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Performance of PCR/electrospray ionization-mass spectrometry on whole blood for detection of bloodstream microorganisms in patients with suspected sepsis

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ABSTRACT

Blood culture (BC) often fails to detect bloodstream microorganisms in sepsis. However, molecular diagnostics hold great potential. The molecular method PCR/electrospray ionization-mass spectrometry (PCR/ESI-MS) can detect DNA from hundreds of different microorganisms in whole blood. The aim of the present study was to evaluate the performance of this method in a multicenter study including 16 teaching hospitals in the USA (n=13) and Europe (n=3). First, on 2,754 contrived whole blood samples, with or without spiked microorganisms, PCR/ESI-MS produced 99.1% true positive and 97.2% true negative results. Secondly, among 1,460 patients with suspected sepsis (sepsis-2 definition), BC and PCR/ESI-MS on whole blood were positive in 14.6% and 25.6% of cases, respectively, with the following result combinations: BC+/PCR/ESI-MS-, 4.3%; BC+/PCR/ESI-MS+, 10.3%; BC-/PCR/ESI-MS+, 15.3%; and BC-/PCR/ESI-MS-, 70.1%. Compared with BC, PCR/ESI-MS showed the following sensitivities (coagulase-negative staphylococci not included): Gram-positive bacteria, 58%; Gram-negative bacteria, 78%; and *Candida* species, 83%. The specificities were > 94% for all individual species. Patients treated with prior antimicrobial medications (n=603) had significantly increased PCR/ESI-MS positivity rates compared with patients without prior antimicrobial treatment, 31% vs 22% ($p<0.0001$), with pronounced differences for Gram-negative bacteria and *Candida* species. In conclusion, PCR/ESI-MS showed excellent performance on contrived samples. On clinical samples, it showed high specificities, moderately high sensitivities for Gram-negative bacteria and *Candida* species, and elevated positivity rates during antimicrobial treatment. These promising results encourage further development of molecular diagnostics on whole blood for detection of bloodstream microorganisms in sepsis.

KEYWORDS: Sepsis, Bacteremia, direct detection, PCR/ESI-MS

INTRODUCTION

The World Health Organization (WHO) recently recognized sepsis as a global health priority, as it is a common and severe disease that can often be cured with adequate treatment, including appropriate antimicrobial therapy [1, 2]. In order to enable targeted antimicrobial therapy with maximum effect, and avoid unnecessary use of broad-spectrum antimicrobials, the microbiological diagnosis of sepsis should be established [3]. However, even in patients with known bacterial sepsis, blood culture (BC) often provides negative results [4]. For improved detection of bloodstream pathogens, a number of commercial molecular methods have been developed [5]. Unfortunately, most methods are limited by a narrow spectrum of detectable microorganisms (e.g. T2 Bacterial panel; T2 Biosystems) [6] or suboptimal sensitivity (e.g. the LightCycler SeptiFast test, Roche) or specificity (e.g. the Magicplex Sepsis Real-time test, Seegene; the Karius test, Karius) [5, 7]

Based on the PCR/electrospray ionization – mass spectrometry (PCR/ESI-MS) technology, Abbott (Carlsbad, California) developed the IRIDICA BAC BSI Assay with capacity to detect DNA from >200 different microorganisms in whole blood samples [8]. Previous clinical diagnostic studies have shown promising results with PCR/ESI-MS positive detections typically exceeding BC positive results [9, 10]. However, the previous studies of PCR/ESI-MS on whole blood have been too small to enable evaluation on individual microorganisms and to compare the performance of the method on patients with and without prior antimicrobial medication [11].

The aims of the present study were 1) to test PCR/ESI-MS on blood samples spiked with known microorganisms (contrived specimens), and 2) to compare PCR/ESI-MS on whole blood with BC in patients with suspected sepsis, in a large multicenter study. The study was

the basis for an application to the US Food and Drug Administration (FDA) regarding the IRIDICA BAC BSI Assay. However, Abbott withdrew the FDA application and ceased producing IRIDICA instruments and IRIDICA test kits in 2017.

MATERIALS AND METHODS

Study design and settings

This was a prospective, multicenter, observational cohort study, with patients enrolled and samples collected from December 2014 through March 2016 at 16 teaching hospitals in three countries; USA (n=13; Baylor College of Medicine, Houston, TX; Harbor-UCLA Medical Center, Torrance, CA; Henry Ford Hospital, Detroit, MI; Johns Hopkins Hospital, Baltimore, MD; Kern Medical Center, Bakersfield, CA; Maricopa Medical Center, Phoenix, AZ; New York Methodist Hospital, New York, NY; Olive View-UCLA Medical Center, Sylmar, CA; eStudySite, Sharp Chula Vista, San Diego, CA; Truman Medical Center, Kansas City, MO; Beaumont Hospital, Royal Oak, MI; University of Alabama, Birmingham, AL; University of Washington, Seattle, WA); Sweden (n=2; Karolinska University Hospital, Stockholm and Örebro University, Örebro); and the United Kingdom (n=1; University College London Hospitals, London).

The study also included four clinical testing sites, each with an installed IRIDICA PCR/ESI-MS system, that included Johns Hopkins Hospital, Baltimore, MD, USA; Karolinska University Hospital, Stockholm, Sweden; med fusion, Lewisville, TX, USA; and AthoGen testing, Carlsbad, CA, USA.

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Clinical whole blood samples from enrolled study patients were collected and stored at -70°C and later sent to the clinical testing sites for analysis with PCR/ESI-MS (IRIDICA BAC BSI). In addition, the clinical testing sites also analyzed contrived specimens with PCR/ESI-MS.

Contrived Whole Blood Specimens

EDTA whole blood lots were collected by Ibis Biosciences, Abbott from 110 healthy adults, 500 mL from each subject. The whole blood lots were pre-screened for contaminating bacterial DNA using the IRIDICA BAC BSI Assay, and contaminated lots were excluded. Each whole blood lot was split into aliquots of 5 mL that were spiked with culture-quantified stocks of 50 different microorganisms (see Table S1 in Supplemental material).

