

# Enhanced Diagnostic Yields of Bacteremia and Candidemia in Blood Specimens by PCR-Electrospray Ionization Mass Spectrometry

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**A prospective study was performed to determine the value of direct molecular testing of whole blood for detecting the presence of culturable and unculturable bacteria and yeasts in patients with suspected bloodstream infections. A total of 464 adult and pediatric patients with positive blood cultures matched with 442 patients with negative blood cultures collected during the same period were recruited during a 10-month study. PCR amplification coupled with electrospray ionization mass spectrometry (PCR-ESI-MS) plus blood culture reached an overall agreement of 78.6% in the detection and species-level identification of bacterial and candidal pathogens. Of 33 culture-negative/PCR-ESI-MS-positive specimens, 31 (93.9%) were judged to be truly bacteremic and/or candidemic based on a medical chart review and analytical metrics. Among the 15 culture-positive specimens in which PCR-ESI-MS detected additional bacterial or yeast species, 66.7% and 20.0% of the additional positive specimens by PCR-ESI-MS were judged to be truly or possibly bacteremic and/or candidemic, respectively. Direct analysis of blood samples by PCR-ESI-MS rapidly detects bacterial and yeast pathogens in patients with bloodstream infections. When used in conjunction with blood culture, PCR-ESI-MS enhances the diagnostics of septicemia by shortening test turnaround time and improving yields.**

**B**acteremia and candidemia are associated with significant morbidity and mortality. Sepsis is estimated to cause >200,000 deaths annually in the United States alone (1, 2). Although various medical interventions are available, the initiation of early antimicrobial therapy remains a major factor in favorable outcomes in cases of sepsis (2, 3). Currently, patients meeting the clinical criteria for sepsis are placed on empirical antimicrobial therapy before any pathogen is identified. This practice is largely driven by studies demonstrating that sepsis-related morbidity and mortality are dramatically increased when antibiotic therapy is delayed (2, 4, 5).

Currently, the gold standard microbial detection method is the time-consuming process of microbiological culture. In general, culture detects bacterial pathogens within 12 to 48 h of inoculation (6, 7). Precise microbial identification and antibiotic susceptibility determination require an additional 6 to 24 h (7). Thus, a patient often is treated with broad-spectrum antibiotics for several days prior to the establishment of more focused therapy. Frequent and extended uses of broad-spectrum antibiotics are contributing factors in the recent increases in antibiotic-resistant organisms (8). Another limitation of culture is its decreased sensitivity for unculturable or fastidious organisms and in instances when the sample is collected from a patient who has already started antimicrobial therapy (9, 10). Blood cultures are reported to be negative in >50% of cases where true bacterial or yeast sepsis is believed to exist based upon other analytical measurements and clinical presentation (11, 12). This has driven the recommendation by the International Sepsis Committee that decisions regarding antibiotic administration, changes in therapy, or discontinuation of antibiotic therapy be based on clinical judgment rather than culture results (10, 11).

More rapid and sensitive methods to identify bloodstream infections are urgently needed (9, 13). Molecular methods that de-

tect microbes utilizing PCR do so by directly detecting and amplifying microbial nucleic acids that are present in the blood (14, 15). A recently developed molecular detection method analyzes the PCR amplification products by electrospray ionization mass spectrometry (ESI-MS) (16). ESI-MS can measure the mass of PCR amplicons with sufficient accuracy to enable the calculation of the base composition of the amplicon. By comparing the base composition of the detected organism to the base compositions of known organisms, the pathogens present in the sample can be identified within 6 to 8 h of obtaining it (13, 17). Accurate PCR-ESI-MS detections would allow for the initial antimicrobial therapy to be based on the organism(s) present, resulting in more optimal outcomes, reduced toxicity, lower costs, and the preservation of existing antimicrobials from bacterial resistance.

