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Comparative Analysis of PCR–Electrospray Ionization/Mass Spectrometry (MS) and MALDI-TOF/MS for the Identification of Bacteria and Yeast from Positive Blood Culture Bottles

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BACKGROUND: Emerging technologies for rapid identification of microbes demonstrate a shift from traditional biochemical and molecular testing algorithms toward methods using mass spectrometry (MS) for the semiquantitative analysis of microbial proteins and genetic elements. This study was performed to assess the diagnostic accuracy of 2 such technologies, PCR–electrospray ionization (ESI)/MS and MALDI-TOF/MS, with respect to phenotypic and biochemical profiling as a reference standard method. A positive challenge set of blood culture bottles was used to compare PCR-ESI/MS and MALDI-TOF/MS performance on a matched set of samples.

METHODS: We performed characterization of bloodstream infections from blood cultures using the Ibis T5000 PCR-ESI/MS and the Bruker MALDI Biotyper 2.0 (MALDI-TOF/MS) platforms for microbial identification. Diagnostic accuracy was determined by independent comparison of each method to phenotypic and biochemical characterization with Vitek2 analysis as the reference standard identification.

RESULTS: The diagnostic accuracy, represented as positive agreement, at the genus level was 0.965 (0.930–0.984) for PCR-ESI/MS and 0.969 (0.935–0.987) for MALDI-TOF/MS, and at the species level was 0.952 (0.912–0.974) with PCR-ESI/MS and 0.943 (0.902–0.968) for MALDI-TOF/MS. No statistically significant difference was found between PCR-ESI/MS and MALDI-TOF/MS in the ability to rapidly identify microorganisms isolated from blood culture.

CONCLUSIONS: Our results demonstrate that PCR-ESI/MS and MALDI-TOF/MS are equivalent in their ability to characterize bloodstream infections with respect to the reference standard, and highlight key differences in the methods that allow for each method to have a unique niche as a tool for rapid identification of microbes in blood cultures.

Identification of microorganisms is of paramount importance to clinical microbiologists for diagnosis and treatment of bloodstream infections (BSIs).[6] Effective management of BSIs requires rapid detection and identification of microorganisms to allow deescalation from broad spectrum to targeted antibiotics, thus reducing overuse of broad spectrum antibiotics. Automated blood culture systems continuously monitor microbial growth and are the primary tools for the identification of BSIs. Their use is followed by routine phenotypic and biochemical evaluation of propagated bacteria and yeast to provide microbial identification *(1, 2)*. Reduction of analysis time would be advantageous, especially for organisms that are fastidious, slow-growing, nonculturable, or occur in polymicrobial infections. Several technologies using molecular methods have developed in recent years [i.e., targeted PCR assays *(3, 4)* and peptide nucleic acid fluorescent in situ hybridization *(5, 6)*], and are practical for a subset of microorganisms, but broader strategies that can characterize bacteria and yeast without prior knowledge of genetic targets are desirable.

To meet the need for an analytical technique capable of rapidly detecting the diversity of microorganisms found in BSIs, 2 emerging techniques are currently in development: PCR–electrospray ionization (ESI)/

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[6] Nonstandard abbreviations: BSI, bloodstream infection; ESI, electrospray ionization; MS, mass spectrometry.

mass spectrometry (MS) and MALDI-TOF/MS. Both apply MS to obtain reproducible, species-specific spectra that can be used to identify microorganisms. PCR-ESI/MS measures the mass/charge ratio (*m/z*) of PCR amplicons generated from several loci on bacterial and fungal genomes, focusing on conserved and species-specific regions, to identify base compositions comparative to a database of microorganisms. MALDI-TOF/MS relies on proteomic profiling of highly conserved proteins generated from direct ionization of a colony of intact organisms or bacterial protein extract, and correlates this spectral signature to a database of spectra collected from reference strains.

PCR-ESI/MS with the Abbott PLEX-ID (previously Ibis T5000) has proven useful for identification of viruses *(7–9)*, bacteria, and associated antibiotic-resistant pathogens *(10–13)* and, most recently, pathogens directly from blood culture bottles *(14)*. Bruker MALDI Biotyper (MALDI-TOF/MS) has also demonstrated utility for the identification of bacteria and yeast. Early investigations that used visual comparison and identification *(15, 16)*, which progressed to the use of pattern-matching algorithms *(17)* and extension of the mass range to detect higher molecular weight proteins, led to the development of the Bruker MALDI Biotyper, with proven utility in the identification of bacteria *(18–20)*, specifically bacteria with limited biochemical reactivity that are typically missed by phenotypic evaluation *(21, 22)*. Results of recent evaluations of bacteria from positive blood cultures, although limited by the need for a large number of cells, show promise for the application of this technique to routine clinical testing *(23, 24)*.

The aim of this study was to estimate the diagnostic accuracy of PCR-ESI/MS and MALDI-TOF/MS independently with respect to reference standard testing via Vitek2 analysis. In addition, we compared PCR-ESI/MS and MALDI-TOF/MS methods directly to evaluate the degree of agreement and comparative diagnostic accuracy of each technique to asses statistical differences between the 2 platforms.