For each microorganism the limit of detection (LOD) was determined. Whole blood aliquots were spiked with microorganisms at 3-10 different concentrations (5 samples at each concentration). The lowest concentrations for which all samples were PCR/ESI-MS positive were then used in a confirmation analysis of additionally 20 spiked samples. The confirmed LOD was defined as the lowest concentration (CFU/mL) for which the detection rate was at least 95% (minimum of 19/20 valid replicates). In Supplementary Table S1, the confirmed LODs of 50 microorganisms are presented.

It is well known that the concentration of bacteria in the bloodstream varies among patients with bloodstream infection [12]. Thus, in order to reflect a patient scenario with different bloodstream concentrations of microorganisms, whole blood aliquots were spiked to the following target levels: 1.5 × LOD (25 aliquots), 3 × LOD (15 aliquots), 10 × LOD (10 aliquots). Altogether, 50 contrived blood samples of each of 50 microorganisms were made, totaling 2,500 specimens.

In addition, from the pre-screened EDTA whole blood lots from healthy adults described above, Ibis Biosciences provided 254 specimens without spiked microorganisms (negative contrived specimens).

Patients

Patients aged ≥ 6 years presenting to the emergency department or who were being cared for in the hospital's intensive care unit (ICU) or other similar units with suspected sepsis according to the sepsis-2 definition, i.e. suspected bloodstream infection and a diagnosis of systemic inflammatory response syndrome (SIRS) [13], motivating standard of care BC, were eligible for inclusion. The SIRS diagnosis required at least two of the following SIRS criteria; body temperature > 38°C or < 36°C, heart rate > 90 beats/minute, respiratory rate > 20/min or a $PaCO_2$ < 32 mm Hg, and white blood cell count of > 12,000 cells/µL or < 4,000 cells/µL. The single exclusion criterion was previous enrollment in the study. Data on antimicrobial medication taken within 14 days prior to enrollment was collected from each patient´s record shortly after enrollment by chart review.

From each study patient, at least 10 mL whole blood was collected in 1-2 EDTA tubes for testing with PCR/ESI-MS, concurrently with standard of care BC.

PCR/ESI-MS

PCR/ESI-MS (IRIDICA BAC BSI) was performed at the clinical testing sites according to the manufacturer's instructions. The assay was designed to identify unique DNA sequences from >200 different bacteria and fungi for species level identification, as well as the antibiotic resistance markers *mecA*, *vanA*, *vanB*, and bla_{KPC}. A negative control was included in every run and a positive control was included at least once per day of analysis. Four different

positive controls, supplied by ZeptoMetrix Corporation (Buffalo, NY), were used on a rotating basis, i.e. whole blood samples spiked with either methicillin-resistant *Staphylococcus aureus* (MRSA) bundled with *Candida albicans*, vancomycin-resistant *Enterococcus faecium* (VRE), vancomycin-resistant *Enterococcus faecalis*, or carbapenem-resistant *Klebsiella pneumoniae* (KPC). The analytic procedure was run in two separate rooms, one room for sample preparation and DNA extraction, and the other room for PCR, desalting, and mass spectrometry. Assay turnaround time was approximately 8 h, and system throughput was 5 patient samples at a time, permitting a maximum of 15 samples per 24 hours. Operating the IRIDICA system required one full-time laboratory technologist.

Briefly, 5 mL of whole blood were lysed using the IRIDICA bead-beater. DNA was extracted with the IRIDICA DNA Prep Kit, using the automated extraction system. Purified DNA in buffer was automatically distributed by the IRIDICA sample prep into 16-well IRIDICA BAC BSI Assay Strips containing PCR reagents and primers for 18 PCR reactions. PCR was performed on the IRIDICA Thermal Cycler using a preloaded PCR amplification protocol. After PCR amplification, the IRIDICA BAC BSI assay strips were loaded onto the IRIDICA desalter, which purified DNA to remove substances that may interfere with mass spectrometry. Following desalting, plates were loaded onto the IRIDICA mass spectrometer. Purified amplicons were injected one well at a time into an electrospray ionization time-of-flight mass spectrometer for determination of the molecular mass of the amplicons. The resulting information was used for species identification by automated database comparison, as previously described [8].

The IRIDICA BAC BSI Assay Strip contained an internal control template at a known concentration, that generated a control amplicon. The ratio between the amplicon of the sample DNA and that of the control amplicon was reported as a “level”, which represented a semi-quantitative marker of the DNA content of the sample.

191 **Blood cultures**

192 BC was collected as standard of care. One or two sets of BC bottles were collected, each of
193 the sets consisting of one aerobic and one anaerobic bottle. The standardized and accredited
194 blood culture systems of each study hospital were used. Identification and susceptibility
195 testing of the species were performed according to the local laboratory standards, including
196 matrix-assisted laser desorption ionization time-of-flight mass spectrometry, VITEK2
197 (BioMérieux, Durham, NC, USA), and disc diffusion and E-test gradient diffusion. No
198 information about blood volume in the BC bottles was available.

199

200 **Statistics**

201 An IBM SPSS Statistics (20.0) software was used for statistical analyses. Chi-square and
202 Fisher´s exact tests were used for comparison of proportions and Mann-Whitney U test was
203 used for comparison of independent groups. A p-value <0.05 was considered significant.

204

205 **Ethics**

206 The study was approved by an ethical board at each study site and was conducted according
207 to the requirements of the individual country´s laws and regulations and the Declaration of
208 Helsinki. All study participants provided written informed consent.

209

210

211 **RESULTS**

212 **Limits of Detection and Contrived Specimens**

213 Table S1 in Supplemental material shows the confirmed LOD for individual microorganisms.
214 There was no significant difference between the LODs of Gram-positive and Gram-negative
215 bacteria, median 48 CFU/mL (interquartile range [IQR], 16-128 CFU/mL) versus median 32
216 CFU/mL (IQR, 16-64 CFU/mL), p=0.24. However, the LODs of *Candida* species, median 8
217 CFU/mL (IQR, 6-12 CFU/mL), were significantly lower than those of Gram-positive
218 (p=0.012) and Gram-negative bacteria (p=0.016). It should be noted that coagulase-negative
219 staphylococci (CoNS) had high LODs, e.g. *Staphylococcus epidermidis* 256 CFU/mL.