In a previous study, we demonstrated the usefulness of PCR-ESI-MS for rapid and accurate detection of *Ehrlichia* species in patients who were clinically suspected to have ehrlichiosis. In addition, we were able to identify additional bacterial pathogens with matched clinical manifestations directly in blood specimens (17). This prospective study evaluates the use of PCR-ESI-MS as an adjunct to traditional blood culture to enhance the speed and yield of bacteremia and candidemia diagnostics.

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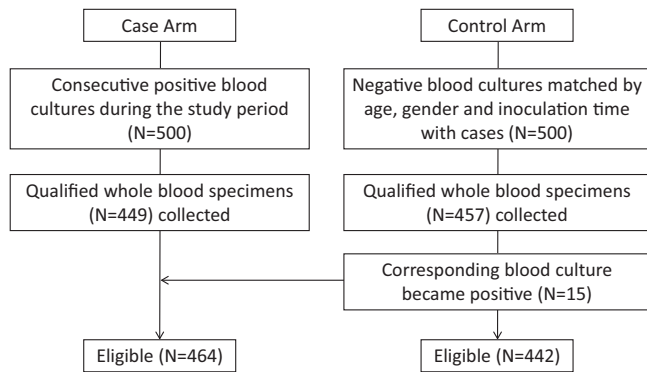


FIG 1 Accountability of specimens collected. See Materials and Methods for inclusion and exclusion criteria.

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## MATERIALS AND METHODS

**Patient samples.** Whole-blood samples were collected at the Vanderbilt University Medical Center (VUMC) between 18 January and 13 November 2010. The samples were representative of those submitted to the VUMC microbiology laboratory for blood culture. When a patient culture was positive, refrigerated surplus whole-blood samples that were drawn within the same 8-h period for other diagnostic assays were collected as cases for PCR-ESI-MS analysis. In addition, refrigerated surplus blood samples from patients whose blood culture result remained negative were selected as controls, matched with the cases by age, sex, and incubation time. Only one whole-blood specimen per patient was included in the study and was aliquoted and stored at  $-80^{\circ}\text{C}$  prior to nucleic acid extraction.

**Blood culture, pathogen identification, and antimicrobial susceptibility.** Patient whole-blood samples were processed with the Bactec 9240 blood culture system (Becton, Dickinson, Sparks, MD). When the positive blood cultures were confirmed by Gram stain, the blood culture bottle contents were subcultured, and identification and differentiation were carried out by a battery of phenotypic methods, including the API commercial biochemical identification strips (bioMérieux, Inc., Durham, NC), which were used according to the manufacturer's instructions (18–20).

TABLE 1 Summary of results for PCR-ESI-MS and culture

Category	Data (no.) by culture result	
	Positive	Negative
PCR-ESI-MS and culture were in agreement in the no. of detections and species identity	155 <sup>a</sup>	398
Culture and PCR-ESI-MS were in agreement on primary pathogen identification with unmatched secondary detections in either method	23 <sup>b</sup>	0
Culture positive, PCR-ESI-MS negative	97 <sup>c</sup>	0
PCR-ESI-MS positive, culture negative	0	33 <sup>d</sup>

<sup>a</sup> See Table 2 for detailed list of organisms.

<sup>b</sup> See Table 3 for details.

<sup>c</sup> See Table 4 for details.

<sup>d</sup> See Table 5 for details.