Methods

PATIENTS AND SAMPLES

Procedures were performed in accordance with ethical standards as reviewed by the University of Arizona Human Subjects Protection Program. In this retrospective study we used banked remnant samples from patients at the University Medical Center in Tucson, Arizona, whose routine care included blood culture. No patient recruitment was required. The 273 positive blood culture broths tested during the course of this study were analyzed by the reference standard method before analysis by both PCR/ESI-MS and MALDI-TOF/MS.

Reference standard testing was performed by 11 medical technologists who were proficient in Vitek2 methods and whose minimum qualifications were Bachelor of Science degrees and medical laboratory science board certification. Evaluation of PCR-ESI/MS and MALDI-TOF/MS was undertaken by 3 laboratory scientists who had expertise in medical microbiology and biochemistry and whose minimum qualification was a Master of Science degree or equivalent.

SAMPLE SELECTION

For this retrospective study, we created a high-prevalence sample set with a diversity of species that could potentially be encountered in the clinical laboratory. Microbes were identified by standard culture methods and Vitek2 analysis before testing. Compared with sets used in previously reported work *(14)*, the sample set used in this study differed by removal of 27 samples that contained mixtures of organisms with different metabolic requirements, to simplify the Biotyper process and to further reduce redundancy, and by addition of 57 organisms, covering 31 additional species. This strategy broadened our scope so that we were able to further assess the accuracy of each method with organisms encompassing a wider range of genus and species diversity. Samples were blinded before evaluation with PCR/ESI-MS and MALDI-TOF/MS. Blood culture broth was used for PCR/ESI-MS; microbial isolates were used for MALDI-TOF/MS.

BLOOD CULTURE BROTHS

Two milliliters of blood from FA and FN blood culture bottles (Fastidious Antibiotic Neutralization, aerobic and anaerobic, respectively; bioMérieux) determined to be positive by the BacT/Alert 3D instrument (bioMérieux) were removed and prepared for cryostorage in the University of Arizona Infectious Disease Research Core's MicroBank biorepository. These specimens were stored at −80 °C within 30 min of bottle positivity until used. PCR-ESI/MS and MALDI-TOF/MS were performed on aliquots from remnant samples. No treatment was given between the reference standard and index testing.

REFERENCE STANDARD METHODS FOR IDENTIFICATION

Aliquots of positive blood culture bottles were subjected to Gram stain and plating on trypticase soy agar supplemented with 5% sheep blood, chocolate agar, MacConkey agar, or Sabouraud agar (Remel), depending on gram-stain results. Phenotypic testing based on determinative protocols described in the *Manual of Clinical Microbiology (25)* and in accordance with guidelines issued by the CLSI *(26–28)* was performed as necessary. Microorganism identifications were determined by the Vitek2 instrument using the GP ID

(gram-positive identification), GN ID (gram-negative identification) and Vitek2 YST ID (yeast identification) card (bioMérieux).

DNA ISOLATION FROM POSITIVE BLOOD CULTURES

DNA was extracted from 200 μL of remnant blood culture broth by using the Qiagen Biorobot EZ1. The DNA Bacteria protocol was used to perform all extractions, along with the associated EZ1 DNA Blood or EZ1 DNA Tissue kits (Qiagen).

PCR-ESI/MS ANALYSIS

The Ibis T5000 instrument and software package, version 2.6.052, was used (Ibis/Abbott Molecular for PCR/ESI/MS testing). The Sterile Fluid Bacteria and *Candida* assay was performed according to the manufacturer's specifications, as previously described *(14)*. Briefly, extracted DNA from blood culture bottles was diluted and distributed into the PCR plate containing primer pairs targeted toward detection and characterization of bloodstream pathogens. The extracted DNA was amplified by PCR on either an MJ Research DNA Engine Tetrad 2 (Bio-Rad Laboratories) or an Eppendorf Mastercycler EP gradient S thermocycler, both of which are approved by Abbott Molecular.

The T5000 was used to obtain the base count of each PCR amplicon, which was compared to a reference database of expected amplicons from each primer pair, which was used to identify bacterial/fungal organisms present. The confidence of identification was determined by the software package. For these experiments, all data meeting a threshold of 87.5% confidence for microbial identification were reported; data with $<$87.5% confidence were recorded as not interpretable and considered discordant.

