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Performance evaluation of InfectID-BSI: A rapid quantitative PCR assay for detecting sepsis-associated organisms directly from whole blood

Lisa A. Simms [a], Corey Davies [a], Nadeesha Jayasundara [a], Sumeet Sandhu [a,b], Alexander Pintara [a], Amorette Pretorius [a], Graeme R. Nimmo [c,d], Jacqueline Harper [c], Matthew Hiskens [e], Karen Smith [e,f], Sarah Boxall [f], Anton Lord [g], Raffaella Giardino [a], David Farlow [a,e], Diane M. Ward [h], Flavia Huygens [a,*]

[a] Microbio Ltd., Translational Research Institute, Brisbane, Queensland 4102, Australia
[b] Queensland Health, Brisbane, Queensland, Australia
[c] Pathology Queensland, Royal Brisbane and Women's Hospital, Brisbane, Queensland 4029, Australia
[d] School of Medicine, Griffith University, Gold Coast, Queensland, Australia
[e] Mackay Institute of Research and Innovation, Mackay Hospital and Health Service, Mackay, Queensland 4740, Australia
[f] Department of Emergency Medicine, Mackay Hospital and Health Service, Mackay, Queensland 4740, Australia
[g] Spectroscopy and Data Consultants, Brisbane, Queensland, Australia
[h] Qserve Consultancy B.V - HQ, Arnhems Bussiness Park, Utrechtseweg 310 Bldg B42, 6812 AR Arnhem, The Netherlands

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ABSTRACT

Background: Bloodstream infections (BSIs) (presence of pathogenic organism in blood) that progress to sepsis (life-threatening organ dysfunction caused by the body's dysregulated response to an infection) is a major healthcare issue globally with close to 50 million cases annually and 11 million sepsis-related deaths, representing about 20% of all global deaths. A rapid diagnostic assay with accurate pathogen identification has the potential to improve antibiotic stewardship and clinical outcomes.

Methods: The InfectID-Bloodstream Infection (InfectID-BSI) test is a real-time quantitative PCR assay, which detects 26 of the most prevalent BSI-causing pathogens (bacteria and yeast) directly from blood (without need for pre-culture). InfectID-BSI identifies pathogens using highly discriminatory single nucleotide polymorphisms located in conserved regions of bacterial and fungal genomes. This report details the findings of a patient study which compared InfectID-BSI with conventional blood culture at two public hospitals in Queensland, Australia, using 375 whole blood samples (from multiple anatomical sites, eg. left arm, right arm, etc.) from 203 patients that have been clinically assessed to have signs and symptoms of suspected BSI, sepsis and septic shock.

Findings: InfectID-BSI was a more sensitive method for microorganism detection compared with blood culture (BacT/ALERT, bioMerieux) for positivity rate (102 vs 54 detections), detection of fastidious organisms (*Streptococcus pneumoniae* and *Aerococcus viridans)* (25 vs 0), detection of low bioburden infections (measured as genome copies/0.35 mL of blood), time to result (<3 h including DNA extraction for InfectID-BSI vs 16 h–48 h for blood culture), and volume of blood required for testing (0.5 mL vs 40–60 mL). InfectID-BSI is an excellent 'rule out' test for BSI, with a negative predictive value of 99.7%. InfectID-BSI's ability to detect 'difficult to culture' microorganisms re-defines the four most prevalent BSI-associated pathogens as *E. coli* (28.4%), *S. pneumoniae* (17.6%), *S. aureus* (13.7%), and *S. epidermidis* (13.7%).

Interpretation: InfectID-BSI has the potential to alter the clinical treatment pathway for patients with BSIs that are at risk of progressing to sepsis.

1. Introduction

Sepsis is a common condition with high morbidity and mortality (Rudd et al., 2020) and currently, blood culture is the gold standard test used for laboratory diagnosis of sepsis (Dellinger et al., 2013). Blood culture has several disadvantages as the gold standard for the diagnosis

* Corresponding author.
E-mail address: fhuygens@microbio.com.au (F. Huygens).

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of sepsis. From a clinical perspective, sometimes a clinical diagnosis of sepsis is obvious. However, in other circumstances, especially when onset is more insidious, diagnosis is often missed. Many patients require critical care and early diagnosis, and prompt treatment is essential as each hour delay in administering antibiotics increases the risk of death by 9% (Liu et al., 2017).

