TITLE: MALDI-TOF Mass Spectrometry for Pathogen Identification: A Review of Accuracy and Clinical Effectiveness

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CONTEXT AND POLICY ISSUES

Timely, appropriate treatment of infection depends on rapid, specific identification of causitive microorganisms. Traditionally, microbial identification involves morphologic characterization by microscopy and staining, growth in culture, phenotypic and metabolic characterization by biochemical tests (e.g., API strips [bioMérieux]) and antigenic labeling, and antimicrobial susceptibility testing.[1] A number of these steps have been automated in the form of equipment such as the VITEK 2 (bioMérieux), and BD Phoenix (Becton Dickinson). Molecular methods such as polymerase chain reaction (PCR) and fluorescence in situ hybridization (FISH) can be used to confirm identity, but these depend upon DNA sequence matching and are therefore organism specific, and not appropriate for identification of completely unknown organisms.[1] Direct gene sequencing of highly variable regions such as the 16S rRNA and 18S rRNA gene regions can be used to identify organisms in the absence of prior knowledge, but is usually undertaken only by specialist laboratories.[1]

Matrix Assisted Laser Desorption Ionization Time of Flight (MALDI-TOF) mass spectrometry (MS) can be applied to biological samples with minimal preparation. The prepared sample is mixed with a low molecular weight compound that strongly absorbs laser light but is stable in the presence of biological samples.[1] The dried matrix-sample is then exposed to multiple pulses of an ultraviolet laser, which causes the matrix to sublime and the sample to ionize. The charged ions are then accelerated under an electrical field towards a detector, and the time of flight under acceleration is used to calculate the mass to charge ratio (*m/z*) of each individual peak. The resulting spectrum can then be algorithmically matched against a database of reference spectra. Microbial identifications may be performed directly from a single colony smeared directly onto the target plate or disposable slide, an aliquot of microbial suspension, or following cell lysis and protein extraction of an aliquot of culture broth or a suspended colony or colonies.

Two MALDI-TOF systems are registered in Canada as Class I Medical Devices, the Bruker Daltonics Flex and Microflex series, and the bioMérieux VITEK MS. Both were approved by the FDA in 2013.[2,3]
The Bruker Daltonics Flex and Microflex series are supported by the BioTyper software and database, and identify spectra by matching them against individual spectra in a reference database and assigning a logarithmic probability score.[1] New spectra can be added and outdated or inaccurate spectra removed by manufacturer's software updates or by user edits.

The bioMérieux VITEK MS is supported by the VITEK MS Plus IVD database, which contains composite spectra for each of the included organisms based on spectra from clonally unrelated isolates verified by sequencing.[1] Its algorithm functions by binning individual peaks, and assigning a probability of a cumulative match.[1] This database is not open to user-modification. The VITEK MS can also be linked to the open source SARAMIS RUO database, which contains multiple reference spectra and can be modified by the user, thereby functioning more like the Bruker BioTyper database.

A previous Rapid Response report[4] published in 2011 on the clinical effectiveness, diagnostic accuracy and reproducibility and cost effectiveness of MALDI-TOF in bacterial species identification reviewed seven diagnostic accuracy studies, of which six used the Bruker Microflex, and one used the Shimadzu Biotech system. Sensitivity and specificity (one study) or identification rates (six studies) were comparable or higher for MALDI-TOF than for the reference method for the majority of organisms that were represented in the MALDI-TOF reference base. Lack of representation of organisms in the database was a recognized limitation. The previous report precedes the versions of the Bruker and SARAMIS RUO databases in current use and the introduction of the VITEK MS Plus IVD.

This Rapid Response report reviews the accuracy and clinical impact of MALDI-TOF MS for the identification of microbial pathogens. This report was reviewed by experts in clinical microbiology and bacteriology and mycology.

RESEARCH QUESTIONS

1. What is the accuracy and reproducibility of MALDI-TOF MS for the identification of organisms from cultures on solid media?

2. What is the accuracy and reproducibility of MALDI-TOF MS for the identification of organisms from blood cultures?

3. What is the clinical effectiveness of using MALDI-TOF MS for pathogen species identification?

4. What is the cost-effectiveness of using MALDI-TOF MS for pathogen species identification?

KEY FINDINGS

The diagnostic accuracy with MALDI-TOF mass spectrometry against established methods of identifying microbes is high and well established, with the exception of a few organisms (depending on the system and database interrogated) that still require additional testing, and those rare organisms not well represented in the various databases in use. MALDI-TOF reduces

the time from positive culture or isolate to identification by at least 24 hours in most cases, though that depends upon the organism, the system and database used, and on individual laboratory workflow modifications. There is limited direct evidence on the effect of this reduction on clinical outcomes, with no randomized controlled trial data and sparse observational evidence on improvement of outcomes such as 30-day mortality, length of hospital stay, length of ICU stay, and recurrence and readmission. In addition, to translate the reduced time to identification to quicker and more specific therapy there must be effective communication between laboratory, ID specialists, and treating physicians. Studies of costs are limited to cost calculations and budget impacts, without considering cost-effectiveness or system updates.

METHODS

Literature Search Methods

The literature search was performed by an information specialist using a peer-reviewed search strategy.

Published literature was identified by searching the following bibliographic databases: The Cochrane Library (2015, Issue 3) via Wiley; and PubMed. The search strategy consisted of both controlled vocabulary, such as the National Library of Medicine's MeSH (Medical Subject Headings), and keywords. The main search concepts were MALDI-TOF Mass Spectrometry and Pathogen Identification

Methodological filters were applied to limit retrieval to Systematic Reviews and health technology assessments (HTAs), randomized controlled trials (RCTs), non-randomized studies, and economic studies. Where possible, retrieval was limited to the human population. The search was also limited to documents published between January 1, 2010 and March 31, 2015.

Grey literature (literature that is not commercially published) was identified by searching relevant sections of the Grey Matters checklist (http://www.cadth.ca/resources/grey-matters). Google and other Internet search engines were used to search for additional web-based materials. These searches were supplemented by reviewing the bibliographies of key papers.

Selection Criteria and Methods

One reviewer screened citations and selected studies. In the first level of screening, titles and abstracts were reviewed and potentially relevant articles were retrieved and assessed for inclusion. The final selection of full-text articles was based on the inclusion criteria presented in Table 1.

Table 1: Selection Criteria	
Population	Patients providing a sample for infectious disease diagnosis, with positive bacteria or fungal culture for identification in a public health care setting, primary microbiology laboratory.
Intervention	Microbial species identification using MALDI-TOF mass spectrometry. Material: pure cultures (colonies on agar), blood cultures Any mass spectrometer instrument available in Canada.
Comparator	Standard reference tests (e.g., 16S rDNA sequencing), conventional biochemical testing.
Outcomes	Analytical accuracy of species identification (including sensitivity, specificity, reproducibility, concordance), diagnostic yield (ability to provide interpretable results). Clinical utility: Time to identification; effect on clinical decisions; clinical outcomes (morbidity, mortality); hospital length of stay; antibiotic prescription rates. Costs and economic analyses
Study Designs	HTAs, systematic reviews/meta-analyses, randomized controlled trials, non-randomized studies, economic evaluations

Exclusion Criteria

Articles were excluded if they did not meet the selection criteria outlined in Table 1, they were duplicate publications, they had been included in a systematic review or previous CADTH Rapid Response report, or they were published prior to 2011. The change from 2010 to 2011 as search cut-off was made between literature search and final selection as the quantity of literature and the need to emphasize most recent database versions became known. Exceptions were made for those papers included in Dixon 2015 (systematic review of time to identification)[5] that also reported accuracy[6-10] and cost[11-13] data. Additional exclusion criteria were: lack of comparison to standard clinical laboratory methods, or studies where the objective was to study methods development and technical improvement. The search used terms specific to the methodology rather to individual systems; systems that had never been marketed in or were not currently available in Canada were excluded on full-text screen.

Critical Appraisal of Individual Studies

The included systematic reviews were critically appraised using AMSTAR,[14] observational studies were critically appraised using the Downs and Black checklist,[15] and diagnostic accuracy studies were appraised using QUADAS II.[16] Summary scores were not calculated for the included studies; rather, the strengths and limitations of each included study were described.

SUMMARY OF EVIDENCE

Rapid Response reports are organized so that the evidence for each research question is presented separately.

Quantity of Research Available

A total of 2620 citations were identified in the literature search. Following screening of titles and abstracts, 2543 citations were excluded and 77 potentially relevant reports from the electronic search were retrieved for full-text review. Two potentially relevant publications were retrieved from the grey literature search. Of these 79 potentially relevant articles, 43 publications were excluded for various reasons, while 36 publications met the inclusion criteria and were included in this report. Appendix 1 describes the PRISMA flowchart of the study selection.

Summary of Study Characteristics

Details of individual study characteristics are presented in Appendix 2.

Study Design

Two systematic reviews were identified, one with narrative synthesis of time to identification across all bacteria,[5] and one a meta-analysis of accuracy across studies reporting identification of fungi.[17]

No randomized studies were identified. Two non-randomized studies[11,13] reported direct or surrogate clinical endpoints using a pre-post approach that compared experience before and after introduction of MALDI-TOF MS with or without an antibiotic stewardship program. One reported sequential modifications to treatment after receipt of initial Gram stain results, followed by the results of MALDI-TOF.[12] Two reported surrogate clinical outcomes from a diagnostic accuracy design.[18,19] Data from some of these reports were included in the systematic review of time to identification.[5]

Twenty-eight diagnostic accuracy studies[6-10,20-42] evaluated database identification and diagnostic accuracy (concordance with routine methods of identification or DNA sequencing) for MALDI-TOF MS in processing of routine laboratory isolates. Twenty-one[6,20-26,28,29,31-37,39-42] described the processing of isolates from solid media, and seven described organism identification from processed blood culture broths.[7-10,27,30,38]

No cost-effectiveness studies were identified. Five studies[10,13,19,33,43] included a comparison of costs between MALDI-TOF MS and conventional methods for routine sample processing.

Country of Origin

Of the five clinical studies, three were conducted in the USA,[11,13,19] one in Switzerland,[18] and one in Belgium.[12]

The 28 diagnostic accuracy studies were carried out in the USA,[9,21,22,25,31,32,34,40] China,[20,23,27,35] Kuwait,[7,24,29] Italy,[8,36,41] Spain,[30] Taiwan,[6] India,[26] France,[28] the UK,[33] Canada,[10] Denmark,[37] the Netherlands,[42] and Australia.[38,39]

The studies that calculated costs did so in the context of the health systems of the USA,[13,19] UK,[33] Canada,[10] and France.[43]

Patient Population

The patient population included patients with infections who supplied specimens for routine culture. The majority of the diagnostic accuracy studies provided minimal patient information.

Four studies reported clinical outcomes on patients with bacteremia,[11-13,18] and one included patients with specimens from all sources cultured on solid media.[19] Of these studies, two included only Gram-negative organisms,[13,18] one excluded anaerobes and filamentous fungi,[11] and the others did not specify.

For the diagnostic accuracy studies, sixteen did not restrict as to genus,[7-10,20,23,24,26-30,33,35,36,39] three selected Gram-negative organisms,[21,25,40] six selected anaerobes,[7,22,32,34,37,42] one selected Gram-positive organisms,[6] one selected yeast and was not included in the systematic review by Ling 2015,[41] and one excluded yeast.[38] Three studies set limits on the number of individual strains to allow for increased representation of less common strains,[21,31,40] and three studies allowed the addition of library specimens of less common strains to augment their routinely collected specimens.[21,31,37]

MALDI-TOF MS Systems and Comparators

The meta-analysis by Ling et al, 2014,[17] included four different MALDI-TOF MS systems, the Biotyper (Microflex), the VITEK MS, the Saramis, and the Andromas. The systematic review by Dixon et al, 2015,[5] included the Bruker Microflex, the VITEK/Saramis, and the Autoflex II.

The five studies reporting clinical outcomes all used the Bruker Microflex with Biotyper software and database.[11-13,18,19]

Three diagnostic accuracy studies[24,27,29] compared the accuracy of the Bruker Biotyper with Bruker database version 3.0 or later with the VITEK MS IVD, database version unspecified. Seven diagnostic accuracy studies used the VITEK MS IVD with VITEK database, three of which identified isolates using the version 2.0 database,[21,22,25] and four of which did not specify the database version used.[20,23,28,31] The remaining eighteen studies used the Bruker Microflex with Bruker database, seven of which matched spectra using the Bruker Biotyper software 1.5, 2.0 and 3.0 and database version 3.0 or later,[6-8,30,32,37,39] and eleven of which identified the Biotyper software version as either 2.0 or 3.0, but not the database used.[9,10,26,33-36,38,40-42]

The studies reporting costs all reported on the Bruker Microflex system, Biotyper 3.0 with database 3.0 or later,[10,19] Biotyper 2.0 with unspecfied database,[33] and unspecified software and database.[13,43]

As comparators, the systematic reviews allowed any standard reference method of identification.[5,17] The clinical and diagnostic studies used combinations of morphology, Gram staining, conventional and rapid biochemical tests (e.g., API[8,10,29,30,33,39]), automated systems (VITEK 2[7,9-11,20,23,24,26-28,39,41] and BD Phoenix[9,13,33,38,40]), and DNA sequencing. DNA sequencing, primarily of the 16S rRNA gene, was used to resolve discrepancies.[10,11,19,20,23,24,27,29,30,39-41] Eleven studies used DNA sequencing as the primary reference method,[6,21,22,25,31,32,34-37,42] particularly those involving subsets of organisms.

Outcomes

One systematic review[17] and the majority of studies reported diagnostic accuracy of MALDI-TOF in the form of concordance with conventional methods, with sequencing for resolution of differences, or by agreement with the reference method of DNA sequencing. One study[11] reported 30-day mortality, recurrence of infection and readmission with the same organism, and two studies reported length of stay in hospital and ICU.[11,13] Two studies reported the effect of MALDI-TOF on antibiotic choice.[12,18] One systematic review[5] and one individual study[19] reported time to identification.

Costs

Five articles estimated associated costs, all of which used the Bruker Microflex.[10,13,19,33,43] One study reported hospitalization costs before and after MALDI-TOF,[12] including room and board, pharmacy, radiology, and laboratory costs. Four studies examined only operational costs for MALDI-TOF compared with conventional methods.[10,19,33,43] Of these studies, three calculated costs per isolate/identification,[10,19,33] and one calculated costs over one year.[43] Of these, one explicitly included the cost of the instrument,[33] and another gave a range of costs for conventional testing, depending on its complexity.[10] .

Summary of Critical Appraisal

Details of the critical appraisal of individual studies are presented in Appendix 3.

Both the systematic reviews involved duplicate study selection and a comprehensive literature search, although the search by Ling et al., 2014,[17] was limited in terms, in that it included only generic terms for fungi rather than specific genera, and did not include terms specific for molds. Both included a list of included studies. Neither study mentioned 'a priori' design or a protocol, and neither listed excluded studies. Ling et al., 2014,[5] reported a meta-analysis, although the meta-analytic dataset was heterogeneous with I^2=72.9% for the random effects analysis of the genus-level data and I^2=90.9% for the species level dataset. Their dataset as a whole was moderate quality, with the majority of studies not being blinded and only a few offering complete identification of all isolates by the reference methods. Dixon et al., 2015,[17] did not document a systematic appraisal of study quality, but included a narrative summary of their overall assessment. Dixon et al., 2015, carried out a narrative synthesis, rather than a meta-analysis. Both sets of authors considered the generally moderate quality of evidence appropriately in their conclusions.

The studies addressing clinical outcomes were generally well reported. Two studies[11,13] involved a pre-post design comparing outcomes before MALDI-TOF implementation with those after implementation. These studies incorporated adjustment for patient covariates that might have affected outcomes. This design is susceptible to the effect of confounding changes between the two study periods, e.g., other institutional initiatives in improving patient care, changing workloads, introduction of new treatments, shifts in organism prevalence due to constantly changing endemnicity and epidemiology, and newly introduced strains that may cause clonal outbreaks. One study[12] compared modifications to treatment after receipt of an initial Gram stain results followed by the results of MALDI-TOF. This study relied on retrospective physician report of the influence of MALDI-TOF on their decision-making, which has a potential for bias. An additional two studies[18,19] involved testing of the same samples, analogous to diagnostic testing, and reported times to identification. For these studies, there was no information on

whether the technologists administering one set of tests were aware of the results of the other tests.

In the diagnostic studies, MALDI-TOF and conventional testing were unavoidably conditional on having a positive culture. Furthermore, when testing involved specific subsets of bacteria, the strains had been at least partially identified before being analyzed by MALDI-TOF. MALDI-TOF identification relies on automated algorithms and where there was an option to define a threshhold for identification (Bruker Microflex) most studies used manufacturer-specified thresholds, reducing the subjectivity involved in identification. Comparator methods are standard laboratory methods, or, in some studies, direct sequencing of 16S rRNA, which would be regarded as definitive identification. The main source of uncertainty was around whether technologists administering each set of tests were unaware of the results of the others. Only one study said so explicitly,[26] and most studies did not provide a description enabling the blinding status to be established. The majority of studies also included a re-run of samples that provided results not concordant with the standard, at which point blinding would not apply. However, given the automation and standardization, there is probably a low risk of bias from contaminating information.