MALDI-TOF/MS ANALYSIS

Microorganisms were subcultured before identification with Bruker MALDI Biotyper (MALDI-TOF/MS). Aliquots of 50-μL remnant blood culture specimens were enriched in 1 mL of trypticase soy broth with 0.5% sodium chloride, Lim Broth (Todd-Hewitt broth with colistin nalidixic-acid, Becton Dickinson), or chopped meat broth (Becton Dickinson), depending on metabolic requirements. The organisms were incubated in either ambient air, 5% CO_2, or in an anaerobic environment using the GasPak™ EZ Anaerobe Container System with Indicator (Becton Dickinson). These enriched cultures were subcultured on trypticase soy agar, chocolate agar, MacConkey agar, Sabouraud agar, or Columbia colistin nalidixic-acid agar (Becton Dickinson) and incubated in the aforementioned conditions. Isolated organisms were frozen in trypticase soy broth containing 20% glycerol (Becton Dickinson) and submitted for MALDI-TOF/MS analysis in batched groupings to the University of Geneva Hospitals, Switzerland. Before analysis by MALDI-TOF/MS, organisms were recultured from trypticase soy broth containing 20% glycerol and incubated at 37 °C for 24 h.

The Bruker MALDI Biotyper version 2.0 platform with library V.3.1.1.0 with 3740 database entries for MALDI-TOF/MS identification was used. A single colony of fresh overnight culture was smeared directly onto a ground-steel MALDI target plate and was covered with 1.5 μL saturated α-cyano-4-hydroxycinnamic acid matrix in 50% acetonitrile–2.5% trifluoroacetic acid, and was allowed to dry. In blood cultures containing multiple organisms, colonies exhibiting variant morphologies were selected and analyzed. Negative blood cultures were not evaluated with the Biotyper, because identification by the Biotyper encompasses the entire sample-processing flow, from positivity through subculture and MALDI-TOF/MS analysis. If no growth was observed after 5 days, the sample was considered negative, and was not analyzed by MALDI-TOF/MS. The plate was inserted into the source of a Bruker MicroFlex MALDI-TOF/MS instrument. Spectra were collected from 2000 to 20 000 Da in linear ion mode, using 240 shots of a 20-Hz nitrogen laser for ionization. All spectra were analyzed using the Bruker Biotyper 2.0 software package, and were compared to reference spectra for identification. A score $\geq$1.7 was considered a confident identification.

Results

Analysis was performed in accordance with STARD (standards for the reporting of diagnostic accuracy studies) guidelines. A total of 273 blood culture bottles were analyzed using PCR-ESI/MS and MALDI-TOF/MS independently. These results were retrospectively compared to Vitek2 biochemical profiles to identify microorganisms. This study was performed from 07/2009 - 10/2010, and remnant blood cultures were collected from June 29, 2008, to April 27, 2010. During this time, there were approximately 2633 patients whose blood was cultured as part of their routine clinical care. A diverse subset of positive cultures was created which were specifically selected by investigators to represent a challenge set to the 2 technologies that contained a range of biologically diverse bacteria and fungi to assess assay specificity. A list of the organisms sampled can be found in Table 1. A flow chart of the testing process is shown in Fig. 1. No adverse events occurred as a result of this testing.

Each patient demonstrated characteristics of BSI, including fever (body temperature $>$38 °C), chills, and hypotension, or had a blood culture ordered at the dis-

Table 1. Bacteria and yeast that were contained within the blood culture bottles evaluated in this study, and the corresponding number of times that organism was present, including those occasions in which the organism was present in a mixture.

Organism	No. of samples	Organism	No. of samples
Abiotrophia sp.	2	*Escherichia coli*	9
Achromobacter denitrificans	2	*Eubacterium lentum*	3
Achromobacter xylosoxidans	2	*Fusobacterium sp.*	2
Acinetobacter baumannii	5	*Haemophilus influenzae*	6
Acinetobacter lwoffii	1	*Klebsiella oxytoca*	3
Actinomyces israelii	1	*Klebsiella pneumoniae*	7
Aeromonas hydrophila	1	*Lactobacillus sp.*	2
Anaerobic diphtheroides	1	*Listeria monocytogenes*	1
Anaerobic gram+ cocci	1	*Micrococcus sp.*	7
Bacillus sp.	3	*Moraxella catarrhalis*	1
Bacteroides capillosus	1	*Moraxella osloensis*	1
Bacteroides fragilis	1	*Morganella morganii*	2
Bacteroides gracilis	1	*Mycobacterium chelonae*	1
Bacteroides thetaiotaomicron	3	*Mycobacterium fortuitum*	1
Bacteroides uniformis	1	*Neisseria elongata*	1
Bacteroides vulgatus	2	*Neisseria sicca*	1
Candida albicans	4	*Pantoea sp.*	3
Candida glabrata	2	*Proteus mirabilis*	1
Candida krusei	1	*Pseudomonas aeruginosa*	6
Candida lusitanae	1	*Pseudomonas fluorescens*	1
Candida parapsilosis	1	*Pseudomonas oryzihabitans*	1
Candida tropicalis	2	*Pseudomonas stutzeri*	1
Capnocytophaga sp.	2	*Rhizobium radiobacter*	1
Citrobacter freundii	1	*Roseomonas sp.*	1
Clostridium difficile	1	*Salmonella sp.*	4
Clostridium paraputrificum	1	*Serratia marcesens*	2
Clostridium perfringens	2	*Staphylococcus sp.* (coagulase negative)	18
Corynebacterium sp.	3	*Staphylococcus aureus*	38
Delftia sp.	1	*Staphylococcus epidermidis*	1
Enterobacter aerogenes	1	*Stenotrophomonas maltophilia*	2
Enterobacter cloacae	9	*Streptococcus gallolyticus*	1
Enterobacter gergoviae	1	*Streptococcus gordonii*	1
Enterobacter sakazakii	1	*Streptococcus mitis*	1
Enterococcus casseliflavus	1	*Streptococcus oralis*	1
Enterococcus faecalis	13	*Streptococcus sp.* (viridans group)	10
Enterococcus faecium	15	*Streptococcus sp.* (β-hemolytic)	10
Enterococcus gallinarum	1		