Blood culture's conventional pathogen identification method is time consuming, requiring 16 h to 5 days (for yeast species) due to the need to culture the pathogen before it can be identified (Schwarzenbacher et al., 2019; Orellana et al., 2020; Baron et al., 2005). Blood cultures are frequently negative (80–89%% of the time) even though other tests and clinical signs suggest sepsis (Previsdomini et al., 2012). Blood culture requires significant blood volume (typically 2–3 sets, amounting to 40–60 mL of blood taken at multiple time points) (Henning et al., 2019; Lee et al., 2007). This can be difficult to obtain from patients with co-morbidities, and from paediatric patients and neonates (Woodford et al., 2021). Blood culture provides an in vitro culture environment where BSI-associated bacteria with fastidious growth conditions (that is, complex or restricted nutritional and/or environmental requirements) are difficult to detect (Rello et al., 2018).

Given the need for a rapid, sensitive test requiring only a small blood volume, we evaluated the clinical applicability of InfectID-BSI in a method comparison study with conventional blood culture. Data from this study were submitted to Europe's In Vitro Diagnostic Medical Devices Directive 98/79/EC (IVDD) in May 2022.

2. Methods

2.1. Clinical samples

Patients ($n = 203$) presenting to both Royal Brisbane and Women's Hospital (RBWH; 929 beds) Emergency and Mackay Base Hospital (MBH; 318 beds) Emergency Departments between July 2019 and July 2020 with suspected BSI, sepsis and septic shock were recruited into the study. Samples were not randomized but collected prospectively only from consenting patients. Patients with cognitive impairment and patients who were unable to give consent were excluded. A 4-mL EDTA whole blood tube was collected from consented patients at the same time as routine blood culture (BacTALERT, Biomerieux; (two bottles - one aerobic and one anaerobic)); 360 blood cultures and 375 EDTA whole blood samples were investigated in this study. As this study was designed as a pilot study, no sample size calculations were done. DNA was extracted from whole blood samples ($n = 375$) (0.35 mL) using the Roche MagNA Pure 96 DNA and Viral NA Large Volume Kit and screened for bloodstream infection-associated bacteria using InfectID-BSI. InfectID-BSI results were compared to blood culture results (blinded). All samples recording an InfectID-BSI-positive result underwent Sanger bidirectional sequencing (SBDS) to confirm the identity of the pathogens detected by InfectID-BSI (referee approach #1). Where blood culture and InfectID-BSI results were discordant, clinical data (referee approach #2) was used to support InfectID-BSI results (Umemneku Chikere et al., 2019). SBDS results were analysed using Sequencher DNA Sequence Analysis software (Gene Codes Corporation, USA) and NCBI blastn (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.2. Clinical metadata

Clinical and diagnostic laboratory data fields for enrolled patients were collected from the integrated electronic medical records (ieMR), Emergency Department Information System (EDIS), Auslab, The Viewer, and/or paper based scanned files. The fields included: heart rate, respiratory rate, blood pressure, Glasgow Coma Score, temperature, lactate levels, and evidence of recent chemotherapy. These seven objective sepsis risk criteria were used to stratify patients as having high, moderate or low risk for sepsis/septic shock according to the Queensland Government's (Australia) Emergency Department Adult Sepsis Pathway guidelines (Fig. S1). In the current study, patients were classified as high risk for sepsis or septic shock if they had any of the following; respiratory rate $\geq$ 25 breaths/min, heart rate $\geq$ 130 beats/min, systolic blood pressure $<$ 90 mmHg (or drop $>$40 from normal), evidence of new or altered mental state (Glasgow Coma Scale $<$15), lactate levels $\geq$2 mmol/L, or recent chemotherapy. Patients with any of the following moderate risk criteria were classified as may having sepsis; respiratory rate 21–24 breaths/min, heart rate 90–129 beats/min, systolic blood pressure 90–99 mmHg, and temperature $<$ 35.5 °C or $\geq$ 38.5 °C. Patients with none of the high or moderate risk criteria listed above were classified as low risk for sepsis.

2.3. Ethics

The study was approved by the Human Research Ethics Committee of the Royal Brisbane and Women's Hospital, Brisbane, Queensland, Australia [HREC/2018/QRBW/47880].

2.4. Contrived samples

The contrived samples contained known amounts of bacteria/yeast species at concentrations ranging from above the limit of detection (LOD) to above the C95 level. (The C95 level is the point at which 95% of samples are above the threshold cut-off and 5% of samples are below it). In particular, there were seven (Previsdomini et al., 2012) samples at the LOD level, seven (Previsdomini et al., 2012) samples at the C95 level and four (Schwarzenbacher et al., 2019) samples above the C95 level. In total, eighteen EDTA whole blood-spiked contrived samples were prepared for InfectID-BSI testing (each species was cultured overnight in 2 mL of Brain Heart Infusion Broth (Oxoid), centrifuged and the pellet resuspended in 1 mL of EDTA blood; DNA was extracted using the Roche MagNA Pure 96 DNA and Viral NA Large Volume Kit); *S. aureus* (2 concentration levels), *E. faecalis* (2 concentration levels), *E. coli* (3 concentration levels), *K. pneumoniae* (3 concentration levels), *P. aeruginosa* (2 concentration levels), *C. auris* (3 concentration levels) and *C. glabrata* (3 concentration levelsThe rationale for including the contrived samples is due to low prevalence of some InfectID-BSI target species in positive blood cultures. The American Tissue Type Collection (ATCC) reference strains used are listed in Table S1.