No formal cost-effectiveness study was identified, and the information on cost calculations in each paper was limited to a summary, preventing formal appraisal. One paper[12] included all hospitalization costs, while the others restricted themselves to laboratory costs.[10,13,19,33,43] One paper explicitly factored in purchase of equipment.[33]

Summary of Findings

Details of individual study findings are presented in Appendix 4.

What is the accuracy and reproducibility of MALDI-TOF MS for the identification of organisms from cultures on solid media?

Bacteria

The overall accuracy of MALDI-TOF MS in identification of bacterial organisms from colonies cultured on solid media is summarized in Table 2. In a comparative study, Jamal et al, 2014,[24] found that the VITEK MS with IVD database identified 99.9% of routine clinical isolates to the species-level and 99.0% to the genus level, while the Bruker Microflex with Biotyper version 3.3 database identified 93.2% and 97.3% to species- and genus-level respectively. In other studies, concordance between MALDI-TOF MS and conventional methods for species-level identification ranged from 45.9% to 100%, and for genus-level identification from 60.8% to 100%. Overall accuracy depended on the organisms tested, showing lower accuracy for anaerobes, and improved noticeably from earlier to later publications, as sample preparation was refined and databases were updated.

Table 2: Summary of Overall Accuracy of MALDI-TOF in Identifying Species and Genus from Solid Media

Study	Organism tested	System and database	Species concordant*	Genus concordant*
Comparative: Bruker Biotyper and VITEK MS IVD				
Jamal, 2014[24]	All	Bruker Microflex 3.0	93.2%	97.3%
	All	VITEK IVD ns	99.0%	99.9%
Jamal, 2013[29]	Anaerobes	Bruker Microflex 3.0	89.1%	99.2%
	Anaerobes	VITEK IVD ns	100%	100%
VITEK MS IVD				
Luo, 2015[20]	All	VITEK IVD ns	92.6%	99.1%
Dubois, 2014[28]	All	VITEK IVD ns	86.7%	94.9%
Guo, 2014[23]	All	VITEK IVD ns	93.4%	99.6%
Manji, 2014[25]	Non-*Enterobacteriaceae* GP aerobic	VITEK IVD 2.0	77.1%	91.8%
Rychert, 2013[31]	GP aerobic	VITEK IVD ns	92.8%	95.5%
Branda, 2014[21]	GN aerobic	VITEK IVD 2.0	96%	97%
Garner, 2014[22]	Anaerobes	VITEK IVD ns	92.5%	91.2%
Bruker Microflex				
Panda, 2014[26]	All	Bruker Microflex 1.1	98.8%	Not reported
El-Bouri, 2012[33]	All	Bruker Microflex ns	89.3%	99.1%
Xaio, 2012[35]	All	Bruker Microflex 1.5	89.6%	95.3%
Justesen, 2011[37]	All	Bruker Microflex 3.1	67.2%	67.2%
Neville, 2011[39]	All	Bruker Microflex 2.0	84.5%	96.4%
Saffert, 2011[40]	All	Bruker Microflex 2.0	82%	93%
Hsueh, 2014[b]	Selected GP aerobic	Bruker Microflex 3.0	39.5%	78.9%
Schmitt, 2013[32]	Anaerobes	Bruker Microflex 3.3	70.8%	91.7%
Fedorko, 2012[34]	Anaerobes	Bruker Microflex ns	79%	89% (Score ≥1.8)
Veloo, 2011[42]	Anaerobes	Bruker Microflex ns	50.6%	60.8%
Spanu, 2011[41]	Yeasts	Bruker Microflex ns	91.3% [(95% CI 87.7% to 93.9%]	Not reported
Bizzini, 2011[36]	Difficult to identify	Bruker Microflex 2.0	45.9%	67.6%

*If missing, 95% Confidence Intervals were not reported
GN = Gram-negative; GP = Gram-positive; ns = not specified

Appendix 5 offers a summary of the reliability of bacterial identification by MALDI-TOF for the more common individual species, based on a reported minimum of 8 isolates per species per study.

In the comparative study by Jamal, 2014,[24] the VITEK MS IVD (database not specified) identified 97.9% of 283 Gram-positive organisms to the species level, and the Bruker Biotyper (Biotyper 3.0, database not specified) identified 88.3% Gram-positive organisms. The enterococci were consistently correctly identified by both the Bruker and the VITEK MS IVD databases. Most staphylococci were correctly identified (with the exception of *Staphylococcus epidermidis* when using the Bruker database) in more recent studies with improved databases. Identification of streptococci, originally unreliable enough that older papers described using biochemical methods to identify *Streptococcus pneumoniae*,[37,39] has improved with updates of both systems.

Identification of common Gram-negative bacteria is more reliable than that of Gram-positive bacteria for both the Bruker and the VITEK IVD databases. In the comparative study by Jamal, 2014,[24] the VITEK MS IVD (database not specified) identified 99.2% Gram-negative isolates to the species level, and the Bruker Biotyper (Biotyper 3.0, database not specified) identified 95.2% Gram-negative isolates. Recent non-comparative studies give similar results; the reports of identification of <90% of Gram-negative isolates mostly come from older papers.

Six studies analyzed only anaerobes,[7,22,32,34,37,42] either from previously stored specimens or as routine clinical isolates, while eight others[20,23,28,31,33,35,39,44] included anaerobes as a minority. In a comparative study of this subset, Jamal et al, 2013,[7] found that the VITEK MS IVD identified 100% of 274 clinical isolates of anaerobes (14 species, 5 genera) to both species- and genus-level, while the Bruker Biotyper (database version 3.3) 89.1% and 99.2% to species- and genus-level, respectively. Accuracy of identification of individual organisms varied between studies, even for the more common species, although again identification improved with updates of the database. There is agreement on the lack of reliability of identification of *Shigella* species, which arises from an homogeneity with *E. coli*.[20,39] Other common *Enterobacteriaceae* were accurately identified by the most recent databases of both systems.

Fungi

In a meta-analysis of 38 studies reported in 33 papers, including a total of 9,977 fungal isolates from blood or other sources (88.6% yeast isolates, 11.4% mold isolates), the overall identification ratio for genus was 97.7% (95% confidence interval [CI] 95.5% to 99.3%, 6 studies), and for species was 95.5% (95% CI 93.9% to 96.7%, 33 studies), compared with identification by morphology, molecular biology, and biochemistry.[17] The identification ratio was defined as the number of isolates correctly identified by MALDI-TOF divided by the total number of isolates. Yeasts were more accurately identified than molds, 95.9% (95% CI 94.3% to 97.3%) over 27 studies compared with 93.4% (95% CI 88.8% to 96.9%) over 8 studies.[17] Systematic review results for individual species were not reported. The majority of studies were described as being of yeasts or molds, but single-genus studies featured *Candida*, *Aspergillus*, *Cryptococcus*, and *Malessezia*.

For clinical isolates only, as opposed to clinical isolates plus reference strains (34 studies), the pooled species-level identification ratio was 95.0%.[17] The VITEK MS had the lowest identification ratio, 93.3% from 4 studies, while the Bruker Biotyper had a pooled identification ratio of 95.4%, from 24 studies. The authors did not report the databases used in each study. The papers considered in this analysis were published 2009 to 2013.

One paper selected for this review (that was not included in the Ling 2015 systematic review) studied the identification of *Candida* species in culture broths,[41] and five studies of routine isolate identification captured small numbers of yeasts.[7-9,23,30]. All but one study involved the testing of culture broths, was published prior to 2013, and used the Bruker database, and reliability tended to be lower than that reported in the meta-analysis,[7-9,30,41] The single study using the VITEK IVD database reported only three isolates.[23]

What is the accuracy and reproducibility of MALDI-TOF MS for the identification of organisms from blood cultures?

The overall accuracy of MALDI-TOF MS in identification of organisms from blood cultures is summarized in Table 3. These represent predominately Gram-positive cocci and Gram-negative

bacilli that grow aerobically with a smaller representation of anaerobes. The results for yeasts have been previous described. In a comparative study, Chen et al, 2014,[24] found that the VITEK MS with IVD database identified 80.7% of routine clinical isolates to the species-level and 92.8% to the genus level, while the Bruker Microflex with Biotyper version 3.3 database identified 81.8% and 97.8% to species- and genus-level respectively. The concordance between MALDI-TOF and conventional methods in identifying species in monomicrobial infections ranged from 59.5% to 94.1% (one study reported only those isolates that had been identified with high confidence), and in identifying genus ranged from 74.8 to 97.8%. Accuracy improved from earlier to later publications, as sample preparation was refined and databases were updated.

Polymicrobial cultures were less readily identified, with single organism species-level identification ranging from 63.4% to 100%, except in an older fungal study, where one organism was identified in six polyfungal samples. In the comparative study by Chen, 2013,[27] the VITEK MS IVD identified one organism in all 21 samples to the species level, while the Bruker Microflex 3.0 identified both in five samples, using a permissive threshold (Score >1.6), and one in the remaining 16.

Table 3 Summary of Overall Accuracy of MALDI-TOF in Identifying Species and Genus from Blood Cultures

Study	Organism tested	System and database	Concordant species*	Concordant genus*
Comparative: Bruker Biotyper and VITEK MS IVD				
Chen, 2013[27]	All	Bruker Microflex 3.0	81.8%	97.8%
Chen, 2013[27]	All	VITEK IVD ns	80.7%	92.8%
Bruker Biotyper				
Jamal, 2013[29]	All	Bruker Microflex 3.0	75.6%	NR
Leli, 2013[8]	All	Bruker Microflex 3.0	77.9%	91.7%
Rodriguez-Sanchez, 2013[30]	All	Bruker Microflex 3.0	79.6%	91.7%
Buchan, 2012[9]	All	Bruker Microflex 3.0	94.1%[a]	97.6%[a]
Lagacé-Weins, 2012[10]	All	Bruker Microflex ns	95.1%[b]	95.1%
Kok, 2011[38]	All	Bruker Microflex 2.0	59.5%	74.8%

* 95% Confidence Intervals not reported
[a] Among isolates identified with Score≥1.7.
[b] At all Scores.
GN = Gram-negative; GP = Gram-positive; NR = not reported; ns = not specified

Appendix 5 offers a summary of the reliability of organism identification in culture by MALDI-TOF for the more common species, based on a minimum of eight isolates per species in each study. Accuracy from blood culture are generally lower than from solid media, as blood cell proteins can interfere with identification, and accuracy may be affected by the prevalence of staphylococci and streptococci in bloodstream infections.

What is the clinical effectiveness of using MALDI-TOF MS for pathogen species identification?

Thirty-day mortality

One observational study[11] reported lower 30-day all-cause mortality for patients with bacteremia or candidemia following the introduction of a MALDI-TOF workflow in conjunction with an

antibiotic stewardship program (20.3% versus 12.7%). On multivariable analysis, with adjustment for significant contributors to mortality of malignancy, bone marrow transplantation, admission to ICU, and older age, this was not significant (Odds ratio [OR] 0.55, 95% CI 0.28 to 1.06).[11]

Length of stay in hospital and in ICU

Two observational studies[11,13] reported reduced length of stay in hospital and ICU for cohorts of patients with positive blood cultures before and after introduction of MALDI-TOF in conjunction with an antibiotic stewardship program. Huang, 2013[11] included patients with bacteremia (intervention group, Gram-positive 55.3%, Gram-negative 38.0%) and candidemia (6.7%), while Perez, 2013[13] selected patients with Gram-negative bacteremia.

In Huang, 2013,[11] pre-intervention hospital length of stay (LOS) was 14.2 ± 20.6 days and with MALDI-TOF/antibiotic stewardship, 11.4 ± 12.9 days, a non-statistically significant difference (P = 0.066). In Perez, 2013,[13] pre-intervention hospital LOS was 11.9 ± 9.3 days and with MALDI-TOF/antibiotic stewardship, 9.3 ± 7.6 days, a statistically significant difference (P = 0.01). On multivariate analysis, the following factors were associated with LOS: antibiotic therapy at 48 hours, MALDI-TOF antimicrobial stewardship intervention, APACHE II score, pre-intervention LOS, and pre-existing lung disease.

In Huang, 2013,[11] pre-intervention ICU LOS was 14.9 ± 24.2 days and with MALDI-TOF/antibiotic stewardship, 8.3 ± 9.0 days, a statistically significant difference (P = .014). In Perez, 2013,[13] pre-intervention ICU LOS was 7.3 ± 8.5 days and with MALDI-TOF/antibiotic stewardship, 6.3 ± 8.7 days, a statistically significant difference (P = 0.05).

Recurrence of infection and readmission with the same organism

One study[11] reported rates of recurrence of bloodstream infection as 15% ± 5.9% and 5% ± 2.0%, for pre-intervention and with MALDI-TOF/antibiotic stewardship respectively. Readmission with the same organism occurred at rates of 9% ± 3.5% and 4% ± 1.6%, respectively.

Time to identification

Dixon et al., 2015,[5] systematically reviewed time to identification of organisms in bloodstream infection using MALDI-TOF. Anticipating heterogeneity, they did not plan a meta-analysis. Ten studies met the inclusion criteria, which required that the studies report clearly defined time-points as well as a defined bloodstream infection cohort. All studies but one used the Bruker Microflex MS with Biotyper 2.0 or 3.0. The remaining study used the bioMérieux VITEK MS RUO. Start time definitions varied across studies, with eight measuring time from a positive culture or removal of the bottle from the incubator, four using time of blood draw or loading of the incubator, and two using other time points (some studies used multiple time points). All studies included time to identification, except one that required antibiotic susceptibility and one that truncated observation of the comparator at 24 hours after positive culture. Batch processing was not consistently reported in the studies, and the effect of time of transport to the laboratory was not discussed in the review.

Per-study times to identification for MALDI-TOF ranged from a median of approximately 20 min (measured from start of processing a positive blood culture) to a mean 56 hours (measured

from blood draw in the single study using the bioMérieux VITEK MS RUO), while time to identification for the comparator ranged from 26 hours (measured from positive culture) to 84 hours (measured from blood draw in the single study using the bioMérieux VITEK MS RUO).[5] There was an average reduction in time to identification with MALDI-TOF of at least 24 hours, but this was highly dependent upon the organism, the method of preparation, and the difficulty in identification, particularly by conventional methods.[5]

Identification of isolates of Gram-negative bacteria required 6 to 16.8 hours from culture positivity by bioMérieux VITEK MS RUO MALDI-TOF versus 36 to 132 hours by conventional methods.[5] The corresponding values for Gram-positive bacteria in the same study were 8.3 to 18.5 hours versus conventional 35.5 to 84.1 hours, and for yeasts 12.8 to 14.8 hours versus 40.5 to 61.8 hours.[5] Identification of isolates of Gram-negative bacteria required 2.2 to 10.4 hours from culture positivity by Bruker Biotyper LT MALDI-TOF versus 48.2 to 50.1 hours by conventional methods.[5] The corresponding values for Gram-positive bacteria in the same study were 5.3 to 10.6 hours versus conventional 29.5 to 48.0 hours, for obligate anaerobes were 4.1 to 10.3 hours versus conventional 70 to 79.3 hours, and for yeasts were 31.1 hours versus 68.0 hours.[5] These studies did not report on whether or not cultures were processed in batches.

Tan et al., 2012,[19] reported that MALDI-TOF identified 87.2% of organisms on the first day after isolation on solid media, compared with 9.4% identified by conventional methods. MALDI-TOF identified isolates an average of 34.8 hours earlier, ranging from 1 hour earlier for *Candida albicans* to 99 hours earlier for less common Gram-positive rods.

Time to treatment adjustment or optimization

Three studies reviewed by Dixon et al., 2015,[5] included a measure of treatment adjustment or optimization with MALDI-TOF compared with conventional methods of identification. Times to adjustment ranged from a median of 17.5 hours (measured from positive blood culture) to a mean of 47.3 hours (measured from blood draw) for MALDI-TOF, compared with a median of 24 hours to a mean of 90.3 hours (measured from blood draw) for conventional methods. There was no discussion of time of transport to the laboratory.

Effect on antibiotic choice

Two studies[12,18] reported the proportion of patients whose antibiotic choice had been influenced by MALDI-TOF according to retrospective expert review[18] or physician response to survey,[12] as being 17.3%[12] and 35.1% (versus 20.8% for Gram stain alone).[18]

What is the cost-effectiveness of using MALDI-TOF MS for pathogen species identification?

There were no formal cost effectiveness studies. One paper compared in-hospital costs[13] and two[19,43] compared laboratory costs before and after implementation of MALDI-TOF. Two papers compared costs per isolate identification.[10,33] Of the five papers, four anticipated a net savings and the fifth, from a Canadian group,[10] estimated a net cost per isolate, although the cost was sensitive to the estimate of the comparator.

Perez, 2013,[13] based in the USA, calculated the total hospital costs for a group of 119 patients with Gram-negative bloodstream infection before and after introduction of a MALDI-TOF/antibiotic stewardship program. The instrument used was the Bruker Microtyper, software version and database unspecified. With the reduced hospital and ICU stay associated with

MALDI-TOF, mean total hospital costs before MALDI-TOF implementation were US$45,709 ± $61,806 (112 patients), and after were $26,162 ± $28,996 (107 patients).

Tan, 2012,[19] also based in the USA, estimated the annual costs of using their MALDI-TOF protocol for identification of isolates as US$87,556, versus $189,969 for conventional methods. Their instrument was a Bruker Microflex, with Biotyper 3.0 and database v. 3.1.2. They estimated laboratory throughput for the 12 months prior to a 12-week study of time to identification (described above), as 47,875 bacteria representing 279 species. Fixed annual costs were estimated at $31,273. The cost for each species was calculated from the prevalence and the cost to identify each isolate in reagents, labour in the form of hands-on time, and repeated tests, as observed in the 12 week study, or estimated for those species not tested. The five most common species (*Pseudomonas aeruginosa*, *E. coli*, *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Enterococcus faecalis*) represent 65.3% of potential savings.

