SA	140 *Aspergillus* sp.	98.6	98.6	1.4	0	CI (124) + Env (16)
Marinach et al., 2009	Sdb	13	Y	SA	49 *Fusarium* sp.	NR	91.8	8.2	0	COLL
de Carolis et al., 2012	EB	3290+ 109	N	1.7/2	103 FF	91.3	88.3	8.7	0	CI 16 months+ COLL
Lau et al., 2013	Sdb+ Biotyper	5118	Y	1.7/2	421 FF	92.2	87.9	7.9	0	NR
Theel et al., 2011	EB	3995+ 20	Y	SA	171 dermatophytes	93.0	59.6	36.2	4.1	CI 1 month + COLL
	Biotyper	3995		1.7/2		22.2	4.7	NR	NR	
Alshawa et al., 2012	Sdb	50	Y	66%	360 dermatophytes	NR	91.9	7.5	2.4	CI 6 months

db, database; EB, enriched Biotyper; Sdb, specific database; SA, specific algorithm E?, extraction?; Y, yes; N, no; P, on plate; CO, cutoff; RS, reference strain; COLL, collection strains; CI, clinical isolates; NR, not reported; FF, filamentous fungi

1.2.7.2.6. Mycology

Both the time- and cost-effectiveness of MALDI-TOF MS explain its potential to revolutionise medical mycology (Putignani et al. 2011, Clark et al. 2013, Westblade et al. 2013, Buchan et al. 2013).

Studies that have evaluated the capacity of MALDI-TOF MS in medical mycology are presented in Table 5.

Studies that have evaluated and compared commercial MALDI-TOF MS systems in the identification of fungal microorganisms have not highlighted major differences between systems but instead confirmed their superiority compared with conventional identification techniques (Bader 2013, Rosenvinge et al. 2012, Lohmann et al. 2013, Iriart et al. 2012, Lacroix et al. 2013, Westblade et al. 2013). The main remaining differences are that an extraction is recommended for the Biotyper system in contrast with other systems (Iriart et al. 2012, Lacroix et al. 2013), although on-plate extraction appears efficient, and no reference spectrum can be added to the VITEK MS database, which is a closed system (Westblade et al. 2013, Mancini et al. 2013). For the identification of non-*Candida* isolates, Mancini et al. reported a higher rate of incorrect identification using the VITEK MS database, whereas the Biotyper database showed a higher rate of unidentified organisms (Mancini et al. 2013).

1.2.7.2.6.1. Yeasts

Ascomycetous and basidiomycetous yeasts are easily processed and identified by MALDI-TOF MS (Bader 2013).

As previously discussed, a crucial parameter for successful identification by MALDI-TOF MS is the quality of the database. As previously observed, isolates of yeast species not included in the database will more frequently generate no identification than misidentification (Stevenson et al. 2010a, Pinto et al 2011). Even if inconvenient, regular updating of MALDI-TOF MS databases will improve the technology's potential and is much easier to perform than updating of the databases for conventional methods (Seyfarth et al. 2011).

Such an update was efficient for the accurate species identification of *Cryptococcus* sp., with an increase of more than 40% in the rate of correct species identification and a significant improvement in the identification score values compared with the commercial database (McTaggart et al. 2011).

Moreover, closely related or unusual species that are not discriminated using conventional identification techniques, such as *Candida ortho-*, *meta-* and *parapsilosis*, *Candida albicans* versus *Candida dubliniensis* or species of the genera *Trichosporon* and *Geotrichum*, are now resolved by MALDI-TOF MS within minutes, which will certainly contribute to a better understanding of the epidemiology and virulence of such species in the future (Bader 2013, Quiles-Melero et al. 2012, Clark et al. 2013, Kolecka et al. 2013).

Standardised procedures regarding cutoff criteria and preanalytical processing are still needed (Bader 2013, Pinto et al. 2011, Cassagne et al. 2013). A recent study suggested that an extraction procedure with one single colony is adequate for further MALDI-TOF MS identification, allowing the rapid identification of yeasts from clinical samples harbouring poor yeast inoculum and avoiding the contamination by neighbouring species in the culture, which are two significant advantages (Goyer et al. 2012). Rapid on-plate extraction procedures have also been evaluated in combination with the use of lowered cutoff criteria (van Herendael et al. 2012, Theel et al. 2012).

A major problem with MALDI-TOF MS, particularly with yeasts that produce colonies of similar morphology and colour onto the traditionally used Sabouraud culture medium, is the inability to detect mixtures. The use of chromogenic medium on which the typical colours of growing yeasts may usually be observed after a 48-h incubation is an alternative (Pinto et al. 2011, Lacroix et al. 2013) given that this incubation time does not seem to influence the quality of the subsequent MALDI-TOF MS identification result (Goyer et al. 2013).

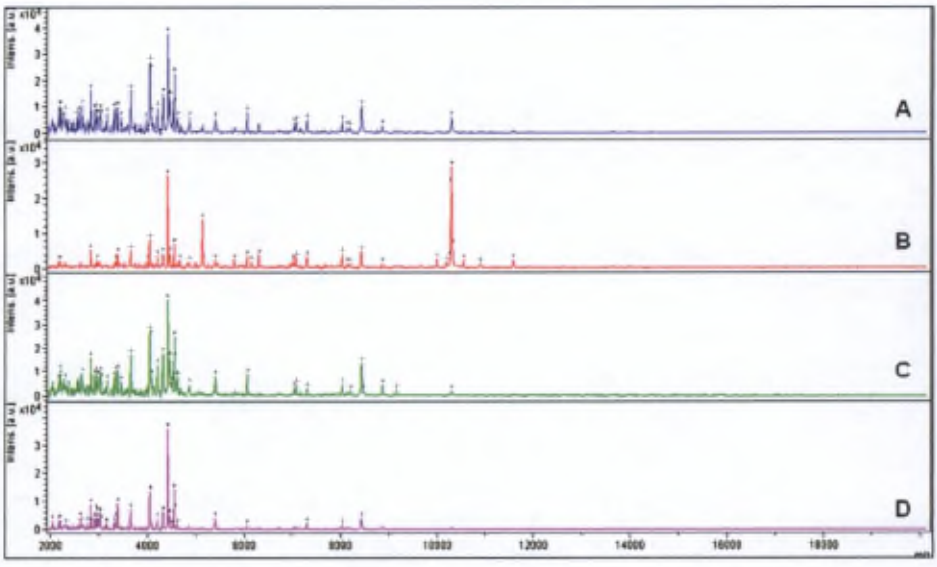


Figure 18. Comparison of mass spectra obtained from four subcultures of a strain of *A. flavus*. The strain was subcultured on four different agar plates. Spectra A, B, C and D display the first spectrum acquired from the subcultures 1, 2, 3 and 4, respectively. Spectra A to D diplay many common peak; however, a few varying peaks are also clearly visible and characteristic of one of the subcultures (from Normand et al. 2013).

1.2.7.2.6.2. Filamentous fungi

In clinical laboratories, the analysis of filamentous fungi cultures is based on micro- and macroscopic characteristics that require subjective evaluation, does not allow discrimination between closely related species and occasionally leads to erroneous identifications (Hettik et al. 2008a,b; Alanio et al. 2011). Again, MALDI-TOF MS technology may offer an alternative to conventional identification techniques if adequate databases are used. However, cultures of filamentous fungi present multiple zones that correspond with different maturation levels, making MALDI-TOF MS identification more challenging (Croxatto et al. 2011, De Carolis et al. 2012a, Alanio et al. 2011, Bader 2013).

For example, Figure 18 shows the heterogeneity of spectra obtained from four subcultures of one *Aspergillus flavus* strain. Such heterogeneity must be taken into account when creating reference spectra and specific databases for filamentous fungi, either by adopting strict and standardised sample preparation procedures or by adding several entries for each species in the database that reflect the genetic and phenotypic heterogeneity due to growth conditions (Bader 2013). Fortunately, a recent study demonstrated that increasing the number of mass spectra acquired from distinct subcultures of the same strain significantly improved database efficiency and could partially offset the relatively low number of specific strains available with which to construct the database (Normand et al. 2013). The development of databases including reference spectra from young and mature cultures of the same strain allows MALDI-TOF MS analysis of fungal isolates regardless of sporulation level, even by direct deposit onto the target plate (De Carolis et al. 2012a, Alanio et al. 2011).

The identification results obtained from a specific database are usually better than those obtained using a commercial database because the reference spectra of the commercial database (Biotyper) were created from liquid cultures that are rarely used in a clinical settings due to the risk of contamination (Lau et al. 2013). However, liquid culture may eliminate identification problems that result from pigmented colonies, as melanin in moulds may inhibit ionisation (Buskirk et al. 2011, Bader 2013).

Different sample preparation procedures have also been evaluated for MALDI-TOF MS analysis of the filamentous fungi *Aspergillus* sp., *Fusarium* sp. and *Penicillium* sp. These procedures include formic acid extraction of scraped colonies, on-plate extraction, boiling and bead-beating and showed variable but promising results (Hettik et al. 2008a,b; De Carolis et al. 2012a, Marinach-Patrice et al. 2009, Cassagne et al. 2011, Lau et al. 2013, Alanio et al. 2001).

Many of these results are presented in Table 5. MALDI-TOF MS allows correct species identification of filamentous fungi in approximately 90% of cases within a few minutes, which is a major advantage compared with conventional identification techniques that require days or even weeks (Marinach-Patrice et al. 2009, Alanio et al. 2011).

1.2.7.2.6.3. Dermatophytes

The diagnosis of dermatomycosis relies on microscopic detection of septate hyphae, which does not provide any genus or species identification, and on micro- and macroscopic analysis of cultures after several weeks of growth. MALDI-TOF MS could replace conventional identification techniques for the identification of dermatophytes and even be implemented in clinical laboratories (Theel et al. 2011, Alshawa et al. 2012, Nenoff et al. 2013).

Before proceeding, databases need to be updated, and the standardisation of cutoff criteria and sample preparation is needed (Theel et al. 2011). Results reported by Theel et al. are promising, with 93% and 59.6% of isolates correctly identified at the genus and species levels, respectively. After creating a specific database that included 17 reference strains covering six dermatophyte species of the *Trichophyton mentagrophytes* complex, we showed that MALDI-TOF MS results were correlated with phylogenetic data and allowed the correct identification of 89% of the studied dermatophyte strains (Packeu et al. 2013; appendix 9.2).

In a study of Theel et al., a rate of 60% correct species identification was reported. The wider range of dermatophytes included in this study may explain the different results observed in a previous study that reported nearly 100% correct species identification (Theel et al. 2011, Erhard et al. 2008).

The clinical context should determine whether species identification is required or if genus identification is sufficient for patient care (Theel et al. 2011).

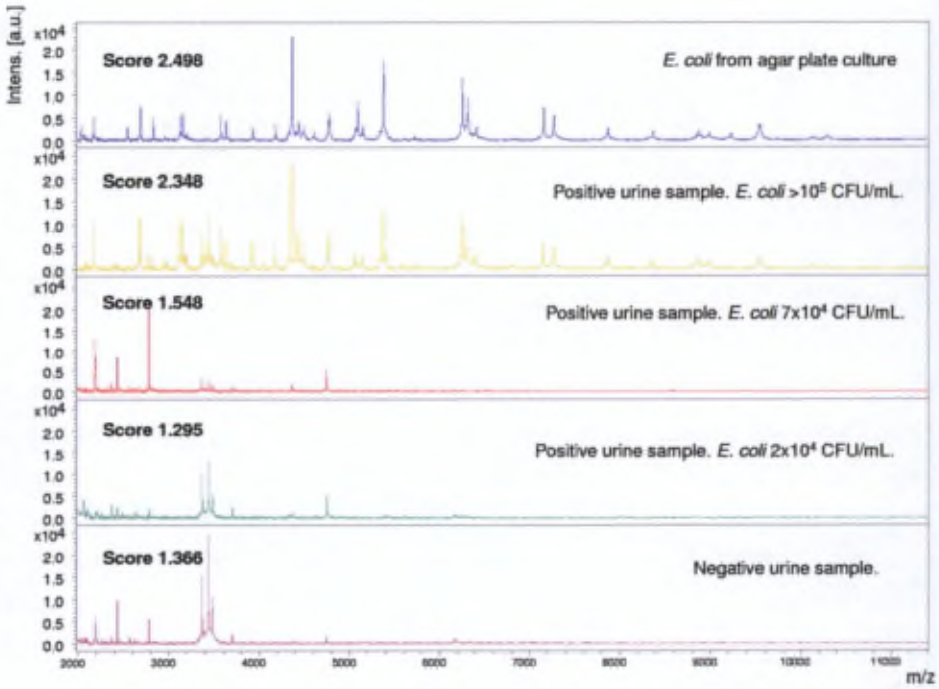


Figure 19. Mass spectra (size 2,000 to 11,000 Da) of *E. coli* cells grown under different conditions (from Ferreira et al. 2010b)

1.2.8. Analysis of clinical samples

The MALDI-TOF MS analysis of microorganisms directly from clinical samples is a major challenge for microbiologists, especially when investigating bloodstream infections that are associated with high rates of mortality and morbidity (Angus et al. 2001, Alberti et al. 2002, Vincent et al. 2006). Because of its low cost and high rapidity, MALDI-TOF MS is an interesting alternative to expensive molecular techniques. Identification of bacteria and yeasts directly from positive blood cultures or other clinical samples using MALDI-TOF MS implies the removal of analytical interferences (proteins, crystals, cells) and the extraction of the microorganism to ensure a sufficient amount of biological material on the target plate and a subsequent good quality spectrum (March-Rossello et al. 2013). The bacterial inoculum is a crucial parameter that clearly affects spectrum quality (Stevenson et al. 2010b, Christner et al. 2010). Based on previous studies, a minimal inoculum of 10^6 to 10^8 CFU/mL appears to be necessary to yield accurate species identifiation (Justesen et al. 2011, Christner et al. 2010). The impact of bacterial inoculum on the MALDI-TOF MS spectrum evaluated using urine samples is shown in Figure 19. The sample volume to be analysed may be adapted to ensure a sufficient concentration on the target plate (Fothergill et al. 2013, Wuppenhorst et al. 2012).

Several types of pretreatment have been reported to help eliminate interference. Cell lysis using saponin, ammonium chloride or Tween 80 (Drancourt et al. 2010) led to satisfactory identification results, and a commercial kit has been produced using this principle (Loonen et al. 2012, Kok et al. 2011, Juiz et al. 2012, Lagacé-Wiens et al. 2012, Meex et al. 2012). Differential centrifugation protocols use sequential centrifugation steps to sediment cells and obtain debris-free supernatants, which are centrifuged a final time to recover the bacterial pellets (March-Rossello et al. 2013). Finally, plasma, which represents up to 55% of total blood volume, is an ideal medium from which to recover propagated bacteria because they are mainly present in the extracellular compartment. Therefore, the use of serum-separator tubes has been evaluated to separate cellular debris and interfering substances from the bacterial pellets (Moussaoui et al. 2010).

Table 6. Major studies evaluating the MALDI-TOF MS direct identification from positive blood cultures bottles.

Study	BC	db	GN (%)	N	GP (%)	N	Yeasts (%)	N	Total (%)	N	PM	N	CO	Procedure
La Scola et al., 2009	B	Biotyper 2881	94	125	37	197	0		59	322	1/2 (18x)	22	SA	DC + TFA
			87	100	67	140			76	240				DC + FA
Prod'Hom et al., 2010	B	Biotyper 2	79	48	42	74	0		57	122	1/2	4	2	NH_4Cl lysis
Ferroni et al., 2010	BA	Andromas	92.6	189	81.5	103	100	20	86.9	312	0		SA	SL Spiked samples
			92.8	139	89.2	223	100	11	90.9	373	1/2 ID	15		SL + slides CI
Stevenson et al., 2010	B	Biotyper 2	79.5	78	56.7	134	0		65.1	212	1/2 ID (9x)	10	SA	SS + lysis
Christner et al., 2010	B	Biotyper 2	NR						95-75	277	1/2 (13x)	16	1.3 -2	DC
Moussaoui et al., 2010	B	Biotyper 3290	98.9	187	92.9	295	excluded		95.2	482	36%	21	SA	SS
Schmidt et al., 2011	B	Saramis V3.3.2	97.7	43	61.7	60	0		76.7	103	0		SA	0.5 µl +DHB+FA
	BA		81.4		21.7				46.6					0.5 µl +DHB+FA
	BA		67.4		13.3				35.9					0.5 µl +DHB+FA
Ferreira et al., 2010	B	Biotyper 3290	83.3	61	31.8	239	5.6 (genus)	18	39.6	318	0		2	DC
Kok et al., 2011	B	Biotyper 2	79.7 87.2	187	46.3 68.4	285	NR		59.4 74.8	507	1/2 (20x)	31	2 1.7	ST
Fuglsang-Damgaard et al., 2011	BA	EB	79.3 89.9	169	6.7 42.4	316	0 0 5.3	19	28.6 55.4	583*	21.6% 67.6%	37	2 none	SS + lysis
Yan et al., 2011	B	Biotyper 3476	NA	NA	NA	NA	100	42	100	42	NA	NA	2 1.7	Adapted ST
Spanu et al., 2012	B	Biotyper 2	NA	NA	NA	NA	91.3	346	91.3	346	NA	NA	SA	DC
Juiz et al., 2012	B	Biotyper 3	87.5	24	44.3	61	0		56.5	85	0		2	In-house
			95.8		83.6				87.1					ST
Loonen et al., 2012	BA	Biotyper 3740	78.7	47	21.2	52	0		48.5	99	1/2 species	2	2	DC
			25.5		5.8				15.2		0			MolYsis
			91.5		42.3				65.7		1/2 genus			ST
Wuppenhorst et al., 2012	BA	Biotyper 3	93.8	64	72.3	148	0		77.6	212	excluded		1.4	SL
Lagacé-Wiens et al., 2012	BA	Biotyper 3	95.0	20	56.4	39	50	2	68.9	61	1/2 ID	2	2	ST
Chen et al., 2013	B	Biotyper 4500	88.7 99.1	106	72 96	75	0		81.8 97.8	181	2/2 (n=5) 1/2 (n=16)	21	2 1.7	ST (+in house)
		VITEK MS >20000	89.8 98.1		68 85.3				80.7 92.9		the majority sp.		98% 90%	
Fothergill et al., 2013	BA	Saramis	82.5 86.0	57	76.0 76.7	150	83.3 88.9	18	78.2 79.9	225	46.4% 1 to species	28	≥ 75%	Lysis-filtration
Foster, 2013	B	VITEK MS 2	83.7 84.7	92	90.1 95.7	161	0		88.1 92.1	253	0		SA	DC + lysis

BC, blood culture; B, Bactec; BA, BacTalert; db, database; EB, enriched Biotyper; GP, Gram-positive bacteria; GN, Gram-negative bacteria; PM, polymicrobial cultures; CO, cutoff; SA, specific algorithm; FA, formic acid; TFA, trifluoroacetic acid; SL, saponin-lysis; SS, serum separator tubes; DC, differential centrifugations; ST, Sepsityper
* 37 strains classified as "miscellaneous" and 5 negative cultures

Numerous studies that have evaluated these protocols in combination with different mass spectrometers, cutoff criteria and databases have been published. A selection of these studies is presented in Table 6. The variety in study designs makes their comparison difficult. However, common observations may be noted.

First, most studies conclude that Gram-negative bacteria are more easily identified at the species level from positive blood cultures than Gram-positive bacteria (Schmidt et al. 2011, Ferreira et al. 2011a, Kok et al. 2011, Jamal et al. 2013b, March-Rossello et al. 2013, Leli et al. 2013b), likely due to the differences in cell wall composition (Chen et al. 2013a). As previously mentioned, the identification of streptococci remains critical (Stevenson et al. 2010b, La Scola et al. 2009, Leli et al. 2013b, Ferreira et al. 2011a). The exclusion of peaks below 4,000 m/z, which correspond with residual blood components could increase the rate of confident MALDI-TOF MS identification, especially for Gram-positive bacteria (Buchan et al. 2012).

Second, mixed cultures result in the failure of MALDI-TOF MS identification from positive blood cultures (Leli et al. 2013b, La Scola et al. 2009, Ferroni et al. 2010, Christner et al. 2010, Wuppenhorst et al. 2012, Vlek et al. 2012, Fothergill et al. 2013). In most cases, only the most abundant organism in the mixture is detected. Organisms that share a minor part of the total biomass (e.g., 10%) will likely be lost in the background noise (Welker et al. 2011). However, several authors have suggested that Biotyper software can occasionally provide additional information about mixtures because several identification results with reliable score values appear among the 10 best identification proposals (Moussaoui et al. 2010, Chen et al. 2013a, Saffert et al. 2012).

The nature of the microorganism may also affect the success of MALDI-TOF MS identification from positive blood cultures, as encapsulated bacteria (*H. influenzae, S. pneumoniae, Klebsiella pneumoniae*) seem to yield poor MALDI-TOF MS identification results (Prod'Hom et al. 2010).

If the incubation time is prolonged, there may be an increase in the number of non-bacterial components, which could impair spectrum quality by masking specific bacterial signals (Christner et al. 2010); moreover, higher bacterial density may also impair the analysis accuracy (Fothergill et al. 2013).

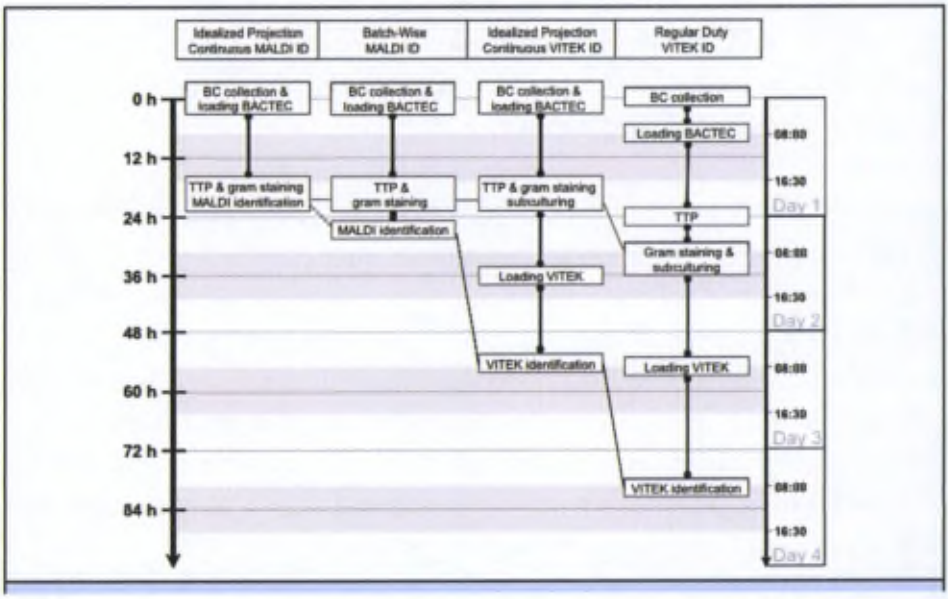


Figure 20. Graphically illustrated comparisons of the investigated workflow concepts demonstrating the lag and processing times of all concepts and consequences for TAT until Gram-staining and bacterial identification report (from Schneiderhan et al. 2013).

Various results have been reported on the identification of yeasts from positive blood cultures, but recent studies focusing on such organisms suggest that MALDI-TOF MS is an efficient method for yeast identification from positive blood cultures and shows excellent results for *C. albicans* in particular (Marinach-Patrice et al. 2010, Yan et al. 2011, Spanu et al. 2012, Lavergne et al. 2013).

In contrast to conventional identification techniques, a misinterpreted Gram stain result has no influence on the MALDI-TOF MS workflow (Buchan et al. 2012). However, such colouration is still mandatory to detect potential mixed cultures (Clerc et al. 2013a), to allow the recognition of organisms poorly identified by MALDI-TOF MS, such as *Propionibacterium* sp. (Stevenson et al. 2010b) or when using adapted cutoff values to exclude discrepant results (Wuppenhorst et al. 2012, Fuglsang-Damgaard et al. 2011).

In the future, improved algorithms and automated purification protocols might increase sensitivity and facilitate sample handling (Christner et al. 2010). Another alternative to quickly identify the microbial agents responsible for bacteraemia is to perfom MALDI-TOF MS identifications in the late afternoon on subcultures prepared from positive blood cultures got earlier during the day (Loonen et al. 2012).

The rapidity of MALDI-TOF MS identification from positive blood cultures as determined by considerable reductions in TATs (from 41 h to more than 112 h depending on the study and identified organisms) compared with conventional identification techniques has been demonstrated many times (Figure 20) (Leli et al. 2013b, Schneiderhan et al. 2013, Buchan et al. 2012). However, the true clinical impact of such rapid identification has been poorly evaluated. The greatest clinical impact may result from the identification of more virulent strains and organisms with predictable resistance to antibiotics, such as *S. maltophilia* (Chen et al. 2013a). Rapid identification should also help find the origin of sepsis if it remains unknown and allow the rapid reduction of broad empirical antimicrobial treatment; however, MALDI-TOF MS cannot replace the susceptibility testing that still must be performed (Croxatto et al. 2012, Emonet et al. 2011, Nori et al. 2013).

Vlek et al. reported that the proportion of appropriate antimicrobial treatments administred within 24 h increased by 11.3% when performing MALDI-TOF MS analysis directly on positive blood cultures (Vlek et al. 2012). In another study, Clerc et al. demonstrated that such rapid identifications affect more than one-third of all episodes of Gram-negative bacteraemia (modification of 35.1% of empirical treatments, although 20.8% of treatments were previously modified based on the Gram stain result) (Clerc et al. 2013a).

Clerc et al. also reported that MALDI-TOF MS identification from positive blood cultures in combination with the GeneXpert molecular test allowed the identification of methicillin-resistant *S. aureus* (MRSA) sepsis within 201 min, which was associated with the decreased unnecessary use of glycopeptides to treat methicillin-susceptible *S. aureus* (MSSA) (Clerc et al. 2013b). More recently, Huang et al. demonstrated that a rapid diagnosis also had an impact on mortality (14.5% vs. 20.3%) and length of stay in the intensive care unit (ICU) (8.3 vs. 14.9 days) (Huang et al. 2013).

It must be noted that improved TAT will have a clinical impact only if used by clinicians without delay; therefore, good communication between health professionals is required (Emonet et al. 2011).

Direct identification from clinical samples other than positive blood cultures has also been reported. As previously discussed, the identification of individual species directly from clinical samples is possible only for species present in a sample in significant abundance, as can be the case for urine samples. In other clinical samples, including respiratory and stool samples, the bacterial background from the commensal flora and degradation products may interfere with the detection of potential pathogens present in comparatively low abundance (Welker et al. 2011).

Identification of the bacteria responsible for urinary tract infections (UTI) using MALDI-TOF MS is controversial. Croxatto et al. suggested that the use of chromogenic medium coupled with simple phenotypic tests is a simpler method to identify most UTI agents (Croxatto et al. 2012), whereas other authors have proposed the selection of candidate samples for MALDI-TOF MS identification using flow cytometers that can quickly identify negative cultures (Wang et al. 2013).

The identification of bacteria from cerebrospinal fluid has been reported (Nyvang Hartmeyer et al. 2010) but is not currently implemented in routine diagnostic laboratories because of the low bacterial load and sample volume available (Croxatto et al. 2012).

Finally, the direct identification of microorganisms from enrichment broths may accelerate pathogen detection, as described by Sparbier et al for *Salmonella* grown in selective liquid media (Sparbier et al. 2012) and more recently by Pereyre et al. for mycoplasmas (Pereyre et al. 2013).

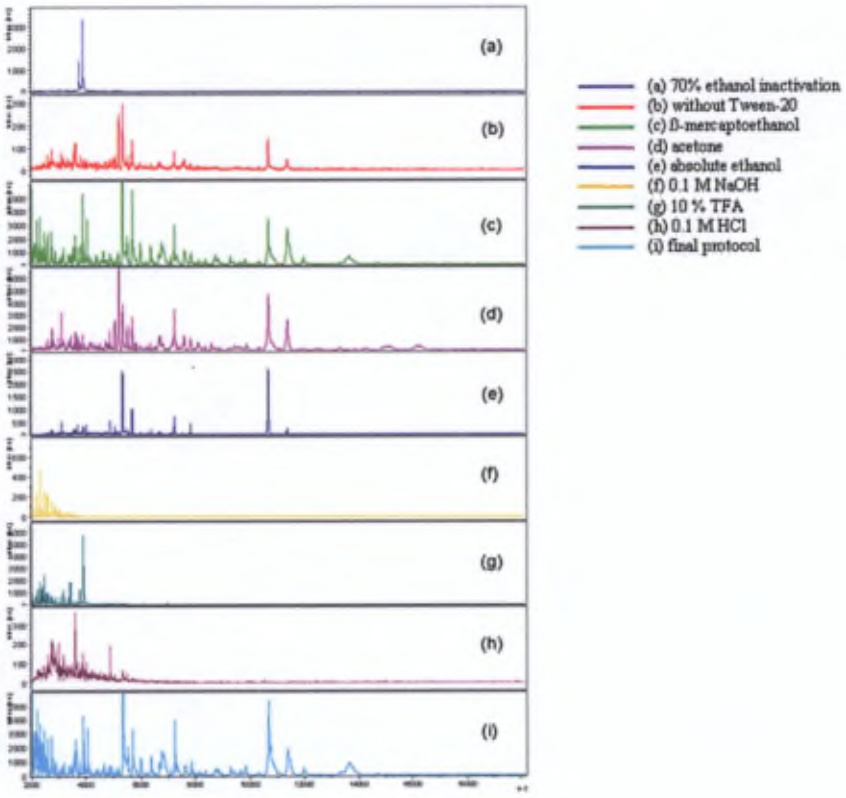


Figure 21. MALDI-TOF MS spectra obtained from one *M. tuberculosis* strain using different protocols (from El Khéchine et al. 2011).

1.2.9. Developments

1.2.9.1. Mycobacteriology

Mycobacteria include approximately 150 different species, many of which are clinically significant. The first studies evaluating MALDI-TOF MS identification of mycobacteria were conducted by Hettik et al. and Pignone et al., who demonstrated the technique's efficiency in discriminating *Mycobacterium tuberculosis* complex species from non-tuberculosis mycobacteria (Pignone et al. 2006, Hettik et al. 2004).

The delay in implementing MALDI-TOF MS for the identification of mycobacteria in clinical laboratories may be due to the need for an efficient inactivation protocol to ensure the technologists' security (Murray et al. 2012). This inactivation is very important given that the mass spectrometer is not usually located in a P3 laboratory (Lotz et al. 2010). Inactivation procedures have been evaluated by several investigators, and heat inactivation (95°C for 30 min – 1 h) appears to be the most efficient procedure still allowing the acquisition of good quality spectra (Figure 21) (Saleeb et al. 2011, El Khéchine et al. 2011). Disruption of the cell envelope for adequate protein extraction is usually achieved using silica beads to preserve the proteins (Saleeb et al. 2011).

Optimal inactivation and extraction procedures associated with database enrichment led to promising results with good identification of both slow- and fast-growing mycobacteria and good discrimination between *Mycobacterium tuberculosis* complex species and non-tuberculosis species, as well as species within the *M. tuberculosis* complex and closely related species, such as *Mycobacterium massiliense* and *Mycobacterium abscessus* (Panda et al. 2013, Shitikov et al. 2012, Teng et al. 2013).

Recently, Balada-Llasat et al. evaluated the Biotyper Mycobacteria library 1.0, which included spectra representing 94 species and 173 strains, for the identification of 178 mycobacterial isolates grown on commonly used solid and liquid media. The results demonstrated successful species and genus identification in 93.8% and 98.3% of cases, respectively. This database leads to grouping at the complex level for the *M. tuberculosis*, *Mycobacterium avium-Mycobacterium intracellulare* and *Mycobacterium fortuitum* complexes and the *M. abscessus* group (Balada-Llasate et al. 2013).

Database completion and standardisation of the pretreatment of solid and liquid cultures will further improve MALDI-TOF MS potential in the mycobacteriology laboratory in the future, as demonstrated in a recent study showing better mycobacterial identification when using the Biotyper database than when using the Vitek MS system (Chen et al. 2013b).

1.2.9.2. Parasitology

In parasitology, MALDI-TOF MS has been limited to fundamental research, including general parasitic proteome studies (Gitau et al. 2011, Wang et al. 2009), and the characterisation of specific biomarkers for the discrimination between environmental and human *Cryptosporidium* and *Giardia* species for water management (Magnuson et al. 2000, Villegas et al. 2006). Diagnostic use of MALDI-TOF MS in parasitology has remained limited and has been used for serum peptide profiling of mice infected with *Leishmania* (Li et al. 2012) and the discrimination between microsporidian isolates grown in cell cultures (Moura et al. 2003).

1.2.9.3. Virology

In virology, the data are also very limited. Mass spectrometry-based assays that combine the benefits of molecular techniques and MALDI-TOF MS have been developed to provide an alternative approach to expensive genotyping methods (Meyer et al. 2011, Ganova-Raeva et al. 2013). This combined system (MassARRAY, Sequenom, San Diego, USA) has been evaluated for the purpose of genotyping various viruses, such as human enterovirus (Peng et al. 2013), hepatitis B virus (Ganova-Raeva et al. 2010) and herpesviruses (Sjoholm et al. 2008). MALDI-TOF MS has also been used in human immunodeficiency virus-infected patients to profile their serum proteins (Van Duyne et al. 2010) and to detect antiretroviral resistance in combination with restriction fragment mass polymorphism (RFMP) (Lee et al. 2013).

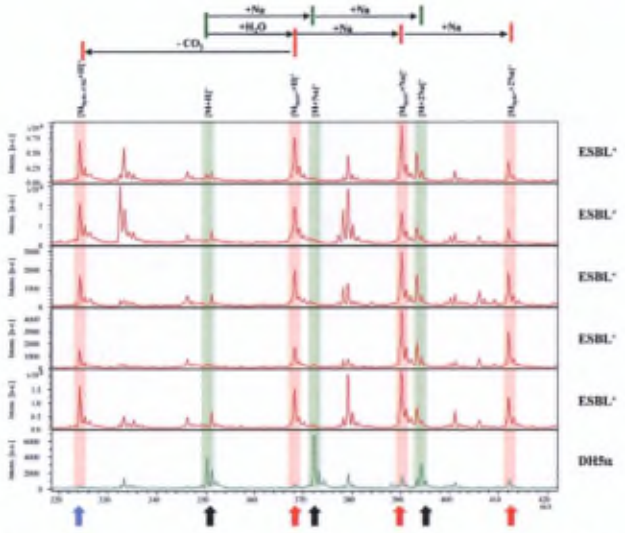
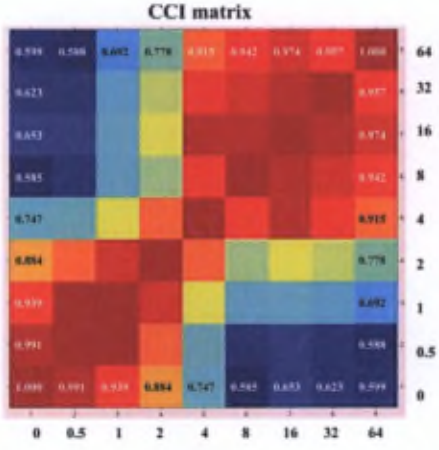


Figure 22. MALDI-TOF mass spectrogram of ampicillin for five ESBL-producing *E. coli* strains and one ampicillin-susceptible strain. The figure demonstrates the accumulation of complete (blue arrow) and partial (red arrow) hydrolysis products in ESBL-producing strains. Accumulation of native ampicillin was present only in the susceptible strain DH5α (black arrow) (from Ledeboer et al. 2011; courtesy of M. Kostrzewa, Bruker Daltonics).



MPCC (μg/ml)	Null CCI	Maximum CCI
64	0.599	1.000
32	0.623	0.957
16	0.653	0.974
8	0.585	0.942
4	0.747	0.915
2	0.884	0.778
1	0.939	0.692
0.5	0.991	0.588
0	1.000	0.599

Figure 23. Representative composite correlation index (CCI) matrix derived from selected mass spectra, which correspond to those for *C. glabrata* DSP155 cells (MIC of 4 μg/ml) exposed for 15 h at 37°C to serial caspofungin concentrations (including the null one), ranging from 0.5 to 64 μg/ml. By comparing spectra with one another at the indicated drug concentrations, numerical correlation indices were obtained, automatically visualised in a CCI matrix view, and translated into a heat map (on the left) in which closely related spectra are marked in hot colors and unrelated ones in cold colors. After matching for each concentration and its spectrum similarity against each of the two extreme concentrations (null or maximum) of the drug, the MPCC was assessed as the CCI value at which a spectrum is more similar to the one observed at the maximum caspofungin concentration (maximum CCI) than the spectrum observed at the null caspofungin concentration (null CCI). The MPCC (4 μg/ml) and its relative null and maximum CCI values are shown on the right in the red box (from De Carolis et al. 2012).

1.2.9.4. Drug susceptibility testing

Antibiotic resistance has increased considerably in recent years and constitutes a major public health problem (Hrabak et al. 2013). Several mechanisms have been recognised in the resistance of microorganisms against antimicrobial agents.
As many of the proteins invloved in drug resistance are not expressed at high levels compared with other bacterial proteins, the direct detection of resistance determinants is not conceivable (Ledeboer et al. 2011).

A first alternative, presented in Figure 22, is to detect peaks of metabolites produced by the enzyme rather than peaks corresponding to the enzyme itself (Ledeboer et al. 2011, Hrabak et al. 2013). This idea is based on spectral acquisition before and after incubation of the bacteria with the antibiotic to be tested to detect peaks corresponding with the antibiotic or its salts and/or degradation products (Hrabak et al. 2013). This approach has been evaluated for the detection of extended spectrum beta-lactamases (Ikryannikova et al. 2008) and carbapenemases (Hrabak et al. 2011, Burkhardt et al. 2011, Alvarez-Buylla et al. 2013). Unfortunately, it does not provide any additional information regarding the presence of other resistance mechanisms, such as porin alteration or efflux mechanisms (Burkhardt et al. 2011).

A second alternative is to consider mass spectral changes while exposing the organism to serial dilutions of the antimicrobial agent to be tested. In 2009, Marinach et al. postulated that the protein composition of *C. albicans* could vary depending on the drug concentration to which it is subjected. They acquired mass spectra from a susceptible strain before and after exposure to serial dilutions of fluconazole and postulated a new endpoint called the minimal profile change concentration (MPCC). This parameter is defined as the lowest concentration at which a change in the mass spectrum can be detected (Marinach et al. 2009). A composite correlation index (CCI)-based method led to a high concordance level between MPCC and minimal inhibitory concentration (MIC). The method revealed promising results for susceptibility testing of caspofungin against both *Candida* and *Aspergillus*; an example is presented in Figure 23 (De Carolis et al. 2012b). In the future, MALDI-TOF MS could accelerate antifungal susceptibility testing, but additional studies are still necessary (Vella et al. 2013).