220 The results of PCR/ESI-MS on 2,500 positive contrived specimens are shown in Table S2 in
221 Supplemental material. PCR/ESI-MS identified the inoculated organism (true positive result)
222 in 2,477 cases (99.1%) and detected other organisms (false positives) in 33 cases (1.3%). The
223 false positive results included *Cutibacterium acnes* (n=7), *Nocardia farcinica* (n=4),
224 *Escherichia coli* (n=3), *S. aureus* (n=3), *S. epidermidis* (n=3), and 9 other species with 1 or 2
225 positive results each (see Table S2, Supplemental material).

226 Among 254 negative contrived specimens, the PCR/ESI-MS system reported true negative
227 results in 247 cases (97.2%). False positive results were noted in 7 cases, one of each of *C.*
228 *acnes, Nocardia* species, *E. coli*, *S. aureus*, *Staphylococcus lugdunensis, Micrococcus*
229 species, and *Mycobacterium* species.

230 There were 255 contrived samples with microorganisms with known resistance markers (143
231 *mecA*, 35 *vanA*, 43 *vanB*, and 34 bla_{KPC}). All of these resistance markers were correctly
232 detected by PCR/ESI-MS. Among 1,143 contrived samples spiked with microorganisms
233 known not to harbor any of the four resistance markers, there were 4 false positives for *mecA*
234 (0.3%), but no false positives for the other resistance markers.

235

236 **Patients**

237 Altogether, 1,501 patients were included in the study, see flowchart (Fig.1). They had a
238 median age of 54 years (range 6-96 years), and 625 patients (41.6%) were females.

239 Forty-one patients had PCR/ESI-MS results that were either invalid (n=33) or not comparable
240 with BC results (n=8), meaning the microorganisms reported by BC were not part of the
241 PCR/ESI-MS organism reporting list. These patients were omitted from the study, and thus
242 the results of 1,460 patients were used in the final analyses. Two sets of BC bottles were
243 obtained in 995 patients (68.2%), one set was obtained in 465 patients (31.8%).

244 Among 1,460 study patients, 603 patients (41.3%) had received any antimicrobial medication
245 within 14 days prior to enrollment (antibiotics in 555 patients, antifungals in 79 patients, and
246 antivirals in 114 patients).

247

248 **Results of blood culture and PCR/ESI-MS in patients and clinical samples**

249 In the study group of 1,460 patients with suspected sepsis, a microorganism was detected by
250 either BC or PCR/ESI-MS or both in 437 patients (29.9%), i.e. by BC in 213 patients (14.6%)
251 and by PCR/ESI-MS in 374 patients (25.6%). The following result combinations were noted:
252 BC+/PCR/ESI-MS- (n=63), BC+/PCR/ESI-MS+ (n=150), and BC-/PCR/ESI-MS+ (n=224),
253 see Fig. 2A. Table 1 shows the combined results of PCR/ESI-MS and BC. Concordant
254 negative results were noted in 1,023 patients. Fully concordant positive results (identical
255 specimens detected by BC and PCR/ESI-MS) were noted in 113 patients, of which 109
256 patients had concordant single microorganisms and 4 patients had concordant multiple
257 microorganisms. Among 150 BC+/PCR/ESI-MS+ patients, fully discordant results (different
258 species detected by BC and PCR/ESI-MS) were noted in 8 cases (5.3%). Fig. 2B shows
259 combined positive results of BC and PCR/ESI-MS among patients with two sets of BC bottles
260 and one set of BC bottles, respectively. As noted, the BC positivity rate was similar between

261 the two categories of patients (15% and 14%), but BC positivity and PCR/ESI-MS positivity
262 combined were significantly more common among patients with one set of BC bottles.

263 Among 25 patients with BC positive for CoNS with two sets of BC bottles analyzed, CoNS
264 was detected in both BCs in 8 cases and in just one BC in 17 cases.

265 Fig. 3 shows the combined positive results of BC and PCR/ESI-MS for Gram-positive and
266 Gram-negative bacteria. As noted, positive BC rate was similar between Gram-positive
267 (7.8%) and Gram-negative bacteria (7.7%). However, as noted in Fig. 3, BC positivity and
268 PCR/ESI-MS positivity combined was significantly more common for Gram-negative than
269 for Gram-positive bacteria. In addition, PCR/ESI-MS positivity was significantly more
270 common for Gram-negative than for Gram-positive bacteria, 243/1,460 (16.6%) vs 145/1,460
271 (9.9%), $p<0.0001$. The same pattern was noted for individual microorganisms, see Fig. 4 and
272 Table S3 in Supplemental material.

273 All non-aureus staphylococci observed in the study were categorized as CoNS, and included
274 *S. epidermidis, Staphylococcus hominis, Staphylococcus capitis, Staphylococcus*
275 *haemolyticus*. CoNS were detected by BC in 45 patients (3.1%) and by PCR/ESI-MS in 10
276 patients (0.68%) (Fig. 4), $p<0.0001$.

277 *Candida* species were detected by BC in 6 patients (0.41%) and by PCR/ESI-MS in 23
278 patients (1.6%), $p=0.0028$, see Fig. 4.

279

280 **Sensitivities and specificities of PCR/ESI-MS compared with blood culture**

281 When results for individual species were considered (CoNS not included), the sensitivity of
282 PCR/ESI-MS compared with BC was 71% (144/203) overall, 58% (45/77) for Gram-positive
283 bacteria, 78% (94/120) for Gram-negative bacteria, and 83% (5/6) for *Candida* species. The

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specificities were > 94% for all individual species. Table 2 shows sensitivities and specificities for the most frequently detected microorganisms.

Results in patients with and without antimicrobial medication prior to enrollment

Fig. 5 shows the results of BC and PCR/ESI-MS in patients without and with any prior antimicrobial medication. The BC positivity rate tended to be lower for patients with prior antimicrobial medication (13%, 77/603) than for those without prior antimicrobials (16% 136/857), p=0.099 (Fig. 5A). However, patients treated with prior antimicrobials had significantly higher BC positivity and PCR/ESI-MS positivity combined for any microorganism (Fig. 5A) and for Gram-negative bacteria (Fig. 5C), but not for Gram-positive bacteria (Fig. 5B), than patients without prior treatment. Accordingly, the PCR/ESI-MS rate was significantly higher for patients with than for patients without prior antimicrobials, for any microorganism ($p<0.0001$) and for Gram-negative bacteria ($p<0.0001$), but not for Gram-positive bacteria (p=16).