Gailliot et al., 2011,[43] calculated costs before and after replacement of conventional testing (primarily VITEK 2 and API strips) by MALDI-TOF (Bruker Microflex, database version not specified) for routine identification of almost all bacterial isolates in a French acute care hospital. Conventional methods were still used for mycoplasma, mycobacteria, *Streptococcus pneumoniae*, beta-hemolytic streptococci, and *Shigella* species, and in the event of breakdown of the MALDI-TOF system (10 days).[43] Following implementation, the running cost over a year was US$21,210: $15,836 for 38,624 isolates at an average 3.4 MS tests per identification (Score ≥ 2.0) plus $5,374 for 960 isolates requiring conventional methods. This compares with $193,754 spent during the corresponding period prior to the adoption of MALDI-TOF to identify 33,320 isolates by conventional methods. There were additional savings in waste disposal ($1794), subculture medium ($1102), and DNA sequencing ($1650).

Canadian authors, Lagacé-Weins et al., 2012,[10] estimated a net additional cost per positive culture of US$3.24, ranging from savings of US$0.28 to a cost US$3.64, when using the Sepsityper kit to prepare positive cultures for MALDI-TOF. In their cost calculations, they included the cost of the kit and formic acid extraction, and assumed that 14.8% of samples would not be identified, the same failure rate as seen in their study. El-Bouri et al., 2012,[33] from the UK, estimated costs per isolate for MALDI-TOF identification of £0.51 to £1.28. Estimated savings per isolate were £1.79 to £2.56, excluding the cost of additional antibiotic susceptibility testing.

Limitations

This Rapid Response report focused on the routine clinical use of MALDI-TOF for analyzing clinical specimens, leading to the following limitations within the study selection:

- The VITEK RUO (research use only) database was eligible for inclusion, but studies using the RUO were excluded on the basis of other criteria, therefore the Rapid Response only describes the Bruker Microflex system and the VITEK MS IVD system.
- Papers testing refinements in methodology as opposed to routine use were excluded. These included papers describing requirements and effectiveness for user updates of the open Bruker or VITEK RUO databases, and studies of the effect of abbreviated incubation times and comparisons of different methods of sample preparation.
- None of the studies concerned the detection of CL3/bioterrorism pathogens for diagnosis and surveillance, which requires a separate database.[40]

The evidence for the diagnostic accuracy of MALDI-TOF in the identification of pathogenic organisms from laboratory specimens is substantial. As the methods are still undergoing refinement, there is heterogeneity in sample preparation and MALDI-TOF systems, algorithms and databases. Earlier publications tend to underestimate accuracy, as ongoing improvements of algorithms and database updates, broadens the range of species that can be identified, and refines the discrimination of others. Furthermore, publication delay means that specimens may have been tested as much as two years before the publication date.

Direct evidence for clinical benefit of the replacement of conventional methods by MALDI-TOF is sparse. The most frequently reported outcome was the surrogate of decreased time to identification, which varied substantially depending upon the exact time-points chosen and how MALDI-TOF was integrated into the laboratory workflow, in particular, whether batch processing or expedited reporting were used. One observational study investigated the direct effect of introduction of MALDI-TOF in the context of an antibiotic stewardship program on mortality and length of stay. These authors and others emphasize that support within the system in the form of timely communications and rapid clinician response to reports are necessary to realize the full benefit of the more rapid identification. Manual manipulation of samples, preparation of reagents, entry of patient information, results verification, and possibly transcription of results into hospital record systems is still required. The requirements for system integration and staffing levels have not been fully studied.

No full cost-effectiveness studies have been done on the implementation of MALDI-TOF, including the assessment of system dependencies of MALDI-TOF, such as the integration and staffing levels required to achieve rapid specimen transport and processing, and real-time communication of results.

CONCLUSIONS AND IMPLICATIONS FOR DECISION OR POLICY MAKING

The diagnostic accuracy with MALDI-TOF against established methods of identifying microbes is high and well established, with the exception of a few organisms that still require additional testing, and those organisms not well represented in the available databases. MALDI-TOF MS reduces the time from positive culture or isolate to identification by at least 24 hours in most cases, though that depends upon organism types encountered, workflow protocols in place, laboratory shift structures, and the MS systems and databases in-use. There is limited direct evidence on the effect of this reduction on clinical outcomes, with no randomized controlled trial data and sparse observational evidence on improvement of outcomes such as 30-day mortality, length of stay, and recurrence and readmission. In addition, the context is important. To translate the reduced time to identification to quicker and more specific therapy requires effective communication between laboratory, ID specialists, and treating physicians on a 24/7 basis, and the costs for these multi-disciplinary services should be weighed against the benefits to patients of earlier initiation of appropriate therapies versus costs to patients due to delayed interventions.

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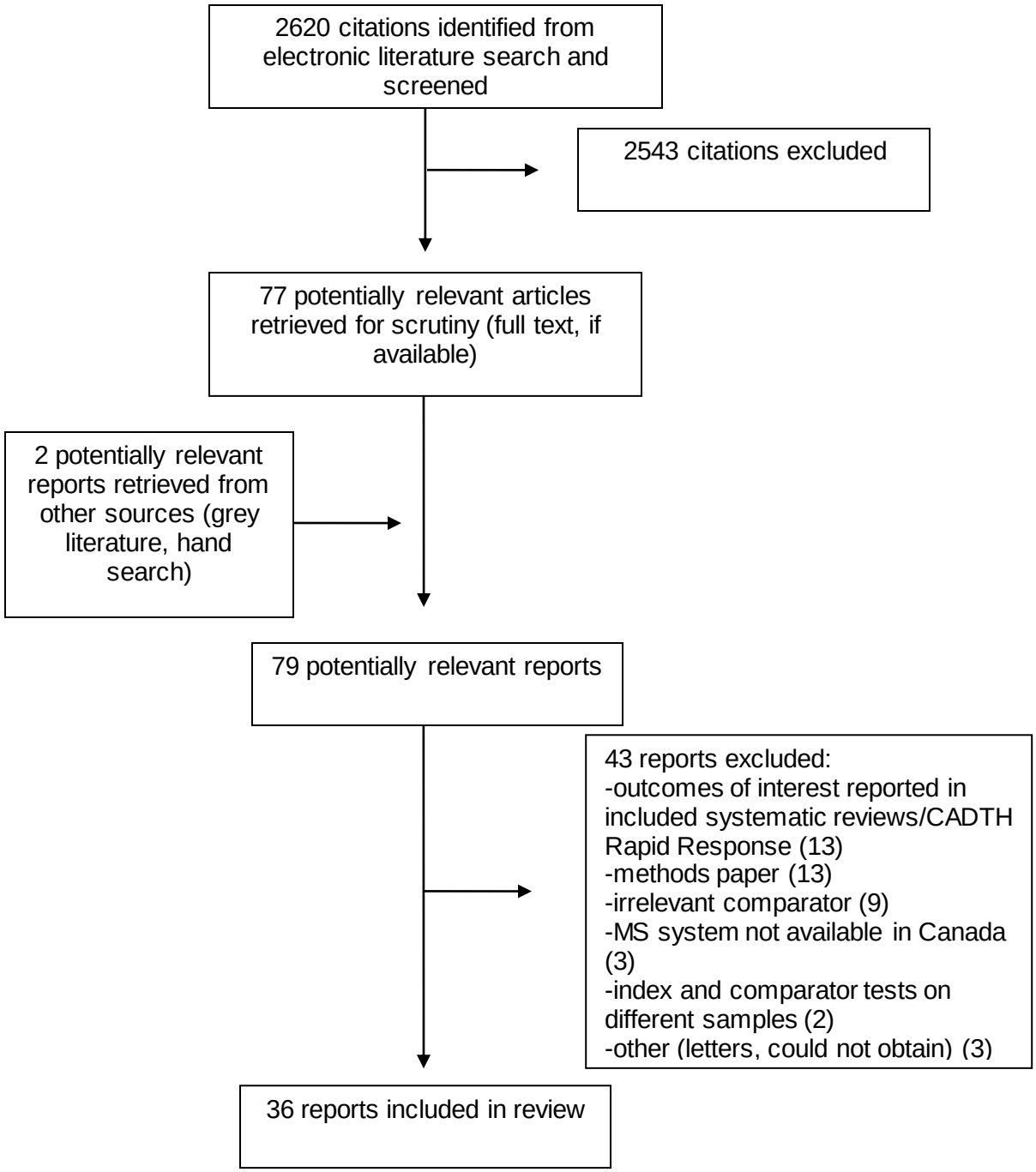
APPENDIX 1: Selection of Included Studies



APPENDIX 2: Characteristics of Included Publications

Table A2-1: Characteristics of Included Systematic Reviews and Meta-Analyses

First Author, Publication Year, Country	Types and numbers of primary studies included	Population Characteristics	Intervention	Comparator(s)	Outcomes
Dixon, 2015[5]	10 studies, all non-RCTs.	Patients with known or suspected microbial bloodstream infection.	MALDI-TOF for identification of microbial pathogen. Any system and sample preparation.	Any other laboratory method for identification of microbial pathogen.	Primary: time to identify. Other: time to appropriate treatment; time to optimal treatment; proportion of patients on optimal treatment; downstream hospital costs.
Ling, 2014[17]	33 articles, 38 studies.	Patients with fungal infection.	MALDI-TOF to identify clinical fungi. Any system and sample preparation.	Reference laboratory methods for identification of fungi (when available).	Primary: proportion of isolates correctly identified.

MALDI-TOF = Matrix Assisted Laser Desorption Ionization Time of Flight; RCT = randomized controlled trial

Table A2-2: Characteristics of Included Studies Reporting Clinical Outcomes

First Author, Publication Year, Country	Study Design, Date of collection	Patient/Sample Characteristics	Sample preparation, testing, and reporting	Comparator(s)/ Reference methods	Clinical Outcomes
Clerc, 2013. Switzerland[18]	Prospective, observational.	First episode of Gram-negative bacteremia (including polymicrobial infections), with ID consultation.	Study isolates selected for MS according to Gram stain prepared from processed pelleted blood culture broth for those positive for Gram-negative bacilli only. Microflex LT MALDI-TOF (Bruker Daltronics, Germany) with MALDI Biotyper 2.0 software.	Gram stain identification.	Effect on choice of empirical antibiotic. (Antibiotic choice with MALDI compared with antibiotic choice with Gram stain alone.)
Huang, 2013[11]. USA.	Prospective, pre-post study. Pre: September to November 2011. Intervention: September to November 2012.	Patient with blood stream infection (bacteremia or candidemia). Exclusions: Transfer patients with active bloodstream infection, patients with organisms not validated for MALDI-TOF, patients with positive culture for skin flora determined to be contaminant.	Isolates directly spotted onto transfer plates, or extracted with formic acid. Bruker Microflex MS with MALDI Biotyper 3.0 software and 3.1.0 database. Scores ≥2.0 and ≥1.7 for species-level and genus-level identification, respectively, and next closest identification >10% different. Results were communicated in real-time between 0600 and 1130 to antibiotic stewardship team member on-call, or by email 24 hours a day.	Biochemical tests, automated systems (VITEK 2) and 16S rRNA gene sequencing. Results were communicated in real-time between 0600 and 1130 to antibiotic stewardship team member on-call, or by email 24 hours a day. .	Clinical outcomes: 30-day all-cause mortality, hospital and ICU LOS, microbiologic clearance, recurrent bacteremia within 30 days of antibiotic discontinuation, 30-day readmission for recurrent bacteremia. Time to effective therapy, time to optimal therapy. (Included in Dixon, 2015).[5]
Martiny, 2013[12] Belgium.	Prospective for diagnostic accuracy, retrospective for clinical. Two tertiary care centres. September 2011 to March	Patients with bacteremia.	In-house protein extraction method. Bruker Microflex LT with MALDI Biotyper 3.0 and database v 3.1.2.0, 3995 spectra. Scores ≥2.0 and ≥1.7 for species-level and genus-level identification, respectively, or Scores ≥1.8 and ≥1.6 with at least 0.3 between first and next match.	Morphology, rapid biochemical tests, 16S rRNA and linker gene sequencing.	Diagnostic yield. Time to identification (reported in Dixon, 2015).[5] Effect on choice of empirical antibiotic.

Table A2-2: Characteristics of Included Studies Reporting Clinical Outcomes

First Author, Publication Year, Country	Study Design, Date of collection	Patient/Sample Characteristics	Sample preparation, testing, and reporting	Comparator(s)/ Reference methods	Clinical Outcomes
	2012.				
Perez, 2013[13] USA.	Retrospective data collection for pre-intervention; prospective for intervention. Pre-intervention: August to November, 2011. Intervention February to May 2012.	Hospitalized patients aged 18 or older, with one or more blood cultures positive for a Gram-negative organism. Excluded: subsequent infections, polymicrobial infections, patient died before blood culture turned positive.	Centrifuged bacteria from broth spotted onto MALDI-TOF testing plate. Bruker Microflex LT with MALDI Biotyper software (version not specified). Lab staff called on-call ID pharmacist; ID pharmacist reviewed prescribed treatment, and if indicated, would make recommendations as to escalation/de-escalation of therapy.	BD Phoenix system, conventional clinical microbiology procedures.	Hospital length of stay, ICU length of stay (survivors). Hospital costs per patient. Time to identification (reported in Dixon, 2015).[5] Appropriateness of antibiotics.
Tan, 2012[19] USA.	Prospective observational. Dates not reported. 12 week period, with rotating comparators.	Patient specimens submitted for bacterial culture: blood, sterile body fluid, urine, stool aerobic wound, anaerobic wound and tissue, respiratory tract, cystic fibrosis respiratory tract, yeasts.	Sample preparation by direct transfer of a colony to the MALDI-TOF plate or by formic acid extraction and spotting of supernatant. Bruker Microflex LT with MALDI Biotyper 3.0 software and database v3.1.2. Scores ≥2.0 and ≥1.7 species-level and genus-level identification in duplicate runs for high confidence. Supplementary tests were included for organisms known to be difficult to identify: *Streptococcus pneumoniae* and *Shigella* species.	Morphology, rapid biochemical tests, 16S rRNA and linker gene sequencing to resolve uncertainties.	Time to identification (number of days between isolate first visualized on primary media and final identification). Cost analysis for each species. Estimated annual savings.

ANC = VITEK 2 ANC ID Card (identification of anaerobic bacteria and coryneform bacteria; GN = VITEK 2 GN ID Card (identification of Gram-negative bacilli); GP = VITEK 2 GP ID Card (identification of Gram-positive bacilli); RCT = randomized controlled trial; MALDI-TOF = Matrix Assisted Laser Desorption Ionization Time of Flight; MS = mass spectrometry; rRNA = ribonuclear ribonucleic acid; YST = VITEK 2 YST ID Card (identification of yeast and yeast-like organisms).

Table A2-3: Characteristics of Included Diagnostic Accuracy Studies

First Author, Publication Year, Country	Study Design, Date of collection	Patient/Sample Characteristics	Sample preparation and testing	Comparator(s)/ Reference methods	Outcomes
Bruker Biotyper and VITEK MS IVD					
Jamal, 2014[24] Kuwait.	Diagnostic accuracy. January to June 2012.	Isolates from routine laboratory processing of clinical specimens.	Colonies transferred directly to disposable target slide, overlaid with matrix. **System 1**. Bruker Microflex MS with MALDI Biotyper 3.0 database. Scores ≥2.0 and ≥1.7 for species-level and genus-level identification, respectively. **System 2**. Spectra acquired with bioMérieux VITEK MS. Analysis with VITEK MS database (version not specified). Reliable identification: ≥90% identity. Samples with discrepant identification between MALDI-TOF and conventional identification rerun, then, if discrepancy persists sequenced.	VITEK 2 GN and GP cards and molecular methods, 16S RNA sequencing.	Identification. Concordance with reference methods.
Chen, 2013[27] China.	Diagnostic accuracy. March to July 2012.	Blood cultures positive by the Bactec system.	Sample preparation by MALDI Sepsityper method. **System 1**. Bruker Microflex MS with MALDI Biotyper 3.0 database (4500 spectra). Scores ≥2.0 and ≥1.7 for species-level and genus-level identification, respectively. **System 2**. VITEK MS with VITEK MS IVD database. Reliable identification: ≥98% and 90 to 98% species and genus, respectively.	Routine identification methods, biochemical tests, automated systems (VITEK 2 and BD Phoenix) and 16S rRNA gene sequencing.	Identification. Concordance with reference methods.

