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Accuracy of LightCycler Septi*F*ast for the detection and identification of pathogens in the blood of patients with suspected sepsis: a systematic review and meta-analysis

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Abstract

Purpose. There is an urgent need to develop diagnostic tests to improve the detection of pathogens causing life-threatening infection (sepsis). Septi*F*ast is a CE-marked multi-pathogen real-time PCR system capable of detecting DNA sequences of bacteria and fungi present in blood samples within a few hours. We report here a systematic review and meta-analysis of diagnostic accuracy studies of Septi*F*ast in the setting of suspected sepsis.

Methods. A comprehensive search strategy was developed to identify studies that compared Septi*F*ast with blood culture in suspected sepsis. Methodological quality was assessed using QUADAS. Heterogeneity of studies was investigated using a coupled forest plot of sensitivity and specificity and a scatter plot in Receiver Operator Characteristic space. Bivariate model method was used to estimate summary sensitivity and specificity.

Results. From 41 phase III diagnostic accuracy studies, summary sensitivity and specificity for Septi*F*ast compared with blood culture were 0.68 (95% CI 0.63-0.73) and 0.86 (95% CI 0.84-0.89) respectively. Study quality was judged to be variable with important deficiencies overall in design and reporting that could impact on derived diagnostic accuracy metrics.

Conclusions. Septi*F*ast appears to have higher specificity than sensitivity, but deficiencies in study quality are likely to render this body of work unreliable. Based on the evidence presented here, it remains difficult to make firm recommendations about the likely clinical utility of Septi*F*ast in the setting of suspected sepsis. We recommend that future studies should include well designed and reported clinical diagnostic accuracy elements measured against all of the features of the STARD criteria to help inform the subsequent design of much needed interventional studies in the management of suspected sepsis.

Word count: 225 241

Introduction

While rapid identification and treatment of sepsis is associated with the best outcomes, sepsis can be difficult to diagnose and to differentiate from other common non-infectious causes of systemic inflammation including trauma, surgery and ischaemia-reperfusion [1]. Confirmation of sepsis, therefore, relies on objective diagnostic evidence for infection, including attempts to detect and identify live pathogens from blood samples by microbiological culture techniques [2, 3].

In the setting of sepsis, blood cultures do not always provide high diagnostic accuracy for infection in a timely manner, routinely taking several days before a positive result is available and are often negative [4]. The time between initial clinical suspicion and confirmation of infection, results in the early use of empirical broad spectrum antimicrobial drugs because treatment delay is associated with substantial increases in mortality [5-7]. Unnecessary and/or prolonged broad spectrum antimicrobial use is an inevitable consequence, which is associated with the development of drug resistant pathogens, *Clostridium difficile* infections, a range of avoidable adverse effects, as well as high costs [5]. An urgent global challenge has emerged [8], therefore, to develop techniques that could provide accurate diagnostic information within a short timeframe of clinical signs appearing and so allow more informed use of antibiotic therapy at an early stage.

Real-time PCR technology can detect minute amounts of pathogen DNA in patient blood samples with results available within hours [9]. Laboratory validation studies have focused on two approaches using PCR for genomic amplification with either (a) broad range detection of bacterial or fungal DNA with universal primers, followed by

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species identification using a post-PCR technique such as gene sequencing or electrospray mass spectrometry or (b) using species-specific hybridisation probes that provide direct confirmation of the species present [10]. While the laboratory analytical accuracy of these techniques for the detection of pathogen DNA in blood has been evaluated, there is a lack of reported clinical trial data to define the utility of such tests in patients [2, 10]. This has been due in part to the lack of standardised technology platforms that meet accepted regulatory standards for clinical diagnosis.