2.5. InfectID-BSI assay

InfectID-BSI comprises two test panels; InfectID-BSI-B1-B6/Y1 (bacteria/yeast) and InfectID-BSI-Y2 (*Candida glabrata*) (Table 1). InfectID-BSI qPCR data was generated using the Rotor-Gene Q real-time PCR cycler (QIAGEN, Germany). qPCR reactions were carried out in a 25-µL reaction volume containing 5 µL of extracted genomic DNA. Melt curve data were analysed using the manufacturer's software. The resulting melt curve profiles and melt temperatures (Tm's) were compared to known positive control samples included in each panel. Product-specific Tm's were used to score samples as positive or negative according to defined thresholds. Non-specific products (i.e. Tm's outside of these temperatures) were excluded. Primers and controls were synthesized by Integrated DNA Technologies, USA.

2.6. Quantitation/measurement of genome copies/0.35 mL of blood

Assay-specific standard curves were generated from serially diluted known concentrations of blood-spiked bacteria or yeast. At the completion of the qPCR melt analysis, the quantity of bacterial/yeast DNA present in a sample was calculated and expressed as genome copies/0.35 mL of blood (http://cels.uri.edu/gsc/cndna.html), whereby the concentration of the pathogen's DNA together with the species' genome size is used in the formula. Low bioburden of pathogens was interpreted as less than ten genome copies/0.35 mL of blood.

Table 1
InfectID-BSI microbial pathogen targets.

Panel Name	Type	Target species
InfectID-BSI-Bacterial (B1-B6/Y1)	Gram-positive bacteria	*Aerococcus viridans*
		Enterococcus faecalis
		Enterococcus faecium
		Staphylococcus aureus
		Staphylococcus epidermidis
		Streptococcus agalactiae
		Streptococcus anginosis
		Streptococcus bovis
		Streptococcus pneumoniae and
		Streptococcus mitis group
		Streptococcus pyogenes
	Gram-negative bacteria	*Acinetobacter baumannii*
		Citrobacter freundii
		Enterobacter cloacae
		Escherichia coli
		Klebsiella spp.
		Morganella morganii
		Proteus mirabilis
		Pseudomonas aeruginosa[*]
		Serratia marcescens
		Stenotrophomonas maltophilia
	Yeast[*]	*Candida albicans*
		Candida auris
		Candida dubliniensis
		Candida parapsilosis
		Candida tropicalis
InfectID-BSI-Yeast (Y2)	Yeast[*]	*Candida glabrata*

[*] Note: at the time the method comparison study (InfectID-BSI vs blood culture) was undertaken, the assays for *P. aeruginosa* and all the *Candida* species were still under development.

2.7. Method comparison analysis

Method comparison was evaluated in two ways, firstly, InfectID-BSI performance compared to blood cultures and secondly, InfectID-BSI performance compared to contrived samples. For the first Method comparison data analysis, positive and negative percent agreement (PPA, NPA) and positive and negative predictive values (PPV, NPV) were calculated by comparing InfectID-BSI results versus blood culture results. The blood culture result (i.e the reference method) was determined to be accurate. Only data that had passed QC for both tests was used in the study analysis. In this analysis, positive percent agreement (PPA) is defined as the proportion of positive observations (across all samples) as detected by the InfectID-BSI assay among all positive observations as determined by blood culture, where a positive outcome is defined as an exact match to blood cultures. Negative percent agreement (NPA) is defined as the proportion of negative observations (across all samples) as detected by the InfectID-BSI assay among all negative observations as determined by blood culture, where a negative outcome is defined as an exact match to blood cultures. PPV is the probability that an individual with a positive test result truly has the disease. NPV is the probability that the disease is absent given a negative test result (Ranganathan and Aggarwal, 2018). A high NPV is considered a good 'rule-out' test for diagnostic tests. For the second Method comparison data analysis (InfectID-BSI results versus contrived samples), PPA, NPA, PPV, NPV were also calculated. A summary of the positive and negative truth criteria for both clinical and contrived samples is shown in Tables S2 and S3. Confidence intervals by modified Wald method (Agresti and Coull, 1998). PPA, NPA, PPV and NPV were calculated as follows:

Positive percent agreement (PPA) $TP/(TP+FN)$ x 100%.