cretion of the physician. Of those selected patient samples, 36.4% were from inpatient wards, 20.8% from the emergency department, 12.6% from the adult intensive care unit, 10.8% from oncology, 6.9% from the pediatric inpatient ward, 3.9% from the pediatric intensive care unit, 7.4% from adult outpatients, and 1.3% from autopsy. The age range of participating patients was 14 days to 93 years, with a mean age of 46.3 years. The sample set consisted of 38.4% female and 61.6% male patients.

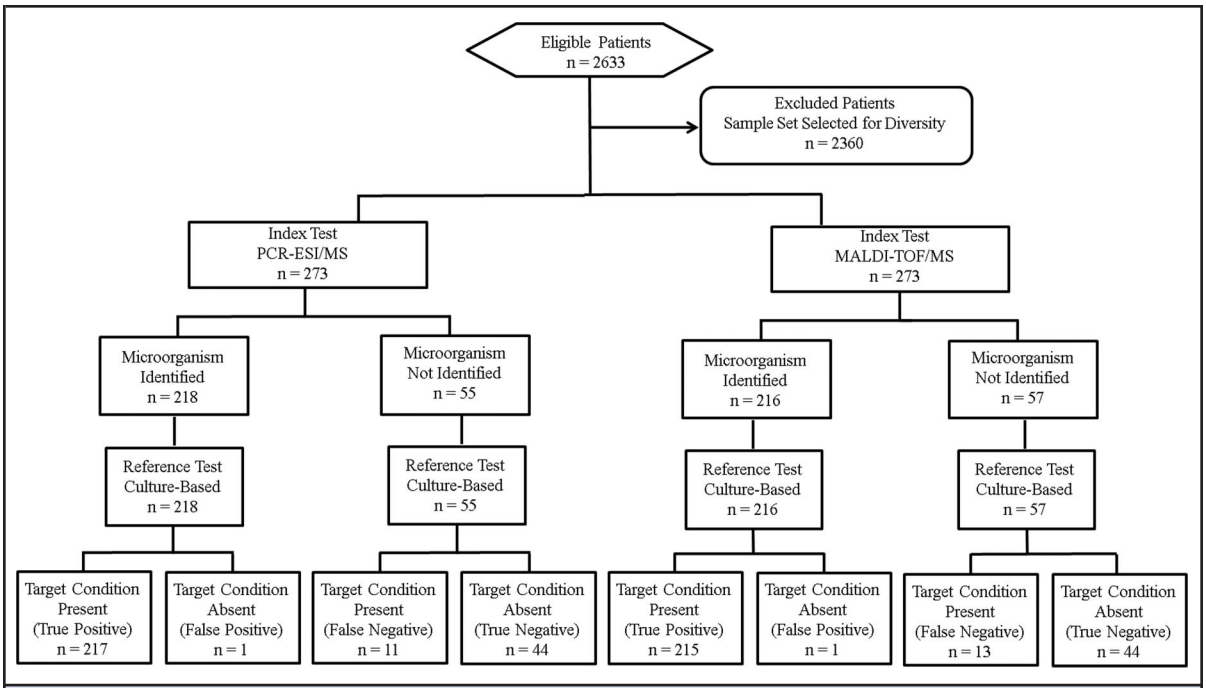

Fig. 1. Flow chart for this study.
All selected blood culture bottles were evaluated by each index test and the reference standard, with no exclusions.

We compared PCR-ESI/MS and MALDI-TOF/MS results directly to reference standard identification to determine the diagnostic accuracy of each method. In doing so, we assessed 3 levels of specificity: gram-stain result, genus identification, and species identification.

PCR-ESI/MS analysis of DNA extracted from 273 blood culture bottles resulted in 100% concordance with the gram-stain classification made by the reference standard. Genus and species concordance are shown in Fig. 2, A and B. Identification was considered concordant if the reference standard identification agreed with the PCR-ESI/MS identification and also when PCR-ESI/MS correctly identified 1 component of the sample in addition to a second identification (false positive with primary ID) or in the case of a missed second organism (false negative with primary ID). These identifications were considered concordant because 1 of the 2 organisms was correctly identified, and the disagreement on the second organism can in most situations be explained, as discussed below. All results that were indeterminate were considered discordant. The combined concordance on the genus level for PCR-ESI/MS was 96.7%, and on the species level was 95.6% (n = 273).