Table A2-3: Characteristics of Included Diagnostic Accuracy Studies

First Author, Publication Year, Country	Study Design, Date of collection	Patient/Sample Characteristics	Sample preparation and testing	Comparator(s)/ Reference methods	Outcomes
Jamal, 2013[29] Kuwait.	Diagnostic accuracy. June to December 2011.	Isolates of clinically significant anaerobic bacteria recovered from routine cultures of clinical specimens (pus, blood cultures, tissues, intra-abdominal samples, wounds).	Colony directly applied to target plate, and layered with matrix. **System 1**. Bruker Microflex MS with MALDI Biotyper 3.0 database. Scores ≥2.0 and ≥1.7 for species-level and genus-level identification, respectively. **System 2**. bioMérieux VITEK MS with VITEK MS database (version not specified). Reliable identification: ≥90%. Isolates with discrepant identification between MALDI-TOF and conventional identification rerun, then, if discrepancy persists sequenced.	API 20AN and molecular methods, 16S RNA sequencing.	Identification. Concordance with reference methods.
VITEK IVD					
Luo, 2015[20] China.	Diagnostic accuracy. March to May 2013.	Isolates recovered from clinical specimens, blood, urine, stool, cerebrospinal fluid, wound swabs throat swabs, sputum, lower respiratory tract.	Isolates recovered on appropriate agar media. Single deposit applied to disposable target slide, layered with VITEK Matrix solution. bioMérieux VITEK MS IVD. System reported best identification matches with confidence level as a percentage. Species-level identification from single result, any confidence level; genus level from multiple results for same genus. No identification from no result offered, or results split across genus.	VITEK 2, with GP, GN, NH, ANC cards (bioMérieux), and biochemical testing. Species-level identification, single result, confidence level ≥85%; genus level, multiple results from same genus. No identification, confidence level	Identification. Concordance with reference methods.

Table A2-3: Characteristics of Included Diagnostic Accuracy Studies					
First Author, Publication Year, Country	**Study Design, Date of collection**	**Patient/Sample Characteristics**	**Sample preparation and testing**	**Comparator(s)/ Reference methods**	**Outcomes**
				<85%, multiple genus, no ID. If no identification, identified by 16S rRNA sequencing.	
Branda, 2014[21] USA.	Diagnostic accuracy, multicentre. December 2011 to August 2012.	Isolates of fastidious Gram-negative bacteria from clinical specimens, with added isolates from culture collections to achieve a minimum of 10 common and 6 less common isolates per species at each centre.	Colonies transferred directly from solid media to disposable target slide, overlaid with matrix. bioMérieux VITEK MS IVD version 2.0. Results reported as single species identification, low discrimination identification (up to four species), no identification. Rerun if no identification or poor quality spectra; not rerun if poor discrimination.	16S rRNA gene sequencing.	Identification. Concordance with reference methods.
Garner, 2014[22] USA.	Diagnostic accuracy. January 2012 to August 2012	Anaerobic strains from clinical specimens collected at participating sites plus manufacturer-provided isolates.	One or more colonies applied to VITEK MS-DS target slide, dried, overlaid with matrix. bioMérieux VITEK MS [IVD] with v2.0 database. Probability score 60% to 100% high discrimination, reliable identification. Probability score <60% low discrimination. If no identification, isolates retested.	16S rRNA gene sequencing at reference laboratory.	Identification. Concordance with reference methods.
Guo, 2014[23] China.	Diagnostic accuracy. 2012.	Isolates from clinical patients.	Colonies transferred directly to disposable target slide, overlaid with matrix.	VITEK 2 GP, GN, YST, ANC, NH cards, with discrepancies	Identification. Concordance with reference methods.

Table A2-3: Characteristics of Included Diagnostic Accuracy Studies

First Author, Publication Year, Country	Study Design, Date of collection	Patient/Sample Characteristics	Sample preparation and testing	Comparator(s)/ Reference methods	Outcomes
			bioMérieux VITEK MS MALDI-TOF (database not specified). Scoring system not defined.	resolved by 16S rRNA gene sequencing.	
Manji, 2014[25] USA.	Diagnostic accuracy. Multicentre. Date not reported.	Fresh clinical isolates of non-*Enterobacteriaceae* Gram-negative bacilli recovered from participating sites plus manufacturer-provided rare isolates (identity confirmed by sequencing). Tester was blinded to the latter.	Isolates recovered for agar plate and/or agar slant cultures. Portion of colony applied to VITEK MS slide wells, overlaid with matrix solution. bioMérieux VITEK 2 MS [IVD] v 2.0. Species-level identification – single match. Genus-level identification – multiple match, same genus. Discordant – single species or genus disagreed with reference. No identification – two or more genera or 'no ID'.	Amplification and 16S RNA sequencing. If no match or LD, phenotypic testing (VITEK GN) or sequencing of *recA*.	Identification. Concordance with reference methods.
Dubois, 2013[28] France.	Diagnostic accuracy. Date not indicated.	Bacterial isolates from clinical specimens (eg, blood, urine, stool, pus, biopsy specimens, cerebrospinal fluid, respiratory tract, wounds, swabs from any site). Representation by any single species was capped at 30 consecutive isolates.	Colony directly applied to disposable plate, and layered with matrix. bioMérieux VITEK MS IVD. Identification: Correct ID to species level – Proposed reference species as single choice or with low discrimination. Correct ID to genus level – Proposed reference species ID among a set of low discrimination results, including same genera. Correct ID above genus level – Proposed ID	VITEK 2 using GP, GN, NH, ANC card. For discrepancies, 16S rRNA gene sequencing.	Identification. Concordance with reference methods.

Table A2-3: Characteristics of Included Diagnostic Accuracy Studies					
First Author, Publication Year, Country	**Study Design, Date of collection**	**Patient/Sample Characteristics**	**Sample preparation and testing**	**Comparator(s)/ Reference methods**	**Outcomes**
			among set of low discrimination results including species of different genera.		
Rychert, 2013[31] USA.	Diagnostic accuracy.	Fresh isolates of Gram-positive aerobic bacteria collected in the course of routine clinical work. A minimum number of isolates (10 or 6) was specified for pre-established lists of common isolates, and sites could add frozen samples to reach the minimum.	Isolated colony applied to disposable target slide, overlaid with matrix solution. bioMérieux VITEK MS with VITEK MS IVD database. Reliable identification: Accurate to species level if single result matching reference. Correct to genus level if multiple results all matched genus of reference method. Incorrect if single non-matching species identification, multiple identifications including different genera, or multiple identifications of a non-matching genus.	Sequencing of 16S rRNA gene, with supplemental sequencing if discrepancy, low discrimination or no result.	Identification. Concordance with reference methods.
Bruker Microflex					
Hsueh, 2014[b] Taiwan.	Diagnostic accuracy.	Selected stored samples of Gram-positive bacilli that grow aerobically, isolated from patient sources.	Colonies supended in 75% ethanol, centrifuged, dried, extracted with formic acid-acetonitrile. Supernatant spotted on Bruker steel target plate, overlaid with matrix, dried. Bruker Microflex LT with FlexControl 1.3 and Biotyper 3.1 (5,627 spectra). Score ≥2.0 and ≥1.7 for species-level and genus-level identification respectively.	For *Nocardia*, *Kocuria*, *Rhodococcus*, and *Tsukamurella* species, 16S rRNA sequencing. For *Nocardia* species, sequencing of *secA1*. For *Gordonia* and *Tsukamurella*	Identification. Concordance with conventional and reference methods.

Table A2-3: Characteristics of Included Diagnostic Accuracy Studies					
First Author, Publication Year, Country	**Study Design, Date of collection**	**Patient/Sample Characteristics**	**Sample preparation and testing**	**Comparator(s)/ Reference methods**	**Outcomes**
			Discrepancies with comparator retested twice.	sequencing of *hsp65*. For *Listeria monocyogenes*, conventional methods, serotypes determined by PCR.	
Panda, 2014[26] India.	Diagnostic accuracy. August to December 2012.	Isolates from routine laboratory processing of clinical specimens.	Sample preparation: Formic acid extraction of 2-3 colonies, placed on target plate and overlaid with matrix. Bruker Microflex MS with FlexAnalysis 2.4 and MALDI Biotyper 1.1 database. Scores ≥2.0 and ≥1.7 for species-level and genus-level identification, respectively.	Routine identification methods, biochemical tests, automated system (VITEK 2). For discrepancies, analysis by external lab using VITEK ID YST considered final.	Identification. Concordance with reference methods.
Jamal, 2013[7] Kuwait.	Diagnostic Accuracy. January to May 2012	Patients with fever and signs and symptoms suspicious of sepsis, providing blood cultures. Exclusions: polymicrobial, no identification by MALDI-TOF or routine.	Sample preparation with Bruker Sepsityper kit, from positive blood cultures, with ethanol-formic acid extraction. Bruker Microflex MS with MALDI Biotyper 3.0 database. Scores ≥2.0 and ≥1.7 for species-level and genus-level identification, respectively. Isolates with discrepant identification between MALDI-TOF and conventional identification	Routine identification methods, biochemical tests, automated system (VITEK 2 and cards GP GN, YST, NH)	Identification. Concordance with reference methods.

Table A2-3: Characteristics of Included Diagnostic Accuracy Studies

First Author, Publication Year, Country	Study Design, Date of collection	Patient/Sample Characteristics	Sample preparation and testing	Comparator(s)/ Reference methods	Outcomes
			rerun, then, if discrepancy persists sequenced.		
Leli, 2013[8] Italy.	Diagnostic accuracy. October 2011 to September 2012.	Samples collected from patients with suspected sepsis and positive on BACTEC incubation.	Cell lysis with Tween 80, centrifugation of bacteria, extraction with formic-acid ethanol. Extract spotted onto steel plate and overlaid with matrix. Bruker Microflex MS with MALDI Biotyper 3.0 database. Scores ≥2.0 and ≥1.7 for species-level and genus-level identification, respectively.	API (bioMérieux) and Phoenix system (Becton Dickinson). Yeasts by germ tube test and API 20C AUX system (bioMérieux).	Identification. Correlation with conventional methods.
Rodríguez-Sanchez, 2013[30] Spain.	Diagnostic accuracy. August 2011 to January 2013.	Positive blood cultures.	Aliquots of culture centrifuged to remove blood cells, re-centrifuged to deposit bacteria. Bacteria spotted directly onto polished steel MALDI target plate, dried, and layered with matrix. Bruker Microflex LT with FlexControl 3.3 and MALDI Biotyper 3.0 database (4613 spectra). Scores ≥2.0 and ≥1.7 for species-level and genus-level identification, respectively. Repeat run with protein extraction for samples that could not be identified.	Microscan panels, API strips, biochemical tests. 16S rRNA sequencing for confirmation of discordant results.	Identification. Concordance with reference methods.
Schmitt, 2013[32] USA.	Diagnostic accuracy. Date not	Clinical isolates of anaerobic bacteria.	Colony mixed with formic acid on plate, dried, overlaid with matrix. Bruker Microflex with Biotyper 3.0	16S RNA sequencing for discrepant results.	Identification. Concordance with reference methods.

Table A2-3: Characteristics of Included Diagnostic Accuracy Studies					
First Author, Publication Year, Country	**Study Design, Date of collection**	**Patient/Sample Characteristics**	**Sample preparation and testing**	**Comparator(s)/ Reference methods**	**Outcomes**
	reported		and library 3.3.1.0 (4,613 entries), plus entries from in-house collection of 87 isolates representing 39 anaerobic species. Scores ≥2.0 and ≥1.7 for species-level and genus-level identification, respectively. Isolates with scores <1.7 retested once.		
Buchan, 2012[9] USA.	Diagnostic accuracy, with prospective sample collection. January to June 2011.	Positive blood cultures, with Gram stain and routine analysis.	Sample preparation with Bruker Sepsityper kit, from positive blood cultures, with ethanol-formic acid extraction. Microflex LT MALDI-TOF (Bruker Daltronics, Germany) with MALDI Biotyper 3.0 (3995 spectra). Scores ≥2.0 and ≥1.7 for species-level and genus-level identification, respectively.	Phoenix automated microbiology system, VITEK 2 (bioMérieux), RaID NM (Remel, Lenexa, KS), latex agglutination, biochemical spot tests.	Identification. Concordance with routine methods. Time to identification (reported in Dixon, 2015).[5]
El-Bouri, 2012[33] UK.	Diagnostic accuracy. 6 months in 2009.	Organisms of clinical significance judged to require identification. From three major acute-care hospitals.	Isolated colony spotted onto steel target plate, overlaid with matrix solution. Bruker Microflex LT with MALDI Biotyper 2.0 software (>3000 spectra). Scores ≥2.0 and ≥1.7 for species-level and genus-level identification, respectively.	BD Phoenix (Becton-Dickinson), API (bioMérieux), biochemical and latex agglutinin tests. *Campylobacter* identified only to genus level.	Identification. Concordance with conventional methods. Cost comparison.
Fedorko, 2012[34] USA.	Diagnostic accuracy	Anaerobic strains collected from clinical isolates and reference	One or more colonies suspended in ethanol, extracted, spotted onto steel plate, overlaid with matrix.	16S rRNA sequencing.	Identification. Comparison to reference methods.

Table A2-3: Characteristics of Included Diagnostic Accuracy Studies

First Author, Publication Year, Country	Study Design, Date of collection	Patient/Sample Characteristics	Sample preparation and testing	Comparator(s)/ Reference methods	Outcomes
		strains.	Bruker Microflex LT with Biotyper version 2.0.4 (database not specified. Updated during study to 3,996 entries). Cut-off score≥1.8.		Turnaround time.
Lagacé-Weins, 2012[10] Canada.	Diagnostic accuracy. Date not reported.	Patient with blood collected at the bedside.	Sample preparation with Bruker Sepsityper kit, from positive blood cultures, with ethanol-formic acid extraction. Microflex LT with MALDI Biotyper 3.0 (database version not specified). Scores ≥2.0 and ≥1.7 for species-level and genus-level identification, respectively. Discordant identifications further characterized or sequenced.	VITEK 2 and API biodchemical test, rapid biochemical tests, 16S rRNA gene sequencing and sequence analysis.	Identification. Concordance with reference methods. Turnaround time. Costs (reported in Dixon, 2015).[5]
Xiao, 2012[35] China.	Diagnostic accuracy. Date not reported.	Patients with community acquired pneumonia.	Protein extraction with ethanol-formic acid. (Direct application of colony to plate was tested, but produced poorer spectra and lower identifications). Bruker Microflex LT with Biotyper 1.5 processing and database (3995 spectra). Scores ≥2.0 and ≥1.7 for species-level and genus-level identification, respectively.	16S rRNA gene sequencing.	Identification. Concordance with reference method.
Bizzini, 2011[36] Italy.	Diagnostic accuracy.	Stored strains from clinical samples previously sequenced due to difficulties in identifying.	Formic acid extraction. Bruker Microflex LT with FlexControl 3.0 and Biotyper 2.0 (3,290 spectra, update of	16S rRNA sequencing.	Identification. Concordance with reference method.

Table A2-3: Characteristics of Included Diagnostic Accuracy Studies					
First Author, Publication Year, Country	**Study Design, Date of collection**	**Patient/Sample Characteristics**	**Sample preparation and testing**	**Comparator(s)/ Reference methods**	**Outcomes**
			September 2, 2008). Score ≥2.0 and ≥1.7 for species-level and genus-level identification respectively.		
Justesen, 2011[37] Denmark.	Diagnostic accuracy. November 2007 to October 2010.	Consecutive clinical isolates of anaerobic bacteria which have been identified by 16S rRNA sequencing, from blood cultures, tissue samples, sterile body fluids, and pus. Plus twelve standard library isolates of rare species.	Isolates cultured on anaerobe agar. Selected colonies inoculated onto ground steel MALDI target place, and covered with matrix. Microflex LT MALDI-TOF (Bruker Daltronics, Germany) with Flex Control 3.0 software and MALDI Biotyper 3.1.1.0. Samples run as duplicates. If Score <2.0 samples rerun. If scores do not qualify, rerun with formic acid extraction.	16S rRNA gene sequencing.	Proportion identified to species and genus level. Concordance with reference methods.
Kok, 2011[38] Australia	Diagnostic accuracy. March to April, 2011.	Culture broths from patients with suspected sepsis, positive by the BacTec FX blood culture system. Exclusion: yeasts.	Sample preparation with Bruker Sepsityper kit, from positive blood cultures, with ethanol-formic acid extraction. Bruker Microflex LT operated by MALDI-Biotyper with Biotyper 2.0. Scores ≥2.0 and ≥1.7 for species-level and genus-level identifications, respectively.	Phoenix automated microbiology system with biochemical and latex agglutinin tests.	Identification against database. Concordance with conventional methods.
Neville, 2011[39] Australia.	Diagnostic accuracy. Date not specified.	All bacteria isolated within one calendar month that would undergo routine diagnostics.	Sample preparation not described. Bruker Microflex with Biotyper 2.0 (software version 3.1.1.0). Scores ≥2.0 and ≥1.7 for species-level and genus-level identification,	VITEK 2, API, biochemical assays. 16S rRNA sequencing to resolve discrepancies.	Identification. Concordance with reference methods.

Table A2-3: Characteristics of Included Diagnostic Accuracy Studies

First Author, Publication Year, Country	Study Design, Date of collection	Patient/Sample Characteristics	Sample preparation and testing	Comparator(s)/ Reference methods	Outcomes
			respectively. Repeated with formic acid extraction to resolve discrepancies.		
Saffert, 2011[40] USA	Diagnostic accuracy. Date not specified.	Gram-negative bacilli collected from clinical sources. Analysis of common isolates limited to increase representation of rarer bacteria.	Colonies applied directly to steel target plate and overlaid with matrix. Bruker Microflex with Biotyper 2.0 (3740 spectra). Scores ≥2.0 and ≥1.7 for species-level and genus-level identification, respectively, with a minimum of 10% difference between the top score and next match. Rerun if Score<2.0; extracted and rerun if still <2.0.	BD Phoenix. Discrepancies resolved by biochemical testing and 16S rRNA sequencing.	Identification. Concordance with reference methods.
Spanu, 2011[41] Italy.	Diagnostic accuracy. Two centres. November 2009 to March 2011.	Fungus-positive BC bactec Mycosis IC/F bottles from routine clinical cultures.	Culture centrifuged, pellet suspended in 0.1% Tween 80, washed with water, 70% ethanol, extracted with formic acid-acetonitrile, supernatant spotted onto MALDI plate in quadruplicate, overlaid with matrix, dried. Bruker Microflex LT with FlexControl software 2.0, database version unspecified. Match with highest score used for species ID. Valid if ≥2 of 4 spectra Scored ≥1.9, or 4/4 scored ≥1.2.	Morphologic identification, bioMérieux VITEK yeast biochemical card. Discordant results, sequence analysis of rRNA gene internal transcribed spacer regions.	Identification. Concordance with conventional methods.