Septi*F*ast, manufactured by Roche Diagnostics, GmbH, Mannheim, Germany and run on their real-time PCR instrument (the LightCycler®), was the first real-time PCR based system to be awarded a Conformité Européenne (CE) mark for simultaneous pathogen detection and identification in suspected bloodstream infection [11]. The system uses a multiplex approach, which allows detection of the most common pathogen species causing bloodstream infection in a single blood sample (Table 1). Identification of the pathogens is based on the use of species-specific probes targeting the internal transcribed spacer region between the 16S and 23S areas of ribosomal DNA of bacteria and between the 18S and 5.8S ribosomal regions of the fungal genome. Septi*F*ast has been assessed at the laboratory level on clinical isolates and shown to have good analytical specificity and exclusivity, confirming its analytical validity [11]. Numerous clinical diagnostic studies have been reported since Septi*F*ast gained its CE-mark in 2006, predominantly focused on suspected sepsis. Chang and colleagues have reported a diagnostic accuracy systematic review of published full journal papers to 2012, with Septi*F*ast as the index test, but with various reference standards used to produce composite overall diagnostic accuracy metrics [12]. Based on their interpretation of the evidence presented, they

recommend that Septi*F*ast can be used to confidently diagnose bloodstream infection [12].

As part of a government commissioned Health Technology Assessment of Septi*F*ast in the United Kingdom [13], we report a more comprehensive diagnostic accuracy systematic review of Septi*F*ast focused on the setting of suspected sepsis and where blood cultures were used as the reference standard. This systematic review was piloted and registered with PROSPERO, the NIHR International Prospective Register of Systematic Reviews, in 2011 [14] and the study protocol was piloted and published in 2012 [15].

Methods

Inclusion criteria of studies:

Participants

Patients suspected of developing sepsis, including adults and children, who required blood cultures irrespective of where their care was being delivered, and including suspected community or hospital acquired infection.

Target conditions

Suspected sepsis, including severe sepsis and septic shock [16].

Index test

LightCycler® Septi*F*ast as the index test on blood specimens for the detection and simultaneous identification of bacterial and fungal pathogens [11].

Comparator test (reference standard)

Blood culture for the detection and identification of bloodstream bacterial and fungal pathogens was used as the reference test [4].

Types of studies

We included any clinical diagnostic accuracy study that compared the index real-time PCR test with standard culture results performed on a patient's blood sample during the management of suspected sepsis.

Search methods for identifying studies:

Electronic searches

We searched the Cochrane Database of Systematic Reviews, the Database of Abstracts of Reviews of Effects (DARE), the Health Technology Assessment Database (HTA), the NHS Economic Evaluation Database (NHSEED), The Cochrane Library, MEDLINE, EMBASE, ISI Web of Science, BIOSIS Previews, MEDION and the Aggressive Research Intelligence Facility database (ARIF). The CE mark for the index test was announced in January 2006; therefore, this systematic review only considered publications from this date in humans. There were no language restrictions in the electronic search for studies.

Search terms/search strategy

Specific search strategies were developed for each electronic database, commencing with MEDLINE (published previously) [14, 15]. The MEDLINE strategy was adapted for each subsequent database. All electronic searches were piloted prior to publication of our review protocol in 2012 and all searches were finally updated at the end of April 2014.

Backward tracking was performed by hand-searching the reference lists of all relevant articles uncovered from the electronic searches and forward tracking using the keyword 'Septi*F*ast' with ISI Citation Indices and Google Scholar and with a conference proceedings search using the Web of Science ISI Proceedings (from January 2006 to April 2014). We requested reference lists held by the only manufacturer of the index test (Roche Diagnostics) and requested any clinical diagnostic accuracy data collected by Roche Diagnostics to file for the CE mark. In addition, we searched for unpublished studies and ongoing trials involving the Septi*F*ast platform in the following online registers: http://www.nlm.nih.gov/hsrproj, http://www.controlled-trials.com/mrct/, http://portal.nihr.ac.uk/Pages/Portfolio.aspx and http://www.who.int/trialsearch, with identified corresponding authors of eligible trials and content experts contacted to identify potentially relevant studies and associated data.

Data collection and analysis:

Selection of studies (Salford, UK)

The selection of titles and abstracts was conducted by review authors (CW, DG, KT and PD) using the inclusion criteria detailed above. The full papers of all abstracts deemed eligible (by any reviewer) were obtained and read to determine their inclusion in the review. Conference abstracts and journal correspondences were included if they met the inclusion criteria and the corresponding author was contacted to request any further information about their study or about full

publications in preparation. Conference abstracts were not included when reporting duplicate data contained in a subsequent paper. We resolved any disagreement for inclusion with discussion between the reviewers.