Diagnostic accuracy was measured as positive and negative agreement values with corresponding 95% CIs calculated by using the score method incorporating continuity correction, which is more accurate at high values *(29)*. Positive agreement was defined as the true-positive rate, calculated as the ratio of "true positives" to the total number of positives (true-positive rate = true positives/positives), and negative agreement was defined as the false-positive rate, calculated as the ratio of false positives to the total number of negatives [false-positive rate = 1 − (false positives/negatives)]. Positive and negative agreement values are shown in Table 2.

MALDI-TOF/MS analysis of subcultured microorganisms from 273 blood culture bottles resulted in 100% concordance with the gram-stain classification made with the reference standard. Genus and species identification concordance can be seen in Fig. 2, C and D. A combined concordance of 97.1% was observed on the genus level, and 94.9% on the species level (n = 273). The diagnostic accuracy was measured as positive and negative agreement values with corresponding 95% CIs, and is shown in Table 2.

No subset analysis was performed with respect to the sample population; test reproducibility was not measured in these experiments owing to feasibility in terms of time and cost of repeating the procedures for such a large sample set. Reproducibility has been previously assessed for MALDI-TOF/MS *(22, 30)*, and for

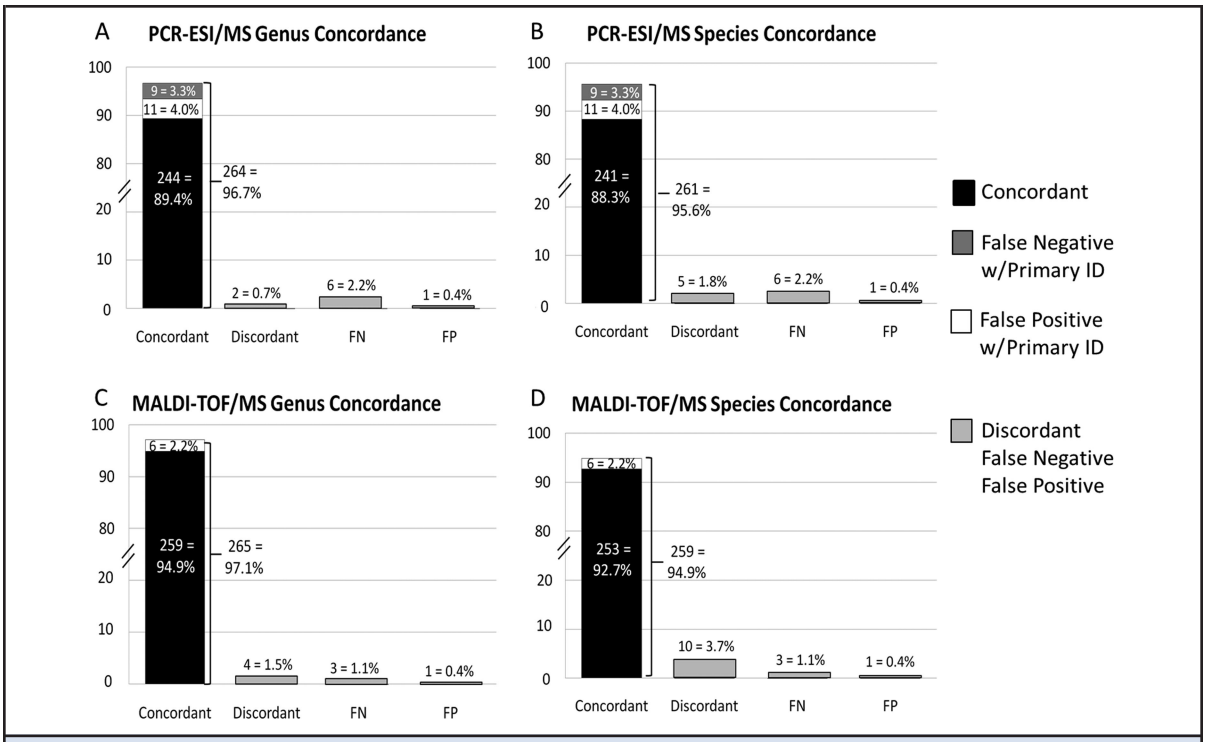

Fig. 2. Concordance seen with respect to the reference standard.

(A), PCR-ESI/MS identification on the genus level. FN, false negative; FP, false positive. (B), PCR-ESI/MS identification on the species level. (C), MALDI-TOF/MS identification on the genus level. (D), MALDI-TOF/MS identification on the species level.

PCR-ESI/MS is measured by correct identification of an internal positive control included in every PCR.