Negative percent agreement (NPA) $TN/(FP+TN) \times 100\%$.

Positive predictive value (PPV) $TP/(TP+FP) \times 100\%$.

Negative predictive value (NPV) $TN/(TN+FN) \times 100\%$.

TP, True positive; TN, True negative; FP, False positive; FN, False negative.

2.8. Role of funder

The funder (Microbio Ltd.) designed the study with assistance from Emergency Department clinicians/academic researchers and Qserve, an independent regulatory consultant company with technical, regulatory, and clinical experience in in vitro diagnostics/medical devices. The funder coordinated sample and data collections, analysis and interpretation of results. The funder was responsible for the report writing. More than one author had full access to the data in the manuscript. The corresponding author had the final responsibility to submit for publication.

3. Results

3.1. Accounting of the data and patient demographics

The intent was to collect an EDTA-whole blood sample from each patient, however, for some patients, multiple matched EDTA and blood culture samples were obtained from various anatomical body sites (eg. left arm, right arm, etc.). This resulted in 375 EDTA-whole blood samples resulting in 9377 identifications/observations. Within the data set 11 data line items were removed due to QC failure resulting in 9366 observations (9377–11 = 9366). Six (Baron et al., 2005) of the remaining 9366 data line items were removed due to QC failure for InfectID-BSI resulting in 9360 remaining observations (Table S4). The final observations that passed and were used in the analysis was 9360 from 360 samples.

A summary of the subject demographics for this study is provided in Table 2. There were 114 males and 89 females in the study with an average age of 58 years and 59 years, respectively.

3.2. Method comparison

Table 3 provides the performance summary results for InfectID-BSI compared to blood culture (reference method). The number of identifications/observations of InfectID-BSI compared to blood culture are listed in Table S5. There was a total of 9360 observations in the evaluable data set. Of these, 54 were deemed positive by BC and of these 30/54 were in agreement for a PPA of 55.6% (95% CI: 42.3% to 68.8%). Of the remaining 9306 negatives determined by BC, 9234/9306 were in agreement for an NPA of 99.2% (95% CI: 99.1% to 99.4%). The two measures of diagnostic accuracy, PPV and NPV were 29.4% (95% CI: 20.6%–38.3%) and 99.7% (95% CI: 99.6%–99.8%), respectively.

Further investigation of the 72 false positive InfectID-BSI

Table 2
Sample and demographics summary for blood culture and InfectID-BSI analysis sets.

	RBWH	MBH	Combined hospitals
Identifications/ observations	7098	2262	9360
Samples	273	87	360
Unique individuals, n	152	51	203
Gender^			
Male	86 (56.6%)	28 (54.9%)	114 (55.6%)
Female	66 (43.4%)	23 (45.1%)	89 (43.4%)
Age^			
Male, mean (yrs)	58	60	58
Female, mean (yrs)	57	63	59
Median (range, yrs)	60 (range, 20–94)	67 (range, 12–91)	62 (range, 12–94)

RBWH, Royal Brisbane and Women's Hospital; MBH, Mackay Base Hospital.

Table 3
Performance summary of InfectID-BSI compared to blood culture.

Performance Summary: InfectID-BSI vs blood culture			
Agreement Statistic	Point Estimate (Percentage)[#]	95% CI Modified Wald^	Acceptance Criteria
PPA	30/54 = 55.6%	(42.3%–68.8%)	LB ≥42.3%
NPA	9234/9306 = 99.2%	(99.1%–99.4%)	LB ≥99.1%
PPV	30/102 = 29.4%	(20.6%–38.3%)	LB ≥20.6%
NPV	9234/9258 = 99.7%	(99.6%–99.8%)	LB ≥99.6%

^# Confidence intervals by modified Wald method (Ranganathan and Aggarwal, 2018).

observations (Table S5) was done by confirming the species identification using Sanger bidirectional sequencing (SBDS). Of the 72 false positive observations in this data set, 100% were found positive by SBDS (Table 4). The 72 false positives were also compared to a second referee approach, which is the Queensland Adult Sepsis pathway clinical quantitative data (Fig. S1). Results from these analyses confirm that 57/72 (79.2%) of the false positives were confirmed as true positives, indicating that the PPA and PPV in Table 3 have been influenced by the lack of sensitivity on the part of blood culture, which is reflected in the literature[1,2]. Table 4 below provides a summary of the analysis of the 72 InfectID-BSI false positives confirmed as true positives by SBDS and sepsis data.

False negative observations were also observed in the dataset (n = 24) (Table S6). The types of sepsis-associated bacteria detected by blood culture and not by InfectID-BSI in this study included *S. epidermidis* (7 detections), *P. aeruginosa* (6 detections), *E. coli* (6 detections), *S. aureus* (2 detections), *E. cloacae* (2 detections), and *Streptococcus viridans* group (1 detection).