Fig. 6 presents the detection rates of individual microorganisms in patients with and without prior antimicrobial medication. *E. coli* was clearly the most commonly detected microorganism in both categories. This bacterium, as well as *Enterococcus* species, *Enterobacter* species, and *Bacteroides* species were significantly more often detected by BC and/or PCR/ESI-MS in patients who received treatment than in patients without prior antimicrobial medication ($p<0.05$ in all cases; Fig. 6). They were also more often detected by PCR/ESI-MS alone ($p<0.05$ in all cases).

Fig. 7 shows that administration of prior antifungal medication was strongly associated with PCR/ESI-MS positivity for *Candida* species. Prior antifungal medication was also strongly associated with BC positivity for *Candida* species, 2.5% vs 0.29% (p=0.038).

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Semi-quantitative results of *Staphylococcus aureus* and *Escherichia coli* DNA

The semi-quantitative levels of *S. aureus* and *E. coli* DNA, produced by the PCR/ESI-MS system, were studied as one representative each for Gram-positive and Gram-negative microorganisms. The levels were significantly higher for BC+/PCR/ESI-MS+ results than for BC-/PCR/ESI-MS+ results for both microorganisms (Fig. 8).

Resistance markers in clinical samples

Among 1,460 study patients, PCR/ESI-MS detected resistance markers combined with relevant bacteria in 29 cases, i.e. *mecA* in 18 cases and *vanA* in 11 cases. No patient was PCR/ESI-MS positive for *vanB* or bla_{KPC} together with relevant bacterial species.

The *mecA* positive cases were PCR/ESI-MS+ for *S. aureus* in 10 cases and PCR/ESI-MS+ for CoNS in 8 cases. Among 10 patients with PCR/ESI-MS+ for *S. aureus* and *mecA*, BC was positive for *S. aureus* in 7 cases, including 3 cases with MRSA and 4 cases of methicillin-susceptible *S. aureus*.

Among 11 patients with PCR/ESI-MS+ for *vanA* and *E. faecium*, two were BC positive for *E. faecium*; one with VRE and one with vancomycin- susceptible *E. faecium*.

bla_{KPC} was identified by standard laboratory methods in a BC isolate of *K. pneumoniae*. The corresponding patient´s whole blood sample was PCR/ESI-MS+ for *K. pneumoniae*, but PCR/ESI-MS- for bla_{KPC}.

DISCUSSION

330 This is the largest study of PCR/ESI-MS performed on either contrived samples or clinical
331 samples. The study showed excellent results of PCR/ESI-MS performed on contrived
332 samples, with very few false negative or false positive results. Evaluation of whole blood
333 samples from patients with suspected sepsis found that PCR/ESI-MS was more often positive
334 than BC, and that trend was more pronounced in those receiving prior antimicrobial
335 medication. While the specificity of PCR/ESI-MS was high relative to BC, sensitivities varied
336 between species, but were generally higher for Gram-negative than for Gram-positive
337 bacteria.

338 It is well known that the BC positivity rate in a sepsis population increases with the number of
339 BC bottles analyzed. Thus, the fact that the patients with one set of BC bottles had almost as
340 high BC positivity rate as patients with two sets of BC bottles (Fig. 2B) indicates that the
341 population with one set may have had a higher rate of true bloodstream infection. A higher
342 frequency of bloodstream infection could be a reason for the high rate of PCR/ESI-MS+
343 results among patients with one BC set collected (Fig. 2B). An alternative possible reason
344 could be more false positives in this patient group. The obvious difference between those with
345 one and two BC sets suggests a possibly biased or unrepresentative sample population.

346 The low rate of PCR/ESI-MS positivity for CoNS in the study (Fig. 4) was unexpected.
347 However, it could perhaps be explained by the high LODs of PCR/ESI-MS for CoNS (Table
348 S1 in Supplemental material).

349 Seven previous studies of PCR/ESI-MS on 5 mL whole blood samples [9, 10, 14-18] reported
350 BC positivity rates of 5.4-34% and PCR/ESI-MS positivity rates of 10.6-37% [19]. Of these
351 studies, there were higher rates of PCR/ESI-MS positive results (versus BC) seen in 5 studies
352 [9, 10, 14, 16, 18] and more BC positive results (versus PCR/ESI-MS) in 2 studies [15, 17]. In
353 the largest previous study (n=616) by Vincent et al. [10], BC was positive in 11% and

354 PCR/ESI-MS was positive in 37% of the cases. In the present study, BC was positive in
355 14.6% and PCR/ESI-MS was positive in 25.6% of patients.

356 An interesting finding of the present study was that although the BC positivity rate for Gram-
357 positive and Gram-negative bacteria was similar (7.8% and 7.7%), the PCR/ESI-MS
358 positivity rate was significantly higher for Gram-negative than for Gram-positive bacteria
359 (Fig. 3). Accordingly, the sensitivity of PCR/ESI-MS compared to BC was higher for Gram-
360 negatives (78%) than for Gram-positives (58%). The reason for this difference is not clear. A
361 possible explanation could be different loads of bacterial DNA in the bloodstream during
362 sepsis. However, the PCR/ESI-MS semi-quantitative levels did not differ significantly
363 between Gram-negative and Gram-positive bacteria, as illustrated by *S. aureus* and *E. coli* in
364 Fig. 8. Similarly, Ziegler et al. [20] found comparable PCR cycle thresholds of bacterial
365 DNA in whole blood from patients with Gram-positive and Gram-negative bloodstream
366 infection, using the LightCycler SeptiFast test. Another possible explanation could be
367 different LODs. However, the present study could not find any general difference in LODs
368 between Gram-positives and Gram-negatives (Table S1 in Supplemental material). Thus, the
369 reason for the difference remains unclear. An interesting finding, however, was that the
370 difference between Gram-negative and Gram-positive detections with PCR/ESI-MS was
371 predominantly noted in patients receiving prior antimicrobial medication (Fig. 5B and 5C).