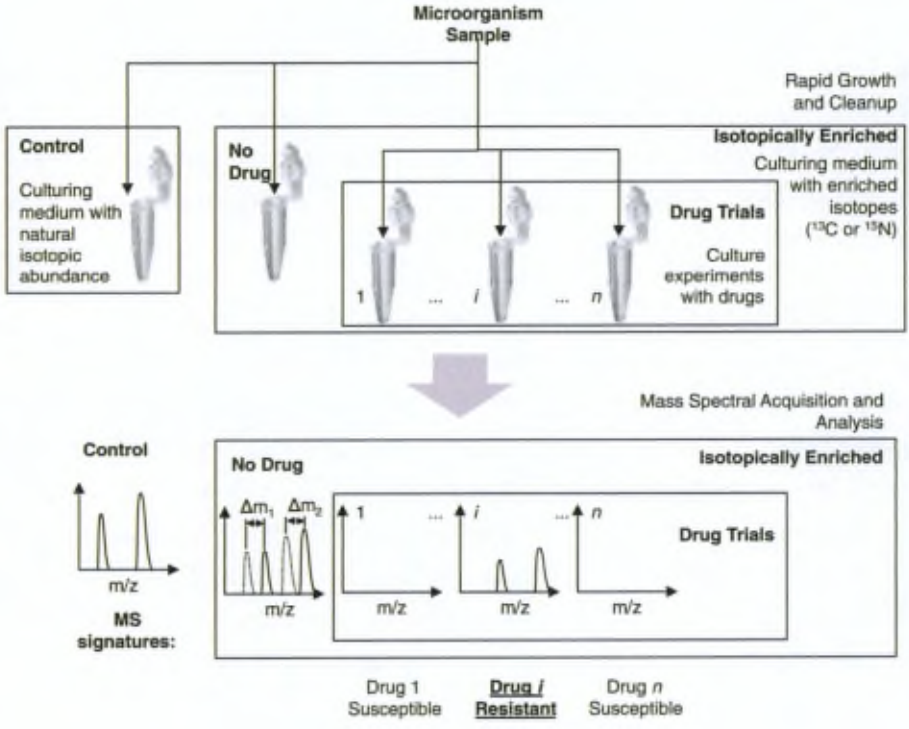


Figure 24. Assay to establish drug resistance by rapid growth and subsequent MS analysis of intact microorganisms. It includes sample growth and processing, MS acquisition, and subsequent bioinformatics analysis. All stages can be automated and performed in a multiplexed array format to establish resistance against multiple drugs/microorganisms (from Demirev et al. 2013).

Using the CCI method, several authors have demonstrated that the differentiation between cfia-negative and cfia-positive *B. fragilis* strains using MALDI-TOF MS was feasible (Wybo et al. 2011, Nagy et al. 2011).

More recently, a new approach based on MALDI-TOF MS and bioinformatics was evaluated using drug-containing isotope-labelled growth medium (Demirev et al. 2013). Comparison of the spectra of microorganisms grown in unlabelled (control) and isotope-labelled media allows the determination of resistance by the detection of mass changes; if the microorganism survives in the presence of drug, it consumes and metabolises the isotope-labelled nutrients, which results in a mass shift compared with the natural isotope-abundance mass value. This approach is illustrated in Figure 24 and requires a 5-h incubation instead of the usual 24-h incubation for conventional AST. Based on the same principle, Sparbier et al. reported satisfactory results for the detection of MRSA using labelled amino acids (Sparbier et al. 2013).

The manual analysis of raw spectra is difficult, and interpretation of the spectra requires adequate software. Moreover, MALDI-TOF MS susceptibility testing only provides partial information. Therefore, such methods will likely complement but not replace standard AST in the future (Burkhardt et al. 2011, Hrabak et al. 2013).

1.2.9.5. Bacterial typing

A comprehensive review focusing on MALDI-TOF MS bacterial typing has recently been published (Sandrin et al. 2013).

Briefly, the three objectives of bacterial typing require low, intermediate and high discriminatory power. Strain categorisation requires low discriminatory power and aims to group strains that share a common trait (e.g., classification of strains according to their membership in a complex or whether they are clinical or environmental in origin; Dubois et al. 2010, Wolters et al. 2011). Strain differentiation requires intermediate discriminatory power and is based on the presence/absence of one or several peaks (e.g., the use of a strain-specific biomarker to differentiate strains of *Streptococcus pyogenes*; Moura et al. 2008). Strain identification requires high discriminatory power and compares the profiles of unknown strains to reference databases (e.g., strain identification of *Streptococcus agalactiae*; Lartigue et al. 2009). Bacterial typing is more demanding than routine identification in terms of reproducibility and discriminatory power (Croxatto et al. 2012, Dieckmann et al. 2008, Josten et al. 2013).
The limit of MALDI-TOF MS typing might be largely determined by the nature of the bacteria profiled, as some bacteria are very different and others are almost indistinguishable at the subspecies level (Karger et al. 2011). Therefore, there will likely be a need for bacterium-specific modules (Sandrin et al. 2013, van Belkum et al. 2012).

Numerous MALDI-TOF MS typing schemes have been evaluated and compared with conventional typing methods.
Wolters et al. reported the elaboration and validation of a hospital-associated MRSA (HA-MRSA) typing scheme that showed a high concordance with the clonal complex deduced by *spa* typing (Wolters et al. 2011). This type of discrimination between *S. aureus* lineages using MALDI-TOF MS was recently confirmed by Josten et al. (Josten et al. 2013).
Other studies have evaluated MALDI-TOF MS as a typing tool for *Listeria* sp., *Stenotrophomonas* sp., *Neisseria meningitidis*, *Legionella* sp. and others compared with pulsed-field gel electrophoresis (PFGE) or multi-locus sequence typing (MLST). Each study revealed promising results (Fujinami et al. 2011, Vasileuskaya-Schulz et al. 2011, Suarez et al. 2013, Barbuddhe et al. 2008).

Satisfactory results investigating outbreaks have also been reported with *Corynebacterium striatum*, *Corynebacterium pseudodiphteriticum*, *S. pneumoniae* and MRSA and *Candida parapsilosis* in neonatal intensive care units (Verroken et al. 2013, Bittar et al. 2010, Williamson et al. 2008, Schlebusch et al. 2010, Pulcrano et al. 2012).

MALDI-TOF MS is usually faster and cheaper than conventional typing methods, but additional studies are still required to confirm these results and to determine the potential role of this technology in epidemiological studies (Lartigue et al. 2013). While it allows the discrimination between bacteria below the species level, it may not always be as discriminatory as other typing methods (Verroken et al. 2013). If MALDI-TOF MS is unsuccessful, other techniques providing more rigorous structural biomarker characterisation might be required to attain the desired level of taxonomic resolution (Sandrin et al. 2013).

MALDI-TOF MS typing is usually achieved using library-based approaches, but bioinformatic-based approaches have also been described and will likely flourish in the future thanks to easier access to hardware and genome sequences (Sandrin et al. 2013).

1.2.9.6. Microbial virulence

The detection of virulence factors using MALDI-TOF MS has been poorly investigated. Specific peaks corresponding with the Panton-Valentine leukocidin (PVL) toxin of *S. aureus* have been reported, but these results have been questioned and rejected by another team that showed that the peaks were also present in PVL-negative isolates (Bittar et al. 2009, Szabados et al. 2011). More recently, the staphylococcal delta toxin, the presence of which is associated with acute infection, has been detected by MALDI-TOF MS (Gagnaire et al. 2012).

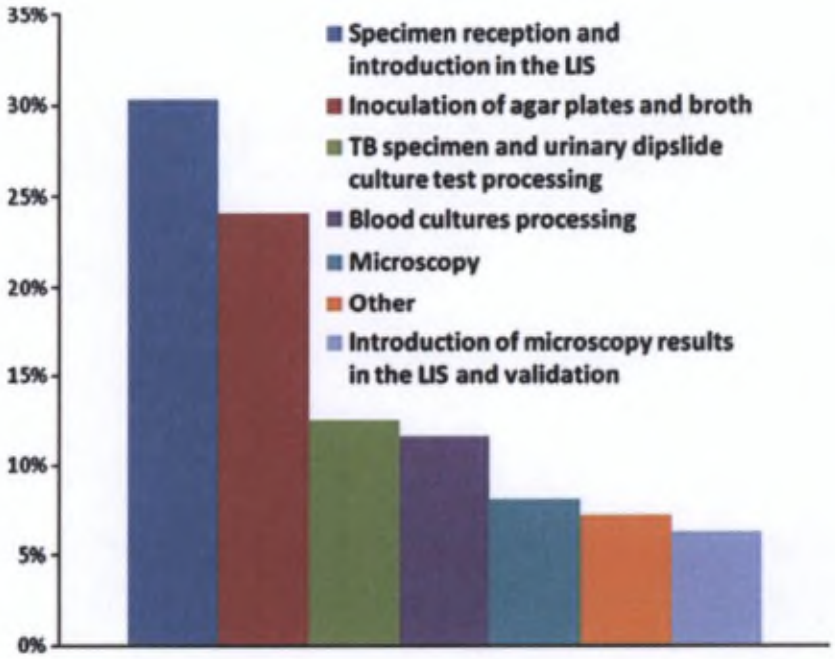


Figure 25. Partition (%) of the workload at the sample reception unit in a university laboratory processing a mean of 300 samples per days and employing ten full-time technologists. Time was assessed by self-reporting activity at 15-min intervals; activities had to be assigned to one of the seven categories presented on the graph (from Greub et al. 2011).

1.3. Evolution of the microbiology laboratory

Today, the clinical laboratory is evolving much faster than in previous centuries (van Belkum et al. 2013, Burnham et al. 2013, Doern et al. 2013).

First, the clinical laboratory has been completely transformed by the development of numerous molecular and biophysical technologies (van Belkum et al. 2013, Emonet et al. 2010, Ho et al. 2011).

Second, the trend towards an increased number of samples is not matched by an increase in human resources due to severe financial restrictions; however, assuring the quality of the results is still required. Automated systems for repetitive and fastidious tasks such as sample inoculation, which represents a quarter of the technologist's activities (Figure 25), could be a solution as they can save 50%-70% of the time spent by a full-time technologist (Dumitrescu et al. 2011, Greub et al. 2011).

Finally, there is move toward large pooled analytical platforms, which usually consist of a central laboratory and one or more remote laboratories that address urgent samples. This change leads to the concentration of resources, amalgamation of services and sub-specialisation (Humphreys et al. 2010, Raoult et al. 2004).

This complete rethinking of clinical microbiology organisation should not be performed without putting the patients' interests in the forefront.

2. Study objectives

2.1. General objective

The aim of this research is to evaluate the contribution of MALDI-TOF MS to the microbiology laboratory.

2.2. Specific objectives

2.2.1. Contribution to routine microbiological diagnosis

In this section, the MALDI-TOF MS technology is evaluated as an analytical tool for both a routine clinical practice and a national reference centre (NRC) practice. Using a large panel of routine isolates, two commercially available IVD MALDI-TOF MS systems are evaluated and compared in terms of analytical accuracy and practicality (see section 4.1). In a second study, MALDI-TOF MS is compared with conventional identification techniques for the identification of *Campylobacter* and related organisms (see section 4.2). Finally, a commercial kit and an "in-house" method for MALDI-TOF MS identification directly from positive blood culture are compared to determine which system would be the best option for use in a routine laboratory (see section 4.3).

2.2.2. Contribution to the clinical management of infected patients

Despite the fact that every hour with inadequate antimicrobial treatment in septic shock is associated with increased mortality, doubts remain about the clinical impact of a rapid identification in these situations. Indeed, an "identification result" does not mean a "susceptibility testing" result, and broad spectrum antimicrobial agents should always be immediately administered in severe sepsis. The aim of this section is therefore to evaluate the impact of the beneficial aspects of MALDI-TOF MS (accurate identification and reduced TTI) on the clinical management of patients with suspected sepsis (see section 4.4).

2.2.3. MALDI-TOF MS in the microbiology laboratory of the future

In Europe, the rationalisation of health costs requires major changes in hospital organisation and the development of strategies for laboratory cost-containment. Large centralised clinical laboratories are now being developed which requires technique harmonisation, automation and workflow adaptation. In the context of pooled analytical platforms, MALDI-TOF MS appears to be a good candidate for the delocalisation of bacterial identification. The feasibility of MALDI-TOF MS networking is evaluated in this third section (see section 4.5).

2.2.4. MALDI-TOF MS in fundamental microbiology research

To date, MALDI-TOF MS has mostly been evaluated as an identification tool in bacteriology and mycology. Research studies that open perspectives for new MALDI-TOF MS applications in microbiology are presented in this last section that include the optimisation of *Campylobacter* growth conditions for further identification by MALDI-TOF MS (see section 4.6) and the evaluation of MALDI-TOF MS for the subtyping of *Blastocystis* sp. (see section 4.7).

3. Materials and methods

3.1. Study sites

3.1.1. iris-Lab

iris-Lab is a multisite clinical laboratory that performs all clinical biology analyses for the following hospitals:

- Saint-Pierre University Hospital: a 450-bed teaching hospital located in the centre of Brussels, Belgium. Saint-Pierre University Hospital offers general and specialised medical services in approximately 20 disciplines, including paediatrics, and is a certified level 1 regional centre for infectious diseases (www.stpierre-bru.be).
- Jules Bordet Institute: a 167-bed teaching hospital. The only autonomous hospital in Belgium (Brussels) totally dedicated to cancer. Jules Bordet Institute covers both cancer screening and clinical care. The institute is also a teaching hospital involved in research activities (www.bordet.be).
- Brugmann University Hospital: an 854-bed hospital organised into three distinct sites in Brussels, Belgium. Brugmann University Hospital provides medical care for 27,700 inpatients and 325,000 outpatients annually (www.chu-brugmann.be).
- Queen Fabiola Children's University Hospital (QFCUH): a 168-bed teaching hospital. The only Belgian university hospital devoted entirely to children's medicine, with over 11,000 children admitted annually (www.huderf.be).

3.1.2. Bruker Daltonics R&D Department

Portions of the experiments were performed on a Microflex LT in the Research and Development Department of Bruker Daltonics, Bremen, Germany.

3.1.3. bioMérieux R&D Department

Portions of the experiments were performed in the Research and Development Department of bioMérieux, Lyon, France.

3.2. Population

The study population consists of the population in the hospitals listed above and therefore includes general and oncological adult patients and paediatric patients. Many of these patients are of low socioeconomic status. Many patients are not covered by any insurance or are political or economic refugees.

3.3. Period

The studies were conducted over a 3.5-year period from November 2009 to April 2013.

3.4. Strain and clinical sample origins

- In the study comparing commercial MALDI-TOF MS systems, all strains included in the "routine" panel (n=986) were prospectively isolated from clinical samples from both Saint-Pierre University Hospital and Jules Bordet Institute. Strains included in the "anaerobe" panel (n=75) were from a collection of prospectively acquired routine strains provided by the Microbiology Department of Universitair Ziekenhuis (UZ) Brussel, Brussels, Belgium. Strains included in the "enteric pathogen" panel (n=53) were either collection or reference strains from Saint-Pierre University Hospital.
- All strains (n=1055) analysed in the study evaluating MALDI-TOF MS networking were routine strains prospectively acquired from clinical samples from both Brugmann University Hospital and Queen Fabiola Children's University Hospital.
- *Campylobacter* strains were routine (n=224) and reference strains from Saint-Pierre University Hospital.
- *Blastocystis* strains (n=19) were cultivated in the Department of Parasitology in the Academic Medical Center of Amsterdam, The Netherlands.

3.5. General methods

3.5.1. Data collection

Microbiological data, routine results extracted from the laboratory information system (LIS) and MALDI-TOF MS results were collected in Excel files. Clinical data were prospectively and/or retrospectively collected on standardised case report forms.

3.5.2. Microbiological diagnosis

- Routine isolates were identified using MALDI-TOF MS and/or conventional identification techniques, including Gram staining, Vitek 2 and API systems (bioMérieux, Marcy l'Etoile, France), biochemical tests (e.g., coagulase, oxidase, catalase, agglutination, indole), the use of chromogenic media and others. Uncertain identifications were confirmed by molecular methods.
- Anaerobic strains were identified by gas-liquid chromatographic analysis of their cellular fatty-acid composition and complementary biochemical and enzymatic tests, if necessary.
- Enteropathogenic strains were identified by biochemical and serological tests or commercial systems (API, Vitek 2). Most strains were also submitted to NRCs.
- *Campylobacter* strains were identified by several PCR-based identification methods using whole-cell protein profiling and DNA-DNA hybridisation experiments, classical biochemical tests and/or partial 16S rDNA sequencing.
- Positive blood cultures were plated onto nonselective agar media, and isolated colonies were identified by MALDI-TOF MS or the conventional identification techniques described above.
- *Blastocystis* STs were determined by the analysis of partial SSU-rDNA sequences.

3.5.3. MALDI-TOF MS analysis

MALDI-TOF MS analyses were performed on a Microflex LT instrument equipped with the Biotyper database and/or in-house databases (Bruker Daltonics, Bremen, Germany) or on an Axima Assurance equipped with the SARAMIS and VITEK MS databases (bioMérieux, Marcy l'Etoile, France).

3.5.4. Statistical analysis

Statistical analysis for the evaluation of the best *Campylobacter* growth conditions for further MALDI-TOF MS identification was performed using SAS/STAT software 9.2 (SAS Institute Inc., Cary, NC, USA). Other statistical analyses were performed using the Statistical Package for Social Sciences (SPSS Inc.; Chicago, IL, USA), the Analyse-it software (Leeds, United Kingdom) and EpiInfo 6.04c PLUS for DOS (Centres for Disease Control and Prevention; Atlanta, GA, USA) software.

4. Results

4.1. Comparison of the MICROFLEX LT and VITEK• MS systems for the routine identification of bacteria by Matrix-Assisted Laser Desorption-Ionisation Time-Of-Flight Mass Spectrometry

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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

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

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

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

An abundant collection of literature now highlights the many benefits that result from the use of this technology. Using only a small portion of a colony and a drop of matrix solution, MALDI-TOF MS can accurately identify bacteria within a few minutes at a moderate cost (25, 30, 31). Although the technique has proven to be valuable for the identification of common bacteria, numerous studies have recently shown that it is a promising tool that can also be used for species that are usually difficult to identify, such as yeasts, anaerobes, and fastidious microorganisms (2, 3, 12, 16, 17, 22, 32). The ability of MALDI-TOF MS to directly identify bacteria in positive blood cultures also enhances the quality of patient management (11, 15, 19, 33, 34).

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

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

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

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

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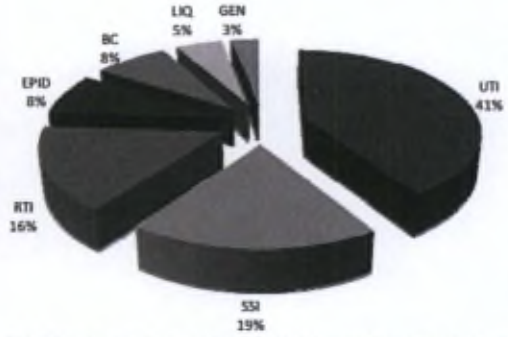


FIG 1 Distribution of the origins of the 1,003 routine isolates tested in this study. RTI, respiratory tract infections; SSI, skin and soft tissue infections; LIQ, normally sterile body fluids; UTI, urinary tract infections; EPID, epidemiological samples (methicillin-resistant *S. aureus* and extended-spectrum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

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

MATERIALS AND METHODS

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

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

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

All of the specimens were incubated overnight at 36 ± 1°C.

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

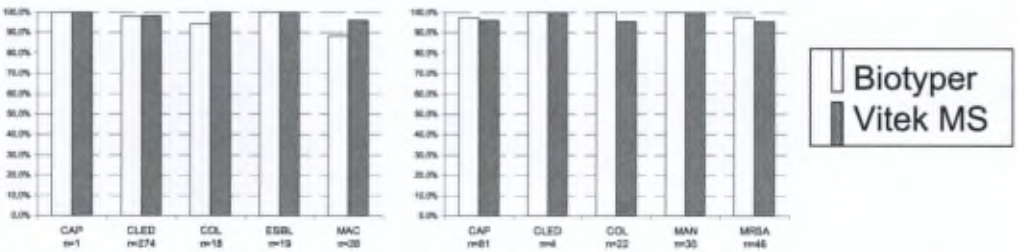
The anaerobe panel, previously collected in a university hospital for a susceptibility survey, included 75 strains covering 28 species of 13 different genera (8). Although the anaerobes were of various origins, they were derived primarily from blood cultures and abdominal samples. After thawing, the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

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

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

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

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



a. *E.coli* (n=338) b. *S. aureus* (n=183)

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

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

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

[a] ID, identification.

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

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

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

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

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

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

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

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

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

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

(Continued on next page)

TABLE 2 (Continued)

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

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

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

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

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

TABLE 4 MALDI-TOF MS results for 53 enteropathogenic strains

Genus and species/serovar	No. (%) of isolates	Saramis					Biotyper					Vitek MS				
		No. with correct genus and species IDs	No. with correct genus ID	No. with no ID	No. misidentified to species level	No. misidentified to genus level	No. with correct genus and species IDs	No. with correct genus ID	No. with no ID	No. misidentified to species level	No. misidentified to genus level	No. with correct genus and species IDs	No. with correct genus ID	No. with no ID	No. misidentified to species level	No. misidentified to genus level
Aeromonas veronii	1 (1.9)	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0
Arcobacter butzleri	1 (1.9)	0	0	1	0	0	0	1	0	0	0	0	0	1	0	0
Campylobacter coli	3 (5.7)	0	0	3	0	0	3	0	0	0	0	3	0	0	0	0
Campylobacter fetus	1 (1.9)	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0
Campylobacter jejuni	5 (0.4)	3	0	2	0	0	5	0	0	0	0	5	0	0	0	0
Campylobacter lari	2 (3.8)	0	0	2	0	0	2	0	0	0	0	2	0	0	0	0
Clostridium difficile	11 (20.8)	6	0	5	0	0	11	0	0	0	0	11	0	0	0	0
Escherichia coli O157 (nontoxinogenic)	2 (3.8)	0	0	2	0	0	2	0	0	0	0	2	0	0	0	0
Plesiomonas shigelloides	1 (1.9)	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0
Salmonella Durban	2 (3.8)	0	1	1	0	0	0	1	0	1	0	0	2	0	0	0
Salmonella Enteritidis	1 (1.9)	0	0	1	0	0	0	1	0	0	0	0	1	0	0	0
Salmonella Newport	1 (1.9)	0	0	1	0	0	0	1	0	0	0	0	1	0	0	0
Salmonella Saint-Paul	1 (1.9)	0	1	0	0	0	0	1	0	0	0	0	1	0	0	0
Salmonella Typhi	5 (9.4)	0	0	5	0	0	0	4	0	1	0	5	0	0	0	0
Salmonella Typhimurium	5 (9.4)	0	0	5	0	0	0	4	0	1	0	0	5	0	0	0
Shigella boydii	1 (1.9)	0	0	1	0	0	0	0	1*	0	0	0	0	1*	0	0
Shigella flexneri	4 (7.5)	0	0	3	0	1	0	0	4*	0	0	0	0	4*	0	0
Shigella sonnei	2 (3.8)	0	0	0	0	2	0	0	2*	0	0	0	0	2*	0	0
Vibrio cholerae	2 (3.8)	0	0	2	0	0	0	2	0	0	0	2	0	0	0	0
Yersinia enterocolitica	2 (3.8)	0	0	2	0	0	2	0	0	0	0	2	0	0	0	0
Total no. (%)	53 (100)	11 (20.8)	2 (3.8)	37 (70.0)	0 (0.0)	3 (5.7)	26 (49.1)	17 (32.1)	7 (13.2)	3 (5.7)	0 (0.0)	35 (66.0)	10 (18.9)	8 (15.1)	0 (0.0)	0 (0.0)

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

TABLE 5 Comparison of the results obtained using the three systems

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

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

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

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

RESULTS

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

The results obtained from the three databases (Biotyper, Saramis, and Vitek MS) for the strains requiring identification to the genus or species level are presented in Tables 1 and 2, respectively. For a comparison of the results obtained by the three systems, see Table 5.

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

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

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

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

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

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

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

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

E. coli ($n = 338$) and *S. aureus* ($n = 183$) were the most frequently isolated species. The rate of correct species identification ranged from 94.4% (Columbia agar, Biotyper database) to 100% and from 95.5% (Columbia agar, Vitek MS database) to 100% for *E. coli* and *S. aureus*, respectively (Fig. 2). The Fisher test indicated that the quality of the identification was not related to the choice of the culture medium.

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

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

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

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

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

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

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

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

In terms of the quality management parameters, both the Vitek MS IVD and Biotyper databases are CE marked. Both manufacturers offer RUO versions that require validation by the laboratory before the data can be reported to clinicians. The Vitek MS RUO (Saramis database) is provided by bioMérieux, whereas Bruker Daltonics offers a research tool that includes the same database as the IVD system (Biotyper database). This manufacturer also offers the possibility of combining both the IVD and RUO versions on the same device. The Vitek MS IVD system has a more highly developed quality management system because it contains dedicated positions for quality controls and a well-defined traceability system.

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

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

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

DISCUSSION

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

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

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

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

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

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

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

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

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

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

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

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

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

MALDI-TOF MS seems to be less effective for the identification of anaerobes than for aerobic organisms. In a recent comparison of the Saramis and Biotyper databases, Veloo et al. found that the Biotyper database performed better when identifying species of the *B. fragilis* group, but they also noted that the analysis of Gram-positive cocci with this technology required a time-consuming extraction step to obtain the same results as those obtained using the Saramis database and direct deposit (32). In the present study, the results obtained with the Saramis database were unsatisfactory. This major difference probably resulted from the different correct-identification cutoffs adopted in the two studies. However, the Biotyper database performed similarly in the identification of anaerobic organisms in both studies. Comparing the Biotyper and Vitek MS systems, the Biotyper database allowed better identification of *Bacteroides* species ($P < 0.05$). The Vitek MS database showed better results for *Fusobacterium* species ($P < 0.01$); however, this difference should not impact clinical management. Indeed, the Gram coloration of these species is often sufficiently informative for their identification. Moreover, the identification of these organisms to the genus level was not statistically significantly different using both databases ($P = 0.125$).

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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4.2. Accuracy of the API Campy System, the Vitek 2 *Neisseria-Haemophilus* (NH) Card and the Matrix Assisted Laser Desorption/Ionisation Time-of-flight Mass Spectrometry (MALDI-TOF MS) for the Identification of *Campylobacter* and Related Organisms

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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

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Abstract

Biochemical identification of *Campylobacter* and related organisms is not always specific, and may lead to diagnostic errors. The API Campy, the Vitek 2 system and matrix-assisted desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) are commercially available methods that are routinely used for the identification of these microorganisms. In the present study, we used 224 clinical isolates and ten reference strains previously identified by multiple PCR assays, whole cell protein profiling and either DNA–DNA hybridization or sequencing analysis to compare the reliability of these three methods for the identification of *Campylobacter* and related pathogens. The API Campy accurately identified 94.4% of *Campylobacter jejuni* ssp. *jejuni* and 73.8% of *Campylobacter coli*, but failed to correctly identify 52.3% of other *Epsilobacteria*. The Vitek 2 *Neisseria–Haemophilus* card correctly identified most *C. jejuni* ssp. *jejuni* (89.6%) and *C. coli* (87.7%) strains, which account for the majority of campylobacterioses reported in humans, but it failed in the identification of all of the other species. Despite a good identification rate for both *C. jejuni* ssp. *jejuni* and *C. coli*, both methods showed poor sensitivity in the identification of related organisms, and additional tests were frequently needed. In contrast to API Campy and Vitek, MALDI-TOF MS correctly identified 100% of *C. coli* and *C. jejuni* strains tested. With an overall sensitivity of 98.3% and a short response time, this technology appears to be a reliable and promising method for the routine identification of *Campylobacter* and other *Epsilobacteria*.

Keywords: API Campy, *Campylobacter*, identification, MALDI-TOF MS, NH card, Vitek
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Introduction

Bacterial species within the class *Epsilobacteria*, especially *Campylobacter jejuni* and *Campylobacter coli*, are the most frequently reported bacterial causes of foodborne illness worldwide. Recent reports suggest that *Campylobacter* infections may be followed by bacteraemia or septicaemia at an estimated rate of 1.5 cases per 1000 intestinal infections. Moreover, 0.1% of *Campylobacter* infections, especially *C. jejuni* infections, are associated with the development of Guillain–Barré syndrome [1].

With recent advances in diagnostic methods, the importance of at least 50 other species as possible causes of gastroenteritis is now recognized [2]. In a previous study, we showed a prevalence of non-*jejuni/coli Campylobacter* of 0.53% in 67 599 stool specimens from 40 995 patients presenting with abdominal complaints [3]. Non-*jejuni/coli Campylobacter* strains also seem to be common in developing nations; in South Africa, Lastovica *et al.* [4] found them in 12.0% of 19 535 stool samples from children with diarrhoea. To date, at least seven species, *Campylobacter upsaliensis*, *C. jejuni* ssp. *doylei*, *Campylobacter fetus* ssp. *fetus*, *Campylobacter concisus*, *Arcobacter butzleri*, *Helicobacter fennelliae* and *Helicobacter cinaedi*, have been identified as emerging human gastrointestinal pathogens [3,5–7].

In most laboratories, the conventional identification scheme for *Campylobacter* and related microorganisms usually consists of a few discriminatory tests, including growth temperature, oxidase and catalase activities, cephalothin and nalidixic acid susceptibility and hippurate hydrolysis. However, such simple schemes may lead to identification errors [8], and, in many laboratories, the identification of *Campylobacter* species is only performed up to the genus level. However, correct identification is mandatory to provide information about the prevalence and antimicrobial susceptibility patterns of the different species, as well as to study specific risk factors.

Alternative identification methods, such as a wide range of PCR assays using either species-specific or multiplex reactions and microarray-based identification tests, have been developed [9]. However, most clinical laboratories do not use these methods, but instead use manual or automatic commercial identification systems for *Campylobacter* identification. For about three decades, API Campy (BioMérieux, Marcy l'Etoile, France), a miniaturized identification system that includes 11 enzymatic and conventional tests and nine assimilation and inhibition tests, has often been used for the routine identification of *Campylobacter* [10,11]. More recently, BioMérieux has commercialized a *Neisseria–Haemophilus* (NH) identification card, which allows the identification of *C. jejuni*, *C. coli* and *C. fetus* with the Vitek 2 system [12,13]. In 2005, Mandrell *et al.* described matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) as a promising identification method for *Campylobacter* species relevant to public health and food safety. This system could therefore be used for the routine identification of *Campylobacter* and emerging *Epsilobacteria* [14–16].

However, to our knowledge, neither the NH card nor MALDI-TOF MS has ever been extensively tested against molecular methods for the routine identification of *Campylobacter* and other *Epsilobacteria*.

From a routine laboratory perspective, we have evaluated the ability of these three commercial methods to identify a random selection of strains obtained during an 8-year surveillance survey. These strains were previously identified in the course of a comparative study of several PCR-based assays and/or sequencing analysis [9].

Materials and Methods

Bacterial cultures

The clinical isolates were obtained during an 8-year surveillance study. During that period, stool samples submitted to two Belgian hospital laboratories were routinely examined macroscopically and microscopically, and cultured for common bacterial pathogens. A total of 1906 *Campylobacter*-like organisms were isolated, and biochemical identification classified 1471 isolates as *C. jejuni* ssp. *jejuni* and 218 isolates as *C. coli* [9].

From these isolates, 224 strains that cover a wide range of gastrointestinal pathogens (123 *C. jejuni* ssp. *jejuni*, 62 *C. coli*, eight *C. upsaliensis*, six *Campylobacter curvus*, five *C. fetus*, five *Campylobacter lari*, five *C. jejuni* ssp. *doylei*, three *A. butzleri*, three *Campylobacter hyointestinalis*, two *Arcobacter cryaerophilus*, one *Campylobacter peloridis* and one *Helicobacter pullorum*) were selected. The collection was further supplemented with ten reference strains (three *C. coli*, three *A. butzleri*, two *C. jejuni* and two *C. fetus*). Strain details are provided in Table S1.

Most of the clinical isolates (185/224) were previously included in an evaluation of several PCR-based identification methods using whole cell protein profiling and DNA–DNA hybridization experiments as reference identification methods [3,9]. The identification results of the latter polyphasic study were therefore used as the reference identification for the present study. The other strains were identified by classical biochemical testing and molecular methods; for a few of them, partial 16S rDNA sequencing was performed in order to confirm the identification (for details, see the Supporting Information).

Before identification by the three commercial methods was performed, frozen isolates (−70°C) were subcultured twice onto Mueller–Hinton agar containing 5% sheep blood (Oxoid, Erembodegem, Belgium) for 24–72 h at 37°C in a micro-aerobic atmosphere.

Methods

API Campy. API Campy identification was performed according to the manufacturer's instructions. The results were interpreted with the computerized automatic API Campy analytical profile index software.

NH card. NH card identification of the strains was performed according to the manufacturer's instructions, as previously described [12]. The results were interpreted on the basis of the software provided.

MALDI-TOF MS. The procedure used to identify the strains by MALDI-TOF MS was adapted from the 'ethanol/formic acid extraction' and 'direct transfer' procedures validated by the manufacturer (Bruker, Bremen, Germany). In order to prevent degradation of the proteins and to store samples for further investigations, ethanol pellets were prepared from fresh colonies in exponential growth phase. After centrifugation and discarding of the ethanol supernatant, inactivated biological material from the pellet was directly smeared onto a MALDI steel target plate. The following steps of the analy-

sis were performed on the Microflex LT (Bruker), according to the manufacturer's instructions. The results obtained were interpreted with the software provided; spectra were compared with the MALDI Biotyper database (Bruker). Among the 3287 entries (1820 species) included in the database at that time, 35 were entries for ten of the 11 species analysed in the present study.

Additional testing

In cases of doubtful identification, additional tests are proposed by the manufacturers of the API Campy and Vitek systems. Most were performed in our laboratory (for details, see Supporting Information). The MALDI-TOF MS final report did not suggest any additional testing in order to discriminate related species; definitive identifications were provided immediately.

Comparison

According to the confidence levels provided by the three software programs and the previously determined identification, the results obtained from the three methods were divided, after initial reading and complementary testing, into the following categories: 'correct identification of genus and species', 'misidentification' and 'no identification'.

Results

Table 1 shows the results obtained by the three methods. API Campy allowed correct identification of 92.8% and 94.4% of *C. jejuni* ssp. *jejuni* strains at the initial reading and after complementary testing, respectively (n = 125). This method showed lower sensitivity for the identification of

TABLE 1. Classification of the results obtained from the three evaluated methods

	After initial reading				After additional testing		
	Correct to genus and species level, no. (%)	Misidentification, no. (%)	No identification, no. (%)	Additional test required, no. (%)	Correct to genus and species level, no. (%)	Total misidentification (%)	No identification (%)
API Campy							
C. jejuni ssp. *jejuni*	116/125 (92.8)	–	7/125 (5.6)	2/125 (1.6)	118/125 (94.4)	–	7/125 (5.6)
C. coli	36/65 (55.4)	3/65 (4.6)	4/65 (6.2)	22/65 (33.8)	48/65 (73.8)	3/65 (4.6)	14/65 (21.5)
C. upsaliensis	3/8 (37.5)	–	–	5/8 (62.5)	8/8 (100)	–	–
C. fetus	2/7 (28.6)	–	5/7 (71.4)	–	2/7 (28.6)	–	5/7 (71.4)
C. curvus[a]	–	2/6 (33.3)	3/6 (50)	1/6 (16.7)	–	3/6 (50)	3/6 (50)
C. lari	2/5 (40)	–	–	3/5 (60)	3/5 (60)	–	2/5 (40)
A. butzleri[a]	–	–	–	6/6 (100)	–	6/6 (100)	–
C. jejuni ssp. *doylei*	5/5 (100)	–	–	–	5/5 (100)	–	–
C. hyointestinalis	1/3 (33.3)	–	2/3 (66.7)	–	1/3 (33.3)	–	2/3 (66.7)
A. cryaerophilus	2/2 (100)	–	–	–	2/2 (100)	–	–
C. pelaridis[a]	–	–	–	1/1 (100)	–	1/1 (100)	–
H. pullorum[a]	–	–	–	1/1 (100)	–	–	1/1 (100)
Total	167/234 (71.4)	5/234 (2.1)	21/234 (9.0)	41/234 (17.5)	187/234 (79.9)	13/234 (5.6)	34/234 (14.5)
NH card							
C. jejuni ssp. *jejuni*	101/125 (80.8)	13/125 (10.4)	–	11/125 (8.8)	112/125 (89.6)	13/125 (10.4)	–
C. coli	47/65 (72.3)	5/65 (7.7)	2/65 (3.1)	11/65 (16.9)	57/65 (87.7)	5/65 (7.7)	3/65 (4.6)
C. upsaliensis[a]	–	4/8 (50)	1/8 (12.5)	3/8 (37.5)	–	7/8 (87.5)	1/8 (12.5)
C. fetus	–	4/7 (57.1)	1/7 (14.3)	2/7 (28.6)	–	6/7 (85.7)	1/7 (14.3)
C. curvus[a]	–	–	–	6/6 (100)	–	–	6/6 (100)
C. lari[a]	–	–	1/5 (20)	4/5 (80)	–	–	5/5 (100)
A. butzleri[a]	–	5/6 (83.3)	1/6 (16.7)	–	–	5/6 (83.3)	1/6 (16.7)
C. jejuni ssp. *doylei*[a]	–	4/5 (80)	1/5 (20)	–	–	4/5 (80)	1/5 (20)
C. hyointestinalis[a]	–	3/3 (66.7)	–	1/3 (33.3)	–	3/3 (100)	–
A. cryaerophilus[a]	–	–	–	2/2 (100)	–	2/2 (100)	–
C. pelaridis[a]	–	1/1 (100)	–	–	–	1/1 (100)	–
H. pullorum[a]	–	1/1 (100)	–	–	–	1/1 (100)	–
Total	148/234 (63.2)	39/234 (16.7)	7/234 (3.0)	40/234 (17.1)	169/234 (72.2)	47/234 (20.1)	18/234 (7.7)
MALDI-TOF MS							
C. jejuni ssp. *jejuni*	125/125 (100)	–	–	–			
C. coli	65/65 (100)	–	–	–			
C. jejuni ssp. *doylei*	5/5 (100)	–	–	–			
C. upsaliensis	8/8 (100)	–	–	–			
C. fetus	7/7 (100)	–	–	–			
C. curvus	5/6 (83.3)	–	1/6 (16.7)	–			
C. lari	5/5 (100)	–	–	–			
A. butzleri	6/6 (100)	–	–	–			
C. hyointestinalis	3/3 (100)	–	–	–			
A. cryaerophilus	–	–	2/2 (100)	–			
C. pelaridis[a]	–	–	1/1 (100)	–			
H. pullorum	1/1 (100)	–	–	–			
Total	230/234 (98.3)		4/234 (1.7)				

MALDI-TOF MS, matrix-assisted desorption ionization time-of-flight mass spectrometry.
[a]Not included in the database.