3.3. Comparative prevalence rates

InfectID-BSI identified 102 pathogens compared to blood culture, which identified 54 pathogens (Table S7). All InfectID-BSI positive samples were confirmed by both methods detected similarly high proportions of *E. coli* (blood culture, 46.3%; InfectID-BSI, 28.4%), *S. aureus* (blood culture, 14.8%; InfectID-BSI, 13.7%) and *S. epidermidis* (blood culture, 12.9%; InfectID-BSI, 13.7%). Rates of other bacteria, however, differed between the two methods; *P. aeruginosa* (blood culture, 11.1%; InfectID-BSI, 0%), *S. pneumoniae* (blood culture, 0%, InfectID-BSI, 17.6%) and *A. viridans* (blood culture, 0%, InfectID-BSI, 6.9%).

Table 4
Analysis of false positive results: Blood culture negative/InfectID-BSI positive (n = 72).

Species	Blood culture neg/ InfectID-BSI pos Total	Referee Approach		
		#1 Sanger Seq confirmed (%)	#2 Strong clinical evidence of Sepsis (High/ Mod Risk Criteria) (%)	#2 No strong evidence of Sepsis (Low Risk Criteria) (%)
E. coli	10	10/10 (100%)	10/10 (100%)	0/10 (0%)
C. dublinensis^	2	2/2 (100%)	2/2 (100%)	0/2 (0%)
C. lapagei^	1	1/1 (100%)	1/1 (100%)	0/1 (0%)
A. viridans	7	7/7 (100%)	6/7 (85.7%)	1/7 (14.3%)
E. cloacae	5	5/5 (100%	4/5 (80%)	1/5 (20%)
S. pneumoniae	18	18/18 (100%)	14/18 (77.8%)	4/18 (22.2%)
S. epidermidis	13	13/13 (100%)	10/13 (77%)	3/13 (23%)
S. aureus	8	8/8 (100%)	6/8 (75%)	2/8 (25%)
S. marcescens	3	3/3 (100%)	2/3 (66.7%)	1/3 (33.3%)
S. putrefaciens^	3	3/3 (100%)	2/3 (66.7%)	1/3 (33.3%)
C. freundii	1	1/1 (100%)	0/1 (0%)	1/1 (100%)
E. cecorum^	1	1/1 (100%)	0/1 (0%)	1/1 (100%)
Total	72	72/72 (100%)	57/72 (79.2%)	15/72 (20.8%)

^Not in the InfectID-BSI target panel but detected by InfectID-PCR melt analysis and identify confirmed by Sanger bidirectional sequencing.

3.4. Detection of fastidious bacteria and polymicrobial infections

Notably, 20/25 (80%) patients with a fastidious infection (*S. pneumoniae* or *A. viridans)* identified by InfectID-BSI where blood culture reported no growth had high and/or moderate risk criteria for sepsis in half of these patients. (Table S8). Mixed bacterial infections were detected by InfectID-BSI and blood culture in 13/203 (6.4%) and 1/203 (0.5%) patients, respectively.

3.5. Quantitation/measurement of genome copies/0.35 mL of blood

Quantitative data was determined for InfectID-BSI-positive patient samples only. The first analysis examined intra-sample quantitation consistency in samples positive for the most prevalent sepsis-associated bacteria detected in the study, *E. coli*. Results from paired blood collections (i.e. same patient, same collection time, two blood samples, two collection sites (e.g. left arm, right arm) from ten patients are shown below in Fig. 1 (Table S9). Quantitation of intra-patient samples demonstrated a very high level of robustness for the InfectID-BSI qPCR test as well as a high level of consistency between samples collected from different body sites at the same collection time-point. Quantitative data from patient MB39, the patient with the highest *E. coli* DNA load, were less consistent (genome copies per 0.35 mL, range 7688–27,478, median 17,583). Quantitative data analysis was also conducted for *S. pneumoniae* and *S. epidermidis*-positive patient samples (Tables S8 and S9). *Streptococcus pneumoniae* was detected in 18 patient samples with the number of genome copies per 0.35 mL ranging from 1 to 410 (median, 69; Table S10). *Staphylococcus epidermidis*, a major species of the coagulase-negative staphylococci (CNS) group and frequent cause of sepsis (Nguyen et al., 2017; Otto, 2017) ranged in genome copy number from 1 to 239 (median 7; Table S11). *S. epidermidis* is also a common skin commensal (Otto, 2009). Of note, the levels of *S. epidermidis* were very low in five of eight patients with quantitative data (<10 genome copies per 0.35 mL blood). InfectID-BSI's ability to detect very low genome copy numbers directly in blood is based on the presence of multiple copies of its genetic targets/bacterial cell.