Table A2-3: Characteristics of Included Diagnostic Accuracy Studies					
First Author, Publication Year, Country	**Study Design, Date of collection**	**Patient/Sample Characteristics**	**Sample preparation and testing**	**Comparator(s)/ Reference methods**	**Outcomes**
Veloo, 2011[42] Netherlands.	Diagnostic accuracy	Anaerobic strains derived from clinical specimens.	*No-pretreatment*: colony spotted directly onto MALDI-plate, overlaid with matrix, dried. *Pre-treatment*: Suspended in ethanol, centrifuged pellet suspended in formic acid-acetonitrile, supernatant spotted onto MALDI-plate, overlaid with matrix. Bruker Microflex LT with FlexControl software 3.0. Database 3476 spectra, to February 2010. Score ≥2.0 and ≥1.7 for species-level and genus-level identification respectively.	16S rRNA sequencing.	Identification. Concordance with reference method.

ANC = VITEK 2 ANC ID Card (Identification of anaerobic bacteria and coryneform bacteria; GN = VITEK 2 GN ID Card (identification of Gram-negative bacilli); GP = VITEK 2 GP ID Card (identification of Gram-positive bacilli); RCT = randomized controlled trial; MALDI-TOF = Matrix Assisted Laser Desorption Ionization Time of Flight; MS = mass spectrometry; rRNA = ribonuclear ribonucleic acid; YST = VITEK 2 YST ID Card (identification of yeast and yeast-like organisms).

APPENDIX 3: Critical Appraisal of Included Publications

Table A3-1: Strengths and Limitations of Systematic Reviews and Meta-Analyses using AMSTAR[14]

Strengths	Limitations
Dixon, 2015[5]	
• There was duplicate study selection and data extraction. • A comprehensive literature search was performed. • A list of included studies was provided. • The characteristics of the included studies were provided. • The scientific quality of the included studies was used appropriately in formulating conditions. • No meta-analysis was carried out, because of heterogeneity. • Any conflict of interest was stated.	• There was no mention of 'a priori' research design • Grey literature and books were excluded from selection • A list of excluded studies was not available. • The scientific quality of the included studies was not systematically assessed. There was a narrative description. • The likelihood of publication bias was not assessed.
Ling, 2014[17]	
• There was duplicate study selection and data extraction. • Literature search was comprehensive in sources. • A list of included studies was provided. • The characteristics of the included studies were provided, although not in great detail. • The scientific quality of the included studies was assessed and documented. • The scientific quality of the included studies was used appropriately in formulating conditions. • The likelihood of publication bias was assessed. • Any conflict of interest was stated.	• There was no mention of 'a priori' research design • Literature search was limited in terms. • A list of excluded studies was not provided. • Meta-analysis was conducted using random-effects models and exploratory subgroup analyses. However, statistical heterogeneity of the pooled dataset was very high, and no subgroups were identified.

Table A3-2: Strengths and Limitations of Observational Studies using Downs and Black[1]

Strengths	Limitations
Clerc, 2013[18]	
• The objective of the study is clearly described. • The main outcome is clearly described. • Inclusion criteria are clearly described. No exclusion criteria are specified. • The intervention (Gram stain plus MALDI-TOF) and comparator (Gram stain) are described in detail. • The invention and comparator are applicable to the diagnosis of infection. • The main findings of the study are clearly described. • Samples were representative of routine laboratory practice.	• It is unclear whether MALDI-TOF or conventional testing was carried out and interpreted by operators blinded to the results of the comparator.
Huang, 2013[11]	
• The objective of the study is clearly described. • The main outcomes are clearly described. • The inclusion and exclusion criteria are clearly described. • The intervention (MALDI-TOF) and comparator (conventional testing methods) are described in detail. • The main findings of the study are clearly described. • The patients were representative of those undergoing testing for infection. • Data for potential confounders for clinical outcomes were collected; the two groups were similar	• The study was of a pre-post design, covering two separate calendar periods. The intervention was not randomized. • Given the pre-post design, there was no blinding to results obtained in the pre-intervention period.
Martiny, 2013[12]	
• The objective of the study is clearly described. • The main outcomes are clearly described. • Inclusion and exclusion criteria are clearly described. • The intervention (MALDI-TOF) is described in detail. • The main findings of the study are clearly described. • The patients were representative of those undergoing testing for infection.	• It is unclear whether MALDI-TOF or conventional testing was carried out and interpreted by operators blinded to the results of the comparator. • Potential confounders for choice of antibiotic (eg, acuity of disease, comorbidities) not reported.
Perez, 2013[13]	
• The objective of the study is clearly described. • The main outcomes are clearly described. • Inclusion and exclusion criteria are clearly described. • The intervention (MALDI-TOF) is described in detail. • The main findings of the study are clearly described. • The patients were representative of those undergoing testing for infection. • Potential confounders for choice of antibiotic	• It is unclear whether MALDI-TOF or conventional testing was carried out and interpreted by operators blinded to the results of the comparator.

Table A3-2: Strengths and Limitations of Observational Studies using Downs and Black[1]	
Strengths	**Limitations**
were considered.	
Tan, 2012[19]	
• The objective of the study is clearly described. • The main outcome is clearly described. • Inclusion criteria are clearly described. No exclusion criteria are specified. • The intervention (MALDI-TOF plus supplementary tests) and comparator (conventional methods of identification) are described in detail. • The invention and comparator are applicable to the diagnosis of infection. • The main findings of the study are clearly described. • Samples were representative of routine laboratory practice.	• It is unclear whether MALDI-TOF or conventional testing was carried out and interpreted by operators blinded to the results of the comparator.

Table A3-3: Strengths and Limitations of Diagnostic Accuracy Studies using the framework of QUADAS II[16]

Strengths	Limitations
Bruker Biotyper and VITEK MS IVD	
Jamal, 2014[24]	
• Samples were collected consecutively over the period of interest. • MALDI-TOF was applicable to the diagnosis of infection. • The threshhold for identification by MALDI-TOF was pre-specified. • The reference test was applied to all isolates. Discrepancies were resolved by definitive DNA sequencing. • Reference test was an established, routine method of identifying pathogens.	• It is unclear whether MALDI-TOF or conventional testing was carried out and interpreted by operators blinded to the results of the comparator.
Chen, 2013[27]	
• MALDI-TOF was applicable to the diagnosis of infection. • The threshold for identification by MALDI-TOF was pre-specified according to the manufacturer. • The reference test was applied to all isolates. • The reference test was an established, routine method of identifying pathogens.	• Method for sample selection is unclear; samples were from routine testing, but it is not stated whether they were consecutive or all samples from the period of interest. • It is unclear whether MALDI-TOF or conventional testing was carried out and interpreted by operators blinded to the results of the comparator.
Jamal, 2013[29]	
• MALDI-TOF was applicable to the diagnosis of infection. • The threshhold for identification by MALDI-TOF was pre-specified. • The reference test was applied to all isolates. • The reference test was an established, routine method of identifying pathogens. Discrepancies after repeat testing were resolved by 16S rRNA sequencing.	• Method for sample selection is unclear; samples were from routine testing, but it is not stated whether they were consecutive or all samples from the period of interest. • It is unclear whether MALDI-TOF or conventional testing was carried out and interpreted by operators blinded to the results of the comparator.
VITEK IVD	
Luo, 2015[20]	
• Samples were collected prospectively, and all routine samples over period of interest were included. • MALDI-TOF was applicable to the diagnosis of infection. • The threshhold for identification by MALDI-TOF was pre-specified. • The reference test was applied to all isolates. • Reference test was an established, routine method of identifying pathogens.	• It is unclear whether MALDI-TOF or conventional testing was carried out and interpreted by operators blinded to the results of the comparator.
Branda, 2014[21]	
• MALDI-TOF was applicable to the diagnosis of infection. • Threshold for identification by MALDI-TOF was pre-specified according to manufacturer's	• Study sample was enriched with rare organisms, distribution is not characteristic of routine. • It is unclear whether MALDI-TOF or

Table A3-3: Strengths and Limitations of Diagnostic Accuracy Studies using the framework of QUADAS II[16]

Strengths	Limitations
standards. • The reference test was applied to all isolates. • Reference test was an established, definitive method of identifying pathogens.	sequencing was carried out and interpreted by operators blinded to the results of the comparator.
Garner, 2014[22]	
• MALDI-TOF was applicable to the diagnosis of infection. • The threshhold for identification by MALDI-TOF was pre-specified. • The reference test was applied to all isolates. • Reference test was an established, routine method of identifying pathogens.	• Previously identified specimens were used, as study was of accuracy of identifying anaerobes.
Guo, 2014[23]	
• MALDI-TOF was applicable to the diagnosis of infection. • The reference test was applied to all isolates. Discrepancies were resolved by definitive DNA sequencing. • Reference test was an established, routine method of identifying pathogens.	• Method for sample selection is unclear. • Threshold for machine identification by MALDI-TOF was not specified. • It is unclear whether MALDI-TOF or conventional testing was carried out and interpreted by operators blinded to the results of the comparator.
Manji, 2014[25]	
• MALDI-TOF was applicable to the diagnosis of infection. • The threshhold for identification by MALDI-TOF was pre-specified. • The reference test was applied to all isolates. • Reference test was an established, routine method of identifying pathogens.	• Previously identified specimens were used, as study was of accuracy of identifying non-*Enterobacteriaceae* Gram-negative bacteria.
Dubois, 2013[28]	
• MALDI-TOF was applicable to the diagnosis of infection. • The threshold for identification by MALDI-TOF was pre-specified according to the manufacturer. • The reference test was applied to all isolates. • The reference test was an established, routine method of identifying pathogens.	• Study sample did not reflect routine practice in that, to obtain sufficient diversity, number of isolates of common organisms was restricted. • It is unclear whether MALDI-TOF or conventional testing was carried out and interpreted by operators blinded to the results of the comparator.
Rychert, 2013[31]	
• MALDI-TOF was applicable to the diagnosis of infection. • The threshold for identification by MALDI-TOF was pre-specified. • The reference test was applied to all isolates. • The reference test was an established, definitive method of identifying pathogens.	• Study sample was restricted to Gram-negative organisms from routine practice, and augmented with library specimens if insufficient numbers of isolates were not retrieved. • It is unclear whether MALDI-TOF or conventional testing was carried out and interpreted by operators blinded to the results of the comparator.

Table A3-3: Strengths and Limitations of Diagnostic Accuracy Studies using the framework of QUADAS II[16]

Strengths	Limitations
Bruker Microflex	
Hsueh, 2014[6]	
• MALDI-TOF was applicable to the diagnosis of infection. • The threshhold for identification by MALDI-TOF was pre-specified. • The reference test was applied to all isolates. • Reference test was an established, routine method of identifying pathogens.	• Previously identified specimens were used, as study was of accuracy of identifying a specific set of species.
Panda, 2014[26]	
• MALDI-TOF was applicable to the diagnosis of infection. • The threshhold for identification by MALDI-TOF was pre-specified. • The reference test was applied to all isolates. • The reference test was an established, routine method of identifying pathogens. Discrepancies were resolved by assay at an independent laboratory. • Individuals conducting MALDI-TOF were blinded to results of reference tests, and vice versa.	• No major limitations
Jamal, 2013[7]	
• Samples were collected consecutively over the period of interest. • MALDI-TOF was applicable to the diagnosis of infection. • The threshold for identification by MALDI-TOF was pre-specified. • The reference test was applied to all isolates. • The reference test was an established, routine method of identifying pathogens.	• It is unclear whether MALDI-TOF or conventional testing was carried out and interpreted by operators blinded to the results of the comparator.
Leli, 2013[8]	
• MALDI-TOF was applicable to the diagnosis of infection. • The threshold for identification by MALDI-TOF was pre-specified according to the manufacturer. • The reference test was applied to all isolates. • The reference test was an established, routine method of identifying pathogens.	• Method for sample selection is unclear; samples were from routine testing, but it is not stated whether they were consecutive or all samples from the period of interest. • It is unclear whether MALDI-TOF or conventional testing was carried out and interpreted by operators blinded to the results of the comparator.
Rodríguez-Sanchez, 2013[30]	
• Samples were collected consecutively over the period of interest. • MALDI-TOF was applicable to the diagnosis of infection. • The threshold for identification by MALDI-TOF was pre-specified according to the manufacturer. • The reference test was applied to all isolates. • The reference test was an established, routine	• It is unclear whether MALDI-TOF or conventional testing was carried out and interpreted by operators blinded to the results of the comparator.

Table A3-3: Strengths and Limitations of Diagnostic Accuracy Studies using the framework of QUADAS II[16]

Strengths	Limitations
method of identifying pathogens.	
Schmitt, 2013[32]	
• MALDI-TOF was applicable to the diagnosis of infection. • The threshhold for identification by MALDI-TOF was pre-specified. • The reference test was applied to all isolates. • Reference test was an established, routine method of identifying pathogens.	• Previously identified specimens were used, as study was of accuracy of identifying anaerobes.
Buchan, 2012[9]	
• Samples were collected prospectively over the period of interest. • MALDI-TOF was applicable to the diagnosis of infection. • The threshold for identification by MALDI-TOF was pre-specified. • The reference test was applied to all isolates. • The reference test was an established, routine method of identifying pathogens.	• It is not clear that all samples collected during the study period were included. • It is unclear whether MALDI-TOF or conventional testing was carried out and interpreted by operators blinded to the results of the comparator.
El-Bouri, 2012[33]	
• MALDI-TOF was applicable to the diagnosis of infection. • The threshold for identification by MALDI-TOF was pre-specified. • The reference test was applied to all isolates. • The reference test was an established, definitive method of identifying pathogens.	• Method for sample selection is unclear; samples were from routine testing, but it is not stated whether they were consecutive or all samples from the period of interest. • It is unclear whether MALDI-TOF or conventional testing was carried out and interpreted by operators blinded to the results of the comparator.
Fedorko 2012 [34]	
• MALDI-TOF was applicable to the diagnosis of infection. • The threshhold for identification by MALDI-TOF was pre-specified. • The reference test was applied to all isolates. • Reference test was an established, routine method of identifying pathogens.	• Previously identified specimens were used, as study was of accuracy of identifying anaerobes.
Lagacé-Weins, 2012[10]	
• MALDI-TOF was applicable to the diagnosis of infection. • The threshold for identification by MALDI-TOF was pre-specified according to the manufacturer. • The reference test was applied to all isolates. • The reference test was an established, routine method of identifying pathogens.	• Method for sample selection is unclear. • It is unclear whether MALDI-TOF or conventional testing was carried out and interpreted by operators blinded to the results of the comparator.
Xiao, 2012[35]	
• MALDI-TOF was applicable to the diagnosis of infection. • Threshhold for identification by MALDI-TOF was pre-specified according to manufacturer's standards.	• Unclear whether all consecutive patients with positive culture were included. • It is unclear whether MALDI-TOF or sequencing was carried out and interpreted by operators blinded to the results of the

Table A3-3: Strengths and Limitations of Diagnostic Accuracy Studies using the framework of QUADAS II[16]

Strengths	Limitations
• The reference test was applied to all isolates. • Reference test was an established, definitive method of identifying pathogens.	comparator.
Bizzini, 2011[44]	
• MALDI-TOF was applicable to the diagnosis of infection. • The threshhold for identification by MALDI-TOF was pre-specified. • The reference test was applied to all isolates. • Reference test was an established, routine method of identifying pathogens.	• Previously identified specimens were used, as study was of accuracy of selected difficult-to-identify specimens.
Justesen, 2011[37]	
• Samples were collected consecutively over the period of interest. • MALDI-TOF was applicable to the diagnosis of infection. • The threshold for identification by MALDI-TOF was pre-specified according to the manufacturer. • The reference test was applied to all isolates. • The reference test was an established, routine method of identifying pathogens.	• It is unclear whether MALDI-TOF or conventional testing was carried out and interpreted by operators blinded to the results of the comparator.
Kok, 2011[38]	
• Samples were collected prospectively over the period of interest. • MALDI-TOF was applicable to the diagnosis of infection. • The threshold for identification by MALDI-TOF was pre-specified according to the manufacturer. • The reference test was applied to all isolates. • The reference test was an established, routine method of identifying pathogens.	• It is not clear that all samples collected during the study period were included. • It is unclear whether MALDI-TOF or conventional testing was carried out and interpreted by operators blinded to the results of the comparator.
Neville, 2011[39]	
• Samples were collected consecutively over the period of interest. • MALDI-TOF was applicable to the diagnosis of infection. • The threshold for identification by MALDI-TOF was pre-specified according to the manufacturer. • The reference test was applied to all isolates. • The reference test was an established, routine method of identifying pathogens.	• It is unclear whether MALDI-TOF or conventional testing was carried out and interpreted by operators blinded to the results of the comparator.
Saffert, 2011[40]	
• MALDI-TOF was applicable to the diagnosis of infection. • The threshold for identification by MALDI-TOF was pre-specified. • The reference test was applied to all isolates. • The reference test was an established, routine	• Study sample did not reflect routine practice in that, to obtain sufficient diversity, number of isolates of common organisms was restricted. • It is unclear whether MALDI-TOF or conventional testing was carried out and interpreted by operators blinded to the results

Table A3-3: Strengths and Limitations of Diagnostic Accuracy Studies using the framework of QUADAS II[16]

Strengths	Limitations
method of identifying pathogens.	of the comparator.
Spanu, 2011[41]	
• MALDI-TOF was applicable to the diagnosis of infection. • The threshold for identification by MALDI-TOF was pre-specified according to the manufacturer. • The reference test was applied to all isolates. • The reference test was an established, routine method of identifying pathogens.	• Previously identified specimens were used, as study was of accuracy of identifying yeasts.
Veloo, 2011[37]	
• MALDI-TOF was applicable to the diagnosis of infection. • The threshold for identification by MALDI-TOF was pre-specified according to the manufacturer. • The reference test was applied to all isolates. • The reference test was an established, routine method of identifying pathogens.	• Previously identified specimens were used, as study was of accuracy of identifying anaerobes.