C. coli: 55.4% of strains after the initial reading and 73.8% of strains after additional testing (n = 65).

The identification of other *Epsilobacteria* by API Campy was much more variable. A sensitivity of 100% was found for the identification of *A. cryaerophilus* (n = 2) and *C. jejuni* ssp. *doylei* (n = 5). Although additional investigations were needed for correct identification, this method also had a sensitivity of 100% for the identification of *C. upsaliensis* (n = 8). Sixty per cent of *C. lari* (n = 5) strains were correctly identified, but the other 40% remained unidentified, even after complementary testing. About one-third of the *C. fetus* strains (n = 7) and *C. hyointestinalis* strains (n = 3) were correctly identified, and no additional testing was suggested to identify the remaining strains.

API Campy was unable to correctly identify the *H. pullorum* strain tested (no identification, n = 1). API Campy misidentified all tested strains of *A. butzleri* (n = 6) and *C. peloridis* (n = 1). Half of the *C. curvus* (n = 6) strains were misidentified, whereas the other half remained unidentified. The total misidentification rate for this method was 5.6%.

Identification of the *C. coli* strains with the NH card rose from 72.3% upon initial reading to 87.7% after further testing. Correct identification of *C. jejuni* ssp. *jejuni* was 80.8% at the initial reading and 89.6% after additional testing. Most of the *C. jejuni* ssp. *doylei* (80%, n = 5), *A. butzleri* (83.3%, n = 6), *C. fetus* (85.7%, n = 7), *C. upsaliensis* (87.5%, n = 8), *C. hyointestinalis* (100%, n = 3) and *A. cryaerophilus* (100%, n = 2) strains, as well as the single *C. peloridis* and *H. pullorum* strains included in the study, were misidentified. The NH card provided no identification for 100% of *C. curvus* (n = 6) and *C. lari* (n = 5) strains. Overall, the total misidentification rate of the NH card was 20.1%. Both methods needed a long incubation time to provide identification.

MALDI-TOF MS correctly identified 98.3% of the *Epsilobacteria* strains tested. Only four of 234 isolates were not identified, including 16.7% of *C. curvus* (n = 6) and 100% of *A. cryaerophilus* (n = 2) and *C. peloridis* (n = 1) strains. However, the last species is not yet included in the database. A complete target plate (96 targets) was analysed in a few minutes.

Discussion

The data presented here demonstrate that three commercial methods were able to correctly identify most *C. jejuni* ssp. *jejuni* and *C. coli* strains, which account for most diagnosed human *Epsilobacteria* infections.

API Campy had significantly better sensitivity than the NH card in the initial identification of *C. jejuni* ssp. *jejuni* isolates. However, after additional testing, the difference was not statistically significant (94.4% vs. 89.6%). Moreover, with API Campy, <2% of the *C. jejuni* ssp. *jejuni* strains needed additional testing, <6% remained unidentified and none were misidentified (vs. 10.4% with the NH card).

In contrast, the NH card was better at identifying *C. coli* than API Campy. The latter was unable to identify more than 25% of the *C. coli* strains, even after additional testing. However, the misidentification rate for *C. coli* with the NH card was about 8%, and 4.6% of the strains remained unidentified by this method.

A lower identification rate of *C. coli* by API Campy was previously reported by Huysmans *et al.* [10], who compared this method with the conventional tests and concluded that the system offered no advantages.

Regarding the performance of the NH card, these results are in disagreement with those obtained by others [12,13], who showed an overall better identification rate for *C. jejuni* (94%, n = 18 [12]; 100% after additional tests, n = 27 [13]). For the identification of *C. coli*, different authors have obtained contradictory results, but the number of tested strains was too small for a significant comparison to be made 75%, n = 4 [12]; 100%, n = 9 [13]).

The performance of MALDI-TOF MS is significantly better, with an excellent sensitivity of 100%. Moreover, no additional testing was required to correctly identify *C. jejuni* ssp. *jejuni* and *C. coli* with this technology, the preparation of samples was at least as easy as for the other methods, and the results were obtained much more quickly (1 h instead of 24–48 h for the other methods).

The results obtained for the identification of the other species (n = 44), which represent more than 11% of all *Epsilobacteria* isolated from stool specimens [3] and may also be recovered from blood samples, show more variations between the three methods.

API Campy allowed the correct identification of all *A. cryaerophilus*, *C. jejuni* ssp. *doylei* and *C. upsaliensis* strains. However, to obtain these results, either a prolonged incubation time is needed, or additional tests must be performed, both extending the time needed for identification. *C. curvus*, *H. pullorum*, *C. peloridis* and *A. butzleri* were not correctly identified by this method, which is expected, as these species are not included in the database. Unexpected poor sensitivity levels were found for the identification of *C. lari*, *C. fetus* and *C. hyointestinalis*, even though these species are included in the manufacturer's identification table. Finally, 22.7% of the non-*jejuni*/*coli* *Campylobacter* strains were misidentified, and 29.5% remained unidentified.

The NH card failed to identify any other *Campylobacter* species, even though *C. fetus* is included in its database. As expected, related genera such as *Helicobacter* and *Arcobacter* could not be identified by this method. Finally, 65.9% of these 44 strains were misidentified by the Vitek technology, and this represents an important rate of diagnostic error.

The poor performances of API Campy and the NH card can be explained by the low number of discriminatory tests included in the devices. Indeed, 15 phenotypic characteristics have to be taken into account to discriminate *Campylobacter* and *Arcobacter* species [2]. Among the ten biochemical tests, only four are included in API Campy (nitrate reduction, hippurate hydrolysis, and urease and H_2S production; catalase production is included in the method, but must be performed separately). Sensitivity to nalidixic acid is also tested by API Campy, but cephalotin resistance has to be evaluated by an additional test. Among the three growth tests, only one, growth at 25°C, is suggested by the API system as part of the additional testing. The NH card includes only one of the ten biochemical tests (urease). Only a few useful additional tests are suggested by the system's manufacturer to discriminate *Campylobacter* and *Arcobacter* species, including catalase production, hippurate hydrolysis and growth at 25°C.

In the case of suspicion of *Campylobacter*, we propose the following rules to interpret identification results obtained by these methods. The identification results categorized as 'excellent identification to species level' or 'very good identification to species level' according to the instructions of the manufacturer, without or after additional tests, should be considered reliable. Other results are not reliable, and, in these cases, molecular tests or MALDI-TOF MS should be performed when accurate identification is of concern. Following this algorithm, the overall misidentification rate in the present study would have decreased from 5.6% to 0% and from 20.1% to 2.5% for API Campy and the NH card, respectively.

MALDI-TOF MS was shown to be superior to the other two methods, rapidly allowing the correct identification of 90.9% of the non-*jejuni/coli Epsilobacteria* strains. Moreover, this method did not lead to any misidentifications. Our observations concerning MALDI-TOF MS are in accordance with previously reported data [15,16] from studies performed on a limited number of reference strains, which also highlight the reliability of this technology.

In conclusion, the identification results for *Campylobacter* and related organisms obtained with API Campy and the NH card, which are widely used in clinical laboratories, must be interpreted with caution. Although both methods give satisfactory results for the identification of *C. coli* and *C. jejuni* ssp. *jejuni*, the overall misidentification rate for related species remains important.

Among the three evaluated commercial systems, MALDI-TOF MS appears to be the method of choice for the identification of *Campylobacter* and related microorganisms.

In addition to its ability to identify a large range of *Epsilobacteria* species with excellent sensitivity (98.3%) and specificity (100%), this reliable technology is also faster than all of the other available methods. Moreover, the analysis is easy to perform and requires few biological and technical materials.

The inability to provide information on bacterial resistance is probably the main weakness of MALDI-TOF MS, followed by the absence of a computerized expert system. Indeed, the suggestion of additional tests to discriminate species is helpful for the microbiologist in routine practice.

In the future, an expansion of the database to include larger numbers of infrequently encountered *Campylobacter* species and other *Epsilobacteria* species, and additional studies on the direct use of the sample, will further improve the usefulness of this technology for microbiologists [7].

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Transparency Declaration

There are no commercial or other associations that might pose a conflict of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1.

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4.3. Comparison of a in house method and the commercial Sepsityper™ kit for bacterial identification directly from positive blood culture broths by matrix-assisted laser desorption-ionisation time-of-flight mass spectrometry

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ARTICLE

Comparison of an in-house method and the commercial Sepsityper™ kit for bacterial identification directly from positive blood culture broths by matrix-assisted laser desorption-ionisation time-of-flight mass spectrometry

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Abstract The identification of bacteria directly from positive blood cultures using matrix-assisted laser desorption-ionisation time-of-flight mass spectrometry (MALDI-TOF MS) is a new challenge to microbiologists. However, the protocols previously described are often difficult to implement in routine and comparisons are not always possible due to the variability of interpretative criteria. This study evaluated the analytical and practical performances of an in-house (IH) method, adapted from previous protocols, and the Sepsityper™ kit (Bruker Daltonics, Bremen, Germany). Positive blood cultures from 63 different patients were prospectively evaluated by both methods. To enhance the sensitivity of these methods, lowered cut-offs were assessed and validated on 66 additional samples. The IH method produced 86.4% and 73.7% correct genus and species identifications, respectively, when using the lowered cut-offs of 1.4 and 1.6 for correct genus and species identifications. The Sepsityper™ kit showed similar results (78.0% and 68.4% correct genus and species identification, respectively). However, the IH method is ten-fold less expensive than the commercial option (0.72 vs. 7.45 €/analysis) and its turnaround time is approximately 20 min versus the nearly 40 min required for the Sepsityper™ kit, which includes an extraction step. Finally, the IH method was introduced twice-daily in our routine practice.

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Introduction

Matrix-assisted laser desorption-ionisation time-of-flight mass spectrometry (MALDI-TOF MS) has recently revolutionised the world of bacterial identification [1, 2]. Two manufacturers, bioMérieux (Marcy l'Etoile, France) and Bruker Daltonics (Bremen, Germany), are marketing efficient in vitro diagnostic-marked (IVD) systems, Vitek MS and Microflex LT, respectively, that allow the identification of bacteria and yeasts in a few minutes instead of the hours required by traditional methods.

The next challenge is the identification of micro-organisms directly from positive samples. Several procedures have been described for the identification of micro-organisms contained in urine samples, enrichment broths or positive blood cultures [3–5].

When treating patients with septic shock, a delay in implementing the appropriate antimicrobial treatment will considerably reduce the average survival of the patient [6]. For many years, the conventional identification techniques used in routine laboratories required at least 48 h. The fast recognition of bacteria directly from positive blood cultures is an ambitious MALDI-TOF MS application. More than 25 years ago, Vitek, API and other commercial systems were evaluated to determine their ability to perform rapid identification and susceptibility testing on bacterial pellets from positive blood cultures [7]. More recently, molecular methods were also developed but these techniques are too expensive to be implemented in routine [8].

The commercialisation of the MALDI-TOF MS technology, allowing the identification of bacteria in a few minutes, was the beginning of a new era. In an attempt to provide identification within a few minutes of determining the positivity of a blood culture, numerous protocols based on

stepwise centrifugations or lysis solutions were proposed [5, 9–16].

However, these protocols showed variable results and were usually too time-consuming to be implemented routinely in laboratories. A commercial kit, the Sepsityper™ (Bruker Daltonics, Bremen, Germany), was recently developed to standardise the preparation of blood culture prior to the spectrometric analysis [17]. The aim of this study was to develop an "in-house" (IH) processing method and to compare it with the commercial kit in terms of analytical performance, practicality and costs, in order to determine which system would be preferable for implementation in a routine laboratory.

Materials and methods

Blood cultures

During a first period of five weeks, positive blood culture broths (BACTEC Plus Aerobic and BACTEC F Lytic Anaerobic, Becton Dickinson, Franklin Lakes, NJ, USA) from patients with suspected sepsis were analysed by both the IH method and the Sepsityper™ kit. For each patient, only the first positive blood culture broth was included in the study (i.e. only one broth was included for each episode). The analyses were performed from 8 am to 5 pm, according to the laboratory organisation. The broths that became positive out of this period were analysed the next morning. For these blood culture broths, the "post-positivity" incubation time was, thus, longer than for the others.

The identification results were compared with those of our conventional methods, including MALDI-TOF MS identification of colonies, biochemical tests, API and Vitek card systems, or transfer to a reference laboratory.

Identification results from these techniques were considered as references.

The time required to provide a definitive identification by classical methods was also evaluated and is presented in a summary table (Table 1). The identification results obtained by both of the tested methods are presented in several tables (Tables 2, 3, 4 and 5).

During a second period of five weeks, positive blood culture broths (BACTEC Plus Aerobic and BACTEC F Lytic Anaerobic, Becton Dickinson, Franklin Lakes, NJ, USA) from patients with suspected sepsis were analysed by the IH method. The collected data constituted a validation set for the use of lowered cut-off values (Table 4).

In parallel, six inoculated but negative blood cultures (one paediatric, two anaerobic and three aerobic bottles), from six patients, were randomly chosen and tested by the IH method in order to determine the blank limit (see Fig. 1).

Table 1 Routine identifications and delays between determining the positivity of the blood culture and the final identification using traditional methods

Final identification	*n*	Time of identification		
		24 h ID	24-48 h ID	>48 h ID
Clostridium septicum	1			1
Enterobacter aerogenes	1	1		
Enterobacter cloacae	1	1		
Enterococcus faecalis	2	2		
Escherichia coli	10	10		
Finegoldia magna	2			2
Fusobacterium nucleatum	1			1
Klebsiella pneumoniae	2	2		
Micrococcus sp.	2	2		
Morganella morganii	1	1		
Prevotella buccae	1			1
Pseudomonas aeruginosa	1	1		
Rhizobium radiobacter	1		1	
Salmonella typhi	2		2	
Serratia marcescens	1	1		
Staphylococcus aureus	4	4		
Staphylococcus capitis	2	2		
Staphylococcus epidermidis	11	10	1	
Staphylococcus haemolyticus	1	1		
Staphylococcus hominis	3	2	1	
Streptococcus mitis/oralis	2	2		
Streptococcus pyogenes	1	1		
Streptococcus anginosus	2	1	1	
Streptococcus gordonii	1		1	
Streptococcus pneumoniae	2	2		
Streptococcus thermophilus	1		1	
Total	59	46	8	5
Total (%)	100	78.0	13.6	8.5

ID: identification

IH method

The IH method was adapted from various previously described methods [10, 15]. Briefly, 1 mL of the positive blood sample was added to 200 µL of a 5% saponin lysis solution. The tube was then thoroughly vortexed. After 5 min of incubation at room temperature, the tube was centrifuged for 1 min at 16,600g, and the supernatant was discarded. Finally, the pellet was washed with 1 mL of deionised water that was discarded after a second short centrifugation at 16,600g (1 min). When dried, the pellet was smeared on a stainless steel target plate before mass spectrometric analysis (see Fig. 2).

Table 2 Identification results by both methods using recommended cut-off values

	Final identification	*n*	In-house method					Sepsityper™ kit method				
			False ID		No ID	Correct genus ID	Correct genus and species ID	False ID		No ID	Correct genus ID	Correct genus and species ID
			Genus	Species				Genus	Species			
Gram-negative	*Enterobacter aerogenes*	1	0	0	0	1	1	0	0	1	0	0
	Enterobacter cloacae	1	0	0	1	0	0	0	0	0	1	1
	Escherichia coli	10	0	0	0	10	9	0	0	2	8	8
	Fusobacterium nucleatum	1	0	0	1	0	0	0	0	1	0	0
	Klebsiella pneumoniae	2	0	0	0	2	2	0	0	1	1	1
	Morganella morganii	1	0	0	0	1	1	0	0	0	1	1
	Prevotella buccae	1	0	0	0	1	1	0	0	1	0	0
	Pseudomonas aeruginosa	1	0	0	0	1	1	0	0	0	1	1
	Rhizobium radiobacter	1	0	0	0	1	1	0	0	1	0	0
	Salmonella typhi	2	0	1	0	2	0	0	2	0	2	0
	Serratia marcescens	1	0	0	0	1	0	0	1	0	1	0
	Total	22	0	1	2	20	16	0	3	7	15	12
	Total (%)	37.3	0.0	4.5	9.1	90.1	72.7	0.0	13.6	31.8	68.2	54.5
Gram-positive	*Clostridium septicum*	1	0	0	0	1	0	0	0	0	1	1
	Enterococcus faecalis	2	0	0	0	2	1	0	0	0	2	2
	Finegoldia magna	2	0	0	1	1	0	0	0	0	2	2
	Micrococcus sp.	2	0	0	2	0	NA	0	0	0	2	NA
	Staphylococcus aureus	4	0	0	0	4	4	0	0	1	3	3
	Staphylococcus capitis	2	0	0	1	1	1	0	0	1	1	0
	Staphylococcus epidermidis	11	0	0	5	6	1	0	0	4	7	1
	Staphylococcus haemolyticus	1	0	0	1	0	0	0	0	0	7	1
	Staphylococcus hominis	3	0	0	0	3	1	0	0	0	3	3
	Streptococcus anginosus	2	0	0	0	2	1	0	0	0	2	1
	Streptococcus gordonii	1	0	0	0	1	1	0	0	0	1	1
	Streptococcus mitis/oralis	2	0	0	1	1	0	0	0	2	0	0
	Streptococcus pneumoniae	2	0	0	2	0	0	0	0	2	0	0
	Streptococcus pyogenes	1	0	0	0	1	1	0	0	0	1	1
	Streptococcus thermophilus	1	0	0	1	0	0	0	0	0	1	0
	Total	37	0	0	14	23	11	0	0	10	27	16
	Total (%)	62.7	0.0	0.0	37.8	62.2	31.4	0.0	0.0	27.0	73.0	45.7
All	Total	59	0	1	16	43	27	0	3	17	42	28
	Total (%)	100.0	0.0	1.7	27.1	72.9	47.4	0.0	5.1	28.9	71.2	49.1

NA: not applicable: ID to the species level was not needed because *Micrococcus* was considered as a contaminant

ID: identification

MALDI Sepsityper™ kit

The preparation of the samples by the Sepsityper™ kit was performed according to the manufacturer's instructions for Gram-positive organisms. For the analysis of Gram-negative bacteria, our preliminary tests suggested that the formic acid extraction step could be eliminated (data not shown). A shorter procedure, which included the deposit of the pellet directly after the washing step, was then implemented.

MALDI-TOF MS analysis

The analyses were performed on a Microflex LT system (Bruker Daltonics, Bremen, Germany). After drying, each deposit was covered with 1 μL of α-cyano-4-hydroxycinnamic

Table 3 Identification results by both methods using lowered cut-off values

	Final identification	*n*	In-house method					Sepsityper™ kit method				
			False ID		No ID	Correct genus ID	Correct genus and species ID	False ID		No ID	Correct genus ID	Correct genus and species ID
			Genus	Species				Genus	Species			
Gram-negative	*Enterobacter aerogenes*	1	0	0	0	1	1	0	0	1	0	0
	Enterobacter cloacae	1	0	0	1	0	0	0	0	0	1	1
	Escherichia coli	10	0	0	0	10	10	0	0	2	8	8
	Fusobacterium nucleatum	1	0	0	1	0	0	0	0	1	0	0
	Klebsiella pneumoniae	2	0	0	0	2	2	0	0	0	2	2
	Morganella morganii	1	0	0	0	1	1	0	0	0	1	1
	Prevotella buccae	1	0	0	0	1	1	0	0	1	0	0
	Pseudomonas aeruginosa	1	0	0	0	1	1	0	0	0	1	1
	Rhizobium radiobacter	1	0	0	0	1	1	0	0	1	0	0
	Salmonella typhi	2	0	1	0	2	0	0	2	0	0	0
	Serratia marcescens	1	0	0	0	1	1	0	1	0	0	0
	Total	22	0	1	2	20	18	0	3	6	16	13
	Total (%)	37.3	0.0	4.6	9.1	90.1	81.8	0.0	13.6	27.3	72.7	59.1
Gram-positive	*Clostridium septicum*	1	0	0	0	1	1	0	0	0	1	1
	Enterococcus faecalis	2	0	0	0	2	2	0	0	0	2	2
	Finegoldia magna	2	0	0	1	1	1	0	0	0	2	2
	Micrococcus sp.	2	0	0	0	2	NA	0	0	0	2	NA
	Staphylococcus aureus	4	0	0	0	4	4	0	0	1	3	3
	Staphylococcus capitis	2	0	0	0	2	1	0	0	1	1	1
	Staphylococcus epidermidis	11	0	0	3	8	6	0	0	2	9	8
	Staphylococcus haemolyticus	1	0	0	0	1	1	0	0	0	1	1
	Staphylococcus hominis	3	0	0	0	3	3	0	0	0	3	3
	Streptococcus anginosus	2	0	0	0	2	2	0	0	0	2	2
	Streptococcus gordonii	1	0	0	0	1	1	0	0	0	1	1
	Streptococcus mitis/oralis	2	0	1	0	2	0	0	0	1	1	0
	Streptococcus pneumoniae	2	0	0	2	0	0	0	0	2	0	0
	Streptococcus pyogenes	1	0	0	0	1	1	0	0	0	1	1
	Streptococcus thermophilus	1	0	0	0	1	1	0	0	0	1	1
	Total	37	0	1	6	31	24	0	0	7	30	26
	Total (%)	62.7	0.0	2.7	16.2	83.8	68.6	0.0	0.0	18.9	81.1	74.3
All	Total	59	0	2	8	51	42	0	3	13	46	39
	Total (%)	100.0	0.0	3.4	13.6	86.4	73.7	0.0	5.1	22.0	78.0	68.4

NA: not applicable: ID to the species level was not needed because *Micrococcus* was considered as a contaminant

ID: identification

acid (CHCA) matrix solution. When dried, the target plate was loaded into the machine, which was equipped with a 337-nm nitrogen laser. The spectra were recorded in linear mode in a mass range of 2 to 20 kDa and were subsequently analysed using MALDI Biotyper automation control and Biotyper 2.0 software. At that time, the MALDI Biotyper database (v3.1.1.0) included 3,740 spectra from 319 genera and 1,946 species.

Classification

The identification results were first evaluated according to the manufacturer's instructions. Scores above 2 suggested a probable identification to the species level, scores between 1.7 and 2 indicated genus identification and scores of 1.7 and below were considered to be unreliable.

Table 4 Identification results obtained by the in-house (IH) method and using the lowered cut-off values for isolates included in the validation set (n=66)

Final identification	n	In-house method				
		False ID		No ID	Correct genus ID	Correct genus and species ID
		Genus	Species			
Acinetobacter baumannii complex	1	0	0	0	1	1
Acinetobacter johnsonii	1	0	0	0	1	1
Bacteroides caccae	1	0	0	0	1	1
Campylobacter fetus	1	0	0	0	1	1
Eggerthella lenta	1	0	0	1	0	0
Escherichia coli	6	0	0	0	6	6
Klebsiella oxytoca	1	0	0	0	1	1
Klebsiella pneumoniae	7	0	0	1	6	6
Pseudomonas aeruginosa	1	0	0	0	1	1
Pseudomonas fluorescens	1	0	0	0	1	0
Salmonella paratyphi	1	0	0	0	1	0
Total	22	0	0	2	20	18
Total (%)	33.3	0.0	0.0	9.1	90.9	81.8
Clostridium perfringens	1	0	0	1	0	0
Corynebacterium sp.*	1	0	0	1	0	NA
Micrococcus sp.*	2	0	0	0	2	NA
Propionibacterium sp.*	2	0	0	1	1	NA
Staphylococcus aureus	3	0	0	0	3	3
Staphylococcus capitis	3	0	0	1	2	1
Staphylococcus epidermidis	17	0	0	8	9	9
Staphylococcus haemolyticus	1	0	0	0	1	1
Staphylococcus hominis	5	0	0	2	3	3
Staphylococcus saprophyticus	1	0	0	0	1	1
Staphylococcus sp.*	1	0	0	0	1	NA
Streptococcus pneumoniae	5	0	0	1	4	2
Streptococcus pyogenes	2	0	0	0	2	2
Total	44	0	0	15	29	22
Total (%)	66.7	0.0	0.0	34.1	65.9	57.9
Total	66	0	0	17	49	40
Total (%)	100.0	0.0	0.0	25.8	74.2	66.7

ID: identification

NA: not applicable

*Species identification was not clinically required

The results were then evaluated according to the arbitrarily lowered cut-offs of 1.4 and 1.6 for the acceptable identification to the genus and species levels, respectively. When using these lowered cut-off values, a difference of at least 0.3 between the first identification match and the first discordant match was also required to validate the identification.

According to these two schemes, the identification results were classified into the following categories: (i) no identification, (ii) correct genus identification, (iii) correct genus and species identification, (iv) genus misidentification and (v) species misidentification (Tables 2, 3 and 4). The identification results obtained for polymicrobial cultures were considered separately and are presented in a summary table (Table 5).

Influence of additional incubation time on the quality of the rapid identification

Because positive blood cultures were not removed from the incubators at night, several samples benefited from additional incubation time. The effect of a prolonged incubation period was, thus, evaluated, comparing the rate of correct species identification for both samples that were immediately analysed and those that benefited from additional incubation hours. The comparison was made for results obtained by both methods and using both cut-off criteria for all isolates but also for Gram-negative and Gram-positive organisms separately (data not shown).

Table 5 Identification results for polymicrobial cultures using both methods

Polymicrobial cultures	In-house method	Score	Sepsityper™ method	Score
More than three organisms, no ID in routine	No reliable identification	1.19	No reliable identification	1.267
S. epidermidis + *E. coli*	*E. coli*	2.119	*E. coli*	2.256
S. epidermidis + *Streptococcus* sp.	*S. epidermidis*	1.917	*S. epidermidis*	2.047
E. faecalis + *E. coli*	*E. faecalis*	2.347	No reliable identification	1.101

ID: identification

Statistical analysis

McNemar's Chi-square or exact binomial test was applied to compare the results obtained by both methods on the same samples. Fisher's test was used in order to evaluate the impact of additional incubation time on the quality of the rapid identifications and to compare both the derivation and validation sets.

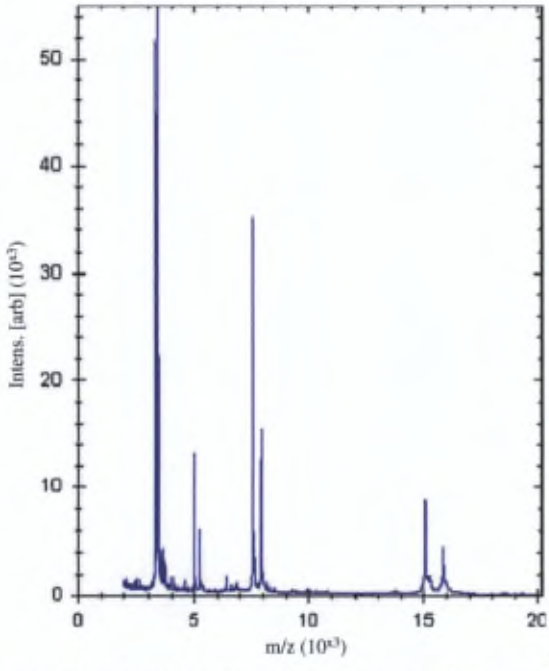
Practical points

To compare the practical strengths and weaknesses of both the IH and the commercial systems, the time required for the analysis, the cost and the ease of use were also taken into account in this evaluation and are presented in a summary table (Table 6).

Results

During the first study period, 140 identifications were prospectively performed, but only one identification per episode was taken into account in order to avoid bias. The final number of identifications (n=63) allowed us to accurately evaluate the performance of the techniques and to rapidly implement the analysis in our routine workflow.

During a period of five weeks, blood culture broths from 63 patients were, thus, prospectively included in the study.



Rank	Matched Pattern	Score value
1	*Staphylococcus pasteuri*	1.083
2	*Rhizobium rhizogenes*	0.838
3	*Myroides odoratimimus*	0.821
4	*Candida albicans*	0.818
5	*Geotrichum silvicola*	0.792
6	*Lactobacillus hammesii*	0.775
7	*Paenibacillus daejeonensis*	0.771
8	*Clostridium inocuum*	0.757
9	*Clostridium acetobutylicum*	0.745
10	*Rhodococcus rhodochrous*	0.731

Fig. 1 Example of the results obtained from an inoculated but negative blood culture (blank)

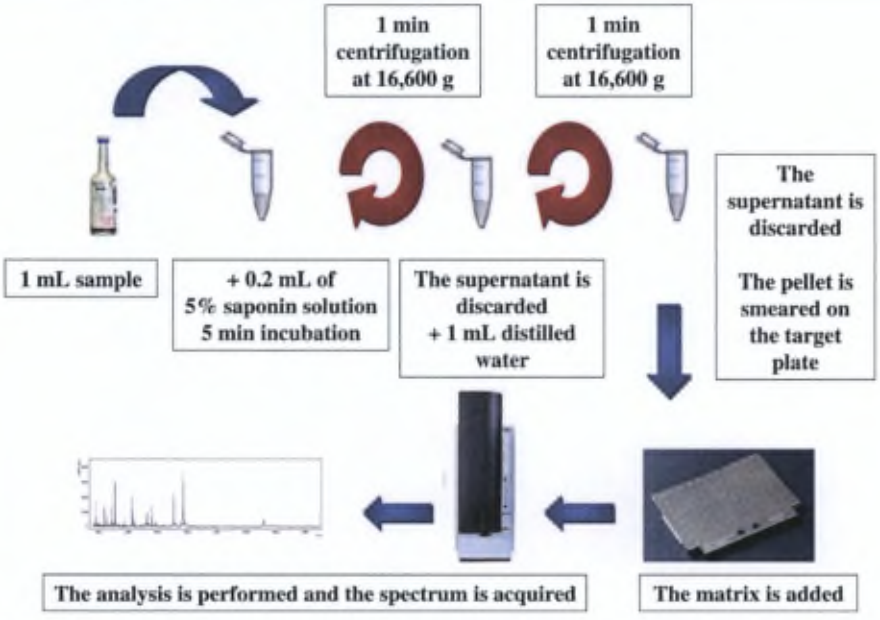


Fig. 2 IH method protocol

The Gram staining and our routine bacterial cultures revealed four polymicrobial (6.3%) and 59 monomicrobial blood cultures (93.7%).

Routine identifications

Of the 59 monomicrobial blood cultures, 22 were Gram-negative rods (37.3%) and 37 were Gram-positive organisms (62.7%). The distribution of the identifications and the time required for the identifications are presented in Table 1. Among the polymicrobial cultures, one culture showed more than three bacterial isolates that were not identified using routine methods, whereas the other three polymicrobial cultures were mixed cultures of two bacterial strains: *Staphylococcus epidermidis*+*Escherichia coli*, *S. epidermidis*+*Streptococcus* sp. and *Enterococcus faecalis*+*E. coli* (Table 5).

The results obtained by both the IH and commercial methods for the monomicrobial cultures using the recommended cut-offs of 1.7 and 2 for correct genus and species identification, respectively, are presented in Table 2.

Among the 22 Gram-negative bacteria, the IH method correctly identified 90.1% and 72.7% of the organisms to the genus and species levels, respectively. Using the Sepsityper™ kit, correct genus identification was reached in 68.2% of the cases, and only 54.5% were correctly identified at the species level. The rates of unidentified organisms were 9.1% and 31.8% using the IH and the Sepsityper™

Table 6 Comparison of practical parameters observed for both methods

	In-house method	Sepsityper™ method	Remark
Cost (€)/analysis	0.72	7.45	Including all chemicals and disposable materials (estimation realised in Belgium, March 2011)
Time to perform analysis (min)	20	40	40 min: including the extraction step, as recommended (Sepsityper™)
		20	20 min: excluding the extraction step (Sepsityper™)
Ease of use	+++	++	Working in the same tube (IH method) avoids accidental loss of the pellet

methods, respectively. With both methods, *Salmonella typhi* ($n=2$) was identified only to the genus level, and serotype misidentifications were observed. No genus misidentification occurred. Several identifications of *E. coli* and other enterobacteria (3/22) were missed by the commercial method, regardless of the cut-off used.

Among the 37 Gram-positive bacteria, the IH method correctly identified 62.2% and 31.4% of the bacteria to the genus and species levels, respectively. Using the commercial kit, 73.0% of these organisms were correctly identified to the genus level. The Sepsityper™ led to 45.7% correct species identifications. The rates of unidentified organisms were 37.8% and 27.0% using the IH and commercial methods, respectively. Half of the streptococci and several coagulase-negative staphylococci were missed by the IH system. No misidentification was observed.

When applied to anaerobic organisms, the IH method correctly identified the species of one *Prevotella buccae* isolate but failed to identify four other isolates, including one *Fusobacterium nucleatum* and one *Finegoldia magna* isolates. The second *F. magna* isolate and the *Clostridium septicum* isolate were only identified to the genus level using this method. The Sepsityper™ kit was unable to identify Gram-negative anaerobic isolates but led to a correct species identification of all Gram-positive anaerobic strains.

Finally, the overall correct species identification rates were 47.4% and 49.1% for the IH method and the Sepsityper™ kit, respectively ($p>0.05$).

To increase the sensitivity of our methods, the impacts of the lowered cut-offs of 1.4 and 1.6 for correct genus and species identification, respectively, and of requiring at least a 0.3 score difference between the first match and the first discordant species were also studied (Table 3).

Using the lowered cut-offs, 90.1% and 81.8% of the Gram-negative bacteria were correctly identified to the genus and species levels, respectively, using the IH method. For these organisms and using the Sepsityper™ method, the lowered cut-offs allowed the correct identification of 72.7% and 59.1% of isolates at the genus and species level, respectively. The rates of correct identification by the IH method for the Gram-positive bacteria were 83.8% and 68.6% at the genus and species levels, respectively. For these organisms, the Sepsityper™ achieved 81.1% and 74.3% correct identification at the genus and species levels, respectively. Lowering the cut-offs resulted in the misidentification of one isolate of *S. mitis/oralis* by the IH method.

However, the lowered cut-offs also enhanced the performance of the IH method in the identification of Gram-positive anaerobic strains, allowing the correct species identification of one *C. septicum* and one *F. magna* additional isolates.

Overall, lowering the cut-off values resulted in the correct identification of 86.4% and 73.7% of isolates by the IH method versus 78.0% and 68.4% of isolates by the Sepsityper™ method at the genus and species levels, respectively. The rate of unidentified organisms using the Sepsityper™ kit was almost twice that observed by the IH method (22.0% vs. 13.6%) ($p>0.05$).

Results obtained for the polymicrobial cultures

The results obtained for the polymicrobial cultures ($n=4$) are presented in Table 5.

The composition of mixed cultures with more than three species was not satisfactorily identified by either method. In the culture with both *E. coli* and *S. epidermidis*, both methods identified *E. coli* with an acceptable score according to the manufacturer's instructions. *S. epidermidis* was also correctly identified in the culture with both *S. epidermidis* and *Streptococcus* sp. (near the recommended cut-off by the IH method). In the culture with both *E. faecalis* and *E. coli*, the IH method correctly identified *E. faecalis*, but no useful information was obtained from the Sepsityper™.

During the second study period, 73 positive blood cultures from 73 sepsis episodes were prospectively analysed by the IH method and included in the validation set. Among the 73 cultures, seven polymicrobial cultures were excluded. Of the 66 monomicrobial cultures, 22 and 44 were Gram-negative (33.3%) and Gram-positive (66.7%) organisms, respectively. For six Gram-positive isolates, accurate species identification was not clinically required (one coagulase-negative *Staphylococcus* isolate, one *Corynebacterium* sp. isolate, two *Micrococcus* sp. isolates and two *Propionibacterium* sp. isolates). The results obtained for the isolates included in the validation set, using the IH method and the lowered cut-off values, are presented in Table 4.

No significant difference was observed between the derivation and validation sets.

Results obtained for the inoculated but negative blood cultures

Among the six negative blood cultures, the IH method allowed the acquisition of five spectra that led to unsatisfactory results when compared to the database. The results were accompanied by a comment explaining the poor quality of the identification ("not reliable identification"). All score values were inferior to 1.1. An example of the spectra and results obtained is presented in Fig. 1. No spectrum was acquired from the paediatric culture bottle.

Influence of additional incubation time

For both the IH and Sepsityper™ methods and for both the recommended and lowered cut-off values, no statistical

difference was observed in the rate of correct species identifications according to the incubation period. The same observation was made considering all of the included isolates together or Gram-negative and Gram-positive organisms separately (data not shown).

Practical points

Practical observations are presented in Table 6.

The cost of analysis was evaluated for both methods according to the prevailing catalogue prices. Our results suggest that the rapid bacterial identification is ten times more expensive with the Sepsityper™ kit than with the IH method (7.45 vs. 0.72 €/analysis), excluding the cost of the technician's time.

Both methods required a similar length of time for the analysis of Gram-negative rods due to the shortened procedure adopted for the Sepsityper™ system; 12 min is needed in order to obtain the biological material and smear it on the target plate. The sample preparation will then be completed approximately 20 min later. To identify Gram-positive bacteria, the complete commercial procedure was followed and included a long formic acid extraction step. In these cases, the entire process was completed in approximately 40 min using the Sepsityper™ kit versus 20 min by the IH method.

In terms of the practicality of the two methods, the IH method was easier to use. The lysis solution provided in the Sepsityper™ kit seemed to enhance the viscosity of the blood; hence, the numerous resuspensions of the bacterial pellet may lead to the accidental loss of the biological material.

Discussion

As demonstrated by the recent and abundant literature, rapid bacterial identification directly from positive blood cultures by MALDI-TOF MS is a challenging goal for microbiologists [5, 9–17]. However, the workload and the cost of most of the described procedures often limit their utilisation and lead microbiologists to seek alternative solutions.

Our experiments found that MALDI-TOF MS analysis directly from positive blood cultures produced 73.7% and 68.4% correct species identifications in less than 1 h by the IH and Sepsityper™ methods, respectively. In contrast, traditional identification methods provide 78%, 91.5% and 100% identification results after 24 h, 48 h and more than 48 h, respectively (Table 1).

Our results are quite similar to those of previous studies, summarised in Table 7, that have evaluated rapid methods of identifying bacteria from positive blood cultures [18]. The first studies described time-consuming procedures for the sample preparation that are difficult to implement in routine practice [5]. Shortened schemes were then evaluated with various results that depended on the tested organisms and the adopted cut-offs [10, 14]. Differences in the interpretative criteria led to substantial variability in the reported results and made comparison of the different methods difficult.

However, a common observation in all studies, independent of their design, is that rapid identification is more successful for Gram-negative organisms than for the Gram-positive species (see Table 7) that cause most cases of septicaemia [19]. This disparity may explain the poorer identification rates in the study by Ferreira et al. because only 20% of the bacteria in that study were Gram-negative [15]. As observed in our prospective study and other studies, the proportion of Gram-negative organisms found in positive blood cultures is usually higher than 20% (37% in the present study) [5, 9–11, 19].

Among the studies that used the interpretative cut-offs recommended by Bruker Daltonics, the range of correct genus and species identifications varies from 68.2 to 89% and from 57 to 79%, respectively [9, 15–17]. The results obtained in the present study show similar performances for genus identification using the manufacturer's cut-offs (72.9% and 71.2% for the IH and Sepsityper™ methods, respectively).

With 47.4% and 49.1% correct species identification for the IH method and the commercial kit, respectively, our successful identification rates seem lower than the results of previous studies.

This discrepancy may be partially explained by the panel of micro-organisms included in our study. An *S. typhi* outbreak appeared during the study period, accounting for the high proportion of *Salmonella* in the panel (n=2, 3.4%). In our routine practice, this bacterium represents only 1% of all pathogens isolated from blood culture, and it is known that the MALDI-TOF MS technology cannot identify these organisms to the serotype level [20].