372 The design of this study enabled analysis of the importance of prior antimicrobial medication
373 on the results of BC and PCR/ESI-MS. Similar to previous studies [21, 22], patients with
374 prior antimicrobials tended to have lower BC positivity rates than patients not receiving
375 antimicrobials (Fig. 5A). However, PCR/ESI-MS was significantly more often positive in
376 patients with than in patients without antimicrobials and consequently, the combined results
377 of BC and PCR/ESI-MS was more often positive in patients with than in patients without

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prior antimicrobials (Fig. 5A). This pattern was pronounced for Gram-negative bacteria (Fig. 5C) but was not noted for Gram-positive bacteria (Fig. 5B).

Notably, differences were observed between individual Gram-negative species (Fig. 6). Prior antimicrobial medication was associated with higher combined positivity rates (BC and PCR/ESI-MS) for *E. coli*, *Enterobacter* species, and *Bacteroides* species, but not for *Klebsiella* species. Among Gram-positives, *Enterococcus* species was more common in patients with prior antimicrobials (Fig. 6). This was an unexpected pattern, as we expected patients with prior antimicrobials to have decreased PCR/ESI-MS positivity rate in line with decreased BC positivity rate. The reason for this pattern is not known. It could perhaps reflect that the cases with and without prior antimicrobials represent different patient populations. Prior antimicrobial medication is, based on clinical practice patterns, likely associated with an increased likelihood of true infection, and to inpatient care prior to enrollment. Bloodstream infections with *Enterobacter* species and *Enterococcus* species have been associated with long hospital durations prior to onset [23]. However, the unexpected pattern with more PCR/ESI-MS positives in patients with prior antimicrobials could also be caused by false positive PCR/ESI-MS results, possibly due to contamination during the extraction step, which could perhaps be more problematic with Gram-negative species.

Candida DNA was detected by PCR/ESI-MS significantly more often than *Candida* species was detected by BC (Fig. 4), similar to the performance of the commercial T2Candida test (T2 Biosystems, Lexington, Massachusetts) [24]. By both BC and PCR/ESI-MS, detection of *Candida* species was linked to prior antifungal medication (Fig. 7). As antifungal medication is usually based on microbiological findings and/or clinical suspicion of fungal infection, this link can reasonably be interpreted as a support for BC-/PCR/ESI-MS+ results for *Candida* species, which would have important value in clinical practice.

A very important question is whether BC-/PCR/ESI-MS+ results represent true infections. It should be noted that in the present study 2.8% of the negative contrived samples were false positive with PCR/ESI-MS. This could represent contamination or false positivity due to non-microbial components or microbial cell free DNA within the blood [25]. Unfortunately, the present study was not designed to evaluate the clinical relevance of BC-/PCR/ESI-MS+ results, as clinical data apart from SIRS data was not collected. However, there are some important findings from previous studies that should be mentioned. Jordana-Lluch et al. [26] identified 80 BC-/PCR/ESI-MS+ microorganisms, of which 41 microorganisms (51%) correlated with clinical findings. In another study, the same group [16] identified 84 BC-/PCR/ESI-MS+ microorganisms, of which 42 micro-organisms (50%) had support from clinical findings. In a European ICU sepsis study [27] (a subgroup study of [10]), the 28-day mortality was found to be higher in patients with BC-/PCR/ESI-MS+ results than in patients with BC-/PCR/ESI-MS- results (42% vs. 26%, p=0.001). This association with disease severity may perhaps be due to true bloodstream infection in a substantial proportion of cases with BC-/PCR/ESI-MS+ results. Proper evaluation of the clinical relevance of BC-/PCR/ESI-MS+ results, requires additional studies designed to evaluate PCR/ESI-MS with detailed clinical data, and should include severely ill patients without infections.

The present study design with BC as the reference standard enabled proper analysis of diagnostic sensitivity. Overall, the sensitivity of PCR/ESI-MS was 71%, which is similar to the pooled sensitivity of 66% that was recently found in the meta-analysis of Huang et al. [28]. This suboptimal clinical sensitivity combined with the low frequency of false-negative results among contrived specimens, indicate that a substantial proportion of patients with bloodstream infection may have bloodstream concentrations of microorganisms below the LODs of the microorganisms. The 5 species with the highest sensitivities compared with BC

in the present study, i.e. *Streptococcus pyogenes*, *E. faecium*, *E. coli*, *Klebsiella oxytoca*, and *Pseudomonas aeruginosa* (sensitivities >92%; Table 2), all had low PCR/ESI-MS LODs (8-16 CFU/mL; Table S1, Supplemental material). As it has been reported that patients with bloodstream infection may have as little as 1-10 CFU/mL of circulating microorganisms [25], the LODs of PCR/ESI-MS may not be clinically optimal for many microorganisms. Accordingly, the concentrations of microorganisms used in the contrived specimens of the present study may have been too high to mimic clinically relevant concentrations. Thus, due to the suboptimal sensitivity, PCR/ESI-MS cannot be used to "rule-out" bloodstream infection.

A disadvantage with PCR/ESI-MS and other molecular methods is the limited information provided about antimicrobial susceptibility. The IRIDICA PCR/ESI-MS panel contains only four resistance markers (*mecA*, *vanA*, *vanB*, and bla_{KPC}). However, on contrived whole blood samples spiked with microorganisms with known presence or absence of resistance in the present study, PCR/ESI-MS showed great performance regarding the resistance markers. On clinical samples, 10 patients were PCR/ESI-MS positive for *S. aureus* and *mecA*, but only 3 of them had culture-proven MRSA in their bloodstream. Accordingly, 11 patients were PCR/ESI-MS+ for *E. faecium* and *vanA*, but only one of them had culture-proven VRE in the bloodstream. These results were not conclusive, as we do not have any additional microbiological data on the patients apart from BC and PCR/ESI-MS. Thus, there is a need for additional evaluations and, in particular, a need for new sensitive methods to determine antimicrobial susceptibility.