3.6. Analysis of contrived samples

Method comparison was additionally evaluated using laboratory contrived samples. For this work, a set of EDTA-whole blood samples were spiked with a single reference bacterial or yeast species. In total, there were 468 observations with 450 negative observations for InfectID-BSI and contrived samples (Table S12) resulting in an NPA of 100% (450/450; 95% CI: 99% to 100%), and NPV of 100% (450/450; 95% CI: 99% to 100%) (Table S13). Within the 468 observations, 18/18 were correctly identified by InfectID-BSI as the same bacterial/yeast species in the contrived samples, resulting in a PPA and PPV of 100% (95% CI: 79.3% to 100%) with a lower 95% CI: of 79.3%. This lower bound meets the acceptance criteria of ≥79.3% (modified Wald method).

4. Discussion

Rapid identification of sepsis-associated pathogens is critical for improving clinical outcomes (Otto, 2009). The earlier a causal pathogen is detected, the earlier appropriate antibiotic therapy can be administered. Traditional blood culture (current gold standard method for blood

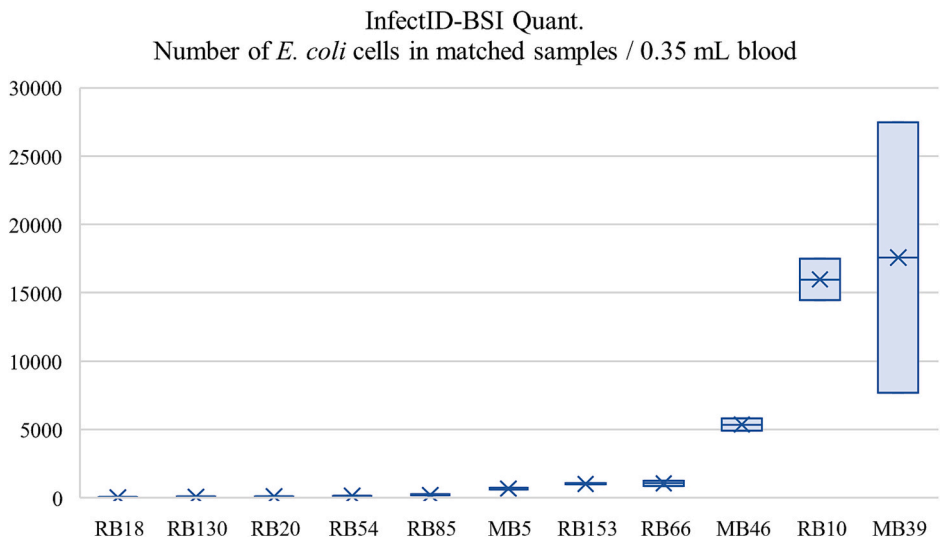

Fig. 1. Box plot showing intra-sample variation of *E. coli* cell numbers per 0.35 mL whole blood. Paired samples were collected from two different body sites from the same patient at the same collection timepoint.

pathogen detection) is imperfect. Blood culture takes approximately 16–24 h to detect bacterial pathogens in patient's blood (Lambregts et al., 2019), and requires a significant volume of blood, which is particularly challenging in difficult-to-bleed, severely ill and paediatric patients. Blood culture also has a relatively low positivity rate of approximately 10–20% (Previsdomini et al., 2012; Lambregts et al., 2019) such that each time a clinician orders a blood culture, a positive result will be detected about 1 in 5–10 times. The aims of our study were to develop a rapid, direct from whole blood qPCR test requiring less blood volume and with a higher sensitivity than conventional blood culture.

To examine the clinical applicability and performance of InfectID-BSI, we conducted a method comparison study comparing InfectID-BSI results to blood culture results. Our results revealed a high NPV of >99% for InfectID-BSI, which translates to a high level of confidence such that when InfectID-BSI is negative, a diagnosis of a BSI is likely to be correct <1% of the time. InfectID-BSI is therefore an excellent 'rule-out' test for BSI. The low PPV and PPA percentage in the study (29.4 and 55.6, respectively) was negatively influenced by the reference method (blood culture) having a lower positivity rate compared to the more sensitive qPCR test, InfectID-BSI. It is worth noting that the low sensitivity of the blood culture method makes it difficult to evaluate a new molecular method. (And this holds true for any new diagnostic test which outperforms the gold standard) (Umemneku Chikere et al., 2019; Hawkins et al., 2001). The low positivity rate of blood culture may be due to low numbers (concentration) of sepsis-associated bacteria (Stranieri et al., 2018), presence of fastidious/non-culturable organisms (i.e. bacteria with complex nutrient/environmental requirements), presence of antibiotics (Scheer et al., 2019) or contamination by ubiquitous skin commensal organisms which suppress the growth of the sepsis bacteria. To address the disagreement between blood culture and InfectID-BSI results, we used two well accepted referee methods, Sanger bidirectional sequencing analysis and patient clinical data.