Moreover, our panel includes five anaerobic organisms (8.5%). MALDI-TOF MS identification for anaerobic organisms still needs to be improved by upgrading the databases and developing a standardised method [21]. The inclusion of these organisms in our study may explain the differences observed between our Sepsityper™ results and those of Kok et al. [17].

Several authors reported up to 90% correct species identifications using short sample preparation procedures. Such results are obtained on the basis of lowered cut-offs and/or the utilisation of additional tests (i.e. Slidex pneumo-kit) [10, 12, 13].

In the present study, lowering the cut-offs to 1.4 and 1.6 for correct genus and species identifications, respectively, and imposing a difference of at least 0.3 between the first match and the first discordant identification as a new

Table 7 Major studies exploring rapid identification directly from positive blood cultures (adapted from Croxatto et al. [18])

Author	Journal	Year	Reference	Blood culture system	MS system	GN (%)	n	GP (%)	n	Yeasts (%)	n	Polymicrobial cultures	n	Total (%)	n	Cut-off	Remarks
La Scola and Raoult	PloS One	2009	[5]	BACTEC	Autoflex	94 87	125 100	37 67	197 140	NA NA	NA NA	1/2 (18×)	22	59 476	322 240	2/4 >1.9 4/4 >1.2	Two different tested protocols
Prod'Hom et al.	J Clin Microbiol	2010	[9]	BACTEC	Microflex	83 90	42	46 77	74	NA	NA	1/2	4	57 79	122	>2 species >1.7 genus	Two *Brevibacterium* sp. poorly identified
Ferroni et al.*	J Clin Microbiol	2010	[10]	BacT/ALERT	Microflex	92.6 1.1	189	81.5 17.5	103	100	20	NA		86.9	312	>1.8 and 0.3 >1.8 and no 0.3 difference between first discordant species matches	Spiked samples
						92.8 2.9	139	89.2 9	223	100	11	1/2 ID	15	90.9 6.4	373		Clinical samples, combined Slidex pneumo-kit
Stevenson et al.*	J Clin Microbiol	2010	[11]	BACTEC	Ultraflex	79.5 87.2	78	58.2 70.1	134	NA	NA	1/2 ID (9×)	10	65.1 76.4	212	>1.9 species >1.7 genus	
Christner et al.	J Clin Microbiol	2010	[12]	BACTEC	Microflex	NR						NR		95 95	277 277	1.3 species 1.4 species	
												1/2 (13×)	16	92	277	1.5 species	
												NR		90	277	1.6 species	
														87	277	1.7 species	
														83	277	1.8 species	
														81	277	1.9 species	
														75	277	2 species	
Moussaoui et al.	Clin Microbiol Infect	2010	[13]	BACTEC	Biflex	98.9	187	92.88	295	NA		36% isolates	21	95.22	482	>1.4	27 contaminant excluded
						91.9	210	89.3	318				50 isolates	89.7	532	4 identical proposals and 4 distinct spectra	Yeasts excluded
Schmidt et al.	Eur J Clin Microbiol Infect Dis	2011	[14]	BACTEC	Shimadzu	74.4 81.4	43	45 58.3	60	NA				57.3 68	103	Superspectrum or top 10 identical ID	4 different tested protocols
						93		75						82.5			
						97.7		61.7						76.7			
				BacT/ALERT SA		58.1		30						41.7			
						69.8		33.3						48.5			
						67.4		30						45.6			
						81.4		21.7						46.6			
				BacT/ALERT FA		30.2		3.3						14.6			
						48.8		3.3						22.3			
						41.9		1.7						18.4			
						67.4		13.3						35.9			
Ferreira et al.	Clin Microbiol Infect	2011	[16]	BACTEC	Autoflex	NR		NR		NR		NR		76 96	68	>2 species >1.7 genus	
Ferreira et al.	Clin Microbiol Infect	2011	[15]	BACTEC	Autoflex	83.3 96.6	61	31.8 64.8	239	5.6 (genus)	18	NA		39.6 68.2	318	>2 species >1.7 genus	
Kok et al.	PLoS One	2011	[17]	BACTEC	Microflex	79.7 87.2	187	46.3 68.4	285	NA		1/2 (20×)	31	59.4 79.8	507	>2 species >1.7 genus	4 anaerobes remaining not identified

NR: not reported; NA: not applicable; GN: Gram-negative bacteria; GP: Gram-positive bacteria; MS system: mass spectrometry system

*Extrapolated results based on published data

security rule resulted in an appreciable improvement of both the IH and Sepsityper™ results, especially for Gram-positive bacteria. These new cut-off criteria were assessed and validated on a validation set including 66 additional isolates.

The revised interpretative criteria did not lead to genus misidentification but did lead to a species misidentification using the IH method for one isolate of *S. mitis/oralis*, which was erroneously identified as *S. pneumoniae*.

Because of the related taxonomy of these two species, this error may appear even when identifying bacteria from a colony and using the recommended cut-offs [22]. The strengths and weaknesses of the rapid technology must be evaluated while taking into account the general limitations of mass spectrometry in bacterial identification. In our routine practice, the results for *S. mitis/oralis* and *S. pneumoniae* are always verified with an optochin test to confirm the identification. A direct identification from a blood culture for which Gram staining showed probable streptococci would not confirm the presence/absence of *S. pneumoniae* but would likely exclude or confirm the presence of an *Enterococcus* species or beta-haemolytic *Streptococcus*.

Our results also suggest that lowered cut-offs may help in the diagnosis of anaerobic isolates, increasing the sensitivity of the IH method in both the identification of Gram-positive and Gram-negative strains. However, as previously mentioned, improvement of the databases is still required in order to enhance the identification of such organisms from blood cultures as well as from isolated colonies [21].

The results of the identification of bacteria from polymicrobial cultures (n=4) were in agreement with the findings of previous studies. When analysing a positive blood culture containing several micro-organisms, the MALDI-TOF MS usually led to the successful identification of the predominant species. There was no misidentification resulting from the concomitant presence of several species of bacteria [5, 9–13, 17]. Interestingly, Ferroni et al. showed that using Gram-negative- and Gram-positive-specific databases when the Gram stain showed organisms of both classes could permit the identification of both the Gram-positive and the Gram-negative bacteria [10].

The analysis of five negative blood cultures led to similar spectral profiles (Fig. 1). This suggests that the development of an algorithm allowing the discrimination of several species from the same spectrum could also be helpful for the direct bacterial identification from positive blood cultures (discrimination of blood proteins from bacterial proteins).

The result observed for the paediatric culture may probably be explained by a lower inoculum.

Interestingly, additional incubation time did not seem to impact the rate of correct species identifications in the present study. However, such an evaluation should preferably be realised on a larger number of isolates and for each species separately in order to draw accurate conclusions.

The major drawback of our study is the absence of fungal identification data resulting from the short study period. We hypothesise that an additional extraction step would be required in order to obtain reliable identification results for yeasts. The Sepsityper™ kit showed promising results in a previous study that evaluated a panel of organisms including 41 species from four *Candida* genera and one isolate of *Cryptococcus neoformans* [23].

In terms of analytical performance, the IH method provided similar results to the Sepsityper™ kit overall. The global performance using the adapted cut-offs was as follows: 86.4% and 73.7% correct genus and species identifications, respectively, versus 78.0% and 68.4% using the commercial method. However, there are some practical points that favour the IH method. Users will certainly appreciate the economic argument for the IH method. Indeed, based on catalogue prices, the IH method is ten times cheaper than the Sepsityper™ kit (0.72 vs. 7.45 €/analysis). These prices include all of the chemicals and disposable materials needed but do not include the technician fee (Table 6).

The time of the analysis is also reduced with the IH method because it does not include a time-consuming extraction procedure, which we found unnecessary because it does not significantly improve the quality of the identifications.

This reduction in the duration of the procedure has also allowed the IH method to be implemented routinely, something that was often considered an unrealistic goal by the microbiologists.

Because non-analytical arguments were in favour of the IH method, we chose to implement this method in our routine practice.

Despite the awareness that earlier identification could lead to more suitable treatments and to substantial costs savings [24], we performed IH in two batches per day, according to the needs of the infectious diseases department.

However, the question remains as to whether a more rapid identification impacts the clinical management of the patients. This question emerged more than 20 years ago during evaluations of the feasibility of rapid identifications and susceptibility testing on positive blood cultures by various commercial systems [7, 24]. Today, the question is still under debate because of the development of new rapid identification assays, such as MALDI-TOF MS or, more recently, polymerase chain reaction (PCR)-electrospray ionisation MS.

Even if identification does not yield information about the susceptibility profile of the organism, a rapid identification technique may offer several advantages. First, it may permit the use of a more narrow-spectrum antimicrobial, even before the acquisition of the susceptibility testing results. Additionally, rapid identification may help the

physician identify the source of the infection, thus, reducing the costs associated with complementary investigations [25, 26].

Our study found another advantage of rapid bacterial identification from positive blood cultures. Indeed, two separate blood cultures over a short period were positive for *Salmonella*. Even though the MALDI-TOF MS did not identify the serotype, the short delay between the two episodes and similarities between the patients' names and nationalities alerted the microbiologist. Infection control specialists were quickly warned of a suspected outbreak, which developed rapidly. Seven confirmed cases were diagnosed in four different hospitals in Brussels and a potential case index was found (data not shown). This example shows that rapid identification might be an additional source of public health information and that the method may be particularly helpful when there is collaboration and communication between health professionals.

Conclusion

The rapid identification of organisms in positive blood cultures is a new challenge for microbiologists, which should be included in a larger process. All pre-analytical, analytical and post-analytical steps must be improved.

In this perspective, an in-house (IH) method has been evaluated in our laboratory. With 86.4% and 73.7% correct genus and species identifications, respectively, using the lowered cut-offs, the IH method showed similar results to the Sepsityper™ kit (78.0% and 68.4% correct identification to the genus and species levels, respectively). However, the IH method is ten times less expensive and is faster and easier to perform than the commercial kit method. Today, the IH method is routinely used in our laboratory. At our institution, a complementary study in collaboration with microbiologists, infectious disease specialists, physicians and pharmacists is in progress to determine the real clinical impact of such a rapid method on the clinical management of the patient.

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4.4. Impact of a rapid microbial identification from positive blood cultures using Matrix Assisted Laser Desorption/Ionisation Time-of-flight Mass Spectrometry on patient management

Martiny D, Debaugnies F, Gateff D, Gérard M, Aoun M, Martin C, Konopnicki D, Loizidou A, Georgala A, Hainaut M, Chantrenne M, Dediste A, Vandenberg O, Van Praet S

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Impact of rapid microbial identification directly from positive blood cultures using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry on patient management

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Abstract

For septic patients, delaying the initiation of antimicrobial therapy or choosing an inappropriate antibiotic can considerably worsen their prognosis. This study evaluated the impact of rapid microbial identification (RMI) from positive blood cultures on the management of patients with suspected sepsis. During a 6-month period, RMI by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was performed for all new episodes of bacteraemia. For each patient, the infectious disease specialist was contacted and questioned about his therapeutic decisions made based on the Gram staining and the RMI. This information was collected to evaluate the number of RMIs that led to a therapeutic change or to a modification of the patient's general management (e.g. fast removal of infected catheters). During the study period, 277 new episodes of bacteraemia were recorded. In 71.12% of the cases, MALDI-TOF MS resulted in a successful RMI (197/277). For adult and paediatric patients, 13.38% (21/157) and 2.50% (1/40) of the RMIs, respectively, resulted in modification of the treatment regimen, according to the survey. In many other cases, the MALDI-TOF MS was a helpful tool for infectious disease specialists because it confirmed suspected cases of contamination, especially in the paediatric population (15/40 RMIs, 37.50%), or suggested complementary diagnostic testing. This study emphasizes the benefits of RMI from positive blood cultures. Although the use of this technique represents an extra cost for the laboratory, RMI using MALDI-TOF MS has been implemented in our daily practice.

Keywords: Blood culture, clinical impact, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, rapid diagnosis, sepsis

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Introduction

Sepsis is a major cause of morbidity and mortality in hospitalized patients. In the USA, 750 000 cases of severe sepsis occur annually [1]. In Europe, sepsis occurs in more than 35% of the patients in the intensive care unit. More than 50% of patients who experience septic shock do not survive [2–4].

The management of bacteraemic patients can be improved by the administration of the appropriate treatment without delay [5–7]. Molecular techniques allow for rapid microbial identification (RMI) from blood samples but have limitations, in particular the high cost per analysis and the need for antimicrobial susceptibility testing [8].

Because it allows the identification of microorganisms in a few minutes instead of the hours required by biochemical techniques, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a promising

alternative diagnostic tool [9]. Since its commercialization at the beginning of the twenty-first century, many new strategies to perform RMI directly from clinical samples have been evaluated [10–13]. Recently, inexpensive strategies that allow RMI within 20 min after the blood culture becomes positive were described [14,15].

Currently, the usefulness of RMI is still debated. Arguments for RMI state that RMI could lead to the faster adoption of the appropriate antibiotic regimen and help to identify the cause of the sepsis if it is unknown. The still limited information concerning the susceptibility of the microorganisms to antimicrobials is a weakness of RMI [16,17].

The primary aim of this study was to prospectively evaluate the theoretical impact of RMI from positive blood cultures on the clinical management of bacteraemic patients in our hospitals. The compliance with the recommendations of the infectious disease specialist (IDS) was also retrospectively evaluated to determine the real clinical impact of the RMI technique.

Materials and Methods

Location

The Saint-Pierre University Hospital and the Jules Bordet Institute are university-affiliated medical centres located in Brussels, Belgium. Saint-Pierre is a public hospital with vast experience in infectious diseases. Jules Bordet is the only hospital in Belgium that is completely dedicated to cancer. Both institutions are served by the same laboratory, which is open on weekdays from 07.30 until 20.00 h and on Saturday and Sunday from 08.00 until 16.00 h. Positive blood culture bottles are analysed during these time periods. During the night, medical microbiologists and IDSs are on call for emergencies.

Collection of blood cultures and inclusion criteria

From September 2011 to March 2012, the first positive blood culture for each bacteraemic episode in patients from both hospitals was prospectively enrolled in the study. All positive cultures for the same patient obtained within 3 days of each other and presenting the same Gram staining results were considered as belonging to the same episode. When staphylococcal morphology was observed in the Gram staining, RMI was always performed to confirm or rule out contamination.

MALDI-TOF MS RMI

The positive blood cultures (Bactec Plus Aerobic and Bactec F Lytic Anaerobic; Becton Dickinson, Franklin Lakes, NJ, USA) were prepared and analysed according to a previously described in-house protocol [14]. The spectra were acquired on a Microflex LT system (Bruker Daltonics, Bremen, Germany) and subsequently analysed using MALDI Biotyper Automation Control and Biotyper 3.0 software. At that time, the database (V3.1.2.0) included 3995 spectra. The analyses were performed in batches twice daily. The RMIs were classified as 'reliable' or 'unreliable' according to previously

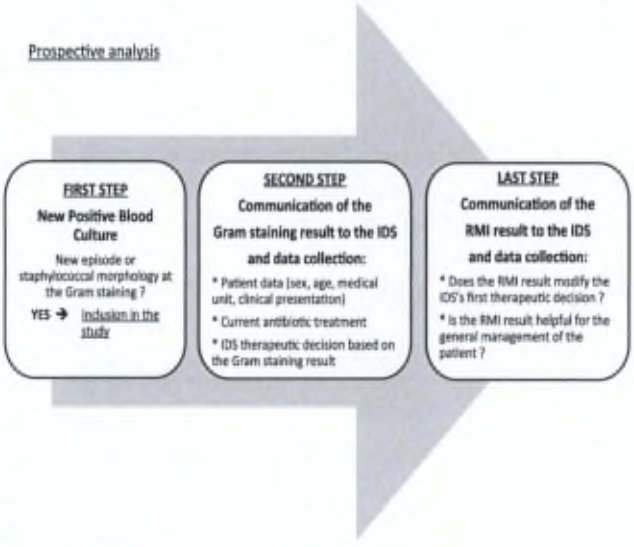


FIG. 1. Design of the prospective analysis.

TABLE I. Rapid microbial identification (RMI) transmitted to the infectious disease specialist for cultures that showed Gram-negative bacteria (n = 71)

Patient no.	Gram staining	RMI	Score	H	Log	Reliable RMI?	TI	CIT	RMI/CIT discrepancy?	From incubator removal to RMI delay (min)	From incubator removal to CIT delay (min)
204	GNR	*A. genomospecies*	1.473	1	0.157	N	WC	*A. faecalis*	Y	50	2950
111	GNR	*A. johnsonii*	1.957	6	0.774	Y	Y	*A. johnsonii*	N	73	3064
90	GNR	*A. genomospecies*	2.025	3	0.651	Y	Y	*A. lowffi*	Y*	50	2975
241	GNR	*A. lowffi*	2.003	7	0.818	Y	Y	*Acinetobacter sp.*	N	151	1479
56	GNR	*K. sedentarius*[a]	1.118	1	0.113	N	WC	*Anaerobic GNR*	Y	MD	MD
97	GNR Campylobacter suspected	*C. fetus*	1.924	7	0.645	Y	Y	*Campylobacter sp.*	N	101	3336
27	GNR	*E. cloacae*	1.938	4	0.339	Y	Y	*E. cloacae complex*	N	93	1559
125	GNR	*E. cloacae*	1.888	10	NA	Y	Y	*E. cloacae complex*	N	54	1437
232	GNR	*E. cloacae*	2.026	4	0.21	Y	Y	*E. cloacae complex*	N	22	1150
262	GNR	*E. cloacae*	2.266	6	0.374	Y	Y	*E. cloacae complex*	N	104	1579
10	GNR	*E. coli*	2.241	10	NA	Y	Y	*E. coli*	N	65	1683
14	GNR	*E. coli*	2	8	0.375	Y	Y	*E. coli*	N	21	1369
25	GNR	*E. coli*	2.254	10	NA	Y	Y	*E. coli*	N	92	1558
30	GNR	*E. coli*	1.806	6	0.273	N	WC	*E. coli*	N	68	1395
38	GNR	*E. coli*	1.979	7	0.492	Y	Y	*E. coli*	N	47	1098
45	GNR	*E. coli*	2.279	8	0.272	Y	Y	*E. coli*	N	75	1061
57	GNR	*E. coli*	2.33	10	NA	Y	Y	*E. coli*	N	61	1497
70	GNR	*E. coli*	1.261	8	0.26	Y	Y	*E. coli*	N	148	1535
95	GNR	*E. coli*	2.073	9	0.574	Y	Y	*E. coli*	N	58	2970
131	GNR	*E. coli*	2.196	9	0.605	Y	Y	*E. coli*	N	80	1180
144	GNR	*E. coli*	1.851	10	NA	Y	Y	*E. coli*	N	71	1676
154	GNR	*E. coli*	2.276	8	0.423	Y	Y	*E. coli*	N	303	1639
155	GNR	*E. coli*	2.291	9	0.578	Y	Y	*E. coli*	N	167	1544
175	GNR	*E. coli*	1.954	10	NA	Y	Y	*E. coli*	N	97	1555
181	GNR	*E. coli*	2307	9	0.522	Y	Y	*E. coli*	N	325	1472
213	GNR	*E. coli*	2.085	10	NA	Y	Y	*E. coli*	N	37	1473
214	GNR	*E. coli*	2.139	9	0.548	Y	Y	*E. coli*	N	34	1161
224	GNR	*E. coli*	2342	10	NA	Y	Y	*E. coli*	N	86	1625
233	GNR	*E. coli*	1.837	10	NA	Y	Y	*E. coli*	N	452	1639
239	GNR	*E. coli*	2.187	6	0.378	Y	Y	*E. coli*	N	168	1552
243	GNR	*E. coli*	1.888	9	0.493	Y	Y	*E. coli*	N	15	1788
250	GNR	*E. coli*	2.367	9	0.46	Y	Y	*E. coli*	N	86	1583
258	GNR	*E. coli*	2.167	10	NA	Y	Y	*E. coli*	N	MD	MD
263	GNR	*E. coli*	2.192	10	NA	Y	Y	*E. coli*	N	105	1516
264	GNR	*E. coli*	2.292	10	NA	Y	Y	*E. coli*	N	106	1517
273	GNR	*E. coli*	2.153	8	0.413	Y	Y	*E. coli*	N	206	1595
281	GNR	*E. coli*	1.798	9	0.636	Y	Y	*E. coli*	N	273	1595
282	GNR	*E. coli*	1.391	6	0.28	N	WC	*E. coli*	N	274	1596
286	GNR	*E. coli*	2.313	9	0.451	Y	Y	*E. coli*	N	187	1529
288	GNR	*E. coli*	2.148	8	0.303	Y	Y	*E. coli*	N	336	1456
296	GNR	*E. coli*	2.314	9	0.361	Y	Y	*E. coli*	N	257	1516
299	GNR	*E. coli*	2.209	8	0.547	Y	Y	*E. coli*	N	60	1101
300	GNR	*E. coli*	3.091	8	0.465	Y	Y	*E. coli*	N	38	1463
255	GNRcc	*H. influenzae*	2.125	10	NA	Y	Y	*H. influenzae*	N	95	3062
259	GNRcc	*H. influenzae*	1.635	10	NA	Y	Y	*H. influenzae*	N	MD	MD
109	GNR	*K. oxytoca*	2.27	3	0.365	Y	Y	*K. oxytoca*	N	73	1553
218	GNR	*K. oxytoca*	2.234	2	0.283	Y	Y	*K. oxytoca*	N	95	1516
13	GNR	*K. pneumoniae*	1.92	4	0.4	Y	Y	*K. pneumoniae*	N	65	1691
41	GNR	*K. pneumoniae*	1.903	6	0.435	Y	Y	*K. pneumoniae*	N	110	1569
58	GNR	*K. pneumoniae*	2.153	4	0.306	Y	Y	*K. pneumoniae*	N	180	1621
77	GNR	*K. pneumoniae*	1.829	3	0.267	N	WC	*K. pneumoniae*	N	56	2199
78	GNR	*K. pneumoniae*	1.325	7	0.426	Y	Y	*K. pneumoniae*	N	55	1648
99	GNR	*K. pneumoniae*	2.239	7	0.411	Y	Y	*K. pneumoniae*	N	100	1646
112	GNR	*K. pneumoniae*	2.22	6	0.369	Y	Y	*K. pneumoniae*	N	74	1572
134	GNR	*K. pneumoniae*	2.088	1	0.258	Y	Y	*K. pneumoniae*	N	136	2075
135	GNR	*K. pneumoniae*	1.786	7	0.362	Y	Y	*K. pneumoniae*	N	136	1515
168	GNR	*K. pneumoniae*	2.199	5	0.323	Y	Y	*K. pneumoniae*	N	62	1654
211	GNR	*K. pneumoniae*	2.071	9	0.402	Y	Y	*K. pneumoniae*	N	43	1209
297	GNR	*K. pneumoniae*	1.932	1	0.237	N	WC	*K. pneumoniae*	N	39	1030
26	GNR	*P. aeruginosa*	2.178	6	1.06	Y	Y	*P. aeruginosa*	N	93	1559
85	GNR	*P. aeruginosa*	2.265	6	0.68	Y	Y	*P. aeruginosa*	N	95	1548
143	GNR	*P. aeruginosa*	2.253	6	0.757	Y	Y	*P. aeruginosa*	N	181	1923
289	GNR	*P. agglomerans*	1.807	3	0.24	N	WC	*P. agglomerans*	N	83	4561
153	GNR	*P. buccae*	2.15	6	1.084	Y	Y	*P. buccae*	N	104	10499
119	GNR	*P. mirabilis*	2.335	9	0.8	Y	Y	*P. mirabilis*	N	468	1523
67	GNR	*P. veroni*	1.613	1	0.109	N	WC	*Pseudomonas sp.*	N	200	1323
268	GNR	*R. ornitholytica*	2.508	1	0.39	Y	WC	*R. planticola*	Y*	154	1507
265	GNR	*S. maltophilia*	1.419	1	0.186	N	WC	*S. maltophilia*	N	259	1384
55	GNR	*Salmonella sp.*	2.242	9	0.36	Y	Y	*S. paratyphi*	N	118	1519
190	GNR	*Salmonella sp.*	1.919	10	NA	Y	Y	*S. typhimurium*	N	217	1512
150	GNR	*Salmonella sp.*	2.321	10	NA	Y	Y	*Salmonella sp.*	N	224	1614

CIT: conventional identification technique; GNR: Gram-negative rods; GNRcc: Gram-negative rods coccobacilli; H: Homogeneity; TI: Transmission of the RMI to the IDS?; Y: yes, N: no; WC: with caution; NA: not applicable; MD: missing or incomplete data; *A. genomospecies*, *Acinetobacter genomospecies*; *A. johnsonii*, *Acinetobacter johnsonii*; *A. lowffi*, *Acinetobacter lowffi*; *A. faecalis*, *Alcaligenes faecalis*; *C. fetus*, *Campylobacter fetus*; *E. cloacae*, *Enterobacter cloacae*; *E. coli*, *Escherichia coli*; *H. influenzae*, *Haemophilus influenzae*; *K. sedentarius*, *Kytococcus sedentarius*; *K. oxytoca*, *Klebsiella oxytoca*; *K. pneumoniae*, *Klebsiella pneumoniae*; *P. agglomerans*, *Pantoea agglomerans*; *P. buccae*, *Prevotella buccae*; *P. mirabilis*, *Proteus mirabilis*; *P. aeruginosa*, *Pseudomonas aeruginosa*; *P. veroni*, *Pseudomonas veroni*; *R. ornithocolytica*, *Raoultella ornithocolytica*; *R. planticola*, *Raoultella planticola*; *S. maltophilia*, *Stenotrophomonas maltophilia*; *S. paratyphi*, *Salmonella paratyphi*; *S. typhimurium*, *Salmonella typhimurium*.
Homogeneity: number of successive matches identical to the first one; log: difference between the score of the first match and the first discrepancy.
[a]Known limitations of the MALDI-TOF MS.
*The medical microbiologist told the IDS that an anaerobic bacterium was suspected (bad MALDI-TOF MS result and anaerobic bottles).

TABLE 2. Rapid microbial identifications (RMIs) transmitted to the infectious disease specialist (IDS) for cultures that showed Gram positive bacteria (n = 107)

Patient no.	Gram staining	RMI	Score	H	Log	Reliable RMI?	T?	CIT	RMI/CIT discrepancy	From incubator removal to RMI delay (min)	From incubator removal to CIT delay (min)
257	GPR	*B. cereus*	1.689	2	0.109	N	WC	*B. cereus*	N	MD	MD
137	GPR	*B. licheniformis*	1.891	5	0.802	Y	Y	*Bacillus sp.*	N	165	1572
141	GPR	*C. amycolatum*	1.387	4	0.234	N	WC	*C. amycolatum*	N	264	1236
106	GPCc	*S. pettenkoferi*	1.716	2	0.568	Y	Y	*CNS*	N	MD	MD
164	GPCc	*E. casseliflavus*[a]	1.204	1	0.126	N	WC	*E. durans*	Y	183	3034
140	GPCpch	*E. faecalis*	1.912	8	0.763	Y	Y	*E. faecalis*	N	35	1524
180	GPCpch	*E. faecalis*	1732	8	0.584	Y	Y	*E. faecalis*	N	54	1519
197	GPCpch	*E. faecalis*	1.673	8	0.565	Y	Y	*E. faecalis*	N	88	1582
287	GPCpch	*E. faecalis*	2.034	8	0.811	Y	Y	*E. faecalis*	N	41	1022
156	GPCpch	*E. faecium*	1.955	9	0.892	Y	Y	*E. faecium*	N	167	1770
272	GPR	*L. crispatus*	1.321	1	0.267	N	WC	*Lactobacillus sp.*	N	76	4459
88	GPCc	*M. luteus*	1.526	2	0.513	Y[b]	Y	*Micrococcus sp.*	N	48	1167
183	GPCc	*M. luteus*	1.391	2	0.512	N	WC	*Micrococcus sp.*	N	47	1315
184	GPCc	*K. sedentarius*	1.63	1	0.526	Y	Y	*Micrococcus sp.*	N	144	3006
194	GPCc	*M. luteus*	1.425	1	0.297	N	WC	*Micrococcus sp.*	N	90	1707
292	GPCc	*M. luteus*	1.798	3	0.499	Y	Y	*Micrococcus sp.*	N	152	1617
102	GPR	*Propionibacterium sp.*	1.684	7	0.607	Y	Y	*Propionibacterium sp.*	N	96	4659
35	GPCpch	*S. agalactiae*	2.147	9	0.704	Y	Y	*S. agalactiae*	N	46	1641
215	GPCpch	*S. anginosus*	1.444	2	0.241	N	WC	*S. anginosus*	N	94	1540
71	GPCc	*S. aureus*	1.624	8	0.59	Y	Y	*S. aureus*	N	170	1506
80	GPCc	*S. aureus*	2.099	10	NA	Y	Y	*S. aureus*	N	78	1515
98	GPCc	*S. aureus*	1.854	10	NA	Y	Y	*S. aureus*	N	233	1518
131	GPCc	*S. aureus*	1.45	7	0.352	Y[b]	WC	*S. aureus*	N	52	1382
167	GPCc	*S. aureus*	1.817	10	NA	Y	Y	*S. aureus*	N	281	1564
285	GPCc	*S. aureus*	1692	9	0.475	Y	Y	*S. aureus*	N	274	1596
75	GPCc	*S. capitis*	1.589	4	0.466	Y[b]	WC	*S. capitis*	N	596	1507
83	GPCc	*S. capitis*	1.987	5	0.743	Y	Y	*S. capitis*	N	125	1192
114	GPCc	*S. capitis*	1.813	5	0.59	Y	Y	*S. capitis*	N	MD	MD
128	GPCc	*S. capitis*	1.637	2	0.604	Y	Y	*S. capitis*	N	85	1579
147	GPCc	*S. capitis*	1.597	4	0.474	Y	Y	*S. capitis*	N	226	1611
148	GPCc	*S. capitis*	1.457	5	0.393	Y[b]	WC	*S. capitis*	N	225	1611
277	GPCc	*S. capitis*	1.732	3	0.35	Y	Y	*S. capitis*	N	77	1457
115	GPCpch	*S. dysgalactiae*	1.824	4	0.188	N	WC	*S. dysgalactiae*	N	87	1152
3	GPCc	*S. epidermidis*	2	8	0.616	Y	Y	*S. epidermidis*	N	131	1554
32	GPCc	*S. epidermidis*	1.99	7	0.63	Y	Y	*S. epidermidis*	N	69	1397
36	GPCc	*S. epidermidis*	1.287	2	0.18	N	WC	*S. epidermidis*	N	47	1360
59	GPCc	*S. epidermidis*	1.728	7	0.65	Y	Y	*S. epidermidis*	N	87	1327
64	GPCc	*S. epidermidis*	2.007	8	0.735	Y	Y	*S. epidermidis*	N	93	1542
76	GPCc	*S. epidermidis*	1.432	4	0.255	N	WC	*S. epidermidis*	N	172	1355
82	GPCc	*S. epidermidis*	1.885	6	0.719	Y	Y	*S. epidermidis*	N	MD	MD
87	GPCc	*S. epidermidis*	1.395	3	0.201	N	WC	*S. epidermidis*	N	96	1526
89	GPCc	*S. epidermidis*	1.233	4	0.384	N	WC	*S. epidermidis*	N	534	1482
96	GPCc	*S. epidermidis*	1.653	6	0.651	Y	Y	*S. epidermidis*	N	65	1492
103	GPCc	*S. epidermidis*	1.88	9	0.844	Y	Y	*S. epidermidis*	N	95	1601
107	GPCc	*S. hominis*[a]	1.978	6	0.934	Y	Y	*S. epidermidis*	Y	MD	MD
110	GPCc	*S. epidermidis*	1.79	6	0.566	Y	Y	*S. epidermidis*	N	73	1553
121	GPCc	*S. epidermidis*	1.91	8	0.615	Y	Y	*S. epidermidis*	N	130	1511
123	GPCc	*S. epidermidis*	1.708	6	0.65	Y	Y	*S. epidermidis*	N	99	1509
126	GPCc	*S. epidermidis*	1.71	5	0.351	Y	Y	*S. epidermidis*	N	41	1155
129	GPCc	*S. epidermidis*	1.262	2	0.162	N	WC	*S. epidermidis*	N	190	1335
133	GPCc	*S. epidermidis*	1.463	1	0.318	Y[b]	WC	*S. epidermidis*	N	MD	MD
136	GPCc	*S. epidermidis*	2.086	7	0.753	Y	Y	*S. epidermidis*	N	MD	MD
139	GPCc	*S. epidermidis*	1.779	7	0.618	Y	Y	*S. epidermidis*	N	36	1525
146	GPCc	*S. epidermidis*	1.865	6	0.658	Y	Y	*S. epidermidis*	N	150	1387
158	GPCc	*S. epidermidis*	1.601	2	0.505	Y	Y	*S. epidermidis*	N	143	1176
160	GPCc	*S. epidermidis*	1.72	6	0.457	Y	Y	*S. epidermidis*	N	244	1324
161	GPCc	*S. epidermidis*	2.187	8	0.966	Y	Y	*S. epidermidis*	N	127	1545
162	GPCc	*S. epidermidis*	1.357	2	0.059	N	WC	*S. epidermidis*	N	57	1129
166	GPCc	*S. epidermidis*	2.05	6	0.502	Y	Y	*S. epidermidis*	N	MD	MD
186	GPCc	*S. epidermidis*	1.509	2	0.352	Y[b]	WC	*S. epidermidis*	N	93	1594
201	GPCc	*S. epidermidis*	1.623	8	0.316	Y	Y	*S. epidermidis*	N	76	1537
216	GPCc	*S. epidermidis*	1.858	8	0.635	Y	Y	*S. epidermidis*	N	93	1516
249	GPCc	*S. epidermidis*	1.774	8	0.481	Y	Y	*S. epidermidis*	N	86	1583
253	GPCc	*S. epidermidis*	1.793	8	0.636	Y	Y	*S. epidermidis*	N	94	1824
256	GPCc	*S. epidermidis*	1.717	4	0.606	Y	Y	*S. epidermidis*	N	MD	MD
260	GPCc	*S. epidermidis*	1.238	1	0.186	N	WC	*S. epidermidis*	N	MD	MD
267	GPCc	*S. epidermidis*	1.866	7	0.606	Y	Y	*S. epidermidis*	N	154	1507
269	GPCc	*S. epidermidis*	1828	8	0.698	Y	Y	*S. epidermidis*	N	153	1506
271	GPCc	*S. epidermidis*	1.378	4	0.297	N	WC	*S. epidermidis*	N	77	1559
275	GPCc	*S. epidermidis*	1.606	2	0.17	N	WC	*S. epidermidis*	N	133	1558
278	GPCc	*S. epidermidis*	1.303	5	0.349	N	WC	*S. epidermidis*	N	119	1381
306	GPCc	*S. epidermidis*	1.698	5	0.622	Y	Y	*S. epidermidis*	N	MD	MD
234	GPCpch	*S. gallolyticus*	1.849	3	0.434	Y	Y	*S. gallolyticus*	N	174	2066
93	GPCc	*S. haemolyticus*	1.931	7	0.62	Y	Y	*S. haemolyticus*	N	39	1434
219	GPCc	*S. capitis*	1.509	6	0.341	Y[b]	WC	*S. haemolyticus*	Y	MD	MD
7	GPCc	*S. hominis*	1.882	6	0.436	Y	Y	*S. hominis*	N	92	1324
11	GPCc	*S. hominis*	1.893	4	0.739	Y	Y	*S. hominis*	N	65	1681
21	GPCc	*S. hominis*	1.706	6	0.621	Y	Y	*S. hominis*	N	273	1583
39	GPCc	*S. hominis*	2.095	6	1.053	Y	Y	*S. hominis*	N	134	1462

Table 2 (Continued)

Patient no.	Gram staining	RMI	Score	H	Log	Reliable RMI?	T?	CIT	RMI/CIT discrepancy	From incubator removal to RMI delay (min)	From incubator removal to CIT delay (min)
72	GPCc	*S. hominis*	1.78	5	0.78	Y	Y	*S. hominis*	N	148	1440
73	GPCc	*S. hominis*	1.84	6	0.833	Y	Y	*S. hominis*	N	177	1439
113	GPCc	*S. hominis*	2.27	6	0.85	Y	Y	*S. hominis*	N	161	1485
122	GPCc	*S. hominis*	2.119	6	0.81	Y	Y	*S. hominis*	N	100	1510
143	GPCc	*S. hominis*	2.128	5	0.8	Y	Y	*S. hominis*	N	71	1596
207	GPCc	*S. hominis*	2.211	6	0.868	Y	Y	*S. hominis*	N	251	1346
212	GPCc	*S. hominis*	1.838	3	0.682	Y	Y	*S. hominis*	N	37	1473
248	GPCc	*S. hominis*	2.229	6	1.004	Y	Y	*S. hominis*	N	54	1563
293	GPCc	*S. hominis*	2.192	6	0.854	Y	Y	*S. hominis*	N	75	1171
305	GPCc	*S. hominis*	2.069	6	0.99	Y	Y	*S. hominis*	N	57	1138
124	GPCpch	*S. pneumoniae*	1.09	2	0.09	N	WC	*S. mitis/oralis*	Y[d]	51	3549
235	GPCpch	*S. salivarius*	1.386	4	0.095	N	WC	*S. mitis/oralis*	N	175	1804
1	GPCpch	*S. pneumoniae*	1.924	7	0.729	Y	WC	*S. pneumoniae*	N	30	1396
18	GPCpch	*S. pneumoniae*	1.735	6	0.513	Y	WC	*S. pneumoniae*	N	94	2933
42	GPCpch	*S. pneumoniae*	1.453	3	0.358	Y[b]	Y	*S. pneumoniae*	N	95	1021
50	GPCpch	*S. pneumoniae*	2.13	7	0.79	Y	WC	*S. pneumoniae*	N	MD	MD
105	GPCpch	*S. pneumoniae*	1.822	4	0.346	Y	WC	*S. pneumoniae*	N	MD	MD
120	GPCpch	*S. pneumoniae*	1.719	6	0.477	Y	WC	*S. pneumoniae*	N	73	1571
185	GPCpch	*S. pneumoniae*	1.341	3	0.193	N	WC	*S. pneumoniae*	N	186	1771
198	GPCpch	*S. pneumoniae*	1.364	4	0.165	N	WC	*S. pneumoniae*	N	87	1627
242	GPCpch	*S. pneumoniae*	1.986	6	0.602	Y	WC	*S. pneumoniae*	N	150	1528
65	GPCpch	*S. pyogenes*	2.15	8	0.571	Y	Y	*S. pyogenes*	N	66	1532
68	GPCpch	*S. pyogenes*	1.896	8	0.635	Y	Y	*S. pyogenes*	N	22	1146
165	GPCpch	*S. pyogenes*	1.847	7	0.613	Y	Y	*S. pyogenes*	N	71	1597
195	GPCpch	*S. pyogenes*	2.006	6	0.637	Y	Y	*S. pyogenes*	N	31	1157
246	GPCpch	*S. pyogenes*	2.27	8	0.678	Y	Y	*S. pyogenes*	N	70	1523
60	GPCc	*S. saprophyticus*	1.761	6	0.631	Y	Y	*S. saprophyticus*	N	197	1608
159	GPCpch	*S. thermophilus*	2.097	6	0.67	Y	Y	*S. thermophilus*	N	MD	MD

CIT, conventional identification technique; GPR, Gram-positive rods; GPCc, Gram-positive cocci in clusters; GPCpch, Gram-positive cocci in pairs and chains; Y: yes, N: no, WC: with caution; NA: not applicable; MD, missing or incomplete data; *B. cereus*, *Bacillus cereus*; *B. licheniformis*, *Bacillus licheniformis*; *C. amycolatum*, *Corynebacterium amycolatum*; *S. pettenkoferi*, *Staphylococcus pettenkoferi*; *E. casseliflavus*, *Enterococcus casseliflavus*; *E. durans*, *Enterococcus durans*; *E. faecalis*, *Enterococcus faecalis*; *E. faecium*, *Enterococcus faecium*; *L. crispatus*, *Lactobacillus crispatus*; *M. luteus*, *Micrococcus luteus*; *K. sedentarius*, *Kytococcus sedentarius*; *S. agalactiae*, *Streptococcus agalactiae*; *S. anginosus*, *Streptococcus anginosus*; *S. aureus*, *Staphylococcus aureus*; *S. capitis*, *Staphylococcus capitis*; *S. dysgalactiae*, *Streptococcus dysgalactiae*; *S. epidermidis*, *Staphylococcus epidermidis*; *S. hominis*, *Staphylococcus hominis*; *S. gallolyticus*, *Streptococcus gallolyticus*; *S. haemolyticus*, *Staphylococcus haemolyticus*; *S. pneumoniae*, *Streptococcus pneumoniae*; *S. mitis*, *Streptococcus mitis*; *S. salivarius*, *Streptococcus salivarius*; *S. pyogenes*, *Streptococcus pyogenes*; *S. saprophyticus*, *Staphylococcus saprophyticus*; *S. thermophilus*, *Streptococcus thermophilus*.
Homogeneity: number of successive matches identical to the first one; log: difference between the score of the first match and the first discrepancy.
[a]The medical microbiologist suggested an 'Enterococcus sp.' identification.
[b]RMI reliable to the genus level.
[c]Contamination by cutaneous flora was not excluded.
[d]Known limitation of the MALDI-TOF MS.

validated cut-offs of 1.4 and 1.6 for the acceptable identification to the genus and species levels, respectively. A difference of at least 0.3 between the first identification match and the first discrepant match was also required to validate the identification [14]. The last criterion was not required if the identification score value met the manufacturer's instructions (cut-off values of 1.7 and 2 for the acceptable identification to the genus and species levels, respectively). The RMIs that showed a discrepancy with the Gram staining results were also classified as unreliable.