At the end of 2014, the PCR/ESI-MS IRIDICA BAC BSI Assay was CE marked and

451 became commercially available for in-vitro diagnostics in Europe. When it was used in
452 routine practice in addition to BC at Karolinska University Hospital [29] it detected BC-
453 /PCR/ESI-MS+ microorganisms that were considered to be clinically relevant [30]. However,
454 in April 2017, Abbott withdrew their application to the FDA regarding the IRIDICA BAC
455 BSI Assay and ceased producing IRIDICA instruments and IRIDICA test kits [29]. Since
456 then, PCR/ESI-MS has not been commercially available. Still, the present study and previous
457 PCR/ESI-MS studies [9, 10, 16, 18] show that in patients with suspected sepsis, bacterial
458 DNA is detected in blood more often than viable bacteria are detected by BC, especially in
459 patients pre-treated. This is encouraging and supportive of the value for further advancing
460 new molecular diagnostics for clinical practice, critical for improved detection of bloodstream
461 microorganisms, with important downstream implications for improved patient outcomes [5].
462 Such development is further motivated by the WHO resolution on sepsis [1] and their global
463 action plan on antimicrobial resistance [31].
464
465 The present study has several strengths. First, the large number of contrived samples, spiked
466 with microorganisms of different concentrations enabled solid conclusions regarding the
467 analytic performance of PCR/ESI-MS. Secondly, the large number of clinical samples
468 enabled comparisons between Gram-positive and Gram-negative bacteria and performance
469 analysis of individual microorganisms. Third, data on prior antimicrobial medication was
470 collected shortly after enrollment, enabling a comparison between patients with and without
471 prior antimicrobial treatment. Altogether, the study provided new knowledge about bacterial
472 DNA in the bloodstream of patients with suspected sepsis.
473
474 The study also has limitations. First, the patient population was heterogenous, as the patients
475 were enrolled at different clinical sites, and were not consecutively enrolled. This design may

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have allowed for bias between variables and may have caused lack of replicability. In addition, due to the lack of a homogenous population, we could not evaluate the additive value of PCR/ESI-MS for the etiologic spectrum of sepsis. Secondly, the lack of standardized blood culturing may have introduced variability regarding blood culture results. Third, standard microbiological tests apart from BC were not registered, and thus we could not properly evaluate BC-/PCR/ESI-MS+ findings. Fourth, severity data apart from SIRS criteria were not registered and thus we could not stratify patients according to the sepsis-3 classification. However, all patients had suspected sepsis according to the sepsis-2 definition, with suspected bloodstream infection and at least 2 SIRS criteria.

In conclusion, PCR/ESI-MS showed excellent performance on contrived whole blood samples. On clinical samples, it showed high specificities, moderately high sensitivities for Gram-negative bacteria and *Candida* species, and elevated positivity rates during antimicrobial treatment. These promising results encourage further development of molecular diagnostics on whole blood for detection of bloodstream microorganisms in sepsis.

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Potential conflicts of interest in addition to Abbott´s above mentioned role in the present study:

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JCM

R.E. Rothman has served on advisory panels for Ibis Biosciences, An Abbott Company, Roche Molecular Systems Inc, Cepheid, Inflammatix, and Qvella Corporation; has been an invited speaker for Roche Molecular Systems Inc.; and has had grant support for research from Abbott and Cepheid.

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TABLE 1 Combined numbers of organisms detected by PCR/ESI-MS and blood culture (BC) in patients with suspected sepsis

No. of organisms detected		All patients analyzed (n=1,460)
PCR/ESI-MS	BC	No. of patients (%)
0	0	1,023 (70)
0	1	53 (3.6)
0	≥ 2	10 (0.7)
1	0	187 (13)
1	1	114 [a] (7.8)
1	≥ 2	8 [b] (0.5)
≥ 2	0	37 (2.5)
≥ 2	1	18 [c] (1.2)
≥ 2	≥ 2	10 [d] (0.7)

[a] The same organism was detected by PCR/ESI-MS and BC in 109/114 cases. Four patients were PCR/ESI-MS+ for *Escherichia coli* and BC+ for coagulase-negative staphylococci. One patient was PCR/ESI-MS+ for *Candia albicans* and BC+ for *Bacteroides* species.

[b] The organism detected by PCR/ESI-MS was also detected by BC in all 8/8 cases.

[c] The organism detected by BC was also detected by PCR/ESI-MS in 15/18 cases. One patient was BC+ for *Peptostreptococcus* species and PCR/ESI-MS+ for *Bacteroides* species and *Clostridium* species; one patient was BC+ for *Eggerthella lenta* and PCR/ESI-MS+ for *Bacteroides* species and *Fusobacterium* species; and one patient was BC+ for *Streptococcus anginosus* and PCR/ESI-MS+ for *Gemella morbillorum* and two anaerobic bacterial species.

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[d] The identical organisms were detected by PCR/ESI-MS and BC in 4/10 cases. At least one organism was detected by both PCR/ESI-MS and BC in 10/10 cases.

660 **TABLE 2** Sensitivity and specificity of PCR/ESI-MS compared with blood culture (BC) in
661 patients with suspected sepsis. Species with more than 5 positive results from PCR/ESI-MS
662 and/or BC are included

Species	Sensitivity % (ratio)	Specificity % (ratio)
Gram-positive bacteria		
Staphylococcus aureus	70 (19/27)	99.2 (1,421/1,433)
Coagulase-negative staphylococci	11 (5/45)	99.6 (1,410/1,415)
Streptococcus mitis/pneumoniae	58 (7/12)	99.4 (1,440/1,448)
Streptococcus pyogenes	100 (4/4)	99.9 (1,455/1,456)
Streptococcus species	27 (3/11)	99.7 (1,445/1,449)
Enterococcus faecalis	57 (4/7)	99.7 (1,449/1,453)
Enterococcus faecium	100 (6/6)	98.8 (1,437/1,454)
Micrococcus species	0 (0/1)	99.6 (1,453/1,459)
Cutibacterium acnes	0 (0/1)	98.8 (1,442/1,459)
Nocardia species	0 (0/0)	98.9 (1,444/1,460)
Gram-negative bacteria		
Escherichia coli	93 (50/54)	94.4 (1,327/1,406)
Klebsiella pneumoniae	80 (20/25)	98.3 (1,411/1,435)
Klebsiella oxytoca	100 (4/4)	99.9 (1,454/1,456)
Enterobacter cloacae complex	80 (4/5)	99.0 (1,441/1,455)
Pseudomonas aeruginosa	100 (5/5)	99.7 (1,451/1,455)
Citrobacter freundii	0 (0/0)	99.6 (1,454/1,460)
Serratia marcescens	62 (5/8)	99.9 (1,451/1,452)