InfectID-BSI outperformed blood culture in the detection of both Gram-positive bacteria (56 versus 19 detections) and Gram-negative bacteria (46 versus 35 detections). qPCR is a sensitive molecular test and could increase the likelihood of false-positive results caused by circulating cell-free (cf) DNA (Liao et al., 2020). We consider the possibility that the performance of InfectID-BSI to be affected by cf-DNA to be very low as (i) InfectID-BSI detects known pathogenic organisms associated with BSI and not DNA from other sources (human, diet, non-BSI related organisms); (ii) detection of Gram-negative organisms (for example from leaky gut syndrome) was not elevated using InfectID-BSI; (iii) InfectID-BSI results correlated strongly with patient clinical data defining moderate and high risk for sepsis and septic shock (data not shown) and (iv) tight InfectID-BSI replicate results (left arm vs right arm) for *E. coli* for example, demonstrates that good clinical and laboratory practice was carried out during patient sample collection, DNA extraction and qPCR testing. The greatest discrepancy in the prevalence rates between methods was observed in the Gram-positive detections with the difference being driven by InfectID-BSI's higher sensitivity for fastidious organisms, in particular *Streptococcus pneumoniae* and *Aerococcus viridians,* compared to blood culture. *S. pneumoniae* ranks at the 16th most prevalent sepsis pathogen identified by blood culture (Opota et al., 2015), however, in our study, *S. pneumoniae* was ranked as the second most prevalent pathogen (17.6%) after *E. coli* (28.4%) and ahead of *S. aureus (13.4%)*. The low *S. pneumoniae* detection rate by blood culture is likely due in part to *S. pneumoniae* being a fastidious bacterium, as well its ability to undergo autolysis (Martner et al., 2009), making it a challenging diagnosis for the clinician. *Aerococcus viridans*, another fastidious sepsis-associated bacteria, was detected by InfectID-BSI with a prevalence rate of 6.9% but not by blood culture. *Aerococcus viridans* are Gram-positive, microaerophilic, and non-motile bacteria that have been associated with arthritis, bacteremia, endocarditis, and meningitis (Ezechukwu et al., 2019). Notably, 80% of patients in our study with a *Streptococcus pneumoniae* or *Aerococcus viridans* infection had one or more moderate/severe sepsis risk criteria (Table S8).

Whilst InfectID-BSI detected greater numbers of BSI-associated pathogens than blood culture, there were 24 detections missed by InfectID-BSI (false negatives) which included seven cases of *S. epidermidis*, six cases of *P. aeruginosa*, six cases of *E. coli*, two cases of *E. cloacae*, two cases of *S. aureus* and one case of *Streptococcus viridans* group (Table S5). Notably, all 7 cases of *S. epidermidis* missed by InfectID but detected by blood culture were in most cases positive for one blood culture set only with the other set recording 'No growth' (suggestive of a contaminant (Hall and Lyman, 2006). During our pilot patient study, the InfectID-BSI assay for *P. aeruginosa* was under development. It is unknown whether the other missed infections (six cases of *E. coli* and two cases of *S. aureus*) were near or below InfectID-BSI's limit of detection. Importantly, InfectID-BSI identified ten *E. coli* and eight *S. aureus* samples that were missed by blood culture.

Fulfilling the other aims of our study, InfectID-BSI detected BSI-associated pathogens from <0.5 mL whole blood. The ability of InfectID-BSI to detect pathogens in low volumes of blood are of particular relevance in cases of neonatal sepsis where the incidence of blood

culture proven sepsis is 10–20%, while 30–50% of very-low-birth-weight infants develop clinical sepsis (Boghossian et al., 2013; Tröger et al., 2014). Furthermore, our study demonstrated high correlation between blood culture and InfectID-BSI identification of *E. coli* and *S. aureus*, two of the most prevalent bacterial aetiological agents of blood stream infection and sepsis. The importance of being able to identify these pathogens in a low volume of blood cannot be understated. The entire procedure, from qPCR set-up to result, took <2 h post-DNA extraction. DNA extraction technologies (not assessed in this study) typically take 30–40 min depending on the extraction platform. Taken together, the time-to-result (TTR) from when a blood sample arrives in the laboratory for testing is therefore estimated to be <3 h.