Clinical impact evaluation

The design of the prospective analysis is presented in Fig. 1. For each positive blood culture, the Gram staining result was transmitted to the IDS, who answered in a blinded manner several questions on the clinical presentation and current antibiotic treatment of the patient and explained his therapeutic decision. All data were registered in a standardized case report form by the medical microbiologist (see Supplementary material, Fig. S1). When the RMI was transmitted, the IDS informed the medical microbiologist of any modification of his initial therapeutic decision. All cases in which the RMI provided another benefit were also recorded. Indeed, RMIs from blood cultures showing staphylococcal forms on the Gram staining were expected to exclude or confirm *Staphylococcus aureus* infections, catheter-related infections and contaminations. The detection of particular organisms could also indicate which additional medical investigations could be conducted to determine the origin of the septic infection if it was unknown.

A retrospective analysis of all cases was then performed. For Saint-Pierre patients, the pharmacy department used billing, medical and nursing files to retrospectively evaluate whether the IDS's therapeutic recommendations were followed. The IDSs of both institutions also retrospectively checked the medical files. In this section, the RMI/conventional identification delay, the IDS contact/adaptation of the treatment delay (when needed) and the influence of the medical

TABLE 3. Rapid microbial identifications (RMIs) transmitted to the infectious disease specialist (IDS) for mixed cultures (n = 18)

Patient nr	Gram staining	RMI	Score	H	Log	Reliable RMI?	TT	CIT	RMI/CIT discrepancy?	From incubator removal to RMI delay (min)	From incubator removal to CIT delay (min)
302	GPCc	*S. hominis*	1.251	1	0.008	N	WC	*S. hominis* *Streptococcus* sp. *Acinetobacter* sp.	Y, IR	135	1176
19	GPCpch GNR	*C. braki*	2.021	1	0.035	Y	Y	*E. faecalis* *C. braki* *P. aeruginosa*	Y, IR	270	2902
20	GNR GPCpch	*E. faecalis*	2.257	8	0.897	Y	Y	*E. coli* *E. faecalis* *S. aureus*	Y, IR	273	1599
40	GPCc	*S. hominis*	1.969	6	0.783	Y	Y	*S. epidermidis* *S. hominis*	Y, IR	46	1629
44	GPCpch GNR	*E. faecalis* *C. freundii*	2.22 2.13	8 5	0.693 0.292	Y	Y	*E. faecalis* *C. freundii*	N[a]	147	1534
46	GPCc	*S. epidermidis*	1.767	6	0.639	Y	Y	*S. epidermidis* *S. epidermidis*	N	119	1532
101	GNR GPCpch	*E. coli*	2.162	8	0.366	Y	Y	*E. coli* *S. mitis/oralis*	Y, IR	94	3115
137	GPCc	*S. capitis*	1.727	4	0.586	Y	Y	*S. capitis* *S. haemolyticus* *S. epidermidis*	Y, IR	MD	MD
138	GNRcc	*H. influenzae*	2.071	10	NA	Y	Y	*H. influenzae* *K. pneumoniae*	Y, IR	136	5853
152	GNR	*K. pneumoniae*	2.078	3	0.325	Y	Y	*K. pneumoniae* *E. coli*	Y, IR	59	1206
176	GNR	*E. coli*	2.109	10	NA	Y	Y	*E. coli* *K. pneumoniae*	Y, IR	98	1556
177	GNR GPR	*K. pneumoniae*	2.277	6	0.407	Y	Y	*K. pneumoniae* *L. lactis*	Y, IR	97	1555
188	GNR	*E. coli*	2.027	10	NA	Y	Y	*E. coli* *E. coli*	N	215	1510
189	GPCc GPCpch	*S. aureus*	2.185	10	NA	Y	Y	*S. aureus* *Streptococcus* sp.	Y, IR	217	3146
193	GPCc GPCpch	*S. haemolyticus*	1.919	7	0.502	Y	Y	*S. haemolyticus* *E. avium*	Y, IR	487	1498
236	GPR GNR	*C. perfringens*[b] *E. coli*	1.872 1.738	Alternate ID	NA	Y	Y	*C. perfringens* *E. coli* *S. salivarius*	Y, IR	175	1578
261	GNR	*S. maltophilia*	2.065	2	0.298	Y	Y	*S. maltophilia* *Ochrobactrum* sp.	Y, IR	115	1415
279	GNR GPCpch	*M. morganii*	2.492	10	NA	Y	Y	*M. morganii* *E. faecalis*	Y, IR	123	1583

CIT, conventional identification technique; GNR, Gram-negative rods; GNRcc, Gram-negative rods coccobacilli; GPR, Gram-positive rods; GPCc, Gram-positive cocci in clusters; GPCpch, Gram-positive cocci in pairs and chains; Y, yes; N, no; WC, with caution; NA, not applicable; IR, incomplete result;
C. braki, *Citrobacter braki*; *C. freundi*, *Citrobacter freundi*; *C. perfringens*, *Clostridium perfringens*; *E. coli*, *Escherichia coli*; *E. faecalis*, *Enterococcus faecalis*; *H. influenzae*, *Haemophilus influenzae*; *K. pneumoniae*, *Klebsiella pneumoniae*; *L. lactis*, *Lactococcus lactis*; *M. morganii*, *Morganella morganii*; *P. aeruginosa*, *Pseudomonas aeruginosa*; *S. aureus*, *Staphylococcus aureus*; *S. capitis*, *Staphylococcus capitis*; *S. epidermidis*, *Staphylococcus epidermidis*; *S. haemolyticus*, *Staphylococcus haemolyticus*; *S. hominis*, *Staphylococcus hominis*; *S. maltophilia*, *Stenotrophomonas altophilia*; *S. mitis*, *Streptococcus mitis*.
Homogeneity: number of successive matches identical to the first one; log: difference between the score of the first match and the first discrepancy.
[a]RMI was performed on two positive blood culture bottles for this patient.
[b]Both identifications appeared alternatively in the ten matches, with acceptable score values.

unit where blood cultures were collected on the utility of the RMI were also determined.

Statistical analysis

The times needed to obtain the RMI and conventional identification results were evaluated and compared using non-parametric Mann–Whitney–Wilcoxon test. A Fisher's exact test was used to compare the proportion of RMIs that led to an altered treatment at each institution.

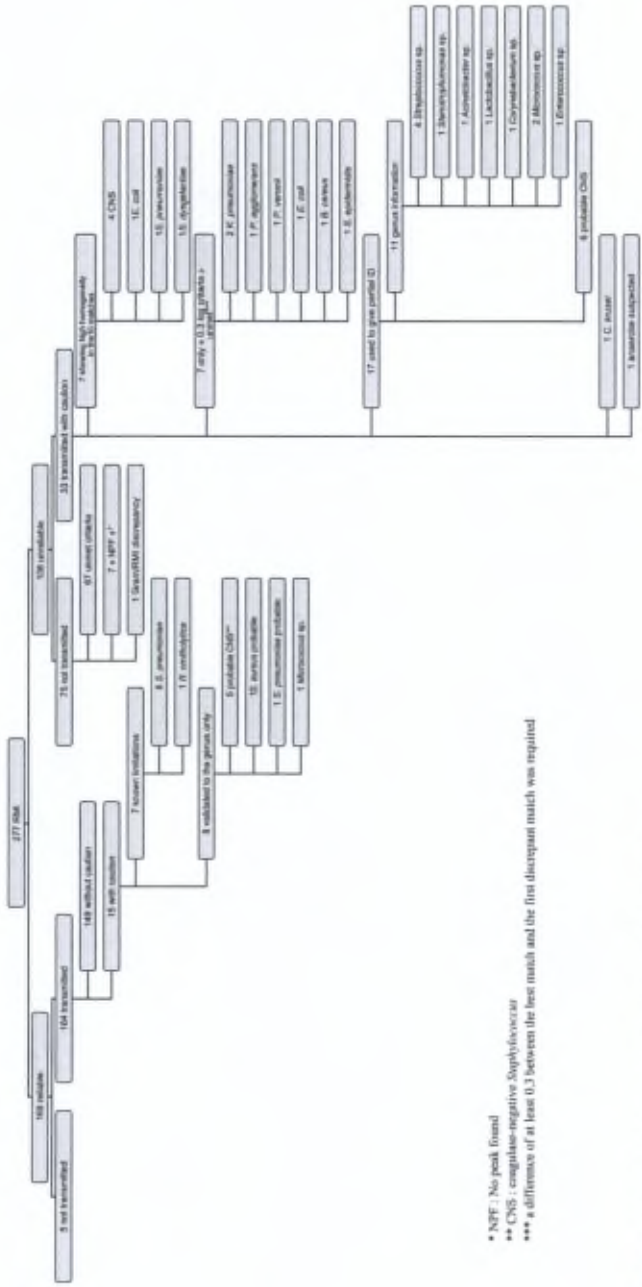
We also used Fisher's exact test to determine whether the RMI had a higher or lower impact on particular patient populations; a lower impact could be expected in medical units where clinicians routinely monitor infectious diseases (e.g. patients from intensive care units and sterile units).

Results

Rapid microbial identifications

During the study period, positive blood cultures from 243 patients were included, and 277 RMIs were performed (see Supplementary material, Table S1).

Based on the cut-off criteria, 61.01% and 38.99% of RMIs (169/277, 108/277) were classified as 'reliable' and 'unreliable', respectively [14]. The medical microbiologist transmitted 71.12% of all RMIs (197/277) to the IDS. All of the bacterial RMIs transmitted to the IDS are presented in Tables 1, 2 and 3 and in Fig. 2. An RMI suggesting a *Candida krusei* fungaemia was



* NPF : No peak found
** CNS : coagulase-negative Staphylococcus
*** a difference of at least 0.3 between the best match and the first discrepant match was required

FIG. 2. Description of the rapid microbial identification (RMI) classification.

TABLE 4. Clinical context and description of cases for whom the Gram result led to a modification of the empirical antimicrobial treatment (*n* = 34)

N°	Gram staining	RMI result	CIT	Current antibiotic treatment	Clinical context	Treatment modification according to the IDS survey	Comment
Saint-Pierre University Hospital							
Adults							
119	GNR	*Proteus mirabilis*	*P. mirabilis*	0	Septic shock <UTI	AMC addition	Community acquired UTI
98	GPCc	*Staphylococcus aureus*	*S. aureus*	0	Dyspnoea, dialysis	Vancomycin addition	Suspicion of CNS infection in a patient with multiple or permanent catheters
38	GNR	*Escherichia coli*	*E. coli*	AMC	Pyelonephritis	Shift to temocillin	Hospital acquired pyelonephritis
44	GPCpch+GNR	*Enterococcus faecalis* *Citrobacter freundii*	*E. faecalis* *C. freundii*	PTZ	Shock of unknown origin	Vancomycin and metronidazole addition	To cover *E. faecium* and Bacteroides sp.
258	GNR	*E. coli*	*E. coli*	0	UTI	Temocillin addition	*E. coli* previously found in urine sample
273	GNR	*E. coli*	*E. coli*	Levofloxacin + oxacillin	Mediastinitis post cardiac surgery + UTI	Shift to PTZ	Hospital acquired infection, broader antimicrobial spectrum
214	GNR	*E. coli*	*E. coli*	AMC	Septic shock, angiocholitis	Shift to PTZ	Severe infection, broader antimicrobial spectrum
97	GNR Campylobacter suspected	*Campylobacter fetus*	*Campylobacter sp.*	PTZ	Diarrhoea	Shift to clarithromycin	According to guidelines
106	GPCc	CNS	CNS	AMC	Pulmonary infection	Shift to PTZ	Treatment modification probably explained by the clinical context more than the Gram result
211	GNR	*Klebsiella pneumoniae*	*K. pneumoniae*	0	Septic shock, brain tumour	PTZ addition	Hospital acquired infection, broad antimicrobial spectrum
42	GPCpch	*Streptococcus pneumoniae*	*S. pneumoniae*	0	Pulmonary infection	AMC addition	*S. pneumoniae* highly suspected
55	GNR	*Salmonella sp.*	*S. paratyphi*	0	Salmonellosis	Levofloxacin addition	*Salmonella highly* suspected, according to guidelines
Paediatrics							
150	GNR	*Salmonella sp.*	*Salmonella sp.*	0	Diarrhoea, fever and nausea	Ceftriaxon addition	*Salmonella highly* suspected, according to guidelines (for paediatric patient)
159	GPCpch	*Streptococcus thermophilus*	*S. thermophilus*	Ampicillin + cefotaxime	Bronchiolitis, fever	Stop ampicillin	Pulmonary origin suspected
246	GPCpch	*Streptococcus pyogenes*	*S. pyogenes*	Temocillin	UTI	Shift to cefotaxim	Pulmonary origin suspected
45	GNR	*E. coli*	*E. coli*	Ampicillin + cefotaxime	UTI	Stop ampicillin	Because of the high ampicillin resistance in GNR
120	GPCpch	*S. pneumoniae*	*S. pneumoniae*	AMC	Suspicion of ethmoiditis	Shift to intravenous administration	According to guidelines
Jules Bordet Institute							
96	GPCc	*Staphylococcus epidermidis*	*S. epidermidis*	0	Lymphoma	Vancomycin addition	Suspicion of CNS infection in a patient with a permanent catheter
180	GPCpch	*E. faecalis*	*E. faecalis*	Temocillin	Endometrial cancer, pyelonephritis, fever	PTZ addition	Genital origin suspected, broader antimicrobial spectrum
197	GPCpch	*E. faecalis*	*E. faecalis*	0	Abdominal surgery	PTZ and vancomycin addition	Abdominal origin suspected, broad antimicrobial spectrum
161	GPCc	*S. epidermidis*	*S. epidermidis*	Cefepim	Acute leukaemia, neutropenia	Vancomycin addition	Suspicion of CNS infection in a patient with multiple or permanent catheters
267	GPCc	*S. epidermidis*	*S. epidermidis*	0	Medullary graft. Systematic blood culture collection for patients receiving high doses of corticosteroids	Vancomycin addition	Suspicion of CNS infection in a patient with multiple or permanent catheters
261	GNR	*Stenotrophomonas maltophilia*	*S. maltophilia* + *Ochrobactrum sp.*	0	Intestinal disease	PTZ addition	Abdominal origin suspected, broad antimicrobial spectrum
262	GNR	*Enterobacter cloacae*	*E. cloacae*	0	Cholangiocarcinoma	PTZ addition	Abdominal origin suspected, broad antimicrobial spectrum
250	GNR	*E. coli*	*E. coli*	Meropenem	Septic shock <UTI	Shift to PTZ	*E. coli* susceptible to PTZ previously found in clinical samples
215	GPCpch	*Streptococcus anginosus*	*S. anginosus*	Oxacillin	Ears, Nose and Throat neoplasy	Shift to moxifloxacin	Better cellular penetration
184	GPCc	*Kytococcus sedentarius*	*Micrococcus sp.*	Meropenem	Acute leukaemia, neutropenia, fever	Vancomycin addition	Suspicion of CNS infection in a patient with multiple or permanent catheters
189	GPCc+GPCpch	*S. aureus*	*S. aureus* *Streptococcus sp.*	TZP	Neutropenia, fever	Vancomycin addition	Suspicion of CNS infection in a patient with multiple or permanent catheters

Table 4 (*Continued*)

N°	Gram staining	RMI result	CIT	Current antibiotic treatment	Clinical context	Treatment modification according to the IDS survey	Comment
41	GNR	*K. pneumoniae*	*K. pneumoniae*	0	Ear, Nose and Throat neoplasy	PTZ and amikacin addition	*P. aeruginosa* was suspected in an infected wound
146	GPCc	*S. epidermidis*	*S. epidermidis*	0	Systematic blood culture collection for patients receiving high doses of corticosteroids	Vancomycin addition	Suspicion of CNS infection in a patient with multiple or permanent catheters
101	GNR+GPCc	*E. coli*	*E. coli* *Streptococcus mitis/oralis*	Cefepim	Acute leukaemia, neutropenia	Vancomycin addition	Suspicion of CNS infection in a patient with multiple or permanent catheters
90	GNR	*Acinetobacter genomospecies*	*Acinetobacter lwoffi*	AMC	Hepatic disease, fever	PTZ+amikacin	Because of the high AMC resistance in GNR
70	GNR	*E. coli*	*E. coli*	0	Acute leukaemia, septic shock	Meropenem addition	Hospital acquired infection in a patient in sterile unit
73	GPCc	*Staphylococcus hominis*	*S. hominis*	0	MD	Vancomycin addition	Suspicion of CNS infection in a patient with multiple or permanent catheters

CIT, conventional identification technique; CNS, central nervous system; PTZ, piperacillin/azobactam; AMC, amoxicillin/clavulanic acid; IDS, infectious disease specialist.

also transmitted to the IDS, with caution because of a poor score value (see Table S1, patient no. 174).

The RMIs that were not transmitted (28.88%, 80/277) included five reliable RMIs that were erroneously not transmitted by the medical microbiologist (oversight) and 75 unreliable RMIs.

Among the unreliable RMIs, 30.56% were nevertheless transmitted to the IDS, with caution (33/108; Fig. 2). These RMIs showed high levels of homogeneity in the ten identification matches, had good score values but failed to meet the 0.3 log difference criterion (a difference of at least 0.3 between the best match and the first discrepant match was required) or were partially informative (genus information or suspicion of 'coagulase-negative' *Staphylococcus*). The decision to transmit data based on unreliable RMIs was made by the medical microbiologist according to his experience.

Among the 197 transmitted RMIs (164 + 33), 88.32% were confirmed by the culture-based identification methods (174/197), 4.06% (8/197) showed erroneous identifications (six RMIs transmitted with caution, one *Acinetobacter* sp.—RMI no. 90—and one *Staphylococcus epidermidis* identified as *Staphylococcus hominis* that most likely resulted from contamination with cutaneous flora). The remaining 7.61% (15/197) were RMIs based on mixed cultures; for eight of them, the Gram staining suggested the presence of several organisms, and for all of them, at least one microorganism remained undetected using the RMI technique. The RMIs for the mixed cultures are presented in Table 3.

Prospective analysis

The transmitted RMIs corresponded to 40 paediatric and 90 adult patients from Saint-Pierre and 67 patients from Jules Bordet. The survey showed that the Gram staining results led to a modification of the patient's treatment in 17.26% of cases (34/197, see Table 4).

All clinical data for patients who benefited from the RMIs according to our survey (confirmation of contamination excluded, $n = 29$) are presented in Table 5.

Of the adult patients, the RMIs led to a modification of the empirical treatment in 11.11% (10/90) and 14.93% (10/67) of cases according to the IDSs of Saint-Pierre and Jules Bordet, respectively (p 0.48).

For adult patients at Saint-Pierre, the modification of the empirical treatment consisted of the addition of a new drug in 80% (8/10). At Jules Bordet, the RMIs led to the addition of a new drug in only 20% of cases (2/10), the cessation of treatment in 20% of cases (2/10) and changes in the antimicrobial treatment regimen in 30% of cases (3/10); in addition, the RMIs prevented the initiation of useless treatments in 30% of cases at this institution (3/10).

In the paediatric population, the IDSs reported a modification of the empirical treatment (escalation) in only 2.50% of cases (1/40).

According to the survey, 37.5% of the RMIs (15/40) for the paediatric population allowed for the rapid confirmation of contamination. In 73.3% of those RMIs (11/15), the RMI highlighted the presence of a coagulase-negative *Staphylococcus* but a blood infection was excluded in all cases because blood cultures were collected at the paediatric emergency department and none of these patients had either catheters or other medical devices. The RMI confirmed contamination for only 11.11% (10/90) and 5.97% (4/67) of adult patients from Saint-Pierre and Jules Bordet, respectively (see Supplementary material, Table S2). The confirmation of contaminated samples never led to the modification of the antimicrobial treatment.

TABLE 5. Clinical context and description of cases for whom rapid microbial identification (RMI) showed a benefit either in terms of antimicrobial treatment or in terms of general management of the patient ($n = 29$)

		N	RMI result	Current AB treatment	Clinical context	Treatment modification according to the IDS survey	IDS retrospective analysis recommendations followed?	Pharmacy retrospective analyses: Based on medical, nurse and billing file	Pharmacy retrospective analyses: Time between communication of the RMI to the IDS / administration of the treatment delay (h)	Other modification according to the IDS survey	IDS retrospective analysis recommendations followed?	Comments and follow-up
Saint-Pierre University Hospital	Adults	1	*S. epidermidis*	moxifloxacin	Respiratory distress syndrome	avoid vancomycin addition	Y	Y	NA	NA		Based on the Gram staining, IDS would have covered MRSA
		12	*S. epidermidis*	AMC	Respiratory distress syndrome cardiac failure	vancomycin addition	Y	Y	14h			Same bacteria previously found in the patient's blood cultures
		44	*E. faecalis* + *C. freundii*	PTZ	Shock of unknown origin	amikacin addition	Y	Y	20 h			Based on the Gram staining, IDS would have chosen vancomycin and metronidazole. According to the RMI amikacin is added to cover *C. freundii*
		76	[illegible]*	meropenem	Septic shock + peritonitis	ornidazol addition	Y	Y	MD**			Anaerobic bacteria suspected
		71	*S. aureus*	moxifloxacin	COPD, iv drug user, fever and cough	vancomycin addition	N	Y	15 h 08			Exclusion of a contamination -> early treatment administration
		80	*S. aureus*	PTZ	Multiple myeloma, raising CRP	vancomycin addition	Y	Y	09 h 50			Exclusion of a contamination -> early treatment administration
		146	*S. aureus*	PTZ + amikacin	Shock, urinary infection, suspected mediastinitis	vancomycin addition	Y	Y	02 h			Exclusion of a contamination -> early treatment administration
		156	*E. faecium*	trimethoprim-sulfamethoxazole	Cirrhosis	vancomycin addition	Y	Y	8 h 30			Because of the identified microorganism
		164	*Enterococcus* sp.	meropenem	Septic shock + abdominal fistula	potential vancomycin addition	N	Y	01 h 28			Because of the identified microorganism, to cover *E. faecium*
		190	*Salmonella* sp.	PTZ	Immunosuppressed, fever	shift to levofloxacin	Y	MD	MD			Because of the identified microorganism
		196	*S. pettenkoferi*	AMC	Pulmonary infection	NA				investigation for CVC infection	Y	Species related to catheter-related infections. Finally considered as a contamination
		234	*S. gallolyticus*	PTZ	COPD, dyspnea and fever					blood cultures control + additional medical investigations	Y	Suspicion of endocarditis, cardiac echography. Patient died
		65	*S. pyogenes*	0	Cardiac arrest					help in excluding patient error	NA	Same Gram staining for blood cultures collected from another patient in the unit suggested ID error. RMI allowed a different identification and misidentification was excluded. Patient died
		280	*S. aureus*	AMC	Pulmonary infection					additional medical investigations	Y	Same bacteria previously found in the patient's blood cultures. Search for complicated *S. aureus* infection -> spondylodiscitis
	Paediatrics	177	*K. pneumoniae*	0	Carbon monoxide poisoning, fever of unknown origin	start ampicillin + cefotaxime	Y	Y	MD**	NA		Exclusion of contamination
Jules Bordet Institute		38	*E. coli*	levofloxacin metronidazole	Patient receiving high doses of corticosteroids	stop metronidazole	Y	ND				Antibacterial spectrum reduction
		284	*A. genomospecies*	ceftriaxone	Prostatic carcinoma, fever	amikacin addition	Y	ND				Because of the identified microorganism
		213	*E. coli*	moxifloxacin IV	Deterioration of the general status, suspicion of pulmonary infection	[illegible] PO shift	Y	ND				Same bacteria previously found in the other sample
		41	*K. pneumoniae*	0	Ears, Nose and Throat neoplasy	avoid amikacin addition	Y	ND				*P. aeruginosa* was suspected in an infected wound. Based on the Gram staining, IDS would have started PTZ and amikacin -> only PTZ
		90	*A. genomospecies*	AMC	Hepatic disease, fever	meropenem instead of PTZ	Y	ND				Based on the Gram staining, IDS would have chosen PTZ
		165	*S. pyogenes*	vancomycin moxifloxacin	Ears, Nose and Throat neoplasy, pulmonary infection	stop vanco	Y	ND				Same bacteria previously found in a drainage fluid
		186	*E. faecalis*	temocillin	Endometrial cancer, pyelonephritis, fever	AMC instead of PTZ	Y	ND				Based on the Gram staining, IDS would have chosen PTZ
		197	*E. faecalis*	0	Abdominal surgery	avoid vancomycin addition	Y	ND				Based on the Gram staining, IDS would have chosen PTZ and vancomycin
		267	*S. epidermidis*	0	Medullary graft, Systematic blood culture collection for patients receiving high doses of corticosteroids	avoid vancomycin addition	Y	ND				Based on the Gram staining, IDS would have chosen vancomycin
		33	*S. agalactiae*	PTZ	Systematic blood culture collection for patients receiving high doses of corticosteroids	aminoglycoside addition	Y	ND		additional medical investigations	Y	Look for pelvic infection symptoms. Finally considered as a post-chemotherapy sepsis
		99	*K. pneumoniae*	cefepime	Ovarian carcinoma, chills	NA				catheter removal	Y	Same bacteria previously found in the patient's blood cultures
		123	*S. epidermidis*	vancomycin	Ovarian carcinoma, fever					catheter removal	Y	Same bacteria previously found in the patient's blood cultures
		271	*S. epidermidis*	0	Multiple myeloma, no fever					blood culture control, early treatment instructions	N	To exclude catheter-related infection. *S. epidermidis* was previously found in blood cultures but no sign of catheter infection was observed. In case of fever, IDS advised to add vancomycin
		300	*S. epidermidis*	MD	Ovarian carcinoma, catheter placement					early CVC control	Y	To check if there are infection signs at the catheter insertion site. This patient had experienced *S. epidermidis* catheter-related infection in the preceding weeks (see 123)

CT, compliance with IDS recommendations (according to the IDS retrospective analysis); Y, yes; N, no; MD, missing or incomplete data; NA, not applicable; ND, not done.
CRP, C-reactive protein; MRSA, methicillin-resistant *Staphylococcus aureus*; VRE, vancomycin-resistant *Enterococcus*; COPD, chronic obstructive pulmonary disease; PTZ, piperacillin/tazobactam; AMC, amoxicillin/clavulanic acid; CVC, central venous catheter.
*The medical microbiologist told the IDS that an anaerobic bacteria was suspected.
**Adaptation was done before getting the identification by conventional methods but the precise administration time is unknown (after 3 days for patient 76).

For adult patients, the IDSs reported other benefits of RMI for 4.44% and 7.46% of patients at Saint-Pierre and Jules Bordet, respectively. Such benefits included requests for new blood cultures ($n = 2$), removal or control of catheters ($n = 4$), additional medical investigations ($n = 3$) and resolving confusion over the samples of two patients ($n = 1$); as presented in Table 5.

Retrospective analysis

Compliance with IDS recommendations, according to the IDS and pharmacy data, is presented in Table 5.

For recommendations regarding the patients at Saint-Pierre ($n = 15$), the pharmacy data suggested that the recommended treatment adaptations were implemented in 9/10 cases before the results of the conventional methods were available. Several discrepancies were observed between the IDS and pharmacy data that may be partially explained by the lack of information in the billing, medical and nursing files.

The IDSs also highlighted an eleventh case not detected by the survey in which the RMI led to the rapid adaptation of the treatment regimen.

For recommendations regarding the patients at Jules Bordet ($n = 14$), with the exception of one patient, the IDS confirmed that all recommendations were respected and that all treatment modifications were implemented the same day as the RMI results became available.

The median times required to obtain an identification result from both the RMI and the conventional methods were evaluated using 178 of the 197 transmitted RMIs (19 were excluded because of missing data) and found to be 1 h 35 min (95 min; minimum–maximum: 15–596 min) and 25 h 43 min (1543 min, 1021–10499 min), respectively. This difference was statistically significant ($p < 0.001$) (see Supplementary material, Table S3).

The delay between the transmission of the RMI to the IDS and the administration of the modified treatment was >4 h in 50% of the cases (4/8, two incomplete or missing data, Table 5).

The Fisher's exact test showed no significant difference in the impact of the RMI according to the medical unit where the samples were collected (see Supplementary material, Fig. S2).

Discussion

MALDI-TOF MS has emerged as a promising tool for the rapid identification of organisms from positive blood cultures [13–15]. Numerous strategies were evaluated to optimize RMI from positive blood cultures. A previous evaluation showed that our RMI protocol was able to correctly identify 73.7% of the blood culture bacteria at the species level in <1 h [14]. The inclusion of a higher proportion of Gram-positive bacteria that are usually less successfully identified by MALDI-TOF MS RMI techniques may explain the lower rate of successful identifications observed in the present study (61.01% vs 73.7%).

To date, the clinical impact of this type of RMI has been poorly investigated [16].

The major strength of our study is the prospective collection of data that led to similar observations for two medical institutions treating different patient populations.

Our results suggest that MALDI-TOF MS may hasten the modification of empirical treatment regimens in 13.38% of cases in the adult population (21/157). The same proportion of treatment regimens were altered (p 0.64) at both Saint-Pierre (11/90, 12.22%, including the eleventh case, which was highlighted during the retrospective analysis) and Jules Bordet (10/67, 14.93%). A recent study by Stoneking *et al.* [18] that retrospectively evaluated the effect of more rapid microorganism identification in bacteraemic emergency department patients suggests that the empirical therapy would remain unchanged in only 23% of cases; the remaining 21.3% and 55.7% of patients would receive treatment with an additional antibiotic for organisms not covered by the initial regimen or the adjustment of the regimen to reduce the spectrum of the antibiotics, respectively. Applying the same analysis (excluding contaminated blood cultures and missing data) to adult patients at Saint-Pierre, 78.95% of treatments would remain unchanged. In our study, the proportion of patients who were infected with pathogens not affected by the initial antibiotic regimen was in agreement with the number reported by Heenen *et al.* [19] in their study of de-escalation in a medico-surgical intensive care unit (16%). We first thought that the high rate of unchanged treatments in our study could be explained by the fact that two-thirds of the patients were hospitalized and received treatment that was based on previous positive samples other than blood cultures. However, the medical unit where the blood cultures were collected did not seem to affect the proportion of treatment modifications in our study. The difference may therefore be explained by the implementation of antimicrobial stewardship programmes in both the Saint-Pierre and Jules Bordet medical centres.

In the Jules Bordet patient population, most modifications of the antimicrobial treatment regimens due to the RMI involved de-escalation. In contrast, most modifications at Saint-Pierre involved treatment escalation. This difference can most likely be explained by the specific population of the Jules Bordet facility, which manages only cancer patients. For such patients, IDSs are confronted with known clinical presentations and must follow rational schemes. Broad-spectrum antibiotics are also most likely to be used in this population.

In both medical centres, the RMI of *Enterococcus* species and β-hemolytic streptococci was responsible for 30% of the modifications of the antimicrobial treatment, which suggests that performing RMI on positive blood cultures showing Gram-positive cocci in pairs or chains may be of interest despite the known limitations of the MALDI-TOF MS technique (no discrimination of *Streptococcus mitis/oralis* from *Streptococcus pneumoniae*).

In the oncological population, the confirmation/exclusion of non-fermenting Gram-negative rod involvement was also responsible for 30% of the observed modifications in the antimicrobial treatment, confirming the previous results of Clerc *et al.* [20], who highlighted the great benefit of the RMI on the clinical management of patients infected with Gram-negative bacteria.

For paediatric patients, MALDI-TOF MS was especially useful for the rapid confirmation of contaminated blood cultures (37.5%). However, these observations never led to a de-escalation of the antimicrobial treatment. As previously suggested, failure to follow the IDSs' recommendations may be a result of physicians' reluctance to modify treatment in patients who are improving [3].

In our study, most changes to the treatment regimen were made before the genus and/or species identification results provided by the conventional methods were available. This high level of compliance certainly results from the design of the study; telephone calls, which allow for a two-way exchange, considerably improved the communication between microbiologists and IDSs [3]. Additionally, it is well known that the implementation of multidisciplinary teams is of major importance for the optimization of antibiotic therapy in clinical settings [21]. However, the delay for the administration of a modified treatment was high (>4 h in 50% of cases), and the communication between other health professionals involved in the antimicrobial treatment administration process (clinicians, nurses, pharmacists) should therefore be improved in our institution. Major improvements of our electronic prescribing system and of the delivery of urgent antimicrobial agents are also awaited. This point will be addressed to our antimicrobial stewardship team members who are particularly skilful at improving such processes.

With a decrease of 26.85 h in the time required for identification and a 13.38% increase in the proportion of patients receiving an appropriate antimicrobial treatment 24 h after the positive blood culture, our results are in perfect agreement with those presented in the recent publication of Vlek *et al.* [22] (28.8 h, 11.3%). The major difference between their study and the present study is the study design: Vlek *et al.* compared a 'standard care' group with an 'intervention' group, whereas we opted for a blinded prospective analysis of a single patient group.

Although it may not always lead to the modification of the treatment regimen, RMI plays an important—and unfortunately difficult to quantify—role in the global management of the patient. The confirmation of a contaminant is of particular interest for non-hospitalized patients and can help to avoid the administration of unnecessary antibiotics. On the other hand, the confirmation of a catheter-related infection—usually made by 'time-to-positivity' determination—will allow for the rapid removal of infected devices and can help to prevent further infections. The detection of particular organisms may also allow the clinician to identify the origin of the sepsis and therefore contribute to cost-saving measures.

From a financial point of view, performing RMI requires an additional cost, primarily because of the need for additional staff and an adapted workflow. In addition, the use of conventional identification methods is still needed to resolve cases of mixed cultures or unreliable RMIs.

Because of these practical considerations and because a case-by-case selection of which RMI to perform in collaboration with the IDS would probably slow down the analytical workflow, we decided to perform the analysis only twice daily, which seems to be the most efficient option for our laboratory. The optimization of the technique, including automation, should lessen the financial impact of the technique in the future.

Our study has several limitations. First, the analysis of the pharmacy data highlighted the difficulty in identifying accurate indicators and revealed discrepancies between the billing, medical and nursing files. At our institution, improvements in computerization in the near future will most likely improve data handling [23]. Second, the observations presented in this study are only valid for similar organizations. Indeed, a higher clinical impact might be observed in institutions where the laboratory and pharmacy are open 24 h per day and an IDS is on site at all times. Both Saint-Pierre and Jules Bordet have antimicrobial stewardship programmes and numerous IDSs. These factors might explain a high rate of appropriate empirical treatment and the rapid adaptation of antimicrobial therapies because of frequent re-evaluations. Medical institutions without IDSs would most likely receive a greater benefit from RMI. The benefit of the RMI could also be different in institutions dealing with a high proportion of antimicrobial-resistant bacteria (e.g. methicillin-resistant *S. aureus* or extended spectrum β-lactamase producers), which is currently not the case in our hospitals.

Conclusion

In an adult population, 13.38% of the MALDI-TOF MS RMIs from positive blood cultures resulted in the faster adaptation of the

antimicrobial treatment regimen. The technique is also able to rapidly confirm contamination, especially in the paediatric population (37.5%), and is able to hasten the removal of infected catheters and suggest complementary diagnostic investigations. Despite the increased cost for the laboratory, RMI analyses are routinely performed twice daily in our laboratory. However, the use of RMI should not be considered unless there is efficient communication between health professionals.

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Medical records of patients were reviewed retrospectively by linking microbiology test results with the respective medical records. Research ethics approval was granted by the Ethics Review Boards of the hospitals.

Transparency Declaration

The authors declare no conflicts of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Case report form.

Figure S2. Impact of the rapid microbial identification according to the medical unit where the blood cultures were collected.

Table S1. Results of all rapid microbial identifications (RMI; $n = 277$)

Table S2. Rapid microbial identification (RMI) that allowed a fast confirmation of contamination ($n = 29$).

Table S3. Data used to determine the identification delay by both conventional techniques and rapid microbial identification (RMI; $n = 178$).