Haemophilus influenzae	0 (0/2)	99.7 (1,453/1,458)
Bacteroides fragilis/thetaiotaomicron	50 (1/2)	99.3 (1,448/1,458)
Fusobacterium nucleatum	0 (0/0)	99.6 (1,454/1,460)
Candida species		
Candida albicans	67 (2/3)	99.4 (1,448/1,457)
Candida glabrata	0 (0/0)	99.6 (1,454/1,460)

663

664

665 **Figure legends**

666

667 **FIG 1** Flow chart of study population

668

669 **FIG 2** Proportion of patients positive by blood culture (BC) and/or PCR/ESI-MS altogether
670 (A) and among patients with two sets and one set of BC bottles, respectively (B)

671

672 **FIG 3** Proportion of patients positive by blood culture (BC) and/or PCR/ESI-MS for Gram-
673 positive or Gram-negative bacteria

674

675 **FIG 4** Individual organisms detected by blood culture (BC) and PCR/ESI-MS

676

677 **FIG 5** Proportion of cases positive by blood culture (BC) and/or PCR/ESI-MS in patients
678 without (n=857) and with prior antimicrobial treatment (n=603), overall (A) and broken down
679 by Gram-positive (B) and Gram-negative (C) detections

680

681 **FIG 6** Proportion of patients without (A) and with (B) any prior antimicrobials, positive for
682 major individual bacteria. * indicates significant ($p<0.05$) differences regarding total
683 proportion of positives (BC and/or PCR/ESI-MS) between cases without and with prior
684 antimicrobials

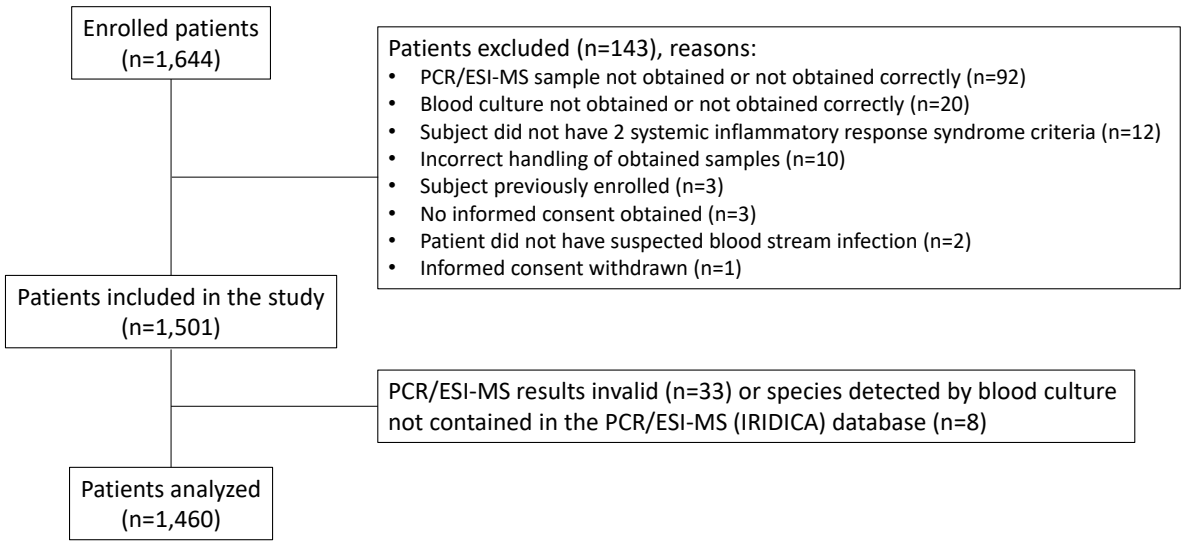
685

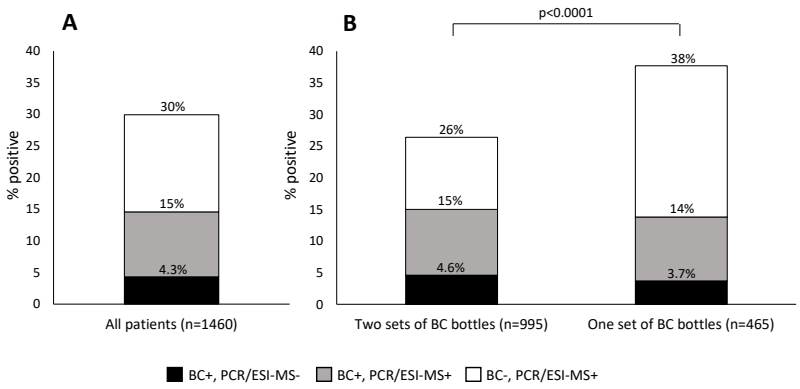
686 **FIG 7** Proportion of patients positive for *Candida* species by blood culture (BC) and/or
687 PCR/ESI-MS, in relation to prior antimicrobial medication