APPENDIX 4: Main Study Findings and Author's Conclusions

Table A4-1: Summary of Findings of Included Systematic Reviews

Main Study Findings	Author's Conclusions
Dixon, 2015[5]	
Studies: 10 non-RCTs. Seven non-randomized, 3 before-and-after. **MS systems/databases**: Bruker Microflex with BioTyper v3.0 and Bruker MS database 3.0+ (4 studies), Bruker Microflex with BioTyper v3.0 and unspecified database (3 studies), Bruker Microflex with BioTyper v2.0 and unspecified database (1 studies), Bruker Microflex with no other information (1 study), bioMérieux MS with SARAMIS RUO (1 study). Narrative summary planned, because of expected high heterogeneity, e.g., 8 studies measured from time of positive blood culture, 4 used time of blood draw, 2 used other measures (some studies used multiple measures). **Time to identification**: MALDI-TOF median 20 min to mean 56 hours. Comparator: 26 hours to 84 hours (10 studies, systems and databases as above). Average reduction in time to identification with MALDI-TOF at least 24 hours. Highly dependent upon organism, preparation, and difficulty identifying. Gram-positive bacteria: MALDI-TOF 5.3 to 18.5 hours versus conventional 29.5 to 84.1 hours (2 studies; Bruker Microflex with BioTyper v3.0 and unspecified database [1 study], bioMérieux MS with SARAMIS RUO [1 study]). Gram-negative bacteria: MALDI-TOF 6 to 16.8 hours versus conventional 36 to 132 hours (3 studies; Bruker Microflex with BioTyper v3.0 and unspecified database [2 studies], bioMérieux MS with SARAMIS RUO [1 study]). Obligate anaerobes: MALDI-TOF 4.1 to 10.3 hours versus conventional 70 to 79.3 hours (1 study; Bruker Microflex with BioTyper v3.0 and unspecified database). Yeast: MALDI-TOF 2.4 to 16.1 hours versus 31.1 to 68.1 hours (2 studies; Bruker Microflex with BioTyper v3.0 and unspecified database [1 study], bioMérieux MS with SARAMIS RUO [1 study]). **Length of stay**: MALDI-TOF hospital 11.4, 9.3 days. Comparator: 14.2, 11.9 days (2 studies; Bruker Microflex with BioTyper v3.0 and Bruker MS database 3.0+ [1 study], Bruker Microflex with no other information [1 study]).	• "The observational studies reviewed provide evidence of potentially substantial time savings of MALDI-TOF in pathogen identification and instigation of appropriate therapy, which may also reduce hospital stay. Due to the small number studies, all at relatively high risk of bias, this cannot be considered as definitive evidence of the impact of MALDI-TOF. More and better evidence, including impact on patient health and cost-effectiveness, is required." (p 1 pre-print version)[5]

Table A4-1: Summary of Findings of Included Systematic Reviews	
Main Study Findings	**Author's Conclusions**
Ling, 2014[17]	
Studies: 33 articles, 38 studies, 16 prospective, 12 retrospective, 5 both. 34 studies used clinical isolates, 4 included reference strains. Total 9977 fungal isolates; 8842 yeast, 1135 mold. **MS systems/databases**: Bruker Biotyper system (24 studies), Saramis system (6 studies), VITEK system (4 studies), Andromas system (4 studies). 5 comparison studies. Databases were not specified. **Pooled identification ratio**: Species-level, random effects (33 studies): 95.5% [95%CI 93.9% to 96.9%], I^2=90.9%. Range 81% to 100%. Genus-level, random effects (6 studies) 97.7% [95%CI 95.5%% to 99.3%], I^2=72.9%. Range 91% to 100%. Clinical isolates only: Species-level 95.0% [95%CI 93.2% to 96.5%). By system: VITEK MS 93.3% [95%CI 88.7% to 96.8%], Biotyper 95.4% [95%CI 93.3 to 97.1%]. Saramis 93.8% [95%CI 88.1% to 97.8%]	• "… MALDI-TOF MS showed high accuracy for the identification of clinical pathogenic fungi in the present meta-analysis. Therefore, future studies to analyze the comprehensive capability of this technology for clinical microbiology diagnostics are warranted." (p2580)[17]

Table A4-2: Summary of Findings of Included Clinical and Cost Studies	
Main Study Findings	**Author's Conclusions**
Clerc, 2013[18]	
202 episodes of bloodstream infection with Gram-negative bacteria. Microflex LT MALDI-TOF (Bruker Daltronics, Germany) with MALDI Biotyper 2.0 software. **Effect on antibiotic choice**: Gram stain plus MALDI-TOF affected choice for 35.1% cases, mainly leading to early appropriate broadening. Gram stain affected choice for 20.8% cases. **Most common strains**: *Escherichia* coli 27.7%, *Klebsiella* species 12.4%, *Pseudomonas* species 10.9%, *Enterobacter* species 8.9%. **Accuracy** Monomicrobial genus-level 86.7%, species-level 72.8%. Polymicrobial, genus-level for at least one pathogen 75.7%.	• "In a low prevalence area for extended spectrum betalactamases (ESBL) and multiresistant gram negative bacteria, MALDI-TOF performed on blood culture pellets had an impact on the clinical management of 35.1% of all Gram-negative bacteremia cases, demonstrating a greater impact than Gram stain reporting. Thus, MALDI-TOF could become a vital second step beside Gram stain in guiding the empirical treatment of patients with bloodstream infection." (p1011)[18]
Huang, 2013[11]	
908 patients with positive blood cultures identified, 501 included in final analysis. Pre-intervention 256 patients, intervention 245 patients. Bruker Microflex MS with MALDI Biotyper 3.0 software and 3.1.0 database. Mean age pre-intervention patients 59.5 years versus intervention 56.5 years. Infectious organisms: Gram-positive 51.7% versus 55.3%, Gram-negative 41.2% versus 38.0%, yeast 7.1% versus 6.7%, polymicrobial 20.3% versus 19.2%, MRSA 3.7% versus 9.7%. **Clinical outcomes**. 30-day all-cause mortality pre-intervention 20.3% versus with MALDI-TOF 12.7% (p=0.021). Time to microbial clearance 3.3 ± 4.8 days versus 3.3 ± 5.7 days. Length of hospitalization 14.2 ± 20.6 days versus 11.4 ± 12.9 days. Length of ICU stay 14.9 ± 24.2 days versus 8.3 ± 9.0 days. Recurrence of BSI 15% ± 5.9% versus 5% ± 2.0%. 30-day readmission with same BSI 9% ± 3.5% versus 4% ± 1.6%.	• "MALDI-TOF with AST [antimicrobial stewardship team] intervention decreased time to organism identification and time to effective and optimal antibiotic therapy." (p1237)[11]
Martiny, 2013[12]	
277 blood cultures from 243 patients; results from 197 transmitted to treating physician. Bruker Microflex LT with MALDI Biotyper 3.0 and database v 3.1.2.0, 3995 spectra. **Accuracy**. 174/197 (88.3%) identifications confirmed by standard methods. 4% misidentified, *Acinetobacter* sp and *Staphylococcus epidermidis*, and mixed cultures.	• MT resulted in faster adaptation of antibiotic regimen in 13.4% of cases. • Technique is able to confirm contamination, especially in pediatric population. • Should not be considered unless there is efficient communication between laboratory and treating physician. Authors noticed delays between transmission and adjustment of >4 h in 50% of cases.

Table A4-2: Summary of Findings of Included Clinical and Cost Studies	
Main Study Findings	**Author's Conclusions**
Effect on antibiotic choice (retrospective). 34/197 (17.3%).	
Perez, 2013[13]	
317 patients with Gram-negative bacteria in culture evaluated for inclusion, 219 included. 112 pre-intervention, 107 intervention. Mean age 66.1 years. Bruker Microflex LT with MALDI Biotyper software (version not specified). **Most common isolates**. *Escherichia coli* 50% and 43% in pre-intervention and intervention groups, respectively. *Klebsiella* spp 23.3% and 19.7%. **Length of stay**. Hospital LOS Pre-intervention versus intervention 11.9±9.3 days versus 9.3±7.6 days. ICU LOS 7.3±8.5 days versus 6.3±8.7 days. Factors associated with LOS on multivariate analysis: active antibiotic therapy at 48 hours, MALDI-TOF antimicrobial stewardship intervention, APACHE II score, pre-infection LOS, pre-existing lung disease. **Costs**. Total hospital costs in survivors US$45,709±$61,806 versus $26,162±$28,996.	• "Integration of rapid identification and susceptibility techniques with antibiotic stewardship significantly improved time to optimal therapy, and it decreased hospital length of stay and total costs. This innovative strategy has ramifications for other areas of patient care." (p1247)[13]
Tan, 2012[19]	
2,217 patient specimens processed; 991 positive, 357 multiple isolates. 952 isolates identified, 824 bacteria, 128 yeast. Bruker Microflex LT with MALDI Biotyper 3.0 software and database v3.1.2. **Overall accuracy** of MALDI-TOF protocol: 98.3% (including supplementary tests for difficult-to-identify strains). **Most common strains**: *Enterobacteriaceae* 31.2%, *Staphylococcus aureus* 12.0%, *Enterococcus* species, 8.6%, *Pseudomonas aeruginosa*, 8.5%. **Time to identification**. (n=911) MT identified 87.2% on first day versus standard protocol 9.4%. On second day, 97.8% versus 61.5%. MT identified an average 1.45 days (34.8 hours) earlier, range 0.04 days (1 hour) *Candida albicans* to 4.13 days (99 hours) other Gram-positive rods. **Estimated annual costs**. MALDI-TOF protocol estimated costs $87,556 versus standard protocol $189,969. Estimation based on 47,845 bacteria representing 279 species tested in the 12 months prior to the study.	• "The MALDI-TOF MS system is robust with different MT users and a broad range of specimens, reflecting the potential as the primary identification system of bacteria and yeasts in a diverse, high-volume clinical laboratory. The MALDI protocol demonstrated reduction of costs and TTI, while maintaining similar accuracy as the standard protocol." (p3307)[19] • "Studies to correlate the reduction of TTI with clinical outcomes and further cost analysis studies in the microbiology laboratory are warranted." (p3307)[19].

Table A4-2: Summary of Findings of Included Diagnostic Accuracy Studies	
Main Study Findings	**Author's Conclusions**
Comparative	
Jamal, 2014[24]	
806 isolates tested from solid media; 70 species, 39 genera. **System 1**. Bruker Microflex MS with MALDI Biotyper 3.0 database. **System 2.** Spectra acquired with VITEK MS. Analysis with VITEK MS database (version not specified). **Most common species**. *Escherichia coli* 22.5%, *Klebsiella pneumoniae* 8.8%, *Pseudomonas aeruginosa* 8.2%, *Staphylococcus aureus* 6.6%. **Accuracy**. Identification Bruker Microflex species-level 93.2%, genus-level 97.3%. bioMérieux VITEK MS 99.9%, 99.0%. VITEK 2 (standard method) 98.6%, 96.4%. Gram-negative: 96.6% by Bruker Microflex, 99.4% identified by bioMérieux VITEK MS and VITEK 2. Gram-positive cocci Bruker Microflex 88.8%, bioMérieux VITEK MS 98.5% versus VITEK 2 95.5%. Gram-negative cocci: Bruker Microflex 50%, bioMérieux VITEK MS 93.8% versus VITEK 2 93.8%. Gram-positive bacilli: Bruker Microflex 81.3%, bioMérieux VITEK MS 87.5% versus VITEK 2 25%. **Misidentified/unidentified**. Gram-negative bacilli: All misidentifications at genus level. Bruker Microflex MS identified 11, bioMérieux VITEK MS misidentified 3. Gram-positive cocci: Bruker Microflex misidentified 30/267 Gram-positive cocci, 18/26 *Streptococcus mitis/oralis* as *Streptococcus pneumoniae*. VITEK MS identified 12 Gram-positive cocci, 8/26 *S. mitis/oralis* as *S. constellatus.* Gram-negative cocci: Bruker Microflex misidentified 8/16, VITEK MS misidentified 1/16. Gram-positive bacilli: Bruker Microflex misidentified 3/16, VITEK MS 2/16, VITEK 2 1 misidentified, 11 not in database.	• VITEK MS performed slightly better than Bruker Microflex MS, especially for Steptococcus species. • MALDI-TOF systems were consistently accurate and produced results much more quickly than conventional system.
Chen, 2013[27]	
202 positive blood cultures; 181 polymicrobial, 21 polymicrobial. Gram-positive 75, Gram-negative 106. **System 1**. Bruker Microflex MS with MALDI Biotyper 3.0 database (4500 spectra). **System 2**. VITEK MS with VITEK MS IVD database. **Accuracy**. Concordance Bruker Microflex 81.8% species-level, 97.8% genus-level. VITEK MS 80.7% species-level, 92.8% genus-level. Gram-positive Bruker Microflex species-level 72.0%, genus-level 96.0%. VITEK MS 68.0%, 85.3%. Gram-negative Bruker Microflex 88.7%, 99.1%, VITEK MS 89.6%, 98.1%.	• Study confirmed the practical advantages of MALDI-TOF MS.

Table A4-2: Summary of Findings of Included Clinical and Cost Studies	
Main Study Findings	**Author's Conclusions**
Misidentified/unidentified. Unidentified Microflex 2.2%, VITEK MS 7.2%. Misidentified at species-level: *Streptococcus constellatus*, *Streptococcus bovis*. *Staphylococcus epidermidis* 7/10, *Streptococcus sanguinis* 3/5. **Polymicrobial cultures**: Bruker Microflex 2 identified 5/21 (23.8%), one identified 16/21 (76.2%). VITEK MS IVD 1 identified 100%.	
Jamal, 2013[29]	
274 isolates on solid media of clinically significant anaerobes; 14 species in 5 genera. **System 1**. Bruker Microflex MS with MALDI Biotyper 3.0 database. **System 2**. bioMérieux VITEK MS with VITEK MS database (version not specified) **Most common species**. *Bacteroides fragilis* 41.2%, *Clostridium difficile* 25.5%, *Prevotella bivia* 11.3%. **Accuracy**. Bruker Microflex 99.2% genus, 99.2% species. bioMérieux VITEK MS 100% genus and species. **Misidentified/unidentified**. Microflex misidentified 2/274. *Bacteroides fragilis, Bacteroides thetaiolaomicron.*	• "MALDI-TOF is a rapid, simple, inexpensive technique." (p543)[29] • "[It] can be incorporated into the routine diagnostic laboratory and used for the identification of anaerobes." (p543)[29]
VITEK IVD	
Luo, 2015[20]	
2266 isolates tested; 56 genera, 127 species. 1581 Gram-negative aerobes, 535 Gram-positive aerobes, 150 anaerobes. bioMérieux VITEK MS IVD. **Accuracy**. Concordance of MALDI-TOF with reference: species-level 96.8% and genus level 99.1%. *Enterobacteriaceae* 97.1% and 99.4%, Non-*Enterobacteriaceae* Gram-negative 94.7% and 99.2%, staphylococci 99.7% and 100.0%, streptococci 92.6% and 100.0%, enterococci 98.8% and 98.8%, Gram-positive bacillae 90%, *Clostridium* spp. 97.3%, *Bacteroides* spp. 100%. Non-*Clostridium* and non-*Bacteroides* anaerobes 50%. **Misidentified/unidentified**. *Enterobacteriaceae*: 2.3% correct genus, incorrect species. Misidentified genus 4 (0.2%), *Shigella* spp. (2), *Raoultella ornithinolytica*, *Proteus mirabilis.* Non-*Enterobacteriaceae*: 4.5% correct genus, incorrect species. Could not be identified, 3. Aerobic Gram-positive bacteria: *Streptococcus* group 7.4% misidentified, *S. bovis*, *S. dysgalaciae*, *S. anginosus*, all	• "VITEK MS is highly accurate and reliable for routine bacterial identification in clinical settings in China." Luo, 2014, p 18.[20] • Equivalent or superior accuracy compared with VITEK in identification of clinically important pathogens. • Biological test panels still needed to aid in a subset of identifications, eg, *Shigella* spp, and non-*Clostridium* and non-*Bacteroides* anaerobes. • Ongoing expansion of the database is needed, particularly for anaerobes.