Discussion

Both PCR-ESI/MS and MALDI-TOF/MS methods showed high diagnostic accuracy, as demonstrated by the positive and negative agreement. A more traditional sensitivity and specificity could not be calculated for this study because the positive set of blood culture bottles was investigator-selected to challenge the 2 techniques. Although these results show clinically applicable positive and negative agreement, it is important to note that agreement rates would likely be higher if only commonly encountered organisms were considered. A closer look at discordant and false-positive results provides more information on the accuracy and common errors associated with the 2 techniques. All discordant, false-positive, and false-negative results are illustrated in Table 3. There was 1 false positive with sample 154, which was discordant utilizing both techniques. PCR-ESI/MS identification of *Ralstonia picketti* is a known blood culture bottle contaminant, resulting in residual DNA following bottle sterilization before use *(31)*, and MALDI-TOF/MS identification of *Staphylococcus warneri* is a common contaminant that is seen in several instances with MALDI-TOF/MS, as discussed below.

The MALDI-TOF/MS discordance list shows false negatives in samples 125, 2027, and 2634 that may be difficult to identify owing to low bacterial biomass in culture, as well as being more resistant to protein extraction and ionization due to cell wall complexity. The false positives seen when the primary identification is correct (samples 145, 171, 177, 221, 226, and 329) were predominantly instances in which a *Staphylococcus sp.* was identified as an additional organism. During the course of these experiments, organisms isolated from representative blood culture bottles underwent multiple passages, offering several opportunities for inadvertent environmental contamination. We believe these false positives were caused by this type of error, and would not be detected if MALDI-TOF/MS was performed immediately after positivity of the blood culture, or in the first pass after removal from cryostorage, as they were not initially seen in subculture before Vitek2 analysis. Discordance observed with MALDI-TOF/MS was due to a limited database, as well as limitations in protein extraction methods for resistant or-

Table 2. Positive and negative agreement values for PCR-ESI/MS and MALDI-TOF/MS on the genus and species level, considering 2 different methods for determining accuracy.[a]

	Method 1[a]		Method 2[b]	
	Value	CI	Value	CI
PCR-ESI/MS				
Genus				
Positive agreement	0.965	(0.930–0.984)	0.922	(0.875–0.952)
Negative agreement	0.978	(0.868–0.999)	0.786	(0.652–0.880)
Species				
Positive agreement	0.952	(0.912–0.974)	0.908	(0.859–0.941)
Negative agreement	0.978	(0.868–0.999)	0.786	(0.652–0.980)
MALDI-TOF/MS				
Genus				
Positive agreement	0.969	(0.935–0.987)	0.969	(0.933–0.986)
Negative agreement	0.978	(0.868–0.999)	0.863	(0.731–0.939)
Species				
Positive agreement	0.943	(0.902–0.968)	0.941	(0.900–0.967)
Negative agreement	0.978	(0.868–0.999)	0.863	(0.731–0.939)

[a] Method 1 considers "false positives with primary ID" and "false negatives with primary ID" as concordant, because at least 1 organism was correctly identified.
[b] Method 2 considers "false positives with primary ID" as false positives and "false negatives with primary ID" as false negatives.

ganisms, (e.g., Actinobacteria, Mycobacteria, and those possessing a strong capsule or slime layer).

The PCR-ESI/MS discordance list shows false negatives in 6 samples (231, 1362, 2096, 2964, 3138, and 3666). Three of these 6 false-negative samples were due to the presence of *Eubacterium lentum* as identified by the reference standard method, an identification that was missed each time this organism was presented. *E. lentum* is environmental in origin and is not commonly isolated from blood culture, thus this result demonstrates another opportunity for database adjustment to ensure identification of this organism in future samples. PCR-ESI/MS demonstrated several instances in which false negatives were seen in a mixture, where 1 organism was correctly identified, as in samples 89, 124, 135, 200, 228, and 271. As discussed in a previous report *(14)*, an error can occur when combinations of staphylococci and streptococci, or staphylococci and enterococci are present. In these situations, 1 organism is preferentially identified. Other missed identifications were possibly due to large differences in bacterial load, such that only 1 of the 2 organisms is detected in the PCR. Additional false negatives with primary ID in samples 223, 242, and 1272 were likely due to large differences in organism burden, resulting in 1 organism being missed in the identification. False-positive results were observed when a second organism was correctly identified (false positive with primary ID) can be split into 2 groups. The first group contains samples 76, 302, and 1647, in which the second identification is due to an incompletely populated database for *Pantoea sp.* and *Hemophilus influenzae*, resulting in a mixed identification. In addition, discordance on both the genus and species levels was seen for *Pantoea sp.* in samples 1033 and 1050, in which the PCR-ESI/MS identification was *Citrobacter braakii*. This identification is very genetically similar, because both organisms belong to the Enterobacteriaceae family, which could be resolved in the future by populating the database with isolates from this family. The remaining group, including samples 276, 309, 323, 341, 482, 1499, and 2889, all contained an organism that was identified by PCR-ESI/MS, but at a significantly lower confidence level than the primary identification. Because PCR-ESI/MS is a DNA-based technique, it is possible to detect organisms that are nonviable by culture or represent DNA contamination. These identifications may be true positives based on DNA present, or may be true false positives in mixture. Discordance was also observed in samples 30, 202, and 2997, and the organisms in each sample were misidentified as genetically similar, possibly because of low copy numbers that resulted in incomplete profiles for identification.