Assessment of methodological quality (Belfast and Warwick, UK)

Independent reviewers from Belfast (DM, RM, BB) and Warwick (GDP) assessed the quality of selected studies using a specific checklist, published previously [15] adapted from the QUADAS tool [17]. Each question on the checklist was answered with a yes/no response or noted as unclear if insufficient information was reported to enable a judgement to be made, and the reasons for the judgement made was documented. Review authors assessed methodological quality independently. Any subsequent discrepancies were then adjudicated and resolved by a systematic review methods expert (BB) and an infection diagnostic expert (RM).

Data extraction

A standard set of data was searched for and extracted where possible from each study using a tailored data extraction form (details published previously) [15] by review authors at Belfast and Warwick. Reviewers extracted data independently and any discrepancies were adjudicated and resolved by a systematic review methods expert (BB) and an infection diagnostic expert (RM).

Statistical analysis and data synthesis:

Statistical analysis and data synthesis were planned and performed independently by a statistician (SG). Estimates of the combined sensitivity and specificity, with 95% confidence intervals, were made using Reitsma's bivariate method [18]. Results were displayed as summary ROC plots, with 95% confidence regions and 95% prediction regions defined by Harbord as "the region within which, assuming the model is correct, we have 95% confidence that the true sensitivity and specificity of a future study should lie" [19]. An overall summary for all studies with useable data was produced, and subgroup analyses separating studies by:

(a) Type of publication: full papers versus abstracts;

(b) Age of participants: adult versus neonate/child (this analysis omitted studies where the population was mixed or unclear);

(c) Hospital setting: emergency department versus other hospital setting, ICU versus other hospital setting (this analysis omitted studies where settings were mixed or unclear);

(d) Co-morbidity: if sufficient data available to allow comparisons;

(e) Commercial sponsorship: stated involvement of Roche Diagnostics versus no statement.

For all subgroup analyses, summary ROC curves were produced with pooled estimates of sensitivity and specificity for each group, and tested whether the sub-grouping explained a significant amount of additional variation using the difference in -2log likelihood statistics between the sub-grouped and overall models.

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No attempts were made to quantify potential sources of study bias in this systematic review as the available methodologies have not been validated for use in relation to diagnostic test meta-analyses (http://srdta.cochrane.org/handbook-dta-reviews).

Analyses were performed using SAS 9.3 (SAS Institute Inc. North Carolina, USA) and Review Manager (RevMan) [Computer program]. Version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2012.

Results

Study inclusion:

We identified 2759 citations in total, of which 66 were considered potentially suitable (Figure 1). After full text review externally at Belfast and Warwick, and having contacted corresponding authors for any extra data in the case of conference abstracts and journal correspondences, 24 studies were excluded as it proved impossible to derive a 2 by 2 table to calculate required diagnostic metrics. In addition, one abstract was removed as the study data were co-reported in a full paper and another abstract was replaced by a full paper that was sent to us by the authors. In total, 41 studies were included in the final analysis (30 papers, 9 conference abstracts and 2 correspondences - summarised in Table 2).

Study quality:

Our independent external reviewers reported variable study quality and, although studies reported as full papers were associated with the best quality measures, there were important deficiencies overall in study design and reporting (Figure 2). Reviewers agreed that all of the studies selected aimed to compare test results from Septi*F*ast with blood culture and that the reported blood sampling for these tests was such that disease progression or regression bias would have been avoided. Blood culture, as the reference standard, appeared to have been applied to patients equally in a way that both partial (work-up) bias and verification bias were likely avoided. However the reference standard was not always adequately described, including blood sampling methods and the prevalence of defined contamination as a potential source of false positive culture results. Comparisons of the results of Septi*F*ast with blood culture at genus/species level were often difficult to adjudicate by reviewers, with no clear standards of reporting followed. The chance of misclassification when comparing the reference and index tests was therefore thought to be likely, impacting on the derived diagnostic accuracy metrics. In some studies, it was not clear how well the CE-marked protocol for Septi*F*ast had been followed, including how blood samples had been stored/handled prior to assay delivery. Assay failure rates were rarely reported. There was a universal lack of reported blinding of both reference standard and index tests such that reviewers believed that incorporation bias was highly likely. Overall, reviewers agreed that none of the included studies, as reported, met the Standards for Reporting Diagnostic Accuracy (STARD) criteria in full [62], and in some cases there were significant deficiencies (Figure 2).