TABLE 2 Concordant detections in PCR-ESI-MS and culture

Organism(s) detected	No. of samples in which organisms were detected
Single organism	
<i>Staphylococcus aureus</i>	64
<i>Enterococcus faecalis</i>	15
<i>Escherichia coli</i>	14
<i>Klebsiella pneumoniae</i>	11
<i>Enterococcus faecium</i>	6
<i>Serratia marcescens</i>	4
<i>Candida glabrata</i>	3
<i>Enterobacter cloacae</i> complex	3
<i>Proteus mirabilis</i>	3
<i>Streptococcus agalactiae</i>	3
<i>Acinetobacter baumannii</i>	2
<i>Candida albicans</i>	2
<i>Candida parapsilosis</i>	2
<i>Enterobacter aerogenes</i>	2
<i>Pseudomonas aeruginosa</i>	2
<i>Staphylococcus lugdunensis</i>	2
<i>Stenotrophomonas maltophilia</i>	2
<i>Streptococcus mitis</i> / <i>Streptococcus pneumoniae</i>	2
<i>Candida tropicalis</i>	1
<i>Enterobacter</i> sp.	1
<i>Citrobacter braakii</i>	1
<i>Klebsiella oxytoca</i>	1
<i>Salmonella enterica</i>	1
<i>Streptococcus pyogenes</i>	1
<i>Streptococcus vestibularis</i>	1
Multiple organisms	
<i>Staphylococcus aureus</i> and <i>K. pneumoniae</i>	1
<i>E. coli</i> and <i>K. pneumoniae</i>	1
<i>S. aureus</i> and <i>P. aeruginosa</i>	1
<i>E. coli</i> and <i>E. aerogenes</i>	1
<i>K. pneumoniae</i> and <i>Aeromonas hydrophila</i>	1
<i>E. faecium</i> and <i>C. albicans</i>	1
Total no. of organisms	155

**Molecular analysis.** Samples for molecular analysis were analyzed using the BAC Spectrum assay (Ibis Biosciences) on the PCR-ESI-MS system (PLEX-ID; Abbott Laboratories), as described previously (17, 21–23). The details of the primer sequences, gene targets, primer coverage of the bacterial and fungal domains, and the organization and multiplexing configuration of the primers on the microtiter plates are described in Table S1 in the supplemental material.

**Lysis and DNA isolation.** A 1.25-ml whole-blood sample was mechanically lysed by bead beating in the presence of SDS and proteinase K. Following bead beating, the tube was incubated for 15 min at  $56^{\circ}\text{C}$  to facilitate enzymatic proteolysis. The DNA was purified on a KingFisher system using the UltraClean DNA isolation kit (22, 23). Briefly, DNA was captured on magnetic silica microparticles in the presence of guanidine isothiocyanate and ethanol and washed once in the same buffer. Three washes in aqueous ethanol were followed by elution in 250  $\mu\text{l}$  of water at  $70^{\circ}\text{C}$ .

**PCR amplification.** Whole-blood DNA was amplified in 16 separate wells in the assay plates. The 16 wells contained a set of 18 primer pairs optimized for the efficient detection and identification of bacterial and *Candida* species, as well as *mecA*, *vanA*, *vanB*, and *Klebsiella pneumoniae* carbapenemase (KPC) antibiotic resistance elements (see Table S1 in the supplemental material). All PCR cycling was carried out on an Eppendorf thermal cycler in plates that were heat sealed with a piercable foil. Loading

TABLE 3 Concordant primary pathogen identification with unmatched secondary detections

Primary organism(s) detected in concordant PCR-ESI-MS and culture results	Secondary detection result by:	
	PCR-ESI-MS	Culture
<i>Acinetobacter baumannii</i>	<i>Serratia marcescens</i>	None
<i>A. baumannii</i>	<i>Proteus mirabilis</i>	<i>Stenotrophomonas maltophilia</i>
<i>Candida albicans</i>	<i>Escherichia coli</i>	None
<i>Candida parapsilosis</i>	None	<i>Enterococcus faecium</i>
<i>Enterobacter cloacae</i> complex	<i>Enterococcus faecalis</i>	None
<i>E. cloacae</i> complex	<i>Leclercia adecarboxylata</i>	None
<i>E. cloacae</i> complex	None	<i>Citrobacter freundii</i>
<i>E. cloacae</i> complex	None	<i>E. faecalis</i>
<i>E. faecalis</i>	<i>E. cloacae</i> complex	None
<i>E. faecalis</i>	<i>Enterococcus durans</i> or <i>Enterococcus hirae</i>	None
<i>E. coli</i>	None	<i>Klebsiella oxytoca</i>
<i>K. oxytoca</i>	<i>Citrobacter koseri</i>	None
<i>Klebsiella pneumoniae</i>	<i>Enterobacter aerogenes</i>	None
<i>K. pneumoniae</i>	<i>Citrobacter amalonaticus</i>	None
<i>K. pneumoniae</i>	<i>S. maltophilia</i>	None
<i>K. pneumoniae</i>	<i>C. albicans</i>	<i>P. mirabilis</i>
<i>Staphylococcus aureus</i>	<i>Candida guilliermondii</i>	None
<i>S. aureus</i>	<i>E. coli</i>	None
<i>S. aureus</i>	<i>Lactobacillus casei</i>	None
<i>S. aureus</i>	<i>Pseudomonas aeruginosa</i>	None
<i>S. aureus</i>	None	<i>E. faecalis</i>
<i>S. aureus, Streptococcus agalactiae</i>	None	<i>E. faecalis</i>
<i>Streptococcus pneumoniae</i>	<i>Streptococcus suis</i>	None