Table 3. List of samples that showed discordance by PCR-ESI/MS and MALDI-TOF/MS compared to the reference standard.

PCR-ESI/MS and MALDI-TOF/MS combined discordance

Sample ID	Discordance	True identification[a]	Discordant identification
154	Both FP[b]	No growth	PCR-ESI/MS: *Ralstonia picketti* MALDI-TOF/MS: *Staphylococcus warneri*

MALDI-TOF/MS discordance

Sample ID	Discordance	True identification	Discordant identification
125	FN	Anaerobic diphtheroides	Unable to identify
2027	FN	*Mycobacterium chelonae*	Unable to identify
2634	FN	*Fusobacterium sp.*	Unable to identify
145	FP w/primary ID	*Clostridium perfringens*	*Bacteroides vulgatus*
171	FP w/primary ID	*Clostridium perfringens*	*Enterococcus faecalis*
177	FP w/primary ID	*Pseudomonas aeruginosa*	*Staphylococcus epidermidis*
221	FP w/primary ID	*Streptococcus sp.* (Viridans)	*Staphylococcus hominis*
226	FP w/primary ID	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
252	FP w/primary ID	*Candida albicans*	*Staphylococcus aureus*
329	FP w/primary ID	*Enterococcus faecalis*	*Staphylococcus saprophyticus*
259	Discordant genus and species	*Streptococcus sp.* (β-hemolytic)	*Staphylococcus aureus*
264	Discordant genus and species	*Actinomyces israelii*	*Clostridium butyricum*
429	Discordant genus and species	*Corynebacterium sp.*	*Actinomyces viscosus*
1609	Discordant genus and species	*Abiotrophia sp.*	*Gamella haemolysans*
249	Discordant species	*Bacteroides thetaiotaomicron*	*Bacteroides fragilis*
2566	Discordant species	*Achromobacter xylosoxidans*	*Achromobacter ruhlandii*
2889	Discordant species	*Aeromonas hydrophilia*	*Aeromonas caviae*
2573	Discordant species	*Achromobacter xylosoxidans*	*Achromobacter ruhlandii*
296	Discordant species	*Streptococcus sp.* (Viridans)	*Streptococcus australis*
440	Discordant species	*Streptococcus oralis/S. mitis*	*Streptococcus pneumoniae*

PCR/ESI-MS discordance

Sample ID	Discordance	True identification	Discordant identification
231	FN	*Candida albicans*	Unable to identify
1362	FN	*Candida lusitanae*	Unable to identify
2096	FN	*Bacteroides uniformis*	Unable to identify
2964	FN	*Eubacterium lentum*	Unable to identify
3138	FN	*Eubacterium lentum*	Unable to identify
3666	FN	*Eubacterium lentum*	Unable to identify
89	FN w/primary ID	*Streptococcus agalactiae*	(*Staphylococcus epidermidis*)
124	FN w/primary ID	*Streptococcus sp.* (Viridans)	(*Staphylococcus aureus*)
135	FN w/primary ID	*Streptococcus sp.* (Viridans)	(*Staphylococcus epidermidis*)
200	FN w/primary ID	*Streptococcus sp.* (β-hemolytic)	(*Staphylococcus sp.* (Coagulase negative) and *Enterococcus sp.*)
218	FN w/primary ID	*Streptococcus sp.* (Viridans)	(*Streptococcus pneumoniae*)
228	FN w/primary ID	*Staphylococcos epidermidis*	(*Enterococcus faecalis*)
271	FN w/primary ID	*Streptococcus agalactiae*	(*Staphylococcus sp.*, coagulase negative)
223	FN w/primary ID	*Klebsiella pneumoniae*	(*Stenotrophomonas maltophilia*)

Continued on page 1065

Table 3. List of samples that showed discordance by PCR-ESI/MS and MALDI-TOF/MS compared to the reference standard. ***(Continued from page 1064)***

		PCR/ESI-MS discordance	
Sample ID	**Discordance**	**True identification**	**Discordant identification**
242	FN w/primary ID	*Staphylococcus epidermidis*	(*Streptococcus pyogenes*)
1272	FN w/primary ID	*Streptococcus sp.* (Viridans)	(*Neisseria sicca*)
76	FP w/primary ID	*Pantoea agglomerans*	*Erwinia tasmaniensis*
302	FP w/primary ID	*Haemophilus influenzae*	*Mannheimia haemolytica*
1647	FP w/primary ID	*Haemophilus influenzae*	*Mannheimia haemolytica*
276	FP w/primary ID	*Micrococcus luteus*	*Acidothermus cellulolyticus*
309	FP w/primary ID	*Staphylococcus aureus*	*Caudatispora biapiculata*
323	FP w/primary ID	*Lactobacillus casei*	*Bifidobacterium animalis*
341	FP w/primary ID	*Citrobacter freundii*	*Clostridium ramnosum*
482	FP w/primary ID	*Moraxella atlantae*	*Methylococcus capsulatus*
1499	FP w/primary ID	*Morganelli morganii*	*Aeromonas hydrophilia*
2889	FP w/primary ID	*Aeromonas hydrophilia*	*Pseudomonas entomophilia*
1033	Discordant genus and species	*Pantoea sp.*	*Citrobacter braakii*
1050	Discordant genus and species	*Pantoea sp.*	*Citrobacter braakii*
30	Discordant species	*Streptococcus gallolyticus*	*Streptococcus pneumoniae*
202	Discordant species	*Staphylococcus sp.* (Coagulase negative)	*Staphylococcus aureus*
2997	Discordant species	*Moraxella catarrhalis*	*Moraxella nonliquifaciens*