Study characteristics and patient populations:

Studies included patient cohorts from a wide range of age and settings (Table 2) representing a total of 7,727 patients contributing 10,493 episodes of suspected sepsis. The median prevalence of blood culture positivity in this group of patients was 17% (inter-quartile range 13-25%). Lack of uniform reporting made it difficult for reviewers to classify studies, with a variety of care settings, outcomes and alternative clinical reference standards reported alongside the direct comparison of Septi*F*ast with blood culture results. However, our external reviewers were able to identify age classes (neonate, child and adult), setting classes (emergency department, hospital setting, and intensive/critical care), commercial sponsorship (stated involvement of Roche Diagnostics or no statement) and a group of studies that focused on haemato-oncology patients. In addition, studies were assigned a diagnostic evidence level III [20] in each case.

Estimated summary diagnostic accuracy of Septi*F*ast:

Figure 3 shows the coupled forest plots of sensitivity and specificity for each study and Figure 4 displays the scatter plot in ROC space (plotting sensitivity against 1 - specificity for each study). Summary sensitivity and specificity for Septi*F*ast compared with blood culture, estimated using the bivariate model method, were 0.68 (95% CI 0.63-0.73) and 0.86 (95% CI 0.84-0.89) respectively, suggesting that a positive blood test at genus/species level returned by Septi*F*ast in the setting of a patient with suspected sepsis could have higher diagnostic value (rule-in) than a negative test result (rule-out) when compared with blood culture.

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Exploration of subgroups:

Subgroups were investigated and estimated pooled diagnostic accuracy metrics produced for each group. Table 3 summaries these results and shows that in each case sub-grouping did not explain any significant amount of additional variation in sensitivity or specificity when compared with the overall models. There were insufficient studies reporting solely in paediatric populations to allow analysis.

Discussion

Our comprehensive systematic review was designed to help understand the estimated combined diagnostic accuracy of SeptiFast in detecting and identifying bacterial and fungal pathogens in the blood of patients with suspected sepsis. We included 41 studies reporting on a total of 10,493 SeptiFast tests when compared with blood culture.

Estimated combined results for sensitivity and specificity suggested that SeptiFast has a higher specificity than sensitivity. For the healthcare team, this implies that positive blood tests returned by SeptiFast at pathogen genus/species level could have higher diagnostic utility (as a rapid rule-in test) than negative results (as a rapid rule-out test), at least when compared with blood culture. The apparent confidence in this statement is greater for specificity than sensitivity (Figures 3 and 4) because the median event rate of 17% BC positivity for the studies means that the majority of reference tests performed was negative.

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The clinical interpretation of these ~~overall~~ diagnostic accuracy results is that negative SeptiFast tests could reasonably be false-negatives (expressed as a low overall diagnostic sensitivity), explained in part by pathogens detected in blood culture that were not on the PCR test panel. In the future, this could suggest that developing molecular tests that incorporate wider test panels of pathogens may be expected to provide greater diagnostic sensitivity (fewer false negative results) giving clinicians greater confidence in a rapid negative test result. In addition, despite a higher estimated overall specificity, when compared with sensitivity, the upper confidence interval did not reach 90%. Specificity of SeptiFast, when compared with culture, will be limited by the presence of false positive results – a positive PCR in the setting of a negative blood culture. In some studies [40,41], a proportion of these false-positive results were reported to be concordant with culture positivity from samples other than blood, suggesting that in some cases ~~of suspected sepsis~~ a positive SeptiFast result may reflect infection not detectable by blood culture. However, overall, there are no clear explanations for all of these false-positive and false-negative SeptiFast results because no systematic investigation has been undertaken linking laboratory performance with clinical diagnostic accuracy. In addition, it remains extremely difficult to speculate what implications these diagnostic accuracy results may have for direct clinical care because SeptiFast does not report antibiotic sensitivity data (other than identifying the *MecA* gene confirming meticillin resistance following detection of *S. aureus* [11]) and there have been no systematic interventional clinical trials reported to date on the ~~efficacy and~~ overall impact on clinical, laboratory and cost effectiveness of supplementing or replacing blood culture with SeptiFast.