of the KingFisher trays and the PCR plates was performed robotically on the PLEX-ID fluid handler (17, 22, 23).

**ESI-MS.** Following amplification, the contents of each well were desalted by a process that included the binding of amplicons to magnetic microparticles, washing, and elution in a volatile buffer. The desalted amplicons were injected into a time of flight mass spectrometer by electrospray ionization. This process separates the two DNA strands of the amplicon without fragmentation, enabling the molecular masses of both strands to be determined separately (17, 22, 23).

**Microbial species identification.** All data analyses were conducted in an automated fashion on the PCR-ESI-MS instrument. The algorithm triangulates to determine a maximum likelihood estimate of the population of microbial species present in the test sample (16). The measured base composition signatures were compared against a very large database, which contained the complete amplicon signatures for 612 reportable clinically relevant bacterial and *Candida* species. Additional amplicon information is fully populated for another 299 species for which complete genomic signatures exist (GenBank, NCBI, Bethesda, MD) but there is not sufficient literature supporting their clinical relevance. These organism base count signatures are utilized by the software algorithm; however, they are not reportable by name. Instead, the user is informed with a message saying, "Bacteria detected—no ID can be provided."

**Data analysis.** Along with organism identification, the ESI-MS analysis includes a level and a Q score. The level indicates the amount of amplified DNA present in the sample. This is reported as genome equivalents/well and is calculated with reference to the internal calibrant, as previously described (24). The Q score, which is a rating between 0 (low) and 1 (high), represents a relative measure of the strength of the data supporting an identification; only Q scores of  $\geq 0.90$  were reported.

**Clinical relevance determination.** The patient medical charts were reviewed by an investigator (C.W.S.) and fell into one of two categories: (i) two or more pathogens were detected and identified by the Ibis PLEX-ID BAC in the case group, and (ii) pathogens were detected and identified by the Ibis PLEX-ID BAC in the control group. The clinical relevance of these findings was determined for each patient by the following criteria: (i) clinical manifestations, (ii) the results of laboratory tests, such as white

blood cell count and differentiation and C-reactive proteins, (iii) blood culture results from this and other specimens collected from the same patient, and (iv) whether specific therapy was initiated and the results of such therapy.

## RESULTS

We performed a prospective study in which we applied a multilocus broad-range PCR amplification coupled with electrospray ionization mass spectrometry (PCR-ESI-MS) to directly identify microorganisms in whole-blood specimens collected from patients with clinically suspected septicemia at the Vanderbilt University Medical Center. During the study period, 500 consecutive cases with positive blood culture results and 500 controls with negative blood culture results matched by age, gender, and incubation time were selected for the study. Soon after the cases and controls were defined, 906 surplus whole-blood samples were collected from these selected patients within an 8-h period for use in other diagnostic assays. Fifteen whole-blood specimens from the control arm were transferred to the case arm, as their blood cultures turned positive later during the incubation period. A total of 464 culture-positive (cases) and 442 culture-negative (control) whole-blood samples were obtained and analyzed by PCR-ESI-MS (Fig. 1). The average age  $\pm$  the standard deviation and the percentage of male participants were  $52.10 \pm 17.75$  years and 60.3% for cases and  $51.74 \pm 17.53$  years and 59.9% for controls, respectively.