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4.5. Feasibility of Matrix-Assisted Laser Desorption/Ionisation Time-of-Flight Mass Spectrometry (MALDI-TOF MS) networking in university hospitals in Brussels

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ARTICLE

Feasibility of matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) networking in university hospitals in Brussels

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Abstract The mutualisation of analytical platforms might be used to address rising healthcare costs. Our study aimed to evaluate the feasibility of networking a unique matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) system for common use in several university hospitals in Brussels, Belgium. During a one-month period, 1,055 successive bacterial isolates from the Brugmann University Hospital were identified on-site using conventional techniques; these same isolates were also identified using a MALDI-TOF MS system at the Porte de Hal Laboratory by sending target plates and identification projects via transportation and the INFECTIO_MALDI software (Infopartner, Nancy, France), respectively. The occurrence of transmission problems (<2 %) and human errors (<1 %) suggested that the system was sufficiently robust to be implemented in a network. With a median time-to-identification of 5 h and 11 min (78 min, min–max: 154–547), MALDI-TOF MS networking always provided a faster identification result than conventional techniques, except when chromogenic culture media and oxidase tests were used ($p<0.0001$). However, the limited clinical benefits of the chromogenic culture media do not support their extra cost. Our financial analysis also suggested that MALDI-TOF MS networking could lead to substantial annual cost savings. MALDI-TOF MS networking presents many advantages, and few conventional techniques (optochin and oxidase tests) are required to ensure the same quality in patient care from the distant laboratory. Nevertheless, such networking should not be considered unless there is a reorganisation of workflow, efficient communication between teams, qualified technologists and a reliable IT department and helpdesk to manage potential connectivity problems.

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Introduction

The introduction of matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) as a new tool for bacterial identification constitutes a major revolution of the 21st century in the field of microbiology [1, 2]. Since its first commercialisation in the mid-2000s, the capabilities of this technology have been widely evaluated, and authors seem to agree that it will soon replace most conventional identification techniques and may often be an efficient alternative to expensive molecular techniques, including polymerase chain reaction (PCR) or sequencing [3–6]. The major advantages of MALDI-TOF MS compared with conventional identification techniques include its ease of use, the small amount of biological material needed (only a loopful of cells is required to prepare the deposit), the execution speed and low time-to-identification delay, its low reagent cost and its high efficiency, which may still be improved by updating the databases [1, 7, 8]. Most studies evaluating the MALDI-TOF MS technology compared with biochemical or molecular tests also

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demonstrate its cost-effectiveness [2, 8–10]. Today, the rationalisation of health costs requires major changes in hospital organisation and the development of strategies for laboratory cost containment. In Europe, large centralised clinical laboratories (CLs) usually organised in one central platform and one or several distant laboratories (DLs) that address urgent analyses are now being developed [11–14]. These centralisations require technique harmonisation, automation and accreditation. In addition, the distance between sites should not alter the clinical management of patients or impede the detection of microbial spread. Because of its numerous advantages, MALDI-TOF MS appears to meet these requirements and is, therefore, a good candidate for the delocalisation of bacterial identification in the context of pooled analytical platforms.

Thus, our study aimed to evaluate the feasibility of performing delocalised bacterial identification from one site to another from financial and organisational points of view in a Belgian laboratory network in Brussels using MALDI-TOF MS technology.

Materials and methods

Location

This study took place at the iris Brussels public hospitals network in both the Porte de Hal Laboratory (CL), which is located at the Saint-Pierre University Hospital, and the laboratory of the Brugmann University Hospital (DL). The DL performs microbiological analysis for both the Brugmann University Hospital and Queen Fabiola Children's University Hospital. The laboratories are located 7.5 km from each other and have recently been combined into a unique analytical platform called iris-Lab.

Organisation

From February 12th, 2013 to March 13th, 2013, all aerobic bacteria isolated from human samples at the DL were identified using conventional identification techniques, and all samples at the CL were identified with MALDI-TOF MS. According to known limitations of MALDI-TOF MS, suspected *Streptococcus pneumoniae* were systematically excluded. Prepared target plates were sent from the DL to the CL using the current available transports between sites, and identification projects and results were transmitted through the INFECTIO_MALDI software (Infopartner, Nancy, France). During weekdays, nine return trips between sites were already scheduled, from 08:00 am to 05:00 pm, for the transportation of clinical samples. Such transport between sites represents a cost of about 165 € daily.

INFECTIO_MALDI

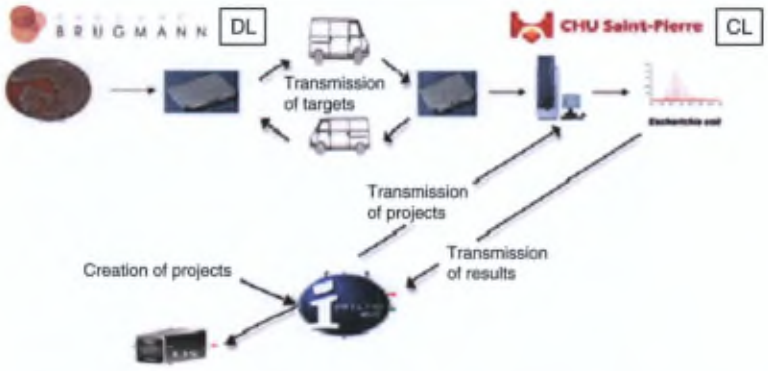
INFECTIO_MALDI software, presented in Fig. 1, was used to create identification projects at every workstation at the DL and send the projects to the MALDI Biotyper system (Bruker Daltonics, Bremen, Germany) located at the CL. After acquisition in the Microflex LT system (Bruker Daltonics, Bremen, Germany) at the CL, all identification results were transmitted to INFECTIO_MALDI at the DL. The identification results were then available for technical validation by the technologist. The software shows all relevant information (e.g. the ten best identification matches, score values, historical results etc.), and validated results may subsequently be sent to the LIS and/or AST instruments. To avoid interference with the clinical laboratory organisation, MALDI-TOF MS results were not transmitted to the LIS instrument during the study period; instead, all data were retained in INFECTIO_MALDI, which offers a full traceability system. Based on catalogue prices, the classical version of the software represents an extra cost of 9,000 € for each laboratory, as well as maintenance costs of 800 € per site and per year.

MALDI-TOF MS analysis

At the DL, each bacterial sample was smeared twice on a target plate and then covered with 1 μL of α-cyano-4-hydroxycinnamic acid (CHCA) matrix solution. After drying, the target plate was sent to the CL. At the CL, the target plate was loaded into the machine, which was equipped with a 337-nm nitrogen laser. The spectra were recorded on a Microflex LT system in linear mode with a mass range of 2 to 20 kDa and subsequently analysed using MALDI Biotyper Automation Control and Biotyper 3.0 software. At that time, the database (v3.1.2.0) included 3,995 spectra. Identification results were classified as reliable or unreliable according to the manufacturer's recommended cut-off values of 1.7 and 2 for validated results for the genus and species levels, respectively. Reliable results with "B" or "C" additional criteria, which are indicative of genus consistency and of no consistency, respectively, were considered individually and reclassified as reliable or unreliable by the medical microbiologist according to his/her experience.

Conventional identification techniques

Conventional identification techniques used at the DL included the Vitek semi-automated identification system and the evaluation of growth requirements, biochemical and specific agglutination tests, and chromogenic culture media for urine samples.



- Training of DL technologists to smear bacteria on the target plates
- Each technologist was responsible for its deposits and identification projects
- Validation of the results is performed on site

- Target plates arriving at the CL are treated as soon as possible according to the analytical workflow
- Identification projects corresponding to the target plates are imported in the MALDI Biotyper
- Technologists and medical microbiologists are not responsible for the validation of the identification results

Fig. 1 Study organisation including the use of the INFECTIO_MALDI software for projects and results transmission between the distant laboratory (DL) and the clinical laboratory (CL)

Organisational impact evaluation

Several indicators were chosen to evaluate the best workflow for both laboratories. Dysfunctions related to the technique, software or network were systematically registered, along with all data related to transport and delays.

For each isolate, the delay between the isolation time (pure colony) and identification time, called the "time-to-identification" (TTI), was determined for both the conventional and MALDI-TOF MS techniques, and defined as the delay between the isolation of the bacteria and identification result confirmation/availability in LIS/INFECTIO_MALDI, respectively. All discrepancies between the conventional and MALDI-TOF MS results were also investigated.

Financial impact evaluation

The identification cost was evaluated for each technique based on bacterial species isolated at least 50 times in 2012 according to the frequency of their isolation during the same period. Reagent costs were evaluated for each technique and, for each bacterial species, the proportion of each conventional technique used for its identification was introduced in the calculation. Three scenarios were considered for the DL: continue with conventional identification techniques, purchase of a MALDI-TOF MS system or sharing the CL MALDI-TOF MS system. For each scenario, the identification cost was evaluated considering a theoretical depreciation of the system over 5 years. Conventional analysers were considered depreciated (mainly Vitek systems), and the Microflex LT of the CL had been in use for 2 years. Technologist times were considered to be the same for MALDI-TOF MS and conventional techniques and were, therefore, neglected, along with transportation costs. Maintenance costs for equipment and computerisation (INFECTIO_MALDI) were also taken into account, as well as a potential laser replacement (the theoretical life expectancy of the laser was about 240,000 identifications).

Statistical analysis

The non-parametric Wilcoxon signed-rank test was used to compare the TTI observed for both the conventional and MALDI-TOF MS identification techniques.

Results

During the study period, 1,055 bacterial isolates from samples of 720 patients, 78.9 % of which were adult patients (568/720) and 65.1 % were female (469/720), were identified using conventional and MALDI-TOF MS techniques in parallel (see supplementary material).

Table 1 Repartition of the bacterial isolates as pathogenic or commensal and classification of the matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) results as reliable or unreliable according to the workstation

		Urinary tract	SSI, fluids and sterile sites	Faeces	Respiratory tract	Genital tract	Screenings	Blood cultures	Total
Pathogenic	Unreliable	87 (19.5 %)	26 (16.3 %)	1 (0.7 %)	20 (15.9 %)	34 (47.2 %)	5 (9.3 %)	11 (23.4 %)	184
	Reliable	354 (79.4 %)	133 (83.1 %)	22 (14.7 %)	101 (80.2 %)	33 (45.8 %)	48 (88.9 %)	36 (76.6 %)	727
Commensal flora	Unreliable	1 (0.2 %)	0	43 (28.7 %)	0	4 (5.6 %)	0	0	48
	Reliable	4 (0.9 %)	1 (0.6 %)	84 (56 %)	5 (4.0 %)	1 (1.4 %)	1 (1.9 %)	0	96
Total		446	160	150	126	72	54	47	1,055

Samples and identifications

The origin of the samples and classification of isolates as pathogenic or commensal is presented in Table 1. The correspondence between the pathogens isolated during the study period and the pathogens isolated during 2012 in the same laboratory is presented in Fig. 2. The nine most frequently isolated pathogens, which represent more than 84.5 % of species isolated in 2012, were the same in both panels.

Among the 1,055 MALDI-TOF MS identifications, 78.0 % were classified as reliable according to the recommended cut-off criteria (823/1,055; Table 1). Among the 232 unreliable identifications, 88.4 % resulted from a poor quality deposit or a technical problem (205/232, "no peak found"). Other unreliable identifications (27/232) were associated with poor score values (<2). Bacterial species that frequently led to unreliable results included *Staphylococcus saprophyticus* (9/9), *Klebsiella oxytoca* (5/8) and *Streptococcus agalactiae* (41/81), among others. However, the rate of unreliable results tended to decrease over time, which was expected, as it has been demonstrated that the quality of MALDI-TOF MS deposits improves with the operator's experience [2, 15].

In six cases (6/1,055, 0.57 %), the MALDI-TOF MS and conventional identification techniques led to discrepant results. In one case, the discrepancy resulted from an inconsistency in the bacterial colonies taken to perform both techniques; in two cases, additional tests concluded that the MALDI-TOF MS provided the correct identification result; in the three remaining cases, MALDI-TOF MS showed a poor discrimination of *Aeromonas* species isolated from stool samples, which is a known limitation of the technique that does not impact on the clinical management of the patient.

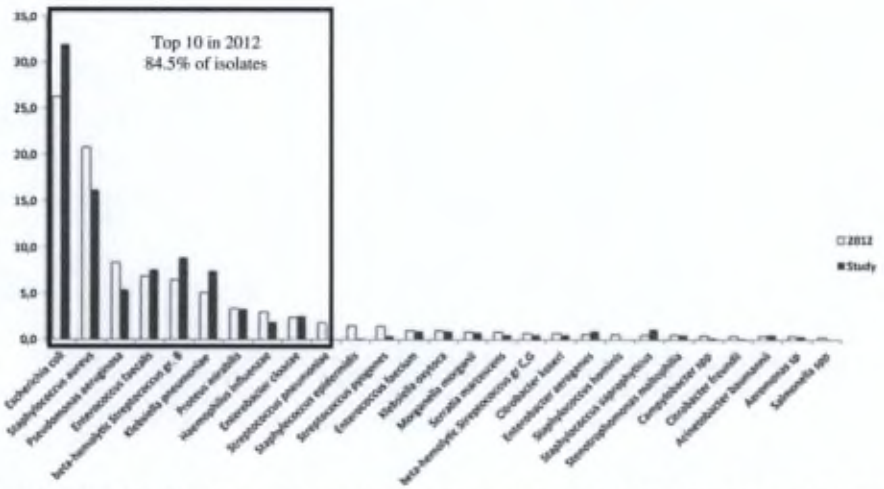


Fig. 2 Correlation between bacterial species isolated during the study period and those isolated during the year 2012. Only species that were isolated at least 50 times in 2012 were considered

Organisational aspects

During the study period, we observed a mean of 2.5 projects, 2 targets and 54.3 identifications per day. The workflow was sequenced in four steps, as presented in Fig. 3. All projects and targets were prepared and ready to be transported before 2:00 pm. The median transport delay was 1 h and 4 min. All identification results were available to the technologist of the DL on the day of isolation. In 85 % of cases, identification results were available before 3:30 pm.

Two types of dysfunctions were observed. First, human errors were noticed in six cases: in one case, the labels of two patients were inverted when creating the project (0.2 %), and in another five cases, the identification results for both smears of the same isolate led to different bacterial identifications (0.5 %), suggesting that different colonies were picked up for smearing onto the target plate. Second, part of the identification results ($n=19$, 1.8 %) was not transmitted into INFECTIO_MALDI because of a transmission defect.

The median TTI observed using the conventional and MALDI-TOF MS techniques were 23 h and 12 min (991 min, min–maximum: 0–5,873) and 5 h and 11 min (78 min, min–max 154–547), respectively. Table 2 shows the TTI observed for each conventional technique and its comparison with the TTI observed for the same bacterial isolate identifications using MALDI-TOF MS. The MALDI-TOF MS results were always available before those provided by conventional techniques, except for bacteria isolated from urine samples that were identified by the use of chromogenic culture media and for *Pseudomonas aeruginosa*, which is still identified using the oxidase test ($p<0.0001$). As presented in Table 2 and Fig. 4, the identification results provided by other conventional techniques were usually available on the night of or the day after the bacterial isolation.

Financial aspects

The estimation of the yearly identification cost for each scenario is presented in Table 3. The annual identification cost using conventional techniques was 35,767 €. In contrast, the annual identification cost was 21,219 and 39,771 € for a shared MALDI-TOF MS system or new MALDI-TOF MS system acquisition, respectively. After the depreciation period, these annual costs decreased to 5,883 € (from 2016) and 8,996 € (from 2018), respectively.

Discussion

The trend towards the mutualisation of analytical platforms requires rethinking the organisation of the routine microbiology laboratory. In this context and thanks to its numerous advantages in terms of financial costs, efficiency and rapidity, the MALDI-TOF MS technology appears to be a good candidate for bacterial identification delocalisation.

Despite the short study period, the nine most frequently isolated bacteria observed during this period was the same as that observed for the entirety of 2012, which suggests that our panel was representative of typical clinical laboratory practice. Moreover, these bacterial species represent more than 84 % of all bacteria isolated annually. The tenth most frequently bacterial species isolated in 2012 was *S. pneumoniae*, which was voluntarily excluded from our evaluation [16].

As previously suggested by Francis et al., the evaluation of how reorganisation affects the quality of the laboratory should include five performance indicators: service standards, analytical quality, finances, productivity and personnel perspective [12]. Because technologists from the DL were not experienced in performing MALDI-TOF MS analyses, we decided to focus only on the first three indicators.

This lack of training may explain the high rate of unreliable MALDI-TOF MS results observed in our study (22 %, 88 % of which were "no peak found" results). The preparation of the smear is a limiting factor that may influence the acquisition of the spectrum and quality of the identification result [17]. This limitation, in combination with the dysfunction of our laser, which was revealed by a higher proportion of unreliable results at the CL, where technologists are fully experienced in preparing MALDI-TOF MS target plates, is a major bias in our study. However, the analytical performance of MALDI-TOF MS for bacterial identification in bacteriology laboratories has been widely evaluated. Our results showed a rate of correct species identification higher than 92 % in a clinical laboratory setting [7].

From an organisational perspective, the INFECTIO_MALDI software is user-friendly. The software allowed good connectivity between both laboratories and made all analytical information available for the DL team. The rare transmission problems observed during the study period (less than 2 % of the identification results) were immediately solved by IT support. Human errors, including label inversion, and erroneous deposits were also anecdotal (<1 % of identifications), which suggests that the combination INFECTIO_MALDI/MALDI-TOF MS is sufficiently robust to be implemented in a network.

Though it is widely acknowledged that automation may lead to workload and positions savings, MALDI-TOF MS implementation and networking did not, in our opinion, lead to such savings [14].

Moreover, in terms of workflow, the networking of MALDI-TOF MS requires communication and reactivity from both teams to avoid loss of the benefits provided by this technology. The CL team must be aware of the consequences of any delay in the management of the target plates sent by the DL team (insufficient time to perform conventional identification in case of an unreliable MALDI-TOF MS result or the

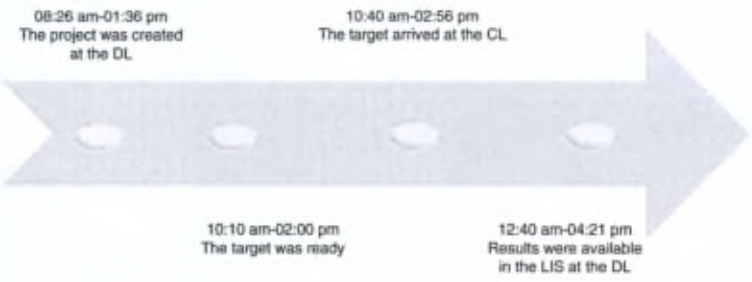


Fig. 3 Timeframe of the four distinct sequences of the workflow

identification result being provided too late to be considered by the medical team). According to the DL activity, the best scenario seemed to send a first target plate for urgent identifications in the morning (at about 10:00 am to get identification results in the early afternoon), followed by a second target plate in the early afternoon (at about 01:00 pm) for less urgent analysis. Of course, each laboratory should evaluate the best option regarding its own activity and transport durations. Such new organisation implies adaptations of the entire workflow that are now in progress at the DL: first, priority should be given more than anything to isolates that require urgent identification (microorganisms isolated from blood cultures and other precious clinical samples, suspicion of highly pathogenic bacteria etc.) in order to benefit from the first MALDI-TOF MS target plate transfer; secondly, antimicrobial susceptibility testing should now be performed in a second step, according to the identification results; thirdly, examination of the agars usually planned in the afternoon should be performed as early as possible in order to benefit from the second MALDI-TOF MS target plate transfer; finally, clinicians and infectious diseases specialists should be informed that part of the identification results are now made available in the afternoon.

In our situation, the management of the transports was easy to implement, as nine return trips between sites were already scheduled daily for the transportation of clinical samples. Several parameters should, nevertheless, be taken into account when implementing such transports between distant sites: the cost of the transport, the number of analyses to perform and the number of target plates to be sent daily. For example, the implementation of transports between our CL and DL—for

Table 2 Comparison of the time-to-identification (TTI, h:min) using conventional and MALDI-TOF MS identification techniques

	n	Minimum	1st Quartile	Median	3rd Quartile	Maximum	Interquartile range	Wilcoxon *p*-value
TTI Vitek card	415	05:15	11:49	22:18	24:26	97:53	12:36	<0.0001
TTI MALDI-TOF MS		02:34	04:30	05:10	05:56	08:11	01:26	
TTI Oxidase test	45	00:00	00:00	00:01	00:01	04:51	00:01	<0.0001
TTI MALDI-TOF MS		02:51	04:10	05:00	05:37	08:02	01:27	
TTI Chromogenic culture media	175	00:00	00:00	00:01	00:01	06:11	00:01	<0.0001
TTI MALDI-TOF MS		03:22	04:03	04:54	05:42	08:03	01:38	
TTI Agglutination + coagulase test	149	06:24	24:00	24:02	25:13	38:50	01:13	<0.0001
TTI MALDI-TOF MS		02:53	04:25	05:22	05:55	09:07	01:30	
TTI *Streptococcus* agglutination	87	00:00	22:17	23:44	25:31	73:49	03:13	<0.0001
TTI MALDI-TOF MS		03:17	04:28	05:02	05:53	07:26	01:24	
TTI API galery	14	06:01	22:47	26:07	27:19	70:59	04:31	<0.0001
TTI MALDI-TOF MS		02:54	03:53	04:12	05:29	06:22	01:36	
TTI Hippurate hydrolysis	12	03:57	05:09	06:49	22:42	25:01	17:33	0.0005
TTI MALDI-TOF MS		03:08	03:36	04:14	04:31	04:32	00:54	
TTI HI-enterotube	134	18:20	24:47	26:43	49:05	97:30	24:17	<0.0001
TTI MALDI-TOF MS		03:22	04:37	05:24	05:39	06:53	01:01	
TTI X, V and XV requirements	21	22:09	23:53	24:02	25:36	48:09	01:42	<0.0001
TTI MALDI-TOF MS		03:45	04:31	05:09	05:47	06:40	01:15	
TTI Routine	1,055	00:00	08:22	23:12	24:54	97:53	16:31	<0.0001
TTI MALDI-TOF MS		02:34	04:27	05:11	05:45	09:07	01:18	

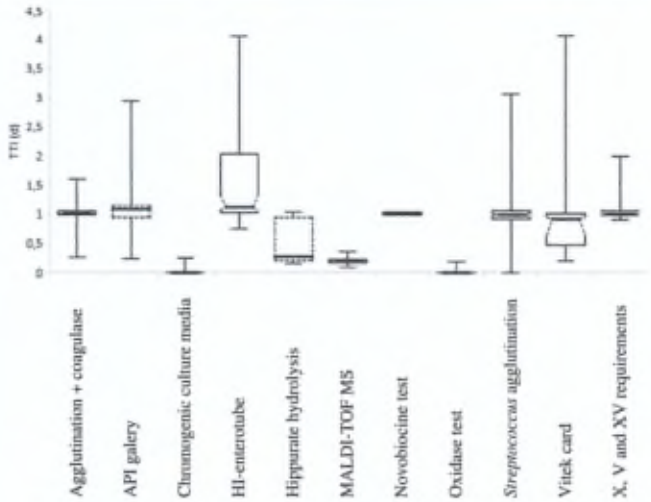


Fig. 4 Median time-to-identification (TTI) and interquartile ranges of each identification technique

the MALDI-TOF MS networking only—would have represented an extra cost of 0.70 € per identification, considering two return trips and 54 identifications daily. We assume that the volume of analyses of the DL and the distance between both laboratories are decisive factors if there is no pre-existing transport between sites.

From a clinical perspective, MALDI-TOF MS networking should not alter patient management. When compared with conventional techniques, the MALDI-TOF MS system frequently provided identification results rapidly. Thanks to the short median transport delay (1 h and 4 min), the median TTI using MALDI-TOF MS was 5 h and 11 min (78 min, min–max: 154–547) versus 23 h and 12 min (991 min, min–max 0–5,873) when using conventional techniques (sub-cultures were sometimes required in order to obtain enough biological material). Moreover, the MALDI-TOF MS results were transmitted in time to be used by the medical team (85 % before 15:30). In contrast, the Vitek cards used in 41.1 % of cases provided results at night or the next morning. Most other conventional techniques required an overnight incubation. According to our observations, the only conventional techniques that could compete with the MALDI-TOF MS technique are chromogenic culture media (median TTI 1 min; IQR 1 min, min–max: 0–371 min) and the oxidase test (median TTI 1 min; IQR 1 min, min–max: 0–291 min).

In a recent study, Tan et al. highlighted deficiencies of previous financial studies comparing MALDI-TOF MS with conventional identification techniques [8]. As suggested by these authors, we considered the auxiliary costs and prevalence of each species encountered in our laboratory in comparing different scenarios. Moreover, we considered the proportion of each conventional technique used to identify a single bacterial species. For example, the annual cost of the *Escherichia coli* identification using conventional techniques is 9,251 €. This number considers the prevalence of the identified *E. coli* in the DL in 2012 and the fact that 57 % of *E. coli* are identified using chromogenic culture media and the remaining 43 % using Vitek cards.

From a financial perspective, our analysis ultimately suggests that MALDI-TOF MS networking could lead to substantial annual cost savings when compared with routine techniques: 14,548 € before depreciation of the Microflex and nearly 30,000 € annually starting in 2016. If the DL decides to buy a new MALDI-TOF MS system to replace routine techniques, the annual cost savings (26,771 €) would be effective in 2018; until then, the new system would represent an annual extra cost of 4,004 €. Buying a new MALDI-TOF MS system instead of sharing the CL MALDI-TOF MS system represents, therefore, an annual extra cost of about 25,000 € considering the depreciation period of the new MALDI-TOF MS system (5 years, 123,432 €).

When considering the species identification cost, it seems obvious that the additional financial cost associated with the use of chromogenic culture media may not be supported by clinical benefits. For example, the shift to using MALDI-TOF MS to identify *E. coli*, which is the most common urinary pathogen and is easily recognised on non-chromogenic culture media, may result in an annual saving of more than 7,000 €.

We assume that, for urine samples, a preliminary result, such as "probable *E. coli*", could be provided until the MALDI-TOF MS result is available. However, the fast result provided by the oxidase test for the identification of *P. Pseudomonas aeruginosa* is not associated with a high additional cost, and, in our opinion, this test could remain a diagnostic tool for the DL.

Table 3 Annual financial evaluation including the three scenarios: continuing with conventional techniques, buying a new mass spectrometer or sharing the Microflex LT of the clinical laboratory (CL). The evaluation considers bacterial species that were isolated at least 50 times in 2012 and takes into account their prevalence and the rate of each technique used for their identification

ID	2012	Routine techniques (according to the study)						Routine	Before depreciation		After depreciation	
	n	Vitek	CM	Oxidase	Agglutination	Agglutination + coagulase	Other		New MALDI-TOF MS	MALDI-TOF MS networking	New MALDI-TOF MS (from 2018)	MALDI-TOF MS networking (from 2016)
Acinetobacter baumannii complex	63	100 %						275 €	149 €	79 €	39 €	28 €
Aeromonas sp.	63	88 %					22 %	263 €	149 €	70 €	40 €	28 €
Campylobacter sp.	84						100 %	−€	198 €	106 €	53 €	37 €
Citrobacter koseri	126	100 %						551 €	297 €	159 €	79 €	56 €
Citrobacter freundii	66	100 %						289 €	156 €	83 €	41 €	29 €
Enterococcus faecium	170	87.5 %					12.5 %	757 €	401 €	214 €	106 €	75 €
Enterococcus faecalis	1,219	100 %						5,330 €	2,877 €	1,535 €	763 €	538 €
Enterobacter aerogenes	106	100 %						463 €	250 €	133 €	66 €	47 €
Enterobacter cloacae	420	100 %						1,836 €	991 €	529 €	263 €	185 €
Escherichia coli	4,635	43 %	57 %					9,251 €	10,941 €	5,837 €	2,902 €	2,045 €
Haemophilus influenzae	527						100 %	188 €	1,244 €	664 €	330 €	233 €
Klebsiella oxytoca	169	100 %						717 €	399 €	213 €	106 €	75 €
Klebsiella pneumoniae	900	100 %						3,935 €	2,124 €	1,133 €	563 €	397 €
Morganella morganii	153	100 %						669 €	361 €	193 €	96 €	68 €
Proteus mirabilis	592	59 %	41 %					1,595 €	1,397 €	746 €	371 €	261 €
Pseudomonas aeruginosa	1,484	11 %		89 %				1,099 €	3,503 €	1,869 €	929 €	655 €
Stenotrophomonas maltophilia	90	100 %						393 €	212 €	113 €	56 €	40 €
Serratia marcescens	146	100 %						638 €	345 €	184 €	91 €	64 €
Staphylococcus aureus	3,665	7 %				93 %		4,371 €	8,651 €	4,616 €	2,294 €	1,617 €
Staphylococcus epidermidis	261	100 %						1,141 €	616 €	329 €	163 €	115 €
Staphylococcus hominis	105	100 %						463 €	248 €	132 €	66 €	46 €
Staphylococcus saprophyticus	93	80 %					20 %	325 €	220 €	117 €	58 €	41 €
Streptococcus agalactiae	1,148	6 %			94 %			888 €	2,710 €	1,446 €	719 €	507 €
Streptococcus pyogenes	250				100 %			134 €	590 €	315 €	157 €	110 €
Streptococcus pneumoniae	314						100	194 €	741 €	395 €	197 €	139 €
Total (*n*)	16,849	6,927	2,885	1,321	1,329	3,408	979	35,767 €	39,771 €	21,219 €	8,996 €	5,883 €
Total (%)	100	41.1 %	17.1 %	7.8 %	7.9 %	20.2 %	5.8 %					

It is important to note that this financial evaluation is limited to frequently isolated species and that less frequently encountered and more difficult to identify bacteria usually require additional conventional and expensive molecular diagnostic tests. Therefore, the difference between identification costs using conventional and MALDI-TOF MS techniques is likely underestimated [18]. Indeed, the panel of pathogens that may be identified by MALDI-TOF MS is much larger than that provided by conventional identification techniques [19]. Moreover, the expansion of the MALDI-TOF MS database will improve the performance of this system in the future. However, the cost of conventional techniques in cases of unreliable MALDI-TOF MS results, which is anticipated to be approximately 5.6 % if excluding suspected *S. pneumoniae* isolates [7], has not been taken into account. In our network, particular attention should be paid to the training of the DL technologists. In the future, the MALDI-TOF MS identification of bacteria directly from positive blood culture could also be implemented at the DL [20–25].

In a recent study, Humphreys et al. discussed the advantages and disadvantages of both the "on-site" and "off-site" laboratories [13]. In our opinion, MALDI-TOF MS networking may provide some of the positive aspects of both the "on-site" and "off-site" situations, such as clinically relevant diagnostics, close contact between laboratories and clinics, integrated service with other disciplines, rationalisation and cost-effectiveness of the bacterial identification, automation and focus on logistics and IT.

Conclusion

Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) networking meets the centralisation goals of clinical biology analyses and appears to be a practical tool in the process of mutualisation of analytical platforms. Few conventional techniques are still required to ensure the same quality in the patient care in the distant laboratory (DL), namely, optochin and oxidase tests. Nevertheless, this networking should not be considered unless there is an efficient reorganisation of the workflow, good communication between laboratory teams, qualified MALDI-TOF MS technologists and a reliable IT department and helpdesk to manage potential connectivity problems.

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Conflict of interest The authors declare that they have no conflicts of interest.

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4.6. Optimization of *Campylobacter* growth conditions for further identification by Matrix-Assisted Laser Desorption/Ionisation Time-of-Flight Mass Spectrometry (MALDI-TOF MS)

Martiny D, Visscher A, Catry B, Chatellier S, Vandenberg O
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Note

Optimization of *Campylobacter* growth conditions for further identification by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS)

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ABSTRACT

Growth conditions – including growth medium and incubation temperature – may influence the identification of *Campylobacter* by MALDI-TOF MS.
For each bacterial species, medical microbiologists should be aware of such potential influences on spectral data before analyzing and interpreting MALDI-TOF MS results.

Campylobacter is the most frequent cause of bacterial enteritis throughout Europe (Jore et al., 2010). This intestinal infection may be followed by bacteremia (1.5 cases per 1000 intestinal infections) and associated with the Guillain–Barré syndrome (1 case per 1000 infections) (Blaser and Engberg, 2008).

The mission of the National Reference Centre for *Campylobacter* is to help microbiologists in identifying *Campylobacter* isolates and determining their antimicrobial susceptibility, but also to evaluate new techniques and give recommendations on the best analytical practices.

After having shown the limitations of traditionally used identification methods for *Campylobacter* and having highlighted the usefulness of the matrix-assisted laser desorption time-of-flight mass spectrometry (MALDI-TOF MS) for their identification, we evaluated the influence of several growth conditions on the accuracy of the MALDI-TOF MS *Campylobacter* identification (Martiny et al., 2011).

Five reference strains including 2 *Campylobacter coli* and 3 *Campylobacter jejuni* (covariate 'species') were cultured on five culture media (covariate 'medium'; Campy-BAP®, Becton Dickinson, Erembodegem, Belgium; Karmali® Thermo Fisher Scientific, Erembodegem, Belgium; Butzler®, BioRad, Nazareth, Belgium; Campylosel® and Columbia, bioMérieux, Marcy l'Etoile, France), applying two temperatures (covariate 'temperature'; 37 °C versus 42 °C) and two systems generating microaerobic conditions (covariate 'atmosphere'; Anoxomat® jars, Mart Microbiology, Drachten, The Netherlands versus Binder CB150 incubators, VWR, Louvain, Belgium). The antimicrobial compositions of each selective medium were as follows: Campy-BAP® amphotericin B 2.0 mg/L, cephalothin 15.0 mg/L, trimethoprim 5.0 mg/L, vancomycin 10.0 mg/L, polymyxin B 2500.0 units/L; Karmali® cefoperazone 32 mg/L, vancomycin 20 mg/L, cycloheximide 100 mg/L; Butzler® cefoperazone, rifampicin, amphotericin B and colistin. The manufacturers provided no information regarding the concentration of the antimicrobial agents included in the Butzler® medium and the antimicrobial composition of the Campylosel® medium. The gaseous mixtures provided by the incubator and jars were 10% CO_2–5% O_2–85% N_2 and 10% CO_2–10% H_2–80% N_2, respectively.

The resulting one hundred bacterial cultures were examined at five incubation times, from 24 to 120 h on an Axima® Assurance mass spectrometer (Shimadzu) according to manufacturer's instructions and the spectra were compared to both IVD (VITEK® MS, bioMérieux) and RUO (SARAMIS, bioMérieux) databases.

A statistical model was built to investigate the effect of the growth condition covariates on the probability of correct bacterial species identification (P(cid)), taking into account the fact that, for each culture plate, repeated measures were obtained at 5 consecutive time points.

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For this longitudinal analysis, we used generalized estimating equation (GEE) models with a positive identification as the binary outcome variable, a logit link for the mean and an AR(1) correlation structure for the 5 repeated measurements on each plate, which were linked by assigning a unique identifier (ID) to each plate. An initial model for the probability of a correct identification was postulated as follows:

$$\begin{aligned}\text{logit}(P(\text{cid})) = {} & \alpha + \beta_1\ \text{Tm} + \beta_2\ \text{Spec.} + \beta_3\ \text{Atm.} + \beta_4\ \text{Medium} \\ & + \beta_5\ \text{Temp.} + \beta_6\ \text{Tm} \times \text{Spec.} + \beta_7\ \text{Tm} \times \text{Atm.} \\ & + \beta_8\ \text{Tm} \times \text{Medium} + \beta_9\ \text{Tm} \times \text{Temp.} + \varepsilon\end{aligned} \quad (1)$$

where α is the intercept, β's are the other model coefficients, ε is the error term and Tm is the time in hours; this model assumes that all trends over time are linear on the logit scale (a quadratic time trend was evaluated but not retained).

As we aim to evaluate all covariates regarding their effect on P(cid), all covariates were included in this model regardless of their significance. However, we applied model selection criteria when expanding the model with two-covariate interactions. After forward selection (with inclusion criterion $p \leq 0.05$), two pairs of interaction terms were retained, yielding the final model:

$$\begin{aligned}\text{logit}(P(\text{cid})) = {} & \alpha + \beta_1\ \text{Tm} + \beta_2\ \text{Spec.} + \beta_3\ \text{Atm.} + \beta_4\ \text{Medium} \\ & + \beta_5\ \text{Temp.} + \beta_6\ \text{Tm} \times \text{Spec.} + \beta_7\ \text{Tm} \times \text{Atm.} \\ & + \beta_8\ \text{Tm} \times \text{Medium} + \beta_9\ \text{Tm} \times \text{Temp.} + \beta_{10}\ \text{Spec.} \\ & \times \text{Atm.} + \beta_{11}\ \text{Tm} \times \text{Spec.} \times \text{Atm.} + \beta_{12}\ \text{Spec.} \\ & \times \text{Temp.} + \beta_{13}\ \text{Tm} \times \text{Spec.} \times \text{Temp.} + \varepsilon.\end{aligned} \quad (2)$$

Although model selection and evaluation were carried out independently for the IVD and RUO database outcomes, one final model was retained for both outcomes.

All analyses were carried out with SAS/STAT software v.9.2 (SAS Institute Inc., Cary, NC, USA), using PROC GENMOD for the GEE analyses.

Averaged over all growth conditions, the observed proportion of correct identifications was higher for the IVD VITEK MS database (85.4%) than for the RUO database (62.6%). We therefore focus on the IVD database results hereafter. The proportion of correct identifications showed a marked decrease when going from 24 h to 120 h, both for IVD (from 98 to 72%) and RUO (from 87 to 40%) databases, which was expected (Williams et al., 2003). The total number of correct species identifications obtained with the IVD system is shown in Table 1, in relation to the values of the four covariates considered in our study.

The estimated evolution in P(cid) according to the final longitudinal model is shown in Figs. 1 and 2 and Table 2. For each covariate, a reference value was determined according to our routine practice (incubator, Butzler medium, 42 °C and *C. jejuni*). In the figures, covariates that are not evaluated are kept at their reference value.

Table 1
Number of correct species identification results obtained by the IVD database, in relation to the values of the four covariates considered in our study.

IVD		n[1]	24 h	48 h	72 h	96 h	120 h
Species	*C. jejuni*	60	60	57	58	41	38
	C. coli	40	38	36	32	33	34
Atmosphere	Jar	50	49	45	48	36	34
	Incubator	50	49	48	42	38	38
Temperature	37 °C	50	49	44	45	40	45
	42 °C	50	49	49	45	34	27
Medium	Columbia	20	20	17	18	15	14
	Campy-BAP	20	20	20	20	19	18
	Campylosel	20	20	20	18	15	15
	Karmali	20	18	20	18	14	16
	Butzler	20	20	16	16	11	9

[1] Number of bacterial cultures.

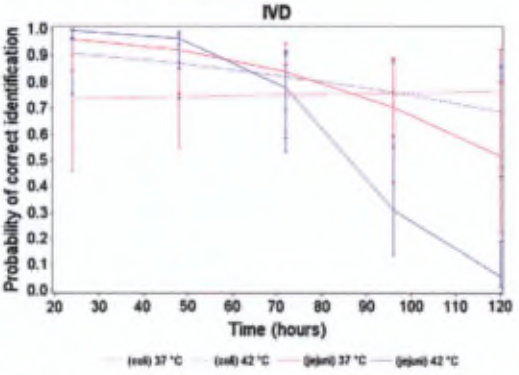

Fig. 1. Estimated evolution of the probability of correct identification (P(cid)) for 37 °C vs. 42 °C (separately for *C. coli* and *C. jejuni*). Other covariates were kept at their reference values (open atmosphere, Butzler medium). Estimated probabilities were obtained from Model (2), bars represent the 95% prediction interval.