All diagnostic metrics were reported using blood culture as the reference standard because this formed the basis of both the original laboratory diagnostic validity studies for Septi*F*ast [11] and the subsequent body of clinical diagnostic accuracy studies reported here. However, we accept that there could be limitations to this standard, particularly in the setting of intercurrent antimicrobial therapy. While antibiotic exposure data were rarely reported in the included studies, it is likely, for example, that patients in intensive care units were likely already exposed to broad spectrum antibiotics [33]. It is interesting to note that included studies recruiting solely from intensive care appeared to have slightly worse diagnostic metrics (both sensitivity, with higher rates of false negatives; and specificity, with higher rates of false positives) when compared with the results of blood culture (Table 3), but not to a significant level. Antimicrobial exposure is not the only factor that will distinguish patients in intensive care from other hospitalised patients, but it does seem particularly pertinent because of the widespread use of blood culture as the reference standard in these studies. The potential impact of antimicrobials in producing positive PCR tests with blood and associated negative blood culture results (false positives in our analysis) leads one to question blood culture as a relevant reference standard and challenges clinicians to consider a positive PCR test in this setting as a true positive, providing additional diagnostic information in patients with suspected sepsis. However, explaining the potential impact of antimicrobials on increasing false negative PCR tests (i.e. where associated blood culture tests are positive) is more problematic. In practice, a wide range of factors are known to influence the diagnostic accuracy of blood cultures [4] and, in our view, it is important to develop further and adopt statistical techniques that avoid the need for comparison to a single reference standard to provide a more meaningful estimate

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of the clinical reliability of emerging molecular diagnostic tests [63]. However, to achieve this aim, diagnostic studies must be designed and reported to accepted international standards [62].

~~However, we do not know the full extent of this problem or indeed whether studies have deliberately included or excluded such patients. In addition, SeptiFast was developed to simultaneously detect and identify a panel of the most common pathogens based on reported international blood culture surveillance data [11]. Therefore, in the absence of an internationally agreed approach to an alternative reference standard for pathogen detection from blood samples at present, we believe that a culture-based reference standard provided the most robust approach for this review.~~

During the implementation of our review, another systematic review has reported on the diagnostic accuracy performance of SeptiFast [12]. Chang and colleagues synthesised improved overall diagnostic accuracy metrics for SeptiFast when compared with our own review: sensitivity 0.75 (95% CI: 0.65-0.83) vs 0.68 (95% CI 0.63-0.73) and specificity 0.92 (95%CI: 0.90-0.95) vs 0.86 (95% CI 0.84-0.89) respectively. However, there were important differences between our reviews. Chang used a basic keyword search strategy (ending in 2012) for journal papers only, risking publication bias. We used a systematic and comprehensive search strategy that aimed to avoid any publication bias. Chang reports SeptiFast results against various reference standards to produce composite overall diagnostic

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accuracy metrics. We report overall diagnostic accuracy metrics from the results of SeptiFast measured against blood culture. These factors may have contributed to inflate the overall diagnostic accuracy metrics for SeptiFast reported by Chang when compared with the results of our own systematic review.

Diagnostic accuracy studies included in our review were performed within the challenging care environments for patients with suspected sepsis. Clinical diagnostic accuracy studies, in general, are difficult to perform well and often fall short in terms of study quality. Our independent reviewers found significant deficiencies when assessing the quality of design and reporting for studies incorporating SeptiFast as the index test. For both papers and abstracts, the application of reference and index tests were the only elements that were reported consistently, raising significant concern about the possible effects of numerous sources of bias on the derived summary estimates of SeptiFast test performance. Indeed, the 95% prediction region in ROC space in Figure 4 shows considerable uncertainty about the likely true sensitivity and specificity of a future study. Therefore, despite the considerable international effort in determining the likely diagnostic accuracy of SeptiFast in the setting of suspected sepsis, we are not confident in the current body of evidence because of the overall weaknesses in study design and reporting outlined in our review. Based on these findings, we do not agree with Chang and colleagues who state that "in the presence of a positive SeptiFast result in a patient with suspected bacterial or fungal sepsis, a clinician can confidently diagnose bacteremia or fungemia and begin appropriate antimicrobial therapy, while forgoing unnecessary additional diagnostic testing" [12]. For example, in a high prevalence population,