Among the total 906 samples, 173 were positive for coagulase-negative staphylococci (CoNS), including 119 detected by culture, 16 detected by PCR-ESI-MS only, and 38 detected by both methods. CoNS are more often than not seen in single-blood culture sets as a result of contamination. Repeatability across multiple sets can be used to increase confidence in the clinical significance of CoNS-positive cultures, but in the absence of this or other evi-

TABLE 4 Culture-positive and PCR-ESI-MS-negative detections

Culture-reported organism(s) when PCR-ESI-MS was negative	No. of culture-positive, PCR-ESI-MS-negative samples
<i>Staphylococcus aureus</i>	28
<i>Streptococcus</i> spp.	13
<i>Escherichia coli</i>	13
<i>Enterococcus faecium</i>	6
<i>Acinetobacter</i> spp.	5
<i>Enterobacter cloacae</i>	4
<i>Klebsiella pneumoniae</i>	3
<i>Pseudomonas aeruginosa</i>	3
<i>Bacillus</i> spp.	2
<i>Citrobacter koseri</i>	2
<i>Enterococcus faecalis</i>	2
<i>Klebsiella oxytoca</i>	2
<i>Proteus mirabilis</i>	2
<i>Burkholderia cepacia</i>	1
<i>Candida albicans</i>	1
<i>C. albicans</i> + <i>E. faecalis</i>	1
Diphtheroids (aerobic <i>Corynebacterium</i> sp.)	1
<i>E. faecalis</i> + <i>Staphylococcus gallinarum</i>	1
<i>Enterococcus</i> sp. + <i>E. faecium</i>	1
<i>Stenotrophomonas maltophilia</i>	1
<i>Veillonella</i> spp. (growth after 4 days)	1
<i>Rhodotorula</i> spp.	1
<i>Zygosaccharomyces fermentati</i>	1
<i>Fusarium</i> spp. (growth after 5 days)	1
Positive culture with no organism name provided	1
Total no. of organisms	97

dence favoring a different conclusion, statistics supports the interpretation of a single CoNS result as the result of a stochastic contamination event (25). In this study, no attempt was made to acquire multiple samples, and therefore these 173 samples were excluded from further analysis.

Of the 733 included specimens, there were 576 specimens (178 in cases and 398 in controls) where culture and PCR-ESI-MS were in agreement on the primary detection, resulting in an overall agreement of 78.6% (Table 1). In 155 of the case specimens, there was perfect concordance at the species level, including in 6 reported dual detections (Table 2). For another 23 samples, the primary identification matched between the two methods, but PCR-ESI-MS found an additional organism in 15 instances, culture found a second organism in six instances, and in 2 instances, each method had a secondary detection that did not match (Table 3). In 97 instances, the culture was positive but no detection was reported by PCR-ESI-MS (Table 4). While a small number of these organisms were outliers, the distribution of the organisms in this group was similar to that shown in Table 2.

Finally, PCR-ESI-MS detected an organism in culture-negative samples in 33 instances (Table 5). The reliability and relevance of these results were further explored by a comparison of the analytical metrics (Fig. 2) and a review of patient medical charts (Table 6). The distribution of the analytic metrics of Q scores and levels for the culture-negative/PCR-ESI-MS-positive samples are illustrated in Fig. 2. The PCR-ESI-MS-positive/culture-negative results are distributed similarly to the results that had a positive corroborative culture, except for two samples that are at the lower limits of the acceptable Q score and genome level. Detailed clinical