Table A4-2: Summary of Findings of Included Clinical and Cost Studies	
Main Study Findings	**Author's Conclusions**
<90% identification.	
Branda, 2014[21]	
226 clinical isolates fastidious Gram-negative bacteria from solid media; 15 species, 9 genera. bioMérieux VITEK MS IVD version 2.0. **Most common pathogens**. *Haemophilus influenzae* 24.3%, *Haemophilus parainfluenzae* 16.3%, *Campylobacter jejuni* 16.4%. **Accuracy.** Concordance VITEK MS species-level 96%, genus-level 97%. Lowest accuracy *Neisseria gonhorrhea* 90%. **Misidentified/unidentified**. No identification for one isolate each of *H. influenzae* and *C. jejuni*. Five isolates (*Haemophilis parainfluenzae, Neisseria gonhorrhoeae*) had low discrimination identification of multiple genera; 4 included correct species.	• "The VITEK MS v2.0 system proviced accurate identifications for fastidious Gram-negative bacteria, compared with nucleic acid sequencing as a reference method." (p 129)[21] • "Clinical laboratories can anticipate that a small fraction of fastidious Gram-negative bacterial isolates (3% in this study) will be unidentifiable using the VITEK MS system, necessitating alternative approaches." (p 129)[21]
Garner, 2014[22]	
651 isolates of anaerobic bateria: 26 species, 11 genera. Gram-positive 265, Gram-negative 357. bioMérieux VITEK MS [IVD] with v2.0 database. **Accuracy**. Correctly identified to species-level 91.2%, genus-level 92.5%. Gram-positive 91.7% species-level, 92.5% genus-level. *Actinomyces* spp 74.1% to species-level. *Clostridium* spp 96.3% to species-level. *Finegoldia magna*, *Parvimonas micra*, *Peptostreptococcus anaerobius*, *Peptostreptococcus asaccharolyticus* all >95%. Gram-negative 90.9% to species-level, 92.5% to genus-level. *Bacteroides* spp 92% (*B. ovatis* 73.3%), *Fusobacterium* spp 81.8%, *Prevotella* spp 91.1%. **Misidentified/unidentified**. No ID 7.5% (no results 5.5%, mixed genera 2%). Gram-positive 7.6%. Gram-negative 7.5%.	• "The VITEK-MS is an accurate system for identifying clinically relevant anaerobic bacteria. The implementation of this technology in the clinical microbiology laboratory will lead to decreased turnaround times for identification. MALDI-TOF MS can also be used in addition to traditional methods (colony morphology and Gram stain) for organisms that are difficult to identify."(p339)[22]
Guo, 2014[23]	
1025 clinical isolates on solid media, bacteria and fungi; 55 species, 25 genera. VITEK MS MALDI-TOF (database not specified). **Most common species**. *Escherichia coli* 31.2%, *Pseudomonas aeruginosa* 13.3%, *Staphylococcus aureus* 8%, *Proteus mirabilis* 8%. **Accuracy**. Concordance species-level 99.6%, genus-level 93.4%.	• Lower identification error rate, better performance than VITEK 2 in identifying bacteria routinely seen in laboratory. • Quick, cost-effectiveness, has potential to replace conventional phenotype methods for identifying common isolates.

Table A4-2: Summary of Findings of Included Clinical and Cost Studies	
Main Study Findings	**Author's Conclusions**
Misidentified/unidentified. 7.4% discordant results between MALDI-TOF and VITEK 2. One strain had no definitive identification. Error rate at genus level MALDI-TOF 0 versus VITEK 2 0.58%. MALDI-TOF species errors included *Enterobacter cloacae*, *Streptococcus mitis*, *Achromobacter xylosoxidans*. Study did not include viridians streptococci, *S. pneumoniae*, or anaerobes.	
Manji, 2014[25]	
558 isolates tested; 18 genera, 33 species. Non-*Enterobacteriaceae* Gram-negative bacilli. Include 187 rare isolates provided by bioMérieux, used to develop database. bioMérieux VITEK 2 MS [IVD] v 2.0. **Accuracy**. Identified 516/558. Correct identification: species-level 77.8%, genus-level 91.8%. Excluding organisms used to develop database: species-level 78.2%, genus-level 91.9%. *Pseudomonas* spp 89.9% species-level, 95.5% genus-level. *Bordetella* spp species-level 56.0%, genus-level 80%. *Acinetobacter* spp 83.3% species-level, 88.0% genus-level. *Bulkholderia cepacia complex* strains 95.0% correct to complex level. *Vibrio* spp 87.9% to 90.9% species-level. **Misidentified/unidentified**. Mis-identified 0.7%, no identification 7.5%. Excluding organisms used to develop database: No identification 7.8%. Four isolates incorrectly identified to genus level: *Alcaligenes faecalis* (1/12) as *Staphylococcus aureus* (possibly technical error orcontamination), *Rhizobium radiobacter* (2/14) as *Obesumbacterium proteus* and *A. denitrificans/xylosoxidans*, *Stenotrophomonas maltophilia* (1/54) as *Ochrobactrum anthropi*.	• "These findings demonstrate that the VITEK MS 2.0 provides accurate results for the identification of a challenging and diverse group of Gram-negative bacteria." (p337)[25]
Dubois, 2013[28]	
767 isolates from solid media; 124 species, 50 genera. bioMérieux VITEK MS IVD. **Most common species**. *Enterococcus faecalis* 5.0%, *Staphylococcus aureus* 4.7%, *Staphylococcus epidermidis* 4.7%, *Pseudomonas aeruginosa* 4.7%. **Accuracy**. Correct identifications. MALDI-TOF species 86.7% and genus 94.9%. *Enterobacteriaceae* 82.2% and 98.2%. Non-fermentative Gram-negative rods 86.2% and 94.7%. Other Gram-negative 80.9% and 83%. *Staphylococci* and related 91.2% and 94.5%. Anaerobes 83% and 83%. **Misidentified/unidentified**. 1.3% (Another 1.3% only identified above genus). 10 misidentified on first test, 3 misidentified on retest: *Shigella*, *Neisseria mucosa*. No identification: 2.5%.	• "The VITEK MS system is a simple, convenient, and accurate method for routine bacterial identification with a single deposit." (p2575)[28]. • "… expanding the spectral database is warranted, particularly for anaerobic, coryneform, and some highly pathogenic bacteria, in order to use almost exclusively this system to isolate IDs in routine medical practice." (p2575)[28]

Table A4-2: Summary of Findings of Included Clinical and Cost Studies	
Main Study Findings	**Author's Conclusions**
Rychert, 2013[31]	
1146 aerobic, Gram-positive isolates on solid media; 16 genera, 42 species. bioMérieux VITEK MS with VITEK MS IVD database. **Most common species**. *Staphylococcus epidermidis* 8.6%, *Enterococcus fecaelis* 5.9%, *Staphylococcus aureus* 5.3%, *Streptococcus agalactiae* 5.1%. **Accuracy**. Concordant species-level 92.8%, genus-level 95.5%. enterococcus species 97% identified, staphylococci 96%, streptococci 82%. **Misidentification**. 1.6% (18 isolates). Misidentified *Listeria monocytogenes* 11/45, *Streptococcus dysgalactiae* 23/47. No identification 2.9%. There was no consistent pattern of failure. Protocol did not include repeat testing.	• "… the VITEK MS system is highly accurate for the identification of Gram-positive aerobic bacteria in the clinical laboratory setting." (p2225)[31]
Bruker Microflex	
Hsueh, 2015[6]	
147 isolates of aerobically growing Gram-positive bacilli. *Nocardia* spp 50.3%, *Kocuria* spp 10.2%, *Rhodococcus* spp 6.8%, *Gordonia* spp 4.8%, *Tsukamurella tyrosinosolvens* 1.3%, *Listeria* spp 26.5%. Bruker Microflex LT with FlexControl 1.3 and Biotyper 3.1 (5,627 spectra). **Accuracy**. Overall correctly identified to species-level 39.5%, genus-level 79.6%. *Nocardia* spp species-level 14.9% (all *N. nova*, *N. otitidiscavarium*), genus-level 68.9%. *Rhodococcus* spp species-level 80%, genus-level 90%. *Kocuria* species species-level 26.7%, genus-level 100%. *Tsukamurella tyrosinosolvens* species-level 0%, genus-level 50%. *Listeria monocytogenes*, species-level 89.7%, genus-level 100%. **Misidentified/unidentified**. Misidentified 0%. Unidentified 20.4%: *Nocardia* other than *N. nova*, *N. otitidiscavarium, Tsukamurella tyrosinosolvens, Gordonia* spp. (*N. beijingensis*, *N. puris*, *N. rhamnosiphila*, *T. tyrosinosolvens*, *G. amicalis* not included in database.)	• "[…] our data sugges that Bruker Biotyper MALDI-TOF system is ineffective for identifying Nocardia and other unusual GPRs [Gram-positive rods] (Gordonia and Tsukamurella species) becuase of the current database limitations […] Therefore it is necessary to continuously update the MALDI-TOF databases. Further expansion of the database with a larger number of recently described isolates of Nocardia species and other unusual GPRs is warranted."(p2378)[6]
Panda, 2014[26]	
82 isolates tested from solid media. Bruker Microflex MS with FlexAnalysis 2.4 and MALDI Biotyper 1.1 database. **Most common species**. *Escherichia coli* 28.0%, *Staphylococcus aureus* 18.2%, *Staphylococcus epidermidis* 9.8%. **Accuracy** Concordance species-level 87.8% with respect to conventional methods. With confirmation from external lab,	• "MALDI-TOF MS was found to be an accurate, rapid, cost-effective and robust system for identification of clinical bacterial isolates." (p770)[26]

Table A4-2: Summary of Findings of Included Clinical and Cost Studies	
Main Study Findings	**Author's Conclusions**
98.8%. **Misidentified/unidentified**. 1 *Streptococcus viridans* as *S. pneumoniae*.	
Jamal, 2013[7]	
Blood cultures from 175 patients, with 160 (91.1%) evaluable (15 excluded). Monomicrobial Gram-positive 55.6%, Gram-negative 33.1%, Yeast 5%. Bruker Microflex MS with MALDI Biotyper 3.0 database. **Most common species**. *Staphylococcus epidermidis* 21.9%, *Escherichia coli* 10.7%, *Staphylococcus aureus* 7.5%, *Klebsiella pneumoniae* 6.9%. **Accuracy**. MALDI-TOF 75.6% versus conventional 93%. Gram-negative 66%. Gram-positive 71.2%. **Misidentified**. 22.5%. 5 with reliable scores ≥2.0. Misidentified: 4/7 *Streptococcus* species including 7/35 *Streptococcus epidermidis,* 6/6 *Staphylococcus warneri*, 3/4 *Acinetobacter baumannii*. 4/8 of yeasts.	• MALDI-TOF provided early diagnosis with shorter turnaround time. • May replace routine identification on colonies, if specificity can be improved for of coagulase-negative staphylococci, viridians streptococci, and yeast.
Leli, 2013[8]	
109 positive blood cultures; 108 monomicrobial. Gram-positive 59.7%, Gram-negative 33.9%, yeasts 6.4%. 1 polymicrobial. Bruker Microflex MS with MALDI Biotyper 3.0 database. **Most common species**. *Escherichia coli* 19.2%, *Staphylococcus epidermidis* 15.6%, *Staphylococcus aureus* 8.2%. **Accuracy**. Overall identification species-level 77.9%, genus-level 91.7%. Gram-positive 85.5% species-level, genus-level 91.9%. Gram-negative species level 96.9%, genus-level 100%. Anaerobes, genus-level 100%. Yeasts, 0%. Concordance with Phoenix system 93.5%. **Misidentified/unidentified**. Gram-positive 2/62. *Streptococcus pneumoniae* 2 isolates with Score <1.7, 2 misidentified at species-level. Yeasts, 3 *Candida albicans*, 1 *Candida glabrata*. **Polymicrobial**. 1 organism identified.	• "MALDI-TOF MS is a valuable tool for identification of pathogens in septic patients. It can replace the Phoenix system, anticipating the bacterial identification results." (p208)[8]
Rodríguez-Sanchez, 2013[30]	
1000 positive blood cultures with 1085 organisms. 68 polymicrobial. Bruker Microflex LT with FlexControl 3.3 and MALDI Biotyper 3.0 database (4613 spectra).	• "Implementation of MALDI-TOF identification directly from the BCB [blood culture bottle] provides a rapid and reliable identification of the causal pathogen within hours." (pO421)[30]

Table A4-2: Summary of Findings of Included Clinical and Cost Studies	
Main Study Findings	**Author's Conclusions**
Most common species. *Escherichia coli* 19.6%, *Staphylococcus epidermidis* 11.6%, *Staphylococcus aureus* 9.1%, *Streptococcus pneumoniae* 5.9%. **Accuracy**. Concordant with reference identification species-level 76.9%, genus-level 81.4%. Protein extraction required for 17.5%. **Misidentified/unidentified**. No identification 18.4% Gram-positive 54.5%: *Staphylococcus epidermidis* 25/126, *Staphylococcus aureus* 31/99, *Streptococcus pneumoniae* 12/64. Yeasts 24.5%, especially *Candida albicans* 25/26. Identified only to genus level, 4.5%, notably *Streptococcus pneumoniae*, and *Salmonella* species. Two discordant identifications: *Streptococcus sanguinis*, *Escherichia coli.* **Polymicrobial**. Both organisms identified to species level in 16.2%, 1 organism in 75%, 1 mis-identified, 7.4% not identified.	
Schmitt, 2013[32]	
253 isolates of anaerobes. Bruker Microflex LT with Biotyper 3.0, library 3.3.1.0 (4613), supplemented with spectra from in-house collection of 87 isolates, 39 species. **Accuracy**. Correct identification to species-level 70.8%, genus-level 91.7%. **Misidentified/unidentified**. Misidentified 4.7%, 11/12 species-level, 1/12 genus-level (3 not in database), *Mogibacterium timidim* (not in database) as *Clostridium halophilium*. No identification 7.9%.	• "Utilization of the MALDI-TOF Biotyper systmem provides accurate, rapid and inexpensive identification of anaerobic bacteria, although use fo the manufacturere's cutoff scores resulted in several misidentifications at the species level and a single misidentification at the genus level. Expansion of the library may improve accuracy."(p786)[32]
Buchan, 2012[9]	
164 positive blood cultures, 134 unique bacteremic episodes. 150 monomicrobial cultures, 67% Gram-positive, 30% Gram-negative, 3% yeast. 9% polymicrobial. Microflex LT MALDI-TOF (Bruker Daltronics, Germany) with MALDI Biotyper 3.0 (3995 spectra). **Accuracy**. Identification, species-level 64.8%, genus-level 85.5%. Gram-positive species-level 53.0%, Gram-negative 91.1%. Concordance with routine methods, species-level 94.1%, genus-level 97.6%. **Misidentified/unidentified**. Gram-negative One isolate not identified by MALDI-TOF *Neissseria gonorrhoeae*; subculture confirmed identification. Mis-identified by MALDI-TOF, one isolate *Enterobacter cloacae*. Gram-positive 20% Score <1.7. *Staphylococcus epidermidis* 34.8% Score <1.7%. Discrepant results: 5 with acceptable confidence scores, all identified as *Streptococcus oralis* or *viridans* by routine methods.	• "The MALDI Biotyper/Sepsityper can be used to directly analyze positive blood cultures in real time and provide definitive species identification within 20 min […] Combined with predictable antibiotic resistance profiles and effective realtime antibiograms, these reduced times to bacterial identification could aid in guidance of antibiotic therapy in patients with bacteremia." (p351)[9]

Table A4-2: Summary of Findings of Included Clinical and Cost Studies	
Main Study Findings	**Author's Conclusions**
Yeasts. None of five isolates identified. **Polymicrobial**. One organism identified in 9/14 (64.3%) polymicrobial cultures.	
El-Bouri, 2012[33]	
928 isolates on solid media from clinical samples; 67 species, 33 genera. Bruker Microflex LT with MALDI Biotyper 2.0 software (>3000 spectra). **Most common species**. *Escherichia coli* 14.5%, *Staphylococcus aureus* 9.2%, *Staphylococcus epidermidis* 5.9%, *Pseudomonas aeruginosa* 4.4%. **Accuracy.** Identification overall species-level 77.7%, genus-level 93.8%. Concordance with conventional methods species-level 89.3%, genus-level 99.1%. Gram-positive bacilli 86.6%, genus-level 100%. Non-fermentative Gram-negative bacilli genus-level 95.9%, species-level 81.6%. **Misidentified/unidentified**. Species-level misidentified 10.7%. No peaks on MALDI-TOF 3.6%. Misidentified: *Streptococcus pneumoniae* 4/10, *Staphylococcus epidermidis* 12/55, *Staphylococcus warneri 2/2. Enterobacter* group 18/38, *Acinobacter* species 13/26. **Costs**. Cost per isolate for MALDI-TOF identification £0.51 to £1.28. Estimated savings per isolate £1.79 to £2.56 without additional disk susceptibility testing.	• "Microbial identification by MALDI Biotyper offers a rare opportunity for significant cost-neutral or even cost-saving quality improvements in medical diagnostics." (p53)[33]
Fedorko, 2012[34]	
152 isolates of anaerobic bacteria. Gram-positive bacilli 69, cocci 35, Gram-negative bacilli 48. Bruker Microflex LT with Biotyper version 2.0.4 (database not specified. Updated during study to 3,996 entries). **Accuracy**. Correctly identified to species-level 79% (Score ≥2), to genus-level 89% (Score ≥1.8). Gram-positive anaerobic bacilli species-level 81.2%, genus-level 92.8%, aerobic cocci 74.3%, genus-level 85.7%, Gram-negative aerobic bacilli species-level 87.5%, genus-level 87.5%. **Misidentified/unidentified**. Misidentified at Score <1.8 5 isolates (11 isolates were correctly identified at <1.8).	• "The present study demonstrates that the Bruker Microflex MALDI-TOF instrument with Biotyper Software and the most recent database compares favourably to 16S rRNA sequencing for the identification of anaerobic bacteria isolated from clinical specimens." (p2261)[34]
Lagacé-Weins, 2012[10]	
63 positive blood cultures; 61 monomicrobial, 2 polymicrobial. Microflex LT with MALDI Biotyper 3.0 (database version not specified).	• "Implementation of a MALDI-TOF-based identification system for direct identification of pathogens from blood cultures is expected to be associated with a marginal increase in operating costs for most laboratories. However, the use of MALDI-TOF for direct