such as a patient admitted to intensive care with septic shock, broad spectrum antibiotics should have been delivered quickly, well before the results of blood culture (or indeed Septi*F*ast) will be available [2]. In this setting, the pre-test probability for circulating pathogens is likely high and there is an increased chance of delivering positive Septi*F*ast and blood culture tests, particularly if blood is sampled before antimicrobial treatment commences. Delivering a positive Septi*F*ast test ahead of a blood culture result in this setting might help the intensive care clinician consider early antibiotic de-escalation based on the detected pathogen species. However, Septi*F*ast does not provide comprehensive antimicrobial susceptibility information and crucial clinical decisions based only on pathogen species may be unwise in this setting and, as described previously, remains untested in carefully controlled clinical trials. Furthermore, In addition, we do not agree with Chang and colleagues that returning a negative Septi*F*ast result, even in a low prevalence population, "may justify withholding antibiotics" [12]. For example, a patient admitted to an emergency department with clinical features of sepsis, in the absence of circulatory compromise or organ dysfunction, is likely to have a low pre-test probability for circulating pathogen. Delivering a negative Septi*F*ast test to the clinical team over the subsequent hours may provide some confidence in excluding serious infection as the cause of symptoms. However, given the high rate of false-negative Septi*F*ast tests identified in our review, the clinician is not completely confident and could miss some cases of bacteraemia and other infections outside the circulation. Based on the evidence presented here, we are concerned that clinical decisions about treatment interventions/adjustments (notably antimicrobial chemotherapy) based on Septi*F*ast results, potentially delivered within hours of the suspicion of sepsis, could expose patients to risk because inadequately managed sepsis is

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associated with high mortality and rapid appropriate antimicrobial choices are crucial for survival [2].

Our views, evidenced by the present systematic review, supports current international guidelines on diagnosis and treatment of sepsis which indicate that there is limited clinical experience with non-culture-based diagnostic methods, such as Septi*F*ast, and that more high-quality clinical studies are needed before any firm recommendations can be made about their potential utility [2]. We recommend that future clinical studies incorporating Septi*F*ast, or any other novel infection/sepsis diagnostic, should include well designed and reported clinical diagnostic accuracy elements measured against all of the features of the STARD criteria [62]. Much needed clinical intervention studies in the setting of suspected sepsis remain difficult to design and justify until higher quality clinical diagnostic accuracy evidence is available.

Manuscript word count: 3,829 3,313

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Table 1: Pathogens detectable using LightCycler® Septi*F*ast test

Gram-negative bacteria	**Gram-positive bacteria**	**Fungi**
Escherichia coli	*Staphylococcus aureus*	*Candida albicans*
Klebsiella (pneumoniae/oxytoca)	Coagulase-negative staphylococci*	*Candida tropicalis*
Serratia marcescens	*Streptococcus pneumonia*	*Candida parapsilosis*
Enterobacter (cloacae/aerogenes)	*Streptococcus spp.*[#]	*Candida glabrata*
Proteus mirabilis	*Enterococcus faecium*	*Candida krusei*
Acinetobacter baumanni	*Enterococcus faecalis*	*Aspergillus fumigatus*
Pseudomonas aeruginosa		
Stentrophomonas maltophilia		

*Single probe detects a group of staphylococcal pathogens including *S. epidermidis, S. haemolyticus*[#] Single probe detects a group of streptococcal pathogens including *S. pyogenes, S. agalacticae, S. mitis*

Table 2: Summary of studies included in review. # indicates haemato-oncology study cohorts.

First author	*Year*	Manuscript type	Study country	Patient setting	Age category	Diagnostic study evidence level[20]	Number of patients	Number of paired blood tests	Bacteraemia prevalence (%)
Raglio [21]	2006	Abstract	Not stated	Not stated	Not stated	III	74	114	15
Klemm [22]	2007	Abstract	Germany	Intensive/Critical Care	Not stated	III	44	56	37
Bingold [23]	2007	Abstract	Germany	Intensive/Critical Care	Not stated	III	21	134	15
Vince [24]	2008	Correspondence	Croatia	In hospital and Intensive/Critical Care	Not stated	III	36	39	21
Mancini [25]	2008	Paper	Italy	In hospital and unclear if Intensive/Critical Care #	Adults	III	34	103	20
Louie [26]	2008	Paper	USA	Emergency Department, In hospital and Intensive/Critical Care	Adults	III	200	200	21
Lodes [27]	2008	Abstract	Germany	Intensive/Critical Care	Not stated	III	137	358	13
Westh [28]	2009	Paper	Germany	Not stated	Not stated	III	359	558	13
Varani [29]	2009	Paper	Italy	In hospital and unclear if Intensive/Critical Care #	Adults and children	III	100	130	29
Palomares [30]	2009	Abstract	Spain	Intensive/Critical Care	Not stated	III	73	76	13