TABLE 5 PCR-ESI-MS-positive and culture-negative detections

Organism(s) detected	No. of PCR-ESI-MS-positive, culture-negative samples
<i>Acinetobacter baumannii</i>	1
<i>Acinetobacter calcoaceticus</i>	1
<i>Brevundimonas diminuta</i>	1
<i>Corynebacterium kroppenstedtii</i>	2
<i>Enterococcus faecalis</i>	2
<i>Escherichia coli</i>	5
<i>Klebsiella pneumoniae</i>	1
<i>Lactobacillus gasseri</i>	1
<i>Mycoplasma hominis</i>	1
<i>Pseudomonas aeruginosa</i>	1
<i>Staphylococcus aureus</i>	6
<i>Staphylococcus lugdunensis</i>	1
<i>Streptococcus mitis</i> / <i>Streptococcus pneumoniae</i>	1
<i>Streptococcus pyogenes</i>	1
<i>Streptococcus salivarius</i> / <i>Streptococcus thermophilus</i>	1
<i>Actinomyces viscosus</i> and <i>P. aeruginosa</i>	1
<i>Corynebacterium striatum</i> and <i>E. faecalis</i>	1
<i>Enterobacter cloacae</i> complex and <i>Leclercia adecarboxylata</i>	2
<i>Streptococcus equi</i> and <i>S. salivarius</i> / <i>S. thermophilus</i>	1
<i>Citrobacter amalonaticus</i> , <i>E. faecalis</i> , and <i>Escherichia vulneris</i>	1
<i>Clostridium perfringens</i> , <i>Clostridium septicum</i> / <i>Clostridium tertium</i> , and <i>Klebsiella oxytoca</i>	1
Total no. of organisms	33

chart review results on 33 PCR-ESI-MS-positive/culture-negative and 15 PCR-ESI-MS-multipositive/culture-single-positive specimens (Table 2) are included in Table S2 in the supplemental material. Of the 33 PCR-ESI-MS-positive/culture-negative specimens, 31 (93.9%) were judged to be truly bacteremic and/or candidemic with or without identification of the correct pathogen (see Table S2a in the supplemental material). While there were no ambiguous results identified, two (6.1%) were judged to be false positives, most likely due to skin contamination, and this corresponded perfectly with the two specimens with low Q scores in Fig. 2. Of the 15 PCR-ESI-MS-multipositive/culture-single-positive specimens, a medical chart review indicated that 10 (66.7%) specimens were truly or possibly bacteremic and/or candidemic with or without identification of the correct pathogen in the medical record (see Table S2b in the supplemental material).

## DISCUSSION

Continuously monitored blood culture is the current gold standard for identifying pathogens associated with bloodstream infections. This study demonstrates that PCR-ESI-MS analysis done directly on blood specimens is a useful adjunct to continuously monitored blood cultures and can provide rapid and helpful microbial information to clinicians in the setting of the suspected bloodstream infection. The majority of the additional positive results provided by PCR-ESI-MS were clinically relevant.

In this study, among the 906 whole-blood specimens collected from patients with clinically suspected bloodstream infections, 78.6% overall agreement was observed between the blood culture and PCR-ESI-MS results. In 33 of 431 (about 7.7%) of the culture-