Because 24 h and 72 h time points represent the daily practice and weekend conditions respectively, observations at these incubation times are our primary interest.

When considering the IVD database, no significant difference on the estimated P(cid) was shown according to the atmosphere generating system used (Table 2). The decision to choose a microaerobic atmosphere generating system instead of another for *Campylobacter* growth therefore can be based solely upon other criteria including isolation performance of such system, economical or practical considerations (Wang et al., 1982).

The combined effect of temperature and species on the estimated P(cid) is given in Fig. 1. For both *C. jejuni* and *C. coli*, which are clinically the most encountered *Campylobacter* species, the P(cid) after 24 h of incubation was higher when the strain was incubated at 42 °C ($p = 0.049$ and $p = 0.048$, respectively; Table 2). This observation was consistent with the optimal incubation temperature for isolation of both *C. jejuni* and *C. coli*. Moreover, oxidation of the substrates seems to be greater at 42 °C than at 37 °C (Line et al., 2010). At 72 h these differences no longer were significant. In our laboratory, selective media are incubated at 42 °C whereas non-selective media used for the filtration method – and subsequent isolation of non-*jejuni/coli Campylobacter* – are incubated at 37 °C (Lastovica, 2006).

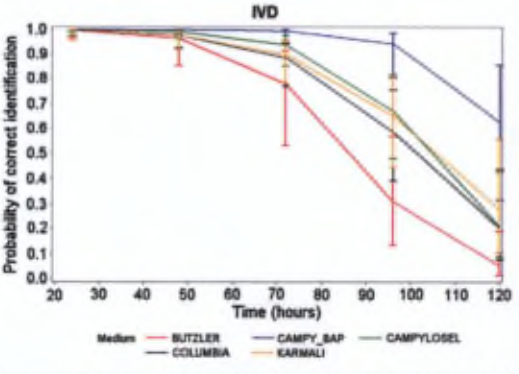

Fig. 2. Estimated evolution of the probability of correct identification (P(cid)) for the 5 growth media, with other covariates at their reference values (open atmosphere, 42 °C, *C. jejuni*). Estimated probabilities were obtained from Model (2), bars represent the 95% prediction interval.

Table 2
Evaluation of the influence of growth parameters on the estimated probability of correct identification (P(cid)) at two incubation times (24 h and 72 h) obtained from Model (2) on the IVD results. Odds ratios are compared to the reference value given; for atmosphere and medium, these depend on the species as well.

		24 h			72 h		
		Odds ratio	Odds ratio confidence interval (95%)	p-Value	Odds ratio	Odds ratio confidence interval (95%)	p-Value
Atmosphere (jar versus incubator)	*C. jejuni*	0.835	[0.127–5.484]	0.85	1.177	[0.419–3.306]	0.76
	C. coli	1.783	[0.538–5.915]	0.34	0.588	[0.286–1.210]	0.15
Temperature (37 °C versus 42 °C)	*C. jejuni*	0.118	[0.014–0.998]	0.049	1.482	[0.486–4.518]	0.49
	C. coli	0.286	[0.083–0.988]	0.048	0.649	[0.310–1.361]	0.25
Medium (versus Butzler)	Campylosel	4.117	[0.994–17.05]	0.051	4.367	[1.830–10.42]	0.0009
	Campy-BAP	79.00	[1.010–5674]	0.045	47.76	[5.639–404.5]	0.0004
	Karmali	0.970	[0.257–3.667]	0.96	2.545	[1.121–5.780]	0.026
	Columbia	1.105	[0.286–4.266]	0.88	2.212	[1.006–4.861]	0.048

The effect of medium on the estimated P(cid) of *C. jejuni* is presented in Fig. 2. There was no species × medium interaction in Model (2), and a similar pattern was obtained for *C. coli* (data not shown). In terms of "medium influence" the Campy-BAP showed a significantly different P(cid) at 24 h of incubation compared to the Butzler medium. Given the high P(cid) observed at 24 h of incubation irrespective of the medium used, for routine daily practice one can choose a selective *Campylobacter* agar upon additional parameters like specificity and sensitivity. For weekend and holiday regimens, all four other tested media had a significantly higher P(cid) compared to the Butzler medium (Table 2).

In summary, significant differences in the probability of correct identification (P(cid)) for *Campylobacter* species using the MALDI-TOF MS were highlighted related to procedures including the growth medium, the incubation temperature, time at interpretation (from 24 h to 120 h), and the database applied. Given the differences between *C. jejuni* and *C. coli*, the effect of altering procedures should be evaluated for every single bacterial species and taken into account for further accurate MALDI-TOF MS research. Standardization of the growth conditions might also be helpful in inter-laboratory comparisons (Horneffer et al., 2004).

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Part of these results was previously presented at the 16th congress on *Campylobacter*, *Helicobacter* and related organisms in Vancouver (CHRO 2011).

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4.7. Subtype determination of *Blastocystis* isolates by Matrix-Assisted Laser Desorption/Ionisation Time-of-Flight Mass Spectrometry (MALDI-TOF MS)

Martiny D, Bart A, Vandenberg O, Verhaar N, Wentink-Bonnema E, Moens C, van Gool T

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ARTICLE

Subtype determination of *Blastocystis* isolates by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS)

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Abstract The pathogenic role of the enteric parasite *Blastocystis* remains controversial. Recent studies have suggested that various subtypes (STs) found in human samples could be correlated to the presence or absence and variability of clinical manifestations, and that STs can differ with respect to drug sensitivity. Polymerase chain reaction (PCR) techniques used to determine these STs are expensive and are usually restricted to research laboratory settings. This study evaluates the potential application of the inexpensive matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) technique to discriminate *Blastocystis* STs. A database of parasitic protein signatures was constructed for five *Blastocystis* STs, and the reference spectra were challenged with those from 19 axenic cultures of ST1, ST2, ST3, ST4 and ST8 and those from nine xenic liquid cultures of ST3 and ST4. Samples from axenic cultures were prepared using standard formic acid extraction and direct deposition procedures. The reference spectra revealed five distinct spectral profiles, and the database library allowed for discrimination between all of the cultures with reliability indices ranging from 2.038 to greater than 2.8 when an extraction was performed. The direct deposition procedure resulted in greater variability in the discrimination and direct MALDI-TOF MS identification from xenic liquid cultures was effective in 3 out of 9 samples. MALDI-TOF MS proved to be an effective technology for efficiently discriminating *Blastocystis* STs in axenic cultures.

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Introduction

Blastocystis spp. are among the most commonly observed intestinal parasites in humans in both industrialised and developing countries, with higher infection rates in the latter population due to poor sanitary conditions [6, 39]. A zoonotic transmission route has been suggested, but anthroponotic faeco-oral transmission appears to be the main transmission route [2, 21, 28]. Despite abundant clinical and epidemiological studies, the clinical significance of *Blastocystis* has long been controversial [7, 27]. For example, *Blastocystis* may provoke various non-specific symptoms, including diarrhoea, abdominal pain, nausea and/or vomiting, constipation, flatulence, fatigue and skin rash, but it is also frequently found in asymptomatic patients. *Blastocystis* may also be involved in chronic gastrointestinal illnesses such as chronic diarrhoea, irritable bowel syndrome and inflammatory bowel disease [9–11, 22, 36, 46]. Conflicting views on its pathogenicity may be related to the amount of infecting parasites, the age of the patient when infection occurs, the nature of infection (acute or chronic) and the strain subtype (ST) or species. In contrast to the earlier concept that only one species, *Blastocystis hominis*, was involved in human infections, it has now been well established that humans can be infected with one or more of nine different *Blastocystis* STs or species [28, 31, 36]. This finding generated a strongly renewed interest in this group

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of parasites, especially with respect to potential differences in pathogenicity, epidemiology and transmission between the different STs [6, 8–11, 24, 27, 35, 46]. The predominant STs observed in humans are ST3, ST1, ST2 and ST4, with a prevalence of 44, 28, 14 and 13 %, respectively, in our hospital setting in Brussels [3, 11, 21, 24, 28]. *Blastocystis* STs have been shown to differ in their in vitro susceptibility to antibiotics; for example, Mirza et al. [19] demonstrated the ST-dependent susceptibility of *Blastocystis* to six different antiprotozoal agents. To date, there is no consensus regarding an appropriate treatment [7, 32, 41].

Microscopy techniques are widely used for the diagnosis of *Blastocystis* in clinical samples [8]. However, the rapid deterioration of the parasite outside the body and the irregular shedding of the parasite in infected patients can lead to false-negative results [42]. The combined use of multiple sampling and SAF Fixative in clinical practice can considerably increase the recovery of *Blastocystis* [40]. Xenic in vitro culture, which amplifies the parasite number, is also a sensitive and cost-effective screening tool [33, 47]. For ST classification, DNA sequence analysis is needed, which is time consuming and requires specific expertise that is not always available in the clinical microbiology laboratory. No consensus regarding subtyping methodology has emerged, although a recent study suggested that SSU-rDNA barcoding is the method of choice [30]. Therefore, *Blastocystis* subtyping to date has been limited to research laboratories. An easy-to-use, standardised method for *Blastocystis* subtyping that can be implemented in routine use would provide a valuable tool for the clinical laboratory.

Recently, clinical microbiology has been revolutionised by the commercialisation of matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) [5, 17, 23]. This technique allows for the rapid, accurate and inexpensive identification of microorganisms from cultures or directly from biological samples. MALDI-TOF MS also presents the advantage of not requiring large amounts of biological materials and is, therefore, highly useful for the identification of slow-growing and exigent organisms [12, 18]. The efficiency of MALDI-TOF MS identification is currently expanding as new database libraries become available and as databases libraries can also be updated by users. This technique is now used with greater frequency in routine laboratories and is expected to soon replace traditional identification tools.

In parasitology, the application of MALDI-TOF MS has been limited to obtaining general parasitic proteome studies [14, 44] and the characterisation of specific biomarkers for discriminating between environmental and human *Cryptosporidium* and *Giardia* species for water management [16, 43]. The diagnostic use of MALDI-TOF MS in parasitology has remained limited, only being used for serum peptide profiling of mice infected with *Leishmania* [15] and the discrimination of microsporidian isolates grown on cell cultures [20].

The main aim of this present study was to evaluate the use of MALDI-TOF MS for the identification and differentiation of *Blastocystis* STs commonly present in clinical samples.

Materials and methods

Blastocystis isolates

Nineteen *Blastocystis* isolates from 19 patients who submitted stool samples for routine parasitological examination to the Clinical Parasitology Laboratory (Academic Medical Center, Amsterdam) were included in the study. *Blastocystis* isolates were axenically cultured on solid media according to a protocol described by Tan et al. [37]. In short, fresh, unfixed stool samples of patients with *Blastocystis* species were initially cultured and subcultured in liquid Isocove's Modified Dulbecco's Medium (IMDM, Gibco) supplemented with 20 % horse serum and antibiotics (100 U/mL penicillin, 2 mg/mL cefotaxime, 0.5 mg/mL amoxicillin and 0.1 mg/mL streptomycin) and incubated in anaerobic jars (Compact Anaerobic Work Station, DW Scientific) at 37 °C. When high loads of *Blastocystis* were observed (>15 cells of vacuolar form of *Blastocystis* per 400× field), 250 μl of the sediment was plated on agar plates with IMDM, 20 % horse serum, 1 % Bacto agar (Becton Dickinson, Breda, The Netherlands) in anaerobic jars, at 37 °C, for 7 days. Colonies of *Blastocystis* repeatedly were transferred to new agar plates until axenic cultures were obtained.

Additionally, nine xenic cultures from 9 patients with *Blastocystis* spp. were examined with MALDI-TOF MS after liquid culture in IMDM medium as described above.

Determination of *Blastocystis* STs

All of the *Blastocystis* STs were identified by the analysis of partial SSU-rDNA sequences and comparison with GenBank sequences using the nomenclature of Stensvold et al. [4, 31]. Five different STs were identified among the 19 isolates cultured on agar, including ST1 ($n=4$), ST2 ($n=4$), ST3 ($n=3$), ST4 ($n=7$) and ST8 ($n=1$) (Fig. 1). The nine *Blastocystis* isolates grown on liquid media corresponded to ST3 ($n=6$) and ST4 ($n=3$).

Sample preparation for MALDI-TOF MS analysis

From agar cultures, deposits were prepared in quadruplicate according to both the ethanol/formic acid extraction and the direct deposition procedures described by the manufacturer.

Using the ethanol/formic acid extraction procedure, colonies for identification were suspended in 300 μl of de-ionised water and 900 μl of absolute ethanol was then added. After a first centrifugation step (2 min at 16,600×g), the pellet was washed with de-ionised water and resuspended in 50 μl of

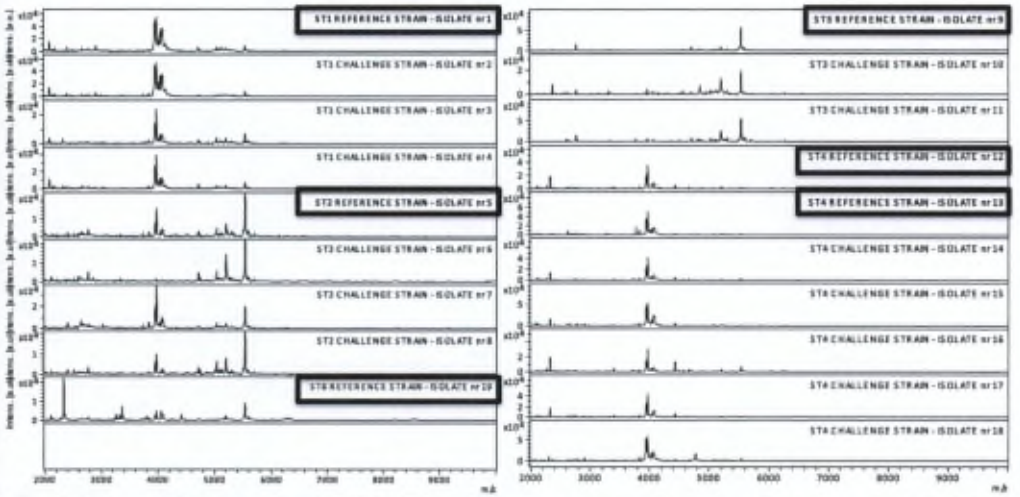

Fig. 1 Spectra acquired from axenic cultures ($n = 19$). Reference strains are shown in the *bases*; challenge strains follow the corresponding reference strain. Left: spectra from ST1, ST2 and ST8; right: spectra from ST3 and ST4. Mass range: 2,000–10,000 m/z

70 % formic acid and 50 µl of pure acetonitrile. After a second centrifugation step (2 min at 16,600 × g), 1 µl of supernatant was spotted onto a target plate.

Using the direct deposition procedure, the colonies for identification were directly smeared onto the target plate without any pre-treatment. When dried, each deposit was covered with 1 µl of α-cyano-4-hydroxycinnamic acid (CHCA) matrix solution.

For each liquid culture medium, a pellet was obtained by the centrifugation of 1 ml of the medium for 1 min at 16,600 × g. The pellet was washed with 1 ml of de-ionised water that was discarded after a second short centrifugation at 16,600 × g (1 min). When dried, the pellet was treated as a colony following the ethanol/formic acid extraction procedure.

MALDI-TOF MS analysis

The analyses were performed at the Porte de Hal laboratory in Brussels on a Microflex LT system using the pre-programmed MBT_FC.par method (Bruker Daltonics, Bremen, Germany). In brief, the spectra were recorded in linear positive mode at a laser frequency of 20 Hz and over a mass range of 2 to 20 kDa. For each spectrum, 240 laser shots in 40-shot steps at different positions of the deposit were acquired in an automatic mode using AutoXecute acquisition control software (flexControl 3.0; Bruker Daltonics, Bremen, Germany). The voltage of ion sources 1 and 2 were set at 20.06 and 16.80 kV, respectively. The lens voltage was set at 7.63 kV. The minimum base peak resolution was set at 400 Da. The spectra were subsequently analysed using MALDI Biotyper Automation Control and Biotyper 2.0 software (Bruker Daltonics, Bremen, Germany). At the time of analysis, the database library included 3,995 spectra. Parasitic signatures were not available in the commercially available database library.

Reference spectra generation

All 19 isolates were candidates for inclusion in a parasite-specific database that included five *Blastocystis* STs: ST1, ST2, ST3, ST4 and ST8. The final selection of reference spectra for integration into the specific database was determined based on the ability of the spectra to correctly discriminate *Blastocystis* STs. To create the reference spectra, the ethanol/formic acid extraction procedure was used as previously described. For each new reference spectra, 1 µl of the supernatant was deposited at eight positions on the target plate. When dried, each deposit was then recovered using 1 µl of the CHCA matrix solution. The acquisition of the spectra was processed three times for each deposit in an automatic mode. The spectral quality of the 24 resulting spectra for each new reference was assessed using flexAnalysis 3.0 software (Bruker Daltonics, Bremen, Germany). Raw spectra were transferred to Biotyper 2.0 (Bruker Daltonics, Bremen, Germany) to smooth the raw intensity spectra and subtract the baseline. The reference spectra were then created using the default settings of the software. Different combinations of reference spectra were tested to create an optimised database.

Evaluation of the MALDI-TOF MS subtyping power

The combination of both the commercial database and the specific database (4,001 reference spectra) was first evaluated

using the reference isolates selected for the creation of the library and was subsequently challenged with the non-reference isolates obtained from agar media. Indicators of the quality of the subtyping power in the present study were the correlation with the expected ST classification, as determined by an analysis of the SSU-rDNA sequence, and the generation of an identification score of ≥2, as recommended by the manufacturer for reliable MALDI-TOF MS identification of a microorganism at the species level from a clinical perspective.

Results

Using the ethanol/formic acid extraction procedure, the 19 *Blastocystis* isolates yielded well-resolved proteic profiles in the studied mass range, suggesting that reliable ST differentiation could be performed using MALDI-TOF MS (Fig. 2).

The combination of reference spectra from six isolates (isolate 38A for ST1, isolate 222 for ST2, isolate 204 for ST3, isolates 25 and 131 for ST4, and isolate 17C for ST8) led to a satisfactory discrimination of isolates for the five *Blastocystis* STs and constituted the optimal database.

This optimised database was queried using spectra acquired from all 19 of the axenically cultured isolates to validate the potential for ST differentiation. For the two best deposits of each isolate, the identification results and associated scores obtained using the ethanol/formic acid extraction procedure and the direct deposition procedure are presented in Tables 1 and 2, respectively. The results obtained for the four deposits are available in the Supplementary material.

As expected, the isolates that were used as references in the specific database led to satisfactory identification results, with scores greater than 2.7 for all four deposits when using the ethanol/formic acid extraction procedure. For these identifications, the gaps between the first and second matches were substantial (>1), resulting in a good discrimination of these organisms from other organisms included in the specific and commercially available databases.

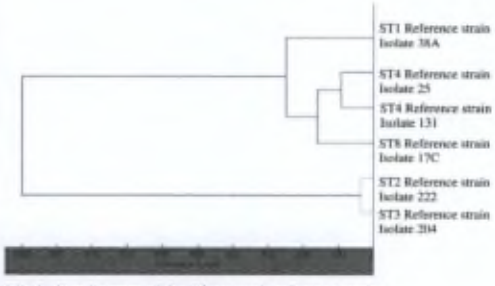

Fig. 2 Dendrogram of the *Blastocystis* reference strains

Using the direct deposition procedure, the isolates that were used to create the reference spectra were correctly assigned to the different STs but with lower score values (ranging from 1.813 to 2.494 for the best of the four deposits).

When the specific database was challenged with 13 non-reference isolates, the MALDI-TOF MS spectrum of each isolate was assigned to the correct ST when using the ethanol/formic acid extraction procedure. In all but one case (isolate 7A, fourth deposit), the observed identification score value was greater than 2. In all cases, no misidentification or confusion with another ST was observed based on the critical gaps between the score values of the first and second identification matches.

The analysis of the same isolates using the more rapid direct deposition procedure again showed satisfactory results with a correct assignment of each tested isolate to its corresponding ST. However, the score values were below the cut-off value of 2 and, in some cases, the gap between the first discrepant identification matches was limited (<0.3), which would not be considered a significant result in a clinical laboratory practice.

The MALDI-TOF MS results were more variable for the nine xenic liquid media samples. Three cultures with abundant *Blastocystis* growth—as observed by microscopy—yielded correct identifications. However, in four cultures with limited *Blastocystis* growth, other bacterial and fungal species were identified. In all cases, the identification score values for spectra obtained directly from the liquid media were lower than those observed from the agar cultures. The identification results of the two best deposits generated from each liquid growth medium are presented in Table 3. The results obtained for the four deposits are available in the Supplementary material.

Discussion

The pathogenicity of *Blastocystis* in humans could be related to the different STs of this parasite [9, 10, 24, 27, 35, 36, 38]. The study of the differences in pathogenicity, epidemiology and transmission between the different *Blastocystis* STs, therefore, presents a new challenge for researchers in parasitology. In the present study, MALDI-TOF MS appears to be an efficient method for the discrimination between *Blastocystis* STs commonly found in humans. To our knowledge, the determination of *Blastocystis* STs with MALDI-TOF MS has not been reported before.

The 19 tested strains were all correctly associated with the correct ST when compared with the SSU-rDNA analysis. In consequence, we assume that MALDI-TOF MS can compete with molecular typing in terms of analytical performance, rapidity and cost. The ethanol/formic acid procedure led to better results than the direct deposition procedure, which was as expected. For example, using the direct deposition procedure,

Table 1 Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) results obtained from axenic cultures using the ethanol/formic acid protocol ($n = 19$). Reference strains are in **bold** (isolates 38A, 222, 204, 25, 131 and 17C); challenge strains (isolates 52, 177A, B17, 102, 189, 215, 112, 9, 131, 208, 31A, 116A, 181A and 7A) follow the corresponding reference strain in the table

				Deposit 1				Deposit 2			
				1st match		2nd match		1st match		2nd match	
	No.	Isolates	ST	ID	Score	ID	Score	ID	Score	ID	Score
Extraction	**1**[a]	**38A**	**1**	**ST1**	**2.876**	**ST4**	**1.703**	**ST1**	**2.852**	**ST4**	**1.770**
	2	52	1	ST1	2.510	ST2	1.770	ST1	2.486	ST4	1.858
	3	177A	1	ST1	2.196	ST2	1.737	ST1	2.141	ST2	1.816
	4	B17	1	ST1	2.308	ST2	1.755	ST1	2.308	ST4	1.683
	5[a]	**222**	**2**	**ST2**	**2.777**	**ST1**	**1.703**	**ST2**	**2.749**	**ST1**	**1.672**
	6	102	2	ST2	2.249	ST3	1.574	ST2	2.194	ST3	1.625
	7	189	2	ST2	2.478	ST1	1.638	ST2	2.456	ST1	1.833
	8	215	2	ST2	2.552	ST3	1.892	ST2	2.522	ST3	1.758
	9[a]	**204**	**3**	**ST3**	**2.836**	**ST2**	**1.542**	**ST3**	**2.824**	**ST2**	**1.661**
	10	112	3	ST3	2.140	ST2	1.389	ST3	2.130	ST2	1.457
	11	9	3	ST3	2.448	ST2	1.921	ST3	2.425	ST2	1.878
	12[a]	**25**	**4**	**ST4**	**2.799**	**ST4**	**1.926**	**ST4**	**2.794**	**ST4**	**1.850**
	13[a]	**131**	**4**	**ST4**	**2.814**	**ST4**	**1.935**	**ST4**	**2.809**	**ST4**	**1.829**
	14	20B	4	ST4	2.372	ST4	1.892	ST4	2.281	ST4	1.848
	15	31A	4	ST4	2.381	ST4	2.168	ST4	2.348	ST4	2.221
	16	116A	4	ST4	2.494	ST4	1.724	ST4	2.450	ST8	1.716
	17	181A	4	ST4	2.443	ST4	1.686	ST4	2.438	ST4	1.676
	18	7A	4	ST4	2.038	ST1	1.691	ST4	2.032	ST4	1.770
	19[a]	**17C**	**8**	**ST8**	**2.863**	**ST4**	**1.764**	**ST8**	**2.808**	**ST4**	**1.664**

[a] Reference strains

metabolite, pigments and/or agar material at the surface of the microorganism may interfere with the crystallisation process and lead to lower score values; this may be avoided by using a more pure extracted protein preparation [1]. A better disruption of the parasitic membrane and concomitant liberation of proteins using a formic acid solution may also explain the differences observed between both procedures, as the finely coated surface of *Blastocystis* has been demonstrated to evolve with the age of the culture [37]. Finally, the differences observed may also be explained by the fact that the reference spectra were created using the extraction procedure and it could be interesting to also evaluate reference spectra created using the direct deposition procedure.

The intra-ST variability, previously reported by Stensvold et al. [26], was highlighted during the selection of the reference spectra to be included in the database, as two reference spectra were required to correctly identify all isolates from ST4. This observation suggests that the created database will likely continue to evolve in the future: reference spectra for ST not included here should be added, although the selection of reference spectra could be modified (spectra addition/restriction) to better fit the diversity of circulating strains. Regardless, this initial database including six reference spectra from five *Blastocystis* STs provided promising results. The culture of *Blastocystis* on agar media remains problematic and is limited to research laboratories; thus, liquid xenic cultures of *Blastocystis* are frequently used for diagnosis from clinical samples [29, 47]. Building upon the satisfactory MALDI-TOF MS identification of *Salmonella* directly from enrichment broths [25], nine liquid xenic cultures of *Blastocystis* were tested by MALDI-TOF MS in the present study. From the nine tested liquid xenic cultures of *Blastocystis*, only two resulted in proper determination of the *Blastocystis* ST. Our results suggested that the identification of *Blastocystis* spp. in such broths is highly dependent on the ratio of parasitic biomass to that of co-cultivated bacterial flora. Similar problems have been reported in other studies that evaluated the use of MALDI-TOF MS for directly identifying organisms from human clinical samples and enrichment broths [13]. As previously demonstrated in other studies, several parameters can affect the quality of the MALDI-TOF MS identification from clinical samples, including the pathogen inoculum, the presence of other microorganisms and the nature of the sample. Our results suggest that it would be useful to submit a selection of the liquid cultures for MALDI-TOF MS analysis to avoid the time-consuming analysis of poorly growing cultures, as has been proposed for the management of urine samples in a clinical practice [45]. A larger study is, nevertheless, required to better evaluate the *Blastocystis* subtyping potential of MALDI-TOF MS analyses from liquid media and the possible merits of adapted cut-off values [34]. The development of an efficient and standardised pre-processing protocol to discard interfering substances is required to allow for the detection of

Table 2 MALDI-TOF MS results obtained from axenic cultures using the direct deposition protocol ($n=19$). Reference strains are in **bold** (isolates 38A, 222, 204, 25, 131 and 17C); challenge strains (isolates 52, 177A, B17, 102, 189, 215, 112, 9, 131, 208, 31A, 116A, 181A and 7A) follow the corresponding reference strain in the table

				Deposit 1				Deposit 2			
				1st match		2nd match		1st match		2nd match	
	No.	Isolates	ST	ID	Score	ID	Score	ID	Score	ID	Score
Direct deposition	**1**[a]	**38A**	**1**	**ST1**	**2.310**	**ST2**	**1.731**	**ST1**	**2.205**	**ST2**	**1.767**
	2	52	1	ST1	2.127	ST4	1.870	ST1	2.095	ST4	1.906
	3	177A	1	ST1	2.097	ST2	1.827	ST1	2.092	ST2	1.885
	4	B17	1	ST1	2.244	ST2	1.820	ST1	2.168	ST2	1.817
	5[a]	**222**	**2**	**ST2**	**1.934**	**ST1**	**1.676**	**ST2**	**1.915**	**ST3**	**1.667**
	6	102	2	ST2	2.072	ST3	1.760	ST2	1.981	ST3	1.582
	7	189	2	ST2	1.952	ST3	1.511	ST2	1.937	ST3	1.516
	8	215	2	ST2	1.954	ST1	1.473	ST2	1.878	ST3	1.657
	9[a]	**204**	**3**	**ST3**	**2.467**	**ST2**	**1.639**	**ST3**	**2.466**	**ST2**	**1.436**
	10	112	3	ST3	1.965	ST2	1.424	ST3	1.797	ST2	1.234
	11	9	3	ST3	2.088	ST2	1.676	ST3	2.014	ST2	1.365
	12[a]	**25**	**4**	**ST4**	**1.813**	**ST4**	**1.705**	**ST4**	**1.705**	**ST4**	**1.701**
	13[a]	**131**	**4**	**ST4**	**2.494**	**ST4**	**1.615**	**ST4**	**2.408**	**ST4**	**1.572**
	14	208	4	ST4	1.805	ST4	1.741	ST4	1.707	ST4	1.470
	15	31A	4	ST4	2.098	ST4	1.732	ST4	1.974	ST4	1.797
	16	116A	4	ST4	1.821	ST4	1.800	ST4	1.631	ST4	1.578
	17	181A	4	ST4	1.837	ST4	1.774	ST4	1.788	ST4	1.422
	18	7A	4	ST4	1.899	ST1	1.569	ST4	1.883	ST4	1.525
	19[a]	**17C**	**8**	**ST8**	**1.918**	**ST4**	**1.263**	**ST8**	**1.836**	**ST1**	**1.485**

[a] Reference strains

Table 3 MALDI-TOF MS results obtained from xenic liquid cultures ($n=9$)

				Deposit 1				Deposit 2			
				1st match		2nd match		1st match		2nd match	
	Ref	ST	Growth in liquid medium[a] (+/++/+++)	ID	Score	ID	Score	ID	Score	ID	Score
Liquid (xenic)	124	3	+++	*Lactobacillus* sp.	1.243	*Pseudomonas* sp.	1.185	*Arthrobacter* sp.	1.227	*Streptococcus* sp.	1.176
	132	3	+++	*E. faecium*	1.762	*E. faecium*	1.618	*E. faecium*	1.687	*E. faecium*	1.593
	135	3	+	*E. faecium*	1.275	*Agromyces* sp.	1.132	*S. maltophilia*	1.228	*E. faecium*	1.038
	136	3	+	*Pseudomonas* sp.	1.393	*Cryptococcus* sp.	1.302	*Candida* sp.	1.391	ST4	1.153
	137B	3	+	*Clostridium* sp.	1.283	*Lactobacillus* sp.	1.199	*Neisseria* sp.	1.250	*Aromatoleum* sp.	1.237
	138A	3	+	*Clostridium* sp.	1.442	*Cryptococcus* sp.	1.320	*Clostridium* sp.	1.412	*Acinetobacter* sp.	1.245
	127	4	+++	ST1	1.628	ST4	1.352	ST1	1.477	ST4	1.184
	130	4	+++	ST4	1.520	ST1	1.246	ST4	1.485	ST1	1.217
	131	4	+++	ST4	1.519	ST1	1.337	ST4	1.408	ST1	1.285

[a] Increasing with the number of crosses

"+": <10 parasites per slide

"+++": >1 parasite per microscopic field

Blastocystis from liquid growth media. In the future, computerisation and statistical algorithms could solve the problem associated with mixed cultures observed when performing the MALDI-TOF MS analyses on clinical samples. Major efforts in terms of optimisation and validation are still needed before implementing such technique in clinical laboratories for daily practice purposes.

The limited number of isolates ($n = 19$) and the resulting small number of isolates per ST (maximum of 7, ST4) are the main limitations of this study. However, to our knowledge, the number of axenic cultures used in this study is, at the moment, the largest set available worldwide.

In the future, the constructed database should be adapted by the addition or removal of reference spectra, to cover the large diversity of *Blastocystis* isolates, and the reproducibility of our preliminary results should be verified using a larger panel of isolates.

Conclusion

Our study demonstrated that matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) can be used for subtyping *Blastocystis* spp. from axenic cultures. Although it may be more time consuming than the direct deposition approach, the ethanol/formic acid extraction procedure is recommended. Larger evaluations are expected to confirm our results and improve the database of parasitic signatures.

Conflict of interest The authors declare that they have no conflicts of interest.

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5. Discussion and perspectives

The main objective of the present work was to evaluate the contribution of MALDI-TOF MS to the microbiology laboratory of four university hospitals.

Contribution of MALDI-TOF MS to routine microbiological diagnosis

First, the performance of MALDI-TOF MS in routine microbiological diagnosis has been evaluated.

Biotyper and VITEK MS were the two main IVD MALDI-TOF MS systems commercially available in Europe at the time of this first study. Their identification processes rely on different algorithms, namely the MSP and ASC methods, respectively (van Belkum et al. 2013).

Both systems were evaluated in comparison with conventional identification techniques commonly used in our laboratory to analyse three bacterial panels that included 1,003 routine isolates, 75 anaerobes and 53 enteric pathogens. With the panel of routine isolates, both systems performed at a similar level, with 92.7% and 93.2% correct identification at the species level by the Biotyper and VITEK MS systems, respectively. Better discrimination between *Bacteroides* species (n=32) was achieved with the Biotyper database, whereas *Streptococcus viridans* (n=8), *Fusobacterium* species (n=12) and enteric pathogens (n=53) were better discriminated by VITEK MS; these differences were the only significant analytical differences observed between the two databases. From a clinical point of view, we postulate that the significant difference observed for *Bacteroides* isolates is the only one that could impact clinical management of the patient. Such a minor difference should not recommend one system over another.

Though both IVD systems use different identification processes, our results suggest that there is currently no best strategy for routine purposes in clinical laboratories. As suggested by Cherkaoui et al., analytical differences between systems result from database construction (Cherkaoui et al. 2010). As databases may be updated, the choice of one system over another should therefore be determined by other parameters including ease of use, cost and integration into the laboratory.

In the future, it would be interesting to see whether the weights attributed to certain peaks in the VITEK MS algorithm allow better discrimination between closely related species compared with when using the Biotyper system; this difference could explain the accurate discrimination between *S. pneumoniae* and *S. mitis* observed in the studies of Branda et al. and Dubois et al. (Branda et al. 2013, Dubois et al. 2012). It should be noted that the VITEK MS system includes a closed database that may not be updated by the user (Westblade et al. 2013). A closed database is required for IVD qualification and/or FDA approval, but RUO systems are of particular interest to university hospitals.

The database is of critical importance and partially explains the supremacy of MALDI-TOF MS over conventional identification techniques. The identification of 234 *Campylobacter* and related organisms revealed major differences between identification systems. MALDI-TOF MS led to 98.3% correct identification at the species level versus only 79.9% and 72.2% after additional testing using the API Campy gallery and the Vitek NH Card (bioMérieux, Marcy l'Etoile, France), respectively. Differences were particularly noticeable for non-jejuni/coli isolates that are less frequently encountered in routine practice. These differences between identification systems result from the differences in the databases, as 10 of the 11 species included in the study were represented in the Biotyper database, whereas only seven and three species were represented in the API Campy gallery and Vitek NH Card databases, respectively. The ease with which MALDI-TOF MS databases can be updated is a major advantage over conventional identification techniques (Seyfarth et al. 2011).

In their study comparing real-time PCR and sequencing, MALDI-TOF MS and conventional identification techniques on a panel of 1,007 *Campylobacter* and related organisms, Bessède et al. observed better performance than us for the identification of *Campylobacter jejuni* (96.7%) and *Campylobacter coli* (97.7%) using conventional identification techniques (*C. jejuni*: 94.4% by API and 89.6% by Vitek; *C. coli*: 73.8% by API and 87.7% by Vitek in our study) (Bessède et al. 2011). These differences may be explained by the fact that these authors combined several biochemical tests, whereas we performed the analysis using commercial systems according to the manufacturer's instructions.

Nevertheless, Bessède et al. also observed a higher rate of misidentification or no identification when using conventional identification techniques compared with MALDI-TOF MS (4.5% vs. 0.4%, respectively) and concluded that the efficient MS technique had now replaced both conventional and molecular techniques in their laboratory for the identification of *Campylobacter* and related organisms. Similar recommendations have been made by our NRC for *Campylobacter* (https://nrchm.wiv-isp.be/fr/centres_ref_labo/campylobacter/Rapports/ Campylobacter%202011.pdf).

Many authors have highlighted the improved performance and other benefits of MALDI-TOF MS compared with conventional identification techniques, especially for less frequently encountered microorganisms (Saffert et al. 2011, Cherkaoui et al. 2011, Kierzkowska et al. 2013). MALDI-TOF MS may considerably improve the microbiological diagnosis and also offers a fast and reliable alternative to expensive molecular techniques, which has previously been demonstrated by Bizzini et al. for "difficult-to-identify" bacterial strains isolated in clinical laboratories (Bizzini et al. 2011). In this study, the authors demonstrated that nearly half of the isolates that usually required sequencing analysis because of identification failure using conventional identification techniques could be identified by MALDI-TOF MS.

Finally, MALDI-TOF MS users should be aware of the technique's limitations (De Bel et al., 2010). Of those limitations, two in particular should be kept in mind. First, the spectra in the database for a given species can inefficiently cover the intraspecies diversity and affect the MALDI-TOF MS patterns (Lartigue et al. 2009). Second, the absence of a species in the database that is closely related to another species present in the database can lead to erroneous identification results (Patel, 2013).

In addition to its analytical benefits, MALDI-TOF MS is also faster and cheaper than conventional identification techniques (Seng et al. 2009). The system requires little biological material, which is particularly important for fastidious and slow growing bacteria. This advantage can be observed when identifying *Campylobacter* and related organisms; a bacterial suspension adjusted to a McFarland standard of 3 was necessary to perform the identification using the Vitek NH Card, whereas a single colony was sufficient to prepare a MALDI-TOF MS deposit.

This difference avoids unnecessary subculturing procedures and saves time. The aspects of workflow adaptation will be discussed later in this work.

Another challenge for microbiologists is the identification of microorganisms directly from clinical samples. To successfully identify bacteria or yeasts from clinical samples by MALDI-TOF MS, several requirements are necessary and include limited interference from the sample matrix and the presence of a single organism with a sufficient concentration. Urine samples and blood cultures are therefore promising candidates for MALDI-TOF MS analysis (Ferreira et al. 2011a,b). Numerous protocols used for the direct identification of bacteria and yeasts from positive blood cultures were not all easily implemented in a routine workflow (Drancourt et al. 2010). Therefore, one of the aims of this study was to develop and evaluate a simple and inexpensive in-house protocol and compare it with the commercial Sepsityper kit (Bruker Daltonics, Bremen, Germany).

Our protocol relies on a saponin-based cell lysis pretreatment adapted from previously described protocols (Ferroni et al. 2010, Ferreira et al. 2011a). Using validated new cutoff criteria, our in-house method led to correct species identification of 73.7% of the isolates within 20 min.
The same analytical performance (68.4%) was observed using the Sepsityper kit. In contrast, conventional identification techniques led to correct species identification in 78% of cases but after a 24-h incubation period.
Although both rapid identification systems showed similar analytical performances, the Sepsityper required a longer preparation time for Gram-positive bacteria and was 10-fold more expensive than the in-house protocol (7.45€ vs. 0.72€ per analysis), which led us to choose the in-house protocol for implementation in our routine workflow.