Table A4-2: Summary of Findings of Included Clinical and Cost Studies	
Main Study Findings	**Author's Conclusions**
Most common species. *Escherichia coli* 15.2%, *Staphylococcus aureus* 10.9%, Coagulase negative *Staphylococcus* 10.6%, *Klebsiella* species 4.4%. **Overall accuracy**. Identified to species level 68.8%, 85.2% to genus-level. 95.1% concordant with final identification, regardless of score. **Misidentified/unidentified**. Discordant: one each *Rothia mucilaginosa*, *Streptococcus mitis*, coagulase negative streptococci. **Polymicrobial**. 1 organism identified in 1 culture, 1 organism identified with score ~1.6 in second. **Costs**. Estimated a net cost/positive culture US$3.24 (range of estimates minus US$0.28 to US$3.64), assuming 14.8% failure to identify with Sepsityper.	identification is accurate and should result in reduced turnaround time to identification." (p3324)[10]
Xiao, 2012[35]	
212 isolates on solid media from 70 patients; 30 species and 12 genera. Bruker Microflex LT with Biotyper 1.5 processing and database (3995 spectra). **Most common species**. *Rothia mucilaginosa* 19.2%, *Streptococcus salivarius* 17.6%, *Streptococcus parasanguinis* 15.7%, *Streptococcus pneumoniae* 12.3%. **Accuracy**. Bruker Microflex LT MALDI-TOF concordant to species level 89.6%, to genus level 95.3%. **Misidentified/unidentified**. Two isolates not identified.	• "As a rapid, accurate, easy to use, inexpensive, and high throughput proteomic technique, the MALDI-TOF MS system is a good tool for CAP-related pathogen identification and epidemiological surveillance." (p306)[35]
Bizzini, 2011[44]	
433 isolates selected from 1405 16S rRNA sequenced over 8 years, with maximum 5 isolates/species, 207 species, 84 genera. 410 produced growth in culture. Gram-positive bacilli 41.2%, Gram-positive cocci 19.5%, Gram-negative bacilli 39.3%. Bruker Microflex LT with FlexControl 3.0 and Biotyper 2.0 (library of 3,290 spectra. Database updated to version 3.1, 3,740 spectra during manuscript revision). **Accuracy**. Overall correctly identified to species-level 45.9%, genus-level 67.6% (species-level 48.8% after database update). Gram-positive bacilli to species-level 30.2%, Gram-positive cocci to species-level 51.3%, Gram-negative bacilli to species-level 59.6%. **Misidentified/unidentified**. Unidentified 133/410 (32.4%). 78 isolates were of species not represented in database. None misidentified to genus level.	• "[...] our study shows that MALDI-TOF MS has the potential to reduce the need for molecular identifcation techniques such as 16S rRNA gene sequencing and might replace these time-consuming and expensive techniques for the majority of difficult-to-identify isolates in the clinical microbiology laboratory [...] Further expansion of the database of the instrument and optimization of extraction protocols for difficult-to-treat samples will undoubtedly increase the accuracy of identification by the MALDI-TOF MS and the diversity of species that might be efficiently identified by this promising approach."(p696)[44]

Table A4-2: Summary of Findings of Included Clinical and Cost Studies	
Main Study Findings	**Author's Conclusions**
Justesen, 2011[37]	
278 clinical isolates, plus 12 library isolates; 80 species from 27 genera. Microflex LT MALDI-TOF (Bruker Daltronics, Germany) with Flex Control 3.0 software and MALDI Biotyper 3.1.1.0. **Most common species**. *Bacteroides fragilis* 21.5%, *Clostridium perfringens* 7.9%, *Bacteroides thetaiotaomicron* 5.8%, *Fusobacterium necrophonum* 5.4%. **Accuracy**. Bruker LT MALDI-TOF identified to species level 67.2%, to genus level 67.2%. Bruker LT *Bacteroides* and *Clostridium* species to species level 84.9% and 93.9%, respectively. Bruker LT identified 83.5% on first run, 9.2% on second, 7.3% post formic acid pretreatment. **Unidentified/misidentified**. Unidentified by Bruker LT 18.3%. Difficulty in identifying metronidazole-resistant Gram-positive rods. Incorrect species identification Bruker LT 7.9%.	• Bruker system identified more samples to species level, but made more incorrect identifications (mainly within the same genus). • Second and third runs of isolates increased the identification in both systems. • Isolates that were not identified despite being in the system database were mainly metronidazole-resistant Gram-positive rods.
Kok, 2011[38]	
507 positive blood cultures identified, 93.9% monomicrobial, 6.1% polymicrobial. In monomicrobial cultures, Gram-positive 59.9%, Gram-negative 39.3%, anaerobic 0.8%. Bruker Microflex LT operated by MALDI-Biotyper with Biotyper 2.0. **Most common species**. Coagulase-negative staphylococci 29.6%, *Escherichia coli* 20.1%, *Staphylococcus aureus* 10.7%. **Accuracy**. Overall MALDI-TOF species-level concordant 59.4%, genus-level identification 74.8%. Gram-positive species-level identification 67.7%, genus-level identification 100%. Gram-negative species-level identification 91.1%. **Unidentified/misidentified**. MALDI-TOF unable to identify 9.3% of *Staphylococcus aureus* and 36% coagulase-negative staphylococci, 4/4 anaerobes, 53.8% enterococci, and 65% Gram-positive bacilli. **Polymicrobial**. 20/32 63.5% one organism identified. 10/32 32.3% none identified, 3.2% (one culture) mis-identified to species level.	• "A diagnostic algorithm that incorporates Gram staining and MALDI-TOF MS should identify the majority of pathogens, particularly to genus level." (p e23285)[38]
Neville, 2011[39]	
927 isolates from solid media included. Bruker Microflex with Biotyper 2.0 (software version 3.1.1.0)	• MALDI-TOF MS is rapid, accurate, and inexpensive." (p2980)[39]

Table A4-2: Summary of Findings of Included Clinical and Cost Studies	
Main Study Findings	**Author's Conclusions**
Accuracy. Concordant species-level 84.5%, genus level 96.4%. **Unidentified/misidentified**. Genus-level misidentifications: 1 isolate of *Micrococcus luteus*, 8/8 *Shigella*. Species-level misidentifications: 6/6 *Streptococcus mitis*, 1 *Streptococcus agalactiae*. **Costs**. Calculated cost savings was $8,395.67, for 927 isolates.	
Saffert, 2011[40]	
440 isolates from solid media. Bruker Microflex LT operated by MALDI-Biotyper with Biotyper 2.0. **Accuracy**. MALDI-TOF identifications to species-level 82%, genus-level 93%. **Unidentified/misidentified**. MALDI-TOF 4% incorrect species, 2% incorrect genus. Misidentified: *Klebsiella oxytoca*, *Raoultella ornithinolytica*, *Shigella*.	• "With the improved turnaround time and cost-effectiveness of the Bruker Biotyper system, MALDI-TOF MS technology provides an advance in bacterial identification in the clinical microbiology laboratory." (p891)[40].
Spanu, 2011[41]	
346 yeast isolates tested from 360 cultures, 6 polyfungal. Bruker Microflex LT with FlexControl software 2.0, database version unspecified. **Accuracy**. Concordant with reference 91.3%. *Candida albicans* 95.9%, non-*albicans Candida* 86.5%. **Misidentified/unidentified**. Unidentified 25/346 (7.2%, Score <1.2). 11/12 organisms in 6 polyfungal cultures. **Polyfungal**. 1 organism identified in 1 culture, no identifications in 5.	• "[…] with direct Bruker Biotyper assay of BC blood broth, physicians can realistically expect (in many cases) to receive species-level ID data for *Candida* isolates causing BSI within 24 h after the BC is drawn […] This advantage, together with other time-saving measures (e.g., prompt collection of BCs when BSI is first suspected, immediate transport of cultures to the laboratory), should allow considerably earlier prescription of effective drug therapy for yeast BSIs, with positive effects on patient outcomes." (p178)[41]
Veloo, 2011[42]	
79 isolates of anaerobic bacteria, 47 species, 19 genera. Bruker Microflex LT with FlexControl software 3.0. Database 3476 spectra, to February 2010. **Accuracy**. Correctly identified to species-level 35.4% and 50.6% without and with pre-treatment, respectively, to genus level, 51.9% and 60.8%, respectively. *Bacteroides fragilis* group species, species-level and genus-level 87%. Gram-positive anaerobic cocci, 12.5% identified to species-level, 37.5% to genus-level.	• "[…] we cannot yet recommend implmenting MALDI-TOF MS for routine identification of anaerobic bacteria in clinical microbiology. However the study is not designed to evaluate the potential advantages of MALDI-TOF identification over currently used methods […] MALDI-TOF systems need optimization …" (p1506)[42]

Table A4-2: Summary of Findings of Included Clinical and Cost Studies	
Main Study Findings	**Author's Conclusions**
Misidentified/unidentified. Misidentified 2/79 (2.5%) with and without pre-treatment (*Actinomyces israelii*, not in database, misidentified as *Lactobacillus catanaformis*, *Actinomyces naeslundii* misidentified as *Neisseria gonorrhoea*). Overall unidentified 41.8% and 30.4% with and without pre-treatment, respectively. Unidentified because not in database 19%.	

APPENDIX 5: Summary of reliability of pathogen identification by MALDI-TOF, for more common pathogens

Studies are represented by their citation numbers.

Table A5-1 Summary of reliability of identification of Gram-positive organisms that grow aerobically

	From solid media				From blood cultures			
	Bruker Biotyper		Vitek MS IVD		Bruker Biotyper		Vitek MS IVD	
	≥90%	<90%	≥90%	<90%	≥90%	<90%	≥90%	<90%
Bacillus cereus/thuringiensis						27		27
Corynebacterium amycolatum		44						
Enterococcus avium			31					
Enterococcus casseliflavus			31					
Enterococcus durans			31					
Enterococcus faecalis	9,24,33	39	20,23,24,31	28	30	38		
Enterococcus faecium	33,39		20,23,28,31			30		
Enterococcus gallinarum			31					
Gardnerella vaginalis			31					
Kocuria kristinae		6						
Listeria monocytogenes	6,39			31				
Micrococcus luteus/lylae			31					
Nocardia cyriacigeorgica		6						
Rothia mucilaginosa		35						
Staphylococcus aureus	9,24,33,39		20,23,24,28,31		7,8,27	30,38		27
Staphylococcus capitis	24	39	20,24,28,31					
Staphylococcus epidermidis	24,33	9,27,39	20,23,24,27,28,31		7,8	30		
Staphylococcus haemolyticus	24		20,23,24,31					
Staphylococcus hominis	39		20,23,31		8	30		
Staphylococcus lugdunensis			20,28,31					
Staphylococcus saprophyticus			31					
Staphylococcus simulans			31					
Staphylococcus warneri			31					
Streptococcus agalactiae	24	39	24,28,31	20		30		
Streptococcus constellatus				28,31				
Streptococcus anginosus		39	28,31					

Table A5-1 Summary of reliability of identification of Gram-positive organisms that grow aerobically

	From solid media				From blood cultures			
	Bruker Biotyper		Vitek MS IVD		Bruker Biotyper		Vitek MS IVD	
	≥90%	<90%	≥90%	<90%	≥90%	<90%	≥90%	<90%
Streptococcus dysgalactiae		39*		20,*31†				
Streptococcus intermedius				31				
Streptococcus mitis/oralis		24	20,24,28	31		38		
Streptococcus parasanguinis		35						
Streptococcus peroris		35						
Streptococcus pneumoniae		35,39	20,28,31					
Streptococcus pyogenes	39		31					
Streptococcus salvarius		35						
Streptococcus sanguinis			31					

***** Subspecies not indicated

† *Streptococcus dysgalactiae ssp equisimilus*

Table A5-2 Summary of reliability of identification of Gram-negative organisms that grow aerobically

	From solid media				From blood cultures			
	Bruker Biotyper		Vitek MS IVD		Bruker Biotyper		Vitek MS IVD	
	≥90%	<90%	≥90%	<90%	≥90%	<90%	≥90%	<90 %
Achromobacter denitrificans				25				
Achromobacter xylosoxidans				25				
Acinetobacter baumannii / Acinetobacter baumannii complex		24	20,23,24	25				
Acinetobacter junii				25				
Acinetobacter lywoffii				25				
Aeromonas hydrohilia/caviae				25				
Aeromonas sobria				25				
Alcaligenes faecalis spp faecalis			25					
Bordetella bronchiseptica				25				
Bordetella pertussis				25				
Burkholderia cepacia				25				
Burkholderia multivorans			25					
Campylobacter jejuni			21					
Citrobacter freundii	40			20,28				

Table A5-2 Summary of reliability of identification of Gram-negative organisms that grow aerobically								
	From solid media				From blood cultures			
	Bruker Biotyper		Vitek MS IVD		Bruker Biotyper		Vitek MS IVD	
	≥90%	<90%	≥90%	<90%	≥90%	<90%	≥90%	<90 %
complex								
Citrobacter koseri	24		20,24	28				
Elizabethkingia meningoseptica			25					
Enterobacter aerogenes	39,40		20,23,28					
Enterobacter cloacae / *Enterobacter cloacae* complex	24,30,39,40		20,24	23,28				
Escherichia coli	24,33,39,40		20,23,24,28		8-10,27,30,38	7	27	
Haemophilus influenzae	33,39,40		21,28					
Hafnia alvei			28					
Haemophilus parainfluenzae			21					
Klebsiella oxytoca	33,39,40		20,23,28			30,38		
Klebsiella pneumoniae / *Klebsiella pneumoniae* complex	24,33,39,40		20,23,24,28		7,27,38	30	27	
Legionella pneumophilia			21					
Moraxella catarrhalis			21					
Morganella morganii			20,28					
Neisseria flavescens	35							
Neisseria gonorrhoeae	39		21					
Neisseria meningitides	39							
Orchobactrum anthropi			25					
Pasteurella multocida			25					
Proteus mirabilis	24,33,39,40		20,23,24			30		
Proteus vulgaris			20,23					
Pseudomonas aeruginosa	24,39,40		23-25,28		38			
Pseudomonas fluorescens				25				
Pseudomonas pudida				25				
Ralstonia pickettii				25				
Rhizobium radiobacter				25				
Salmonella species	24		24			30		
Salmonella enterica	33							
Salmonella typimurium		39						
Serratia marcescens	24,33	40	20,24,28				23	
Sphingobacterium spiritivorum			25					
Sphingomonas paucimobilis			25					
Stenotrophomonas matophilia	40		20,25,28					
Vibrio cholerae			25					

Table A5-2 Summary of reliability of identification of Gram-negative organisms that grow aerobically								
	From solid media				From blood cultures			
	Bruker Biotyper		Vitek MS IVD		Bruker Biotyper		Vitek MS IVD	
	≥90%	<90%	≥90%	<90%	≥90%	<90%	≥90%	<90 %
Vibrio parahaemolyticus				[25]				
Vibrio vulnificus			[25]					

Table A5-3 Summary of reliability of identification of anaerobes				
	From solid media			
	Bruker Biotyper		Vitek MS IVD	
	≥90%	<90%	≥90%	<90%
Actinomyces europaeus		[32]		
Actinomyces graevenitzii	[32]			
Actinomyces neuii				[22]
Actinomyces odontolyticus	[32]			
Bacteroides caccae			[22]	
Bacteroides fragilis	[20,29,32,37]		[22,29]	
Bacteroides ovatus				[22]
Bacteroides thetaiotaomicron	[29,37]		[22,29]	
Bacteroides uniformis				[22]
Bacteroides vulgatis		[29]	[22,29]	
Clostridium difficile	[33]	[29]	[20,22,29]	
Clostridium perfringens	[33,37]	[29]	[20]	[29]
Clostridium ramosum		[37]	[22]	
Finegoldia magna	[32]		[22]	
Fusobacterium necrophorum	[37]		[22]	
Lactobacillus rhamnosus		[37]		
Parvimonas micra			[22]	
Peptistreptococcus anaerobius			[22]	
Prevotella bivia	[32]	[29]	[22,29]	
Prevotella intermedia				[22]
Prevotella melaninogenica				[22]
Propionibacterium acnes	[32]	[37]		[22]
Staphylococcus saccharolyticus		[32]		