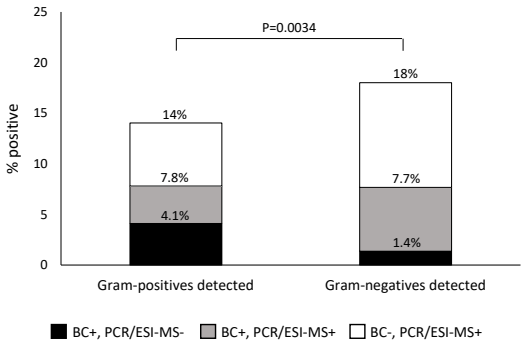
688

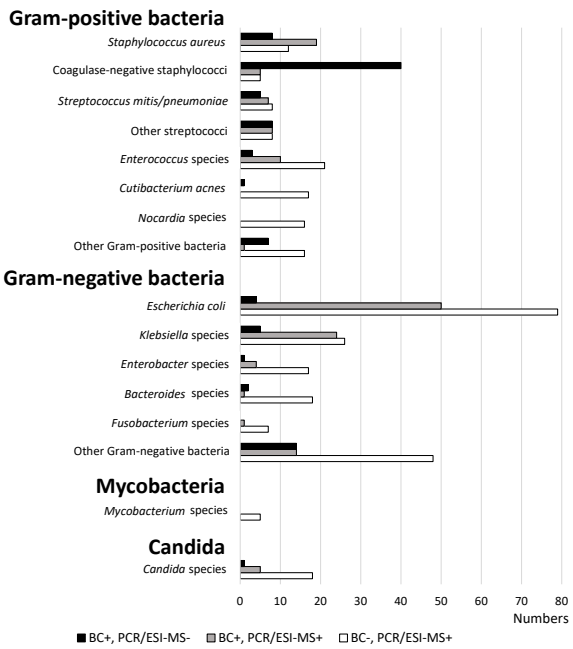
689 **FIG 8** Semi-quantitative levels for *Staphylococcus aureus* DNA (A) and *Escherichia coli*
690 DNA (B) related to blood culture (BC) results in patients with PCR/ESI-MS positive for *S.*
691 *aureus* and *E. coli*

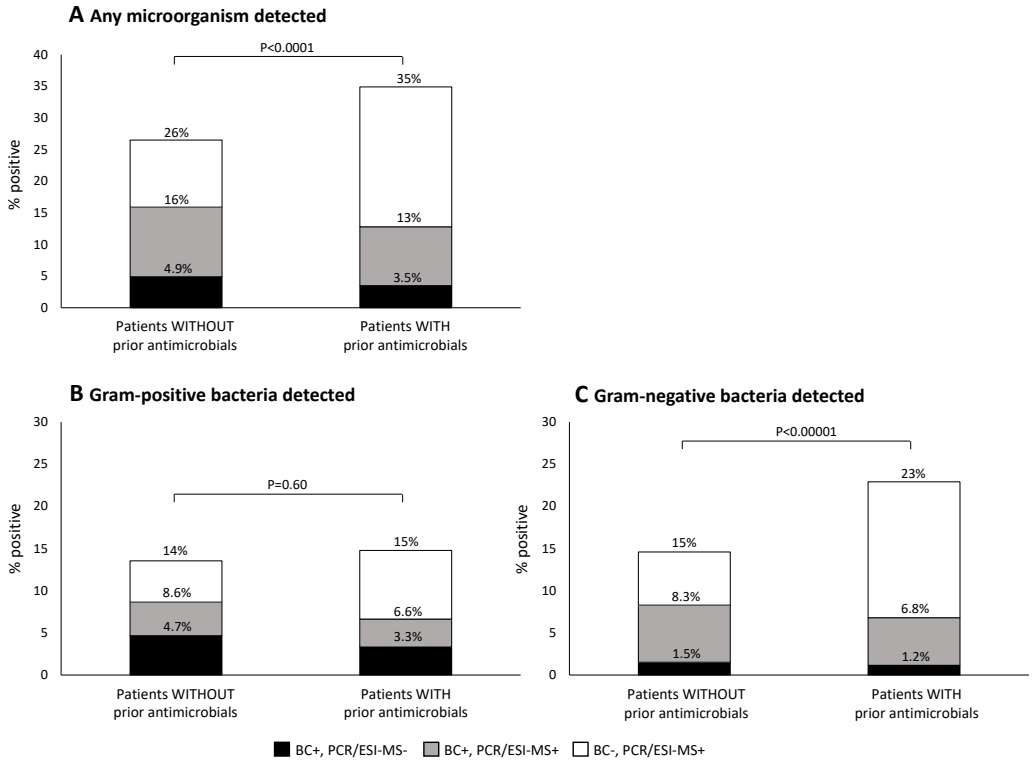
692

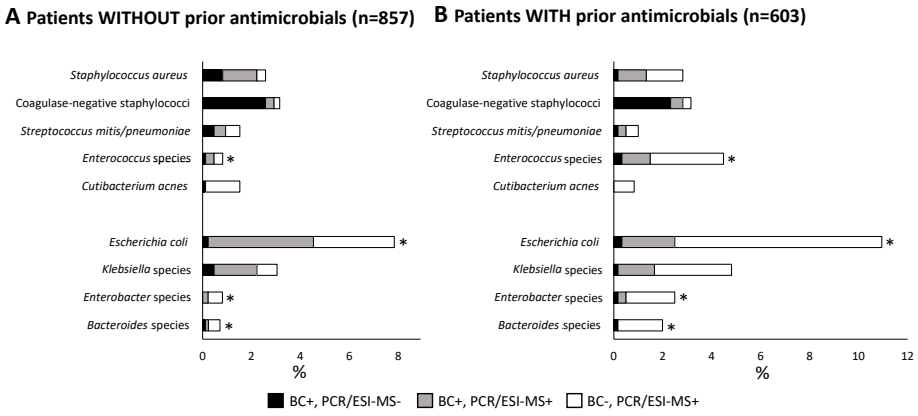












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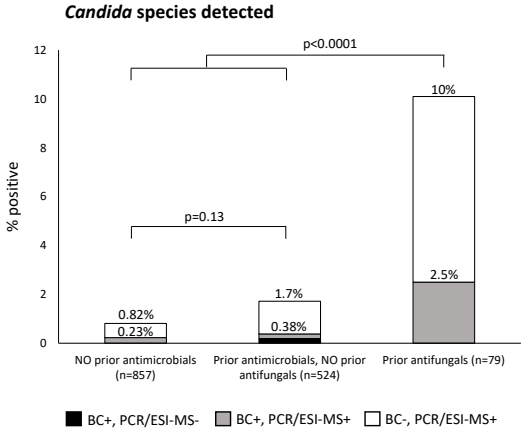

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