Our results are in agreement with other studies evaluating the use of MALDI-TOF MS for direct identification of bacteria from positive blood cultures. Better results were obtained for Gram-negative bacteria than Gram-positive bacteria, and only partial information was obtained from mixed cultures (Kok et al. 2011, Loonen et al. 2011, Lagacé-Wiens et al. 2012).

Recently, our protocol was slightly modified by other investigators (addition of a formic acid extraction after the wash steps) and compared with the Sepsityper kit using an updated Biotyper database (4,500 entries) and the VITEK MS database (Chen et al. 2013a). The authors showed no analytical difference between the Sepsityper kit (correct species identification in 81.5% of cases) and the modified in-house protocol (80.4%) and implemented the latter in their laboratory because of its attractive price (1 US$ vs. 15 US$ using Sepsityper).

While MALDI-TOF MS technology is efficient in the identification of microorganisms from urine and blood samples, direct MALDI-TOF MS identification from other clinical samples remains challenging. Sophisticated pretreatment procedures and other MS technologies outside the scope of this work will likely meet these objectives in a near future (Emonet et al. 2010, van Belkum et al. 2013).

Contribution of MALDI-TOF MS to the clinical management of infected patients

After demonstrating the benefits and performance of MALDI-TOF MS compared with conventional and molecular identification techniques for routine and fastidious bacteria and highlighting its potential to identify bacteria from clinical samples, our second objective was to evaluate the impact of the implementation of such technology in our institution on the clinical management of the patient. As sepsis is a major cause of mortality and morbidity, this study focused on patients with positive blood cultures. The theoretical and actual clinical impacts of rapid microbial identification (RMI) from positive blood cultures using our in-house protocol have been evaluated.

It appears clear that improving TTI has no clinical impact if the use of RMI is delayed by the clinician (Emonet et al. 2011); however, only a few studies have evaluated the true impact of RMI from positive blood cultures on the management of suspected sepsis patients (Vlek et al. 2012, Clerc et al. 2013a, Huang et al. 2013).

The prospective analysis of 277 new episodes of sepsis revealed that the Gram stain result led to the modification of patient treatment in 17% of cases and that the RMI may hasten the use of appropriate antimicrobial treatment in more than 13% of cases in the adult population.

Most modifications involved treatment escalation in the general population and treatment de-escalation in the oncological population, which is likely because broad-spectrum antibiotics are most likely used in cancer patients. In the paediatric population, RMI was particularly helpful in quickly confirming contamination by cutaneous bacteria, but it never led to a de-escalation of treatment, likely because there is a reluctance to stop treatment in a patient who is improving (Farrington et al 1990). There is certainly a room for impact improvement that will correlate with better experience and confidence in the RMI approach.

The retrospective analysis revealed good compliance with the IDS's recommendations, as most changes were made before receiving the identification result provided by conventional identification techniques. However, the delay in administering the modified treatment was high (>4 h in 50% of cases), which suggests that communication between health professionals in our institution still must be improved. Huang et al. observed an impact of RMI on the mortality rate (20.3% vs. 14.5%), length of ICU stay (14.9 days vs. 8.3 days) and bacteraemia recurrence rate (5.9% vs. 2.0%). As in our study, the authors also stressed the significant impact of antimicrobial stewardship initiatives (Huang et al. 2013). These observations emphasise the important role of clinical pharmacists who contribute to better coordination of care (Toklu et al. 2013).

The results of our study are in agreement with those reported by Vlek et al. and Huang et al., who performed case-control studies and showed a decrease of 28.8 h and 28.1 h in the TTI, respectively (26.85 h in our study) (Vlek et al. 2012, Huang et al. 2013). An 11.3% increase in the proportion of patients receiving adequate antimicrobial treatment within 24 h was also reported by Vlek et al., which is in agreement with the 13.85% observed in our work (Vlek et al. 2012).

Despite the higher laboratory cost, RMI has been adopted in our daily practice and is still performed twice a day on weekdays because of the clinical benefits for patients. In the future, it would be particularly interesting to further explore the clinical impact of a rapid identification combined with rapid AST.

From a general point of view, Tan et al. reported that MALDI-TOF MS provided identification from routine isolates an average of 1.45 days earlier than conventional identification techniques (Tan et al. 2012). We assume the rapid identification of microorganisms, even if isolated from less precious samples than positive blood cultures, may also improve the clinical management of patients in daily practice.

MALDI-TOF MS in the microbiology laboratory of the future

Rationalisation of health costs requires major changes in hospital reorganisation and the development of strategies for laboratory cost-containment. In Europe, large centralised clinical laboratories are now being developed. The aim of the third part of this work was to determine whether MALDI-TOF MS could be integrated into the microbiology laboratory of the future by evaluating the feasibility of MALDI-TOF MS networking in the university hospitals in Brussels.

From a clinical point of view, we observed a significant decrease in the median TTI, 23 h 12 min (interquartile range 991 min, min-max 0-5,873), using conventional identification techniques versus 5 h 11 min (interquartile range 78 min, min-max 154-547) using MALDI-TOF MS. All identification results were obtained earlier when sharing the MALDI-TOF MS system of the central laboratory (CL) than when performing conventional identifications at the distant laboratory (DL), except when chromogenic culture media or oxidase tests were used. The extra costs related to the use of chromogenic culture media for urine samples, more than 7000€ annually for *E. coli*, is not supported by the clinical benefits, as it is known that *E. coli* is the most frequent pathogen responsible for UTI and that it may be easily recognised by its morphology when grown on classical culture medium. We hypothesise that a preliminary result, such as "probable *E. coli*", could be provided until the MALDI-TOF MS result is available.

From an organisational point of view, only minor transmission (<2%) or human (<1%) errors were observed, which suggests that the INFECTIO_MALDI software in combination with the MALDI-TOF MS technique is sufficiently robust for networking. Moreover, the INFECTIO_MALDI software allows the microbiologists and technologists of each site to be responsible for their own analyses and validations.

In terms of workflow, good communication between the teams in both laboratories is necessary, and the technologists in the CL must be aware of the consequences of any delay in the management of the analyses sent by the DL, which may include insufficient time to perform additional tests in the case of an unreliable MALDI-TOF MS result or a result provided too late to be considered by the clinician.

From a financial point of view, three scenarios were evaluated for the DL: continue with conventional identification techniques, purchase a MALDI-TOF MS system or share the CL MALDI-TOF MS system. The conventional identification annual budget was determined to be approximately 35,000€ versus 40,000€ and 9,000€ for a new MALDI-TOF MS and 21,000€ and 6,000€ for the networking system, before and after depreciation of the instrument, respectively. This result reveals that the use of MALDI-TOF MS would be preferred over conventional identification techniques and that sharing the instrument of the CL would represent a financial benefit of approximately 125,000€ based on the depreciation period of the new system (5 years).

Finally, a few conventional identification techniques, such as optochin and oxidase tests, would still be required to ensure the same quality of patient care from the DL.

To our knowledge, this study is the first to evaluate the feasability of using one MALDI-TOF MS instrument for several hospitals in a network. In an environment characterised by shared processes and laboratory centralisation, this work presents innovative perspectives on clinical laboratories, demonstrating that networking is possible without altering clinical management of the patient and may lead to substantial cost savings.

Our observations also highlight the feasibility of organising a MALDI-TOF MS backup on another clinical site in case of defect or structure relocation, which is now mandatory in terms of quality standards.

MALDI-TOF MS in fundamental microbiology research

To date, most publications have evaluated the performance of MALDI-TOF MS in bacterial and yeast identification. This section describes perspectives for future research and new applications of MALDI-TOF MS in microbiology

First, we evaluated the influence of growth conditions on the quality of MALDI-TOF MS identification of *Campylobacter*. Five reference strains were cultured under different culture conditions in terms of culture medium, temperature, atmosphere and incubation time.

A statistical model was built to determine the effect of the growth condition covariates on the probability of correct species identification taking into account the fact that repeated measurements were obtained on five consecutive days.
This model revealed significant differences in the probability of correct species identification related to the growth procedure used, which demonstrates the need for strict standardisation of growth conditions in the future, especially for further typing scheme evaluations. Our experience working with *Salmonella* strains suggests that storage conditions may also affect the MALDI-TOF MS spectral quality (unpublished data), and a recent study highlighted the ability of MALDI-TOF MS to successfully classify *E. coli* samples according to the growth time of the bacterial cultures, which confirms that this parameter affects MALDI-TOF MS profiles (Momo et al. 2013).

As previously mentioned, bacterial typing is more demanding than routine identification in terms of reproducibility and discriminatory power (Struelens et al. 1996, Croxatto et al. 2012, Dieckmann et al. 2008, Josten et al. 2013), and its limitations are mainly determined by the nature of the bacteria profiled, as some bacteria are highly different and others are almost indistinguishable at the subspecies level (Sandrin et al. 2013).

This study provides an overview of the growth parameters that could be optimised to develop MALDI-TOF MS typing schemes for *Campylobacter* in the future in the frame of our NRC. Despite first promising results for *Campylobacter jejuni* (Martiny et al. 2012; appendix 9.3), we theorise that MALDI-TOF MS typing of such microorganisms will require better standardisation and will remain particularly challenging.

In the last study of this work, we aimed to identify and subtype *Blastocystis* STs commonly found in clinical samples using MALDI-TOF MS, which has not been performed previously.

Blastocystis is the most frequent parasite responsible for enteric diseases in industrialised countries (Vandenberg et al. 2006). It has been well established that one or more of the nine different *Blastocystis* STs can infect humans, which has generated renewed interest in this parasite, especially regarding potential differences in pathogenicity, epidemiology and transmission between the different STs. Moreover, Mirza et al. found that *Blastocystis* STs differ in their susceptibility to antimicrobial agents (Mirza et al. 2011). For ST classification, DNA sequence analysis is needed, which is expensive and requires specific expertise not always available in clinical laboratories.

To evaluate the potential of MALDI-TOF MS for discriminating between *Blastocystis* STs, 19 axenic strains were analysed, six of which were selected as reference strains to build a specific database of parasitic protein signatures. This database thus included six reference strains covering five *Blastocystis* STs commonly found in clinical samples: ST1, ST2, ST3, ST4 and ST8. In humans, ST3, ST1, ST2 and ST4 are the predominant STs, with prevalences of 44%, 28%, 14% and 13%, respectively, in our hospital setting in Brussels.

When selecting the reference spectra for the creation of the specific database, two reference spectra appeared to be necessary to correctly identify all ST4 isolates included in the study as the correct ST. This result illustrates the intra-ST variability previously reported by Stensvold et al. and suggests that our database will likely continue to evolve in the future to better fit with the diversity of circulating strains (spectra addition/restriction) (Stensvold et al. 2012).

Using the ethanol/formic acid extraction procedure, all 19 isolates were correctly identified as the correct ST and showed reliable score values (>2). No misidentification or "doubtful" ST determination was observed.

Additionally, nine liquid xenic cultures were tested, yielding more variable results. Larger studies are necessary to better evaluate the *Blastocystis* subtyping potential of MALDI-TOF MS analysis from liquid media, the benefits of adapted cutoff criteria and standardisation of preprocessing protocols.

This study highlights the potential for MALDI-TOF MS identification of microorganisms other than bacteria and fungi. However, it also stresses the need for standardised procedures and robust databases that will always need to be reconsidered and adapted. The improvement of MALDI-TOF MS typing applications will contribute to improved epidemiology knowledge and will help correlate clinical and environmental data in the future.

6. Conclusions

Our studies confirmed that MALDI-TOF MS is a powerful technology for the identification of frequently and less frequently encountered microorganisms, as well as fastidious microorganisms. Spectral databases are approximately 10-fold broader than those of conventional identification techniques, which may explain the superiority of MALDI-TOF MS over these techniques (Seng et al. 2013, Sogawa et al. 2012). We are convinced that MALDI-TOF MS will soon replace conventional techniques in the clinical laboratory, likely as a first identification approach, followed by sequencing to resolve ambiguities (Biswas et al. 2013, Bader 2013, Ford et al. 2013, Bertelli et al. 2013).

Though they include different data processors and databases, VITEK MS and Biotyper, the two most marketed systems in Europe, offer the same analytical quality. At this point in time, we suggest that the choice of one system over another should likely be based on practical parameters.

Database construction and availability are key elements in the future of MALDI-TOF MS in clinical laboratories and microbiology in general (Bizzini et al. 2010a). The development of a universal database and the creation of a committee of experts for the control of entries would make the relevant and required data accessible to all laboratories (Biswas et al. 2013, Blondiaux et al. 2010). In this vein, several investigators have already made their in-house databases available to clinical laboratories (Karger et al. 2013, Lau et al. 2013).

When performing RMI from positive blood cultures in our laboratory, MALDI-TOF MS has been shown to improve the clinical management of patients, as indicated by more than 13% of RMI leading to an adaptation of antimicrobial treatments within 24 h. Moreover, the technique is particularly helpful for the quick confirmation of contamination, especially in the paediatric population. The implementation of such a MALDI-TOF MS application, which constitutes an extra cost for the laboratory, should not be considered unless there is efficient communication between health professionals.

We also demonstrated that the MALDI-TOF MS system could be introduced as the main identification platform in a laboratory network. While there is a transportation time between the CL and DL, the identification results are provided 18 h earlier when sharing the MALDI-TOF MS instrument of the CL compared with using conventional identification techniques at the DL. In addition to the significant decrease in the TTI, the MALDI-TOF MS network is also associated with significant cost savings for both laboratories.

Researchers await future developments that will further enhance the practicability of the MALDI-TOF MS technique, such as mechanisation of sample preparation, miniaturisation of mass spectrometers and possible interfacing of MALDI-TOF MS instruments directly with smart phones and personal electronic information devices (Gaillot et al. 2011, van Belkum et al. 2012).

Improved knowledge of the preanalytical parameters that can influence MALDI-TOF MS spectra, including growth and conservation conditions and improved sample preparation, will help optimise and standardise procedures for subtyping, detection of resistance and toxicity mechanisms and analysis directly from clinical samples (Demarco et al. 2013). The combination of molecular and biophysical technologies will increase the mass spectrometry applications in microbiology, as previously described for virology (Fenselau 2013, Ganova-Raeva et al. 2013).

Finally, our promising results on *Blastocystis* subtyping by MALDI-TOF MS suggest that this technology may still yield unanticipated benefits for the microbiology laboratory.

7. Bibliography

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8. Web ressources

- **Superstars of Science**
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  (13 september 2013, last date accessed)

- **Washington-Baltimore Mass Spectrometry Discussion Group**
  "2002 Nobel Prize in Chemistry Awarded to Professor John Fenn of Virginia Commonwealth University and Dr. Koichi Tanaka of Shimadzu Corporation"
  http://wbmsdg.org/wordpress/?s=tanaka
  (13 september 2013, last date accessed)

- **Westfälische Wilhelms-Universität Münster**
  http://www.unimuenster.de/Rektorat/exec/upm.php?rubrik=Alle&neu=1&monat=200312&nummer=04827
  (13 september 2013, last date accessed)

- **Institute of Public Health**
  National reference center for *Campylobacter*, annual report (2011)
  https://nrchm.wiv-isp.be/fr/centres_ref_labo/campylobacter/Rapports/Campylobacter%202011.pdf
  (13 september 2013, last date accessed)

- **Saint-Pierre university hospital**
  http://www.stpierre-bru.be
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- **Jules Bordet Institute**
  http://www.bordet.be
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- **Brugmann university hospital**
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- **Queen Fabiola Children's University Hospital**
  http://www.huderf.be
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9. Appendix

9.1. Specifications of the Microflex LT (Bruker Daltonics, Bremen, Germany) and the VITEK MS (bioMérieux, Marcy l'Etoile, France) IVD systems

This table compares the specifications of the IVD Microflex LT and IVD VITEK MS systems.

	MICROFLEX LT (BRUKER DALTONICS)	VITEK MS (BIOMERIEUX)
SYSTEM		
Dimensions (L x W x H) (mm)	510 x 680 x 1093	700 x 850 x 1920
Weight (kg)	84 + 25 (data system)	325
ENVIRONMENTAL REQUIREMENTS		
Temperature (°C)	10-30	18-26
Humidity (%)	15-85, non condensing at 30°C	<70, non condensing at 26°C
Maximum altitude (m)	3000	2000
ELECTRICITY REQUIREMENTS		
Electrical power	100-240 VAC, 50/60 Hz	200-230 VAC, 50/60 Hz
SPECTRAL ACQUISITION		
Laser shooting	240 satisfactory peaks 40 shots steps quit after 20 failed	1 profile = 5 shots, 100 good profiles if <100 → reshoot on "sweet spots" (max 5) if 60 consecutive failed profiles→failed if <30 profiles acquired→failed
Mass List Find and Binning process	Detection: centroid mode S/N:2 Relative intensity treshold: 0% Minimum intensity treshold: 600 Maximal number of peaks: 300 Peak width: 4 m/z Height: 80% Baseline substraction: Top Hat	Binning process Take uncertainty into account Acquisition area: 2000-20000 Da Spectral area used for ID: 3000-17000 Da Mass range divided in 1300 bins Bin size increases with mass location 1 bin=1300 ppm Peak position replaced by central value of the bin Only highest peak intensity per bin is kept
CALIBRATION		
Calibrator	Spiked *E. coli* (Bacterial Test Standard)	Reference strain *E. coli* (ATCC8739)
Number of peaks in the calibration	8 peaks	11 peaks
Covered mass range	3500-17000 Da	4000-13000 Da
Peak assignment tolerance	1000 ppm	700 ppm
Resolution	> 400	> 600

9.2. Identification of the *Trichophyton mentagrophytes* complex species using MALDI-TOF mass spectrometry

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Identification of the *Trichophyton mentagrophytes* complex species using MALDI-TOF mass spectrometry

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Dermatophytes are fungi capable of invading keratinized tissues and are responsible for the most common fungal infection worldwide: dermatophytosis. Identification of these organisms to the species level is often necessary for the correct treatment of these infections, and is always recommended from an epidemiological point of view. Since the identification of dermatophytes is sometimes problematic, we assessed whether Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) could provide a useful tool to identify dermatophytes of the *Trichophyton mentagrophytes* complex. A reference database was constructed with 17 strains of six different species belonging to this complex. A total of 54 dermatophyte strains of the Belgian co-ordinated collections of micro-organisms, Scientific Institute of Public Health, Brussels, Belgium (BCCM™/IHEM) collection were used to challenge this database; 89% of the tested strains (not used as reference strains in the database) could readily be identified. When incorrect identifications were encountered, the confusion was always between phylogenetically closely related taxa which indicates that observations made by MALDI-TOF MS correlate with phylogenetic data. To assess this observation, a dendrogram outlining the similarities between the obtained spectra was constructed. Strikingly, the relationships found in this dendrogram were highly similar to the ones observed in the phylogenetic tree recently reported by Beguin and co-workers. In conclusion, MALDI-TOF MS is a fast and reliable tool for the identification of dermatophytes, since it can even discriminate between the closely related species of the *T. mentagrophytes* complex. Moreover, our data indicate that the data obtained by MALDI-TOF MS correlate with phylogenetic data.

Keywords Dermatofytes, MALDI-TOF mass spectrometry, identification

Introduction

Dermatophytes cause superficial infections in humans and animals as they have the capacity to invade keratinized tissues such as skin, nails and hair [1]. Dermatomycosis is undoubtedly the most common mycosis worldwide; it is thought to affect 20–25% of the world's population [2]. Dermatophytes can be classified into three anamorphic genera: *Epidermophyton*, *Microsporum* and *Trichophyton*. Depending on their primary habitat, species can be divided into geophilic, zoophilic or anthropophilic.

In general, treatment of infectious diseases relies on the identification of the etiological agent. For dermatomycosis, treatment that is effective against one agent is generally active against all members of this group, though some infections are more persistent than others [3]. However, in 2003 resistance of *Trichophyton rubrum* against terbinafine was reported, indicating the importance of species-level identification [4]. Nonetheless, from an epidemiological point of view, identification up to the species level is essential.

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Identification of dermatophyte strains is traditionally done by correlating the clinical manifestation of the infection with a microscopical examination of the morphological characteristics of the isolated strain, eventually in combination with physiological tests. In the last decades, molecular methods have been used to gain insight in the taxonomy of dermatophytes, and they have led to an enormous number of taxonomical changes, all which have complicated matters further. The correctness of these changes and the 'golden standard' to use for the identification of dermatophytes has become the subject of an ongoing debate [5].

Dermatophytes of the *T. mentagrophytes* complex are particularly hard to identify on the basis of their morphological features and the inter-species relationships within this group are unclear. According to Takashio [6], this complex consists of three perfect states: *Arthroderma vanbreuseghemii*, *A. simii* and *A. benhamiae*, the latter one subdivided in an Americano-African and an African race. Furthermore *T. interdigitale*, a species only known by its conidial state, was included [6,7]. Next to these species, other anamorphic states can be found in this complex: *T. schoenleinii* and *T. quinckeanum*. A recent study by Beguin *et al.* summarized the taxonomic changes within the *mentagrophytes* complex and reanalyzed the species delineation in this complex using multilocus phylogenetic analysis [8].

Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) can routinely be used in clinical laboratories for the identification of bacteria and yeasts, where it seems to be a rapid, cost-effective and reliable tool [9–11]. For filamentous fungi, such as dermatophytes, the utility of this approach for their identification is still under investigation but may represent an alternative to conventional dermatophyte identification [12–15]. The approach is based on the acquisition of a proteic profile (between 2 and 20 kDa) and its comparison with reference spectra included in a database [16].

Strains belonging to the *T. mentagrophytes* complex are very closely related and very hard to distinguish with conventional identification techniques. This study investigated whether MALDI-TOF MS could provide a useful tool for the discrimination of dermatophytes of this complex. First, a reference spectra database was constructed and next, we used a panel of well identified strains of the Belgian co-ordinated collections of micro-organisms, Scientific Institute of Public Health, Brussels, Belgium (BCCM™/IHEM) collection to challenge the robustness of this database. In a second part of the study, a dendrogram was created with the main spectrum profiles (MSPs) from the strains used for the construction of the spectral database. The obtained results were compared with the phylogenetic analyses performed on the *T. mentagrophytes* complex by Beguin *et al.* [8].

Materials and methods

Strains

The 54 dermatophytes used in this study were preserved and referenced in the BCCM™/IHEM Culture Collection (Table 1). A reference spectra library was built up and included isolates of *Trichophyton quinckeanum*, *T. schoenleinii*, *T. interdigitale*, *Arthroderma benhamiae* (African and Americano-European race), *A. vanbreuseghemii* and *A. simii*. All isolates used in this study can be considered as reference material (Table 1), since they were either type or reference strains or they were subjected to rigorous identification by molecular and morphological methods [8].

MALDI-TOF MS sample preparation

Strains were cultivated on agar slants containing diluted (1/10) Sabouraud medium supplemented with 2% yeast extract (BD Biosciences) for 12 days at 25°C before analysis with a Microflex LT (Bruker Daltonics, Germany). The spores and filaments of the colonies were scraped out using a toothpick (Hygostar), transferred in 300 µl of sterile water and mixed thoroughly. Next, absolute ethanol (900 µl) was added and the cells were centrifuged at 13,000 *g* for 5 min at room temperature (RT). After discarding the supernatant, the pellet was air dried during 30 min, re-suspended in 50 µl of 70% formic acid (Sigma-Aldrich), vortexed and incubated at RT for 15 min. Pure acetonitrile (Sigma-Aldrich) was added to the suspension, followed by an incubation of 15 min at RT. Finally, the mixture was centrifuged at 13,000 *g* for 2 min, and 1 µl of the supernatant was placed onto an MALDI 96 polished steel target plate (Bruker Daltonics) and allowed to dry at RT. Each sample was overlaid with 1 µl of α-cyano-4-hydroxycinnamic acid HCCA matrix solution (in 50% acetonitrile, 47.5% water and 2.5% trifluoroacetic acid). After complete drying, MALDI-TOF MS analyses were performed using the default settings. Spectra were recorded in the positive linear mode in a mass range from 2–20 kDa and using the MALDI Biotyper Automation Control software. Instrument calibration was performed with BTS (Bacterial Test Standard, Bruker Daltonics).

Spectrum library entries

In the present study, a reference spectra library was created and challenged. This library contained six dermatophyte species (Table 1). Isolates used were cultured and extracted

Table 1 List of dermatophyte strains used in this study.

Species	IHEM number	Source	Pathogenicity
Trichophyton quinckeanum	13570*	Dog	Favus
	13572*	Rodent	Favus
	13573*	Rodent	Favus
	13574	Rodent	Favus
	13575	Rodent	Favus
	13576	Rodent	Favus
	13577	(RV7234)	
	13697	Mouse	Favus
	19665	Human toenail	
Trichophyton schoenleinii	5232*	Human scalp	Tinea capitis
	13512	Human	Favus
	13513*	Human	Favus
	13515	Human	Favus
	13517	Human scalp	Tinea capitis
	13820	Human scalp	Tinea capitis
	13821	Human	Favus
Trichophyton interdigitale	584*	Human foot	Tinea pedis
	620*	Human skin	Tinea pedis
	1104*	Floor (swimming pool)	
	1210	Floor (swimming pool)	
	1237	Floor (swimming pool)	
	1723	Floor (swimming pool)	
	2497	Human foot	Tinea pedis
	2562	Human skin	Tinea pedis
	2792	Human skin	Tinea pedis
	3227	Human skin	Dermatomycosis
	3290	Human foot	Tinea pedis
	3720	Floor (swimming pool)	
	7493	Floor (tropical swimming pool, changing room)	
	13244	Human nail	Onychomycosis
Arthroderma benhamiae (African race)	4032*	Human	Dermatomycosis
	4033*	Human	Dermatomycosis
Arthroderma benhamiae (Americano-European race)	3287	Monoascospore isolate from RV 23302 × RV 23303	
	3288	Monoascospore isolate from RV 23302 × RV 23303	
	4710*	Type strain (monoascospore isolate from TM20 × TM17)	
	19621	Human	
	19623	Human skin	
	19627	(RV32947)	
	24908*	Type strain (monoascospore isolate from TM20 × TM17)	
Arthroderma vanbreuseghemii	4028*	Human	Dermatomycosis
	4412	Human	Circinate herpes
	10162	Chinchilla's hair	Dermatomycosis
	14193*	Type strain (monoascospore isolate nr 181 (RV 24720 × RV 25645) × RV 27436)	
	19643	Human foot	Tinea pedis
	19644	Human leg	Tinea corporis
	19645	Human scalp	Tinea capitis
	19701*	Type strain (monoascospore isolate nr 187 (RV 24720 × RV25645) × RV 27436)	
Arthroderma simii	3256	monoascospore isolate from RV 15726 × RV 15727	
	3257	monoascospore isolate from RV 15726 × RV 15727	
	4420*	Type strain (monoascospore isolate from IMI98944)	
	4421*	Type strain (monoascospore isolate from IMI98944)	
	13372	Human nail	Onychomycosis
	15729	Domestic fowl	
	19927	Human skin	Tinea corporis

*Strains used to create the reference spectra.

as described above. Each extract was spotted onto eight individual wells, and spectra were collected in triplicate, yielding a total of 24 spectra per isolate.

Visualization of the relationship between the MSPs was displayed in a dendrogram, carried out with the standard settings of the MALDI BioTyper 3.0 software, where

distance values are relative and always normalized to a maximum value of 1000.

Identifications

A total of 37 other strains were used to challenge this database. To ensure reproducibility, mass spectra from three biological replicates and two technical replicates were collected for each isolate. Identifications were considered as correct if the majority of the runs gave a correct identity.

Spectra of dermatophytes isolates were analyzed using the reference spectra library and MALDI BioTyper 3.0 software (Bruker Daltonics, Germany), with logarithmic scores from 0–3. Identification scores were divided in three categories according to the manufacturer's recommendations: ≥ 2.0, 1.70–1.999 and < 1.70. To evaluate the correctness of identification, we compared the result obtained by MALDI-TOF MS with the taxonomic name of the strains in the BCCM™/IHEM collection, which is based on molecular, physiological, sequencing and phylogenetic data.

Results

A database composed of MSPs from reference strains representative of six species [*T. quinckeanum* ($n = 3$), *T. schoenleinii* ($n = 2$), *T. interdigitale* ($n = 3$), *A. benhamiae* (African race, $n = 2$ and Americano-European race, $n = 2$), *A. vanbreuseghemii* ($n = 3$) and *A. simii* ($n = 2$)] was constructed (Table 1). The similarity between the spectra was analysed by the construction of a dendrogram (Fig. 1).

Identifications

To test the robustness of the database, it was challenged with other strains of the same species (Table 1, 2 and Supplementary Table 1, available online at http://informahealthcare.com/doi/abs/10.3109/13693786.2013.770605). Three biological and two technical replicates for each strain were analyzed, and the obtained results are summarized in Table 2. No isolate failed to generate spectra during the MALDI-TOF MS run.

Of the 37 strains used to challenge the database, 89% allowed an immediate correct identification, while 11% resulted in a misidentification. For the isolates *T. quinckeanum*, one of the six resulted in wrong identification, (*T. schoenleinii* instead of *T. quinckeanum* in the case of IHEM 13575). For *A. benhamiae* (Americano-European race), one of the five resulted in an incorrect identification (*A. benhamiae* [African race] instead of *A. benhamiae* [Americano-European race] in the case of IHEM 19621). For *A. vanbreuseghemii* two of the five were wrongly identified (*T. interdigitale* instead of *A. vanbreuseghemii* for IHEM 10162 and IHEM 19643). For *T. schoenleinii*, *T. interdigitale* and *A. simii* no misidentification were seen (Table 2).

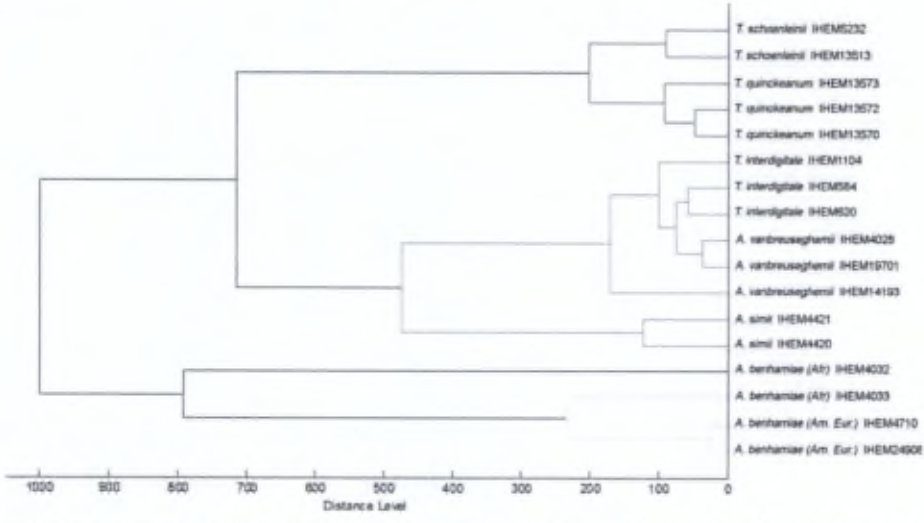

Fig. 1 Dendrogram: representation of the distance level of the dermatophyte strains that were used for the creation of the reference spectra library.

Table 2 Identification of the 37 dermatophytes isolates with MALDI-TOF MS technology.

Group and species	No. (%) of isolates		
	Total	Correct identifications	Incorrect identification
Trichophyton quinckeanum	6	5 (83)	1 (17)
T. schoenleinii	5	5 (100)	0
Arthroderma benhamiae (Americano-European race)	5	4 (80)	1 (20)
A. vanbreuseghemii	5	3 (60)	2 (40)
T. interdigitale	11	11 (100)	0
A. simii	5	5 (100)	0
Total	37	33 (89)	4 (11)

When taking for each isolate all repeats into consideration (three biological and two technical replicates) it could be observed that some misidentifications occurred. For *T. quinckeanum* IHEM 19665 was misidentified as *T. schoenleinii* and *T. schoenleinii* IHEM 13517 and IHEM 13821 were wrongly identified as *T. quinckeanum*. For *A. vanbreuseghemii* isolate IHEM 4412 was misidentified as *T. interdigitale* and *T. interdigitale* IHEM 2792 as well as *A. simii* IHEM 3256 were both faulty recognized as *A. vanbreuseghemii*. It is surprising that not all repeats gave a correct result for the reference strains IHEM 14193 (*A. vanbreuseghemii*) and 4421 (*A. simii*), though the identification is considered as correct. This outcome highlights once more the importance of technical repeats for the preparation of reference spectra, since until presently no general standard procedure has been accepted for the identification of dermatophytes via the MALDI-TOF MS technology (see online Supplementary Table 1 at http://informahealthcare.com/doi/abs/10.3109/13693786.2013.770605).

Discussion

The identification of dermatophytes, and in particular the taxa belonging to the *T. mentagrophytes* complex, is not straightforward. Next to a morphological analysis, multilocus gene sequencing is sometimes required. In this work, we evaluated the use of MALDI-TOF MS for the identification of dermatophytes of the *T. mentagrophytes* complex. Our results showed that, by creating a solid reference database using reference material, 89% of the tested strains (not used as reference strains in the database) led to a rapid and correct identification. Interestingly, in 26% of these cases, identification scores were lower than 2, indicating that even with low log scores, correct identifications can be obtained. In all cases; spectra could be obtained by the MALDI-TOF MS analysis, but in 11% of the cases, this led to an incorrect identification. It should be noted however, that when using a total of six repeats per strain, and based on their molecular, physiological, sequencing and phylogenetic data considering the identification found in the majority of the repeats as correct, only four out of the 37 strains (11%) were wrongly identified.

The wrong identifications observed between *T. schoenleinii* and *T. quinckeanum* indicates that the obtained spectra were very similar between the mistaken taxa. Species delineation and taxonomy in this complex is unclear and was recently re-evaluated by Beguin *et al.* by a multilocus phylogenetic approach using sequences of the Internally Transcribed Spacer region (ITS), Beta Tubulin (BT) and Actin (Act) [8]. In this study, species-level misidentifications for *T. quinckeanum* and *T. schoenleinii* were observed. Strains of the former taxon were identified as the other and vice versa. The work performed by Beguin *et al.* showed in fact that these two taxa are not only part of the *T. mentagrophytes* complex, but are phylogenetically also very closely related, which could explain the wrong identification by MALDI-TOF MS. The close relationship of these taxa is also reported by Makimura and co-workers [17]. Indeed, despite the fact that both dermatophytes differ in morphology, habitat and clinical picture, they have common characteristics such as the production of scutula [18,19].

In the case of *A. simii*, the misidentifications were determined as *A. vanbreuseghemii*. These results underline once more the strength of MALDI-TOF MS to distinguished closely related taxa since Beguin *et al.* demonstrated that *A. simii* is a sister group of the clade containing *T. quinckeanum* [8].

Based on their ITS sequence [17] and by analysis of their CHS1 gene and mitochondrial DNA [20–22], the Americano-European and African races within the *A. benhamiae* clade were clearly distinguished. This observation was also seen when using the MALDI-TOF MS technology, where only one misidentification was seen for *A. benhamiae* Americano-European race, which was identified as *A. benhamiae* African race.

The fact that *A. vanbreuseghemii* and *T. interdigitale* could clearly be distinguished from each other with the MALDI-TOF MS technology, confirms the view of Beguin *et al.* that these taxa are clearly genetically different from one another [8]. The authors motivate that *T. interdigitale* can therefore no longer be recognized as the anamorf of *A. vanbreuseghemii* and that the taxon *T. interdigitale* forms a subspecific group of the latter [8]. Our data provides an additional argument for this view. In general, in all cases were an incorrect identification was observed, a phylogenetically closely related taxon was recognized instead. These data indicate that the observations made by MALDI-TOF MS analysis correlate with phylogenetic data. Indeed, the dendrogram (Fig. 1) that outlines the similarities between the obtained spectra is very similar to the phylogenetic tree [8].

Based on the obtained results, the present study can be considered as a proof-of-principle for identifications of phylogenetically closely related dermatophytes with the MALDI-TOF MS technology.

To conclude, our study indicates that MALDI-TOF MS is a fast, straightforward and user-friendly technique that allows identification of dermatophytes. In this study, 89% of the runs could readily be identified. The minor amount of erroneous identifications is, in our opinion, made up for by the reduction in analysis time and cost. Taking into account the difficulty of identification of dermatophytes, and the relatedness of the taxa in our study group, MALDI-TOF MS provides a powerful alternative for the identification of dermatophytes.

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Supplementary material available online

Supplementary Table 1.

9.3. Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF-MS) for strain-typing of *Campylobacter jejuni*

Martiny D, Ehrard M, Chatellier S, Perrot N, Cornelius A, Vlaes L, van Belkum A, Vandenberg O

15th International Congress on Infectious diseases, Bangkok, Thailand, 2012

55.025

Matrix-Assisted Laser Desorption/Ionisation Time-of-Flight Mass Spectrometry (MALDI-TOF-MS) for strain-typing of *Campylobacter jejuni*.

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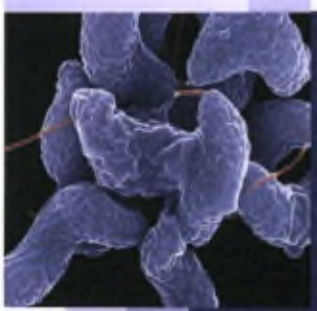


BACKGROUND

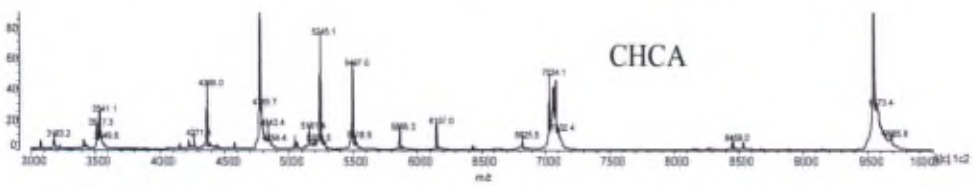
Multilocus sequence typing (MLST) and flagellin gene typing are methods currently used for the typing of *Campylobacter jejuni*. However, these molecular methods are expensive and time consuming; their implementation in a routine laboratory is therefore difficult. This work was designed to explore matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF-MS) as a new typing system for *Campylobacter* directly from culture on agar plates

ATERIALS AND METHODS

his study, 20 randomly selected human *Campylobacter jejuni* isolates belonging to 8 quence types (ST) and 4 clonal complexes (CC) were investigated on a VITEK MS RUO system -Mérieux, Marcy l'Etoile, France).
e presence or absence of distinct spectral peaks as potential strain-specific biomarkers for CC d ST was analysed. A formic acid extraction was performed and all isolates were smeared in adruplicate. One µL of CHCA matrix solution was applied on each bacterial deposit and posable MALDI targets were used. The studied masses ranged from 2.000 to 20.000 Da.

RESULTS

Considering the 80 different spectra obtained by the VITEK MS RUO system, 60 peaks were detected in all spectra. Several specific peaks were found for CC021, ST356, ST572 and ST021.



CONCLUSION

This preliminary study suggests that a standard MALDI-TOF MS procedure is a promising tool for the typing of *Campylobacter jejuni*.

The major advantage of this potential new subtyping system is that it requires the same MALDI-TOF MS platform as the one used for bacterial identification at the species level.

The testing will be expanded to a larger panel of strains covering additional ST and CC to confirm the potential of spectral peak signatures as a rapid subtyping screening tool.