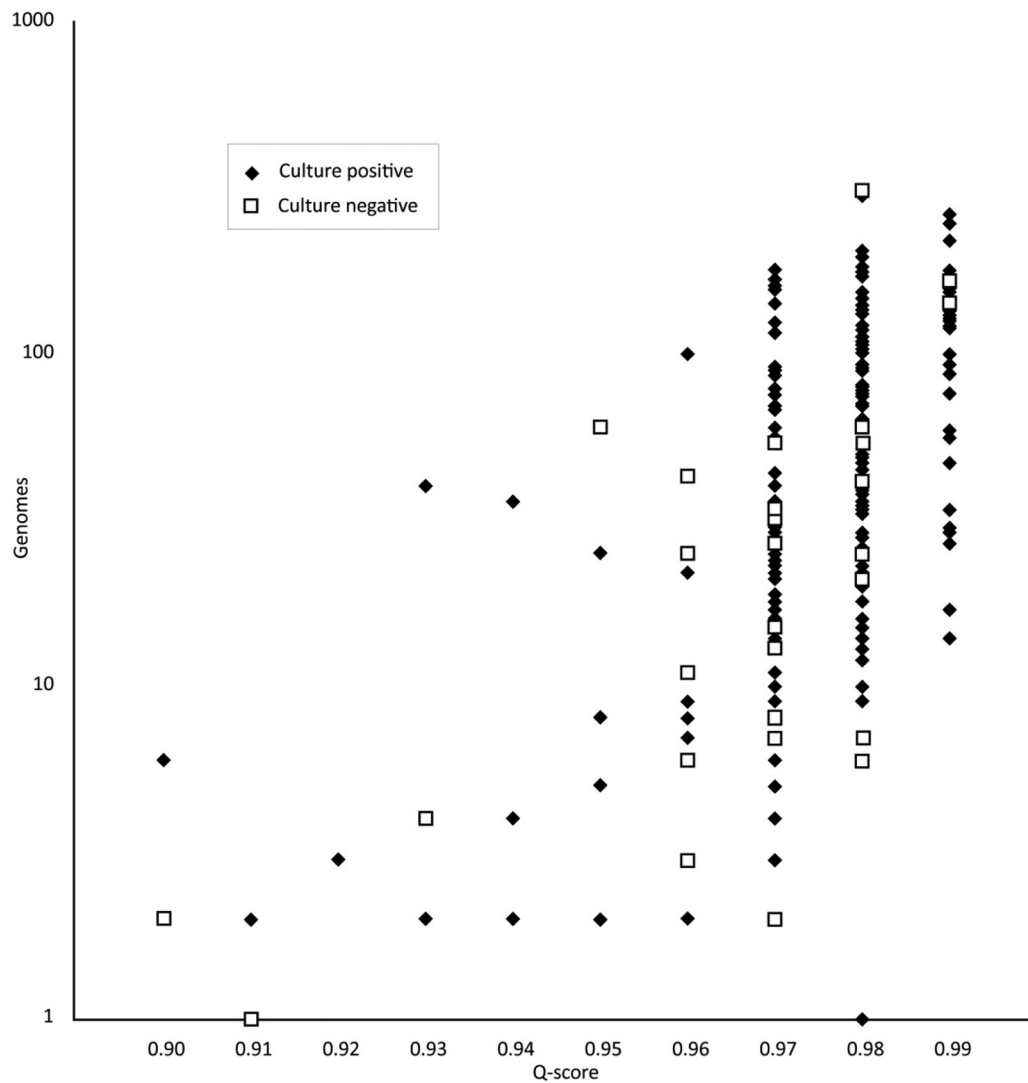


FIG 2 Distribution of Q scores and genome levels for the culture-negative PCR-ESI-MS-positive samples. A Q score of 0.9 is required for reporting a positive detection, and higher Q scores are indicative of higher confidence results.

negative samples, pathogen DNA was detected by PCR-ESI-MS. When 8% was used as the overall positive blood culture rate at the Vanderbilt University Medical Center (19), the 464 culture-positive samples represent the culture-positive yield from a total population of 5,800 blood cultures taken, of which 5,336 were negative by culture. If all 5,800 samples had been evaluated, an additional 409 ( $33/431 \times 5,336$ ) would have tested positive using

the PCR-ESI-MS method. Using positivity by either culture or PCR-ESI-MS as a standard, the sum of all the culture positives and the projected PCR-ESI-MS positives is 711. Of these, 97 would be positive by culture only, 202 would be positive by both methods, and 409 samples would be positive by PCR-ESI-MS in the projected culture-negative population. The estimated sensitivities of PCR-ESI-MS and culture were 85.9%  $[(202 + 409)/711]$  and

TABLE 6 Clinical relevance of PCR/ESI-MS detections in culture-negative samples

Clinical relevance of detections	No. (%) of samples with results:	
	PCR-ESI-MS positive, culture negative ( $n = 33$ ) <sup>a</sup>	PCR-ESI-MS multipositive, culture single-positive ( $n = 15$ )
True bacteremia and/or candidemia with correct pathogen identification	10 (30.3)	3 (20.0)
True bacteremia and/or candidemia	21 (63.6)	7 (46.7)
Possible bacteremia and/or candidemia	0 (0.0)	3 (20.0)
False positive most likely due to skin contamination	2 (6.1)	2 (13.3)

<sup>a</sup> See Table 5 for pathogen identification by PCR-ESI-MS.