Finally, InfectID-BSI's quantitative information in addition to species identification has the potential to considerably alter the clinical treatment pathway for sepsis patients. Our quantitative data (together with the precision of InfectID-BSI's melt curve analysis) demonstrated the sensitivity of InfectID-BSI in detecting very low genome copies in patient samples. Quantitative data can be used to monitor antibiotic efficacy, rule-in or rule-out the common skin contaminants as causal agents (e.g. coagulase-negative staphylococci) and identify the most likely cause of sepsis in mixed bacterial infections. In our study, the majority of *S. epidermidis*-positive samples contained less than ten genome copies per 0.35 mL whole blood. Three patients, however, had higher numbers of *S. epidermidis* with one patient having as high as 239 genome copies per 0.35 mL whole blood. For these three cases, quantitative data together with patient clinical data supports an association with BSI rather than a skin contaminant.

4.1. Limitations

Currently the InfectID-BSI test is limited for use by routine diagnostic laboratories with qPCR capability, and hence is not a random access/point of care test, which is similar to all other qPCR tests utilized for pathogen identification in laboratories. As stated above, InfectID-BSI is an important diagnostic test for guiding appropriate antimicrobial therapy, however, in its current design, it does not detect specific antimicrobial resistance markers.

5. Conclusion

This study found that InfectID-BSI's performance met and exceeded the acceptance criteria compared to blood culture. InfectID-BSI's high NPV value is indicative of its high specificity as a diagnostic 'rule-out' test for sepsis. Adoption of InfectID-BSI rapid qPCR-based test (as a companion test to blood culture) will very likely have a considerable impact on the clinical management of bloodstream infections/sepsis. The full benefits of InfectID-BSI will be determined following future clinical trials globally.

Contributors

F.H. Conceptualization, Project administration, Supervision, Writing - review & editing. C.D., N.J., S.S., A.P and A.B. Methodology, Investigation, Data curation, Formal analysis, Validation. M.H., K.S., S.B., G.N., J.H., and D.F. Investigation, Data curation, Resources. L.A.S., A.P., A.L., D.M.W., and F.H. Methodology, Data curation, Formal analysis. L.A.S. Formal analysis, Data curation, Investigation, Validation, Writing - original draft. R.G. Writing - review & editing.

Research in context

Evidence before the study.

Prompt treatment for bloodstream infection, sepsis and septic shock is essential as each hour delay in administering antibiotics increases the risk of death from septic shock by close to 9%. Conventional pathogen identification using blood cultures is time consuming, requiring 16 h to 5 days (for yeast species) due to the need to culture the pathogen before it can be identified. Blood cultures are frequently negative (~82% of the time) even though other tests and clinical signs suggest BSI, sepsis and septic shock. Blood culture ideally requires significant blood volume (typically 2–3 sets, amounting to 40–60 mL of blood taken at multiple time points). This can be difficult to obtain from patients with co-morbidities, and from paediatric patients and neonates. BSI-associated bacteria with fastidious growth conditions (that is, complex or restricted nutritional and/or environmental requirements) are also difficult to detect by blood culture.

Added value of this study.

We evaluated the clinical applicability and performance of InfectID-BSI (a highly-specific qPCR test for the detection of BSI-associated bacterial species from whole blood without need for pre-culture) compared to conventional blood culture. Three hundred and seventy-five whole blood samples from 203 patients with suspected BSI, sepsis and septic shock were included in the study. InfectID-BSI outperformed blood culture in positivity rate, detection of mixed infections, detection of difficult to culture bacteria, volume of blood required for testing, and time to result. Additionally, InfectID-BSI's high negative predictive value (NPV) of >99% translates to a high level of confidence such that when InfectID-BSI is negative, a diagnosis of BSI is likely to be correct <1% of the time. InfectID-BSI is therefore an excellent 'rule-out' test for BSI.

Implications of all the available evidence.

Our study demonstrated high correlation between blood culture and InfectID-BSI identification of *E. coli* and *S. aureus*, two of the most prevalent bacterial aetiological agents of BSIs. A key feature of InfectID-BSI is the ability to identify pathogens low blood volumes (<0.5 mL), which is of particular relevance in cases of neonatal sepsis where the incidence of blood culture proven sepsis is 10 to 20%, while 30 to 50% of very low birthweight infants develop clinical sepsis. Adoption of InfectID-BSI (as a companion test to blood culture) will very likely have a substantial impact on the clinical management of BSI that progress to sepsis, with earlier diagnosis leading to earlier use of optimal antibiotic therapy.

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Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Data availability

The de-identified data presented in this manuscript can be made available upon reasonable request following publication of this work. Data access proposals should be directed to the corresponding author and requestors will need to sign a data access agreement. Data contained within this study were collected during routine clinical care in a busy, clinical care setting, and their use for research was secondary to their use in clinical care.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mimet.2023.106783.

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