[a] True identification indicates that the organism was identified by the reference standard. Discordant identification indicates that the organism was identified by the index test. Organism name in parentheses indicate the organism that was missed in a mixture.
[b] FP, false positive; FN, false negative.

COMPARISON OF PCR-ESI/MS TO MALDI-TOF/MS

The clearest comparison of PCR-ESI/MS and MALDI-TOF/MS is demonstrated when positive and negative agreement are compared for each technique. These values were calculated by using samples in which false negatives were detected in the case of positive agreement, and samples in which false positives were detected in the case of negative agreement. The performance of each technique was compared by using the McNemar test, and results indicated that PCR-ESI/MS and MALDI-TOF/MS were not statistically different on either the genus or species level ($P > 0.05$).

Although both techniques show similar performance, there are several findings of merit that must be considered in the comparison. Although the resulting identification of a microorganism is the same regardless of the technique used, the information required to come to these data is very different for each technique. PCR-ESI/MS uses genetic information whereas MALDI-TOF/MS uses proteomic information to identify organisms present; this difference highlights the varying utilities of each instrument. Genetic identification allows for discrimination down to the representative strain type of each organism during routine work, detects silent mutations, and allows access to antibiotic resistance genes, providing a mechanism for rapid antibiotic susceptibility testing. Proteomic information however, in many instances discriminates down to species-level functioning to provide organism identification. Although several studies have been performed using MALDI-TOF/MS to discriminate clonal/strain types *(32–34)*, this procedure is not performed routinely and would not be applied if used for high-throughput clinical identification. PCR-ESI/MS and MALDI-TOF/MS show similar performance for routine use, but PCR-ESI/MS offers extended utility for epidemiology and infection control. Furthermore, utilizing genetic information offers the potential for identifying uncultivatable organisms.

An additional aspect that should be considered regarding these methods is the time to result. PCR-ESI/MS requires approximately 4–6 h from the time of blood culture positivity, and can resolve mixtures in 1 assay, but this method requires the batching of 6 samples at a time, and may allow direct analysis on whole blood, removing the requirement for blood culture. Conversely, MALDI-TOF/MS currently requires subculture before identification. The time required for this

step varies from an additional 12 h from positivity up to and beyond 72 h *(35)*, depending on the organism. MALDI-TOF/MS does not require batching, although owing to the subculture requirement, a batched format may be more convenient. Several recent reports have described the use of MALDI-TOF/MS for identification of microorganisms without subculture *(23, 24, 36, 37)*, and the recently released Sepsityper by Bruker Daltonics (www.bdal.com) has demonstrated the potential to reduce the time to identification. In these studies results have shown successful identification for approximately 80% of blood cultures and therefore demonstrate that more developmental work is required to increase this identification rate before clinical implementation. Although PCR-ESI/MS is currently the faster technique for microorganism identification, recently described MALDI-TOF/MS methods may soon reduce the time to identification to less than an hour from blood culture positivity. The identification times observed for these 2 MS techniques offer a stark comparison to current reports of requirements of approximately 40 h for identification with gold standard methods *(38)*.

The costs of these 2 techniques also differ substantially. Both require the purchase of a dedicated mass spectrometer and software package at a cost that can be considered equivalent for the purposes of this discussion, despite the expected differences in the overall costs of each method. However, the day-to-day cost for consumables greatly varies. PCR-ESI/MS requires DNA extraction and a kit that contains reagents necessary for a PCR, including buffers, enzyme, and primers, resulting in a cost of $50 to $100 per sample, which is comparable to the cost of existing molecular assays. Alternatively, MALDI-TOF/MS requires only media to culture the organism, <1 μg matrix, and tips, the cost of which is approximately $3 to $7 per sample.

In conclusion, the results of this study, overall, demonstrate that both PCR-ESI/MS and MALDI-TOF/MS show high diagnostic accuracy compared to biochemical and phenotypic identification as the reference standard used in the clinical laboratory. Both methods show promise for routine and, in some cases, epidemiological use in hospital settings. These 2 techniques show no statistically significant differences in performance. The results we report highlight key differences between the 2 techniques, both of which will fill unique niches in the clinical microbiology laboratory.

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