41.2% [(202 + 91)/711], respectively. Our data support previous assessments that culture is believed to miss half of the cases of bacteremia (11, 12).

The PCR-ESI-MS results in the culture-negative cases are not corroborated by any direct analytical method. This conundrum is difficult to resolve. In the PCR-ESI-MS assay, nine primer pairs were used to amplify relatively short (~100 bp) regions of the bacterial genome, and amplicons were not required from all the primer pairs in order to report a positive result (17, 22, 23). Nonetheless, each positive result depended on multiple positive PCRs. Corroboration by an independent 16S amplification followed by sequencing is a possible strategy for discrepancy resolution, but this requires that a single PCR generate an amplicon of >1,000 bp, which is inherently less sensitive than PCR generating shorter amplicons. In addition, if more than one organism is present, cloning or use of a next-generation sequencing technology would be required for identification. Here, we used two indirect methods to corroborate the PCR-ESI-MS-positive results: comparison of the analytical metrics obtained in the PCR-ESI-MS and patient chart review. In 33 PCR-ESI-MS-positive but culture-negative specimens, medical chart review suggested true bacteremia in 31 cases. The medical chart review findings correlated well with the PCR-ESI-MS analytical metrics. Figure 2 is an illustration of the system-reported Q score and the estimated microbial genome level (based on comparison to an internal calibration standard) for each PCR-ESI-MS-positive sample. The two samples considered to be false positives from the patient chart review show relatively lower Q score and microbial genome levels, suggesting that the remaining 31 cases detected by the PCR-ESI-MS method that were negative by culture are clinically significant.

There were three notable limitations of this study. First, the whole-blood specimens analyzed by PCR-ESI-MS were not collected at the same time and in the same manner as those used for blood culture. Although we strictly limited the study period to 8 h, the organism distributions and quantification in the samples evaluated by culture and by PCR-ESI-MS might have varied. Additional processing of these specimens in other laboratories might induce additional errors and/or contamination. Second, the study was performed at the Vanderbilt University Medical Center, where the false-contamination rate in blood culture has been high, mainly due to the use of persons other than phlebotomists for blood collection (19). Samples in which CoNS was detected were excluded from the data analysis to minimize the effect of contaminated samples. Finally, PCR-ESI-MS was performed on a single 1.25-ml specimen of whole blood from each patient without repeats. Numerous studies have addressed the issue of reproducibility of culture results (26–28), and the data suggest that two or three sequential consecutive samplings of blood are needed to obtain acceptable results, primarily due to sampling error (29, 30). The 97 specimens for which culture was positive with negative PCR-ESI-MS results showed an organism distribution very similar to the cases in which the detection matched between the two methods. This suggests there was nothing specific about the nature of the organisms to result in missed detections, but perhaps a combination of one or more of the above limitations caused them. Future molecular studies should analyze multiple serial samples of blood in higher volumes, as is done routinely with culture, to minimize errors and get a better estimate of clinical sensitivity.

Using the PCR-ESI-MS assay described here, the majority of pathogens can be detected and identified within 8 h directly from

whole-blood specimens, which can significantly shorten the time between empirical therapy and evidence-based practice. In addition, the PCR-ESI-MS assay detected an additional 7% (31 in 431) of clinically relevant organisms in blood that yielded negative culture results. Direct analysis of patient blood samples by PCR-ESI-MS thus has the potential to enhance the diagnostic yield of bacteremia and candidemia in patients with bloodstream infections.

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