Lodes [31]	*2009*	Paper	Germany	Intensive/Critical Care	Adults	III	52	258	12
Dierkes [32]	*2009*	Paper	Germany	Intensive/Critical Care	Adults	III	77	99	23
Dark [33]	*2009*	Correspondence	UK	Intensive/Critical Care	Adults	III	50	90	12
Yanagihara [34]	*2010*	Paper	Japan	In hospital and Emergency Department	Not stated	III	212	400	8
Wallet [35]	*2010*	Paper	France	Intensive/Critical Care	Adults	III	72	102	10
Tsalik [36]	*2010*	Paper	USA	Emergency Department	Adults	III	306	306	22
S'oki [37]	*2010*	Abstract	Hungary	In hospital and Intensive/Critical Care	Not stated	III	159	162	24
Regueiro [38]	*2010*	Paper	Spain	In hospital and Intensive/Critical Care	Adults	III	72	106	25
Maubon [39]	*2010*	Paper	France	In hospital and unclear if Intensive/Critical Care #	Not stated	III	110	110	29
Lehmann [40]	*2010*	Paper	Germany	Intensive/Critical Care	Adults	III	108	453	13
Bloos [41]	*2010*	Paper	Germany, France	Intensive/Critical Care	Adult	III	142	236	17
Berger [42]	*2010*	Abstract	Austria	Neonatal Unit	Neonates	III	38	38	45
Avolio [43]	*2010*	Paper	Italy	Emergency Department	Adult	III	144	144	30
Vrioni [44]	*2011*	Abstract	Greece	Not stated	Not stated	III	33	33	24
Sitnik [45]	*2011*	Abstract	Brazil	Intensive/Critical Care	Not stated	III	114	114	14

Obara [46]	2011	Paper	Japan	Emergency Department, In hospital and Intensive/Critical Care	Adults	III	54	78	15
Lucignano [47]	2011	Paper	Italy	In hospital and Intensive/Critical Care	Neonates and children	III	811	1553	10
Josefson [48]	2011	Paper	Sweden	In hospital	Adults and children	III	1093	1141	12
Hettwer [49]	2011	Paper	Germany	Emergency Department	Adults	III	153	113	45
Bravo [50]	2011	Paper	Spain	In hospital and Intensive/Critical Care	Adult	III	53	53	47
Tschiedel [51]	2012	Paper	Germany	In hospital and Intensive/Critical Care	Adults and children	III	75	110	17
Rath [52]	2012	Paper	Germany	Intensive/Critical Care	Adults	III	170	225	36
Pasqualini [53]	2012	Paper	Italy	In hospital and unclear if Intensive/Critical Care	Not stated	III	391	391	15
Mauro [54]	2012	Paper	Italy	In hospital and unclear if Intensive/Critical Care #	Adult and children	III	79	79	41
Lodes [55]	2012	Paper	Germany	Intensive/Critical Care	Adults	III	104	148	20
Guido [56]	2012	Paper	Italy	In hospital and unclear if Intensive/Critical Care #	Adults	III	166	166	14

Grif [57]	2012	Paper	Austria	In hospital and Intensive/Critical Care	Not stated	III	61	71	7
Leitner [58]	2013	Paper	Austria	Not stated	Not stated	III	57	75	15
Herne [59]	2013	Paper	Estonia	In hospital and Intensive/Critical Care	Adults	III	144	160	21
Burdino [60]	2014	Paper	Italy	In hospital and Intensive/Critical Care	Adults	III	1024	1186	15
Avolio [61]	2014	Paper	Italy	Emergency Department and Intensive/Critical Care	Adults	III	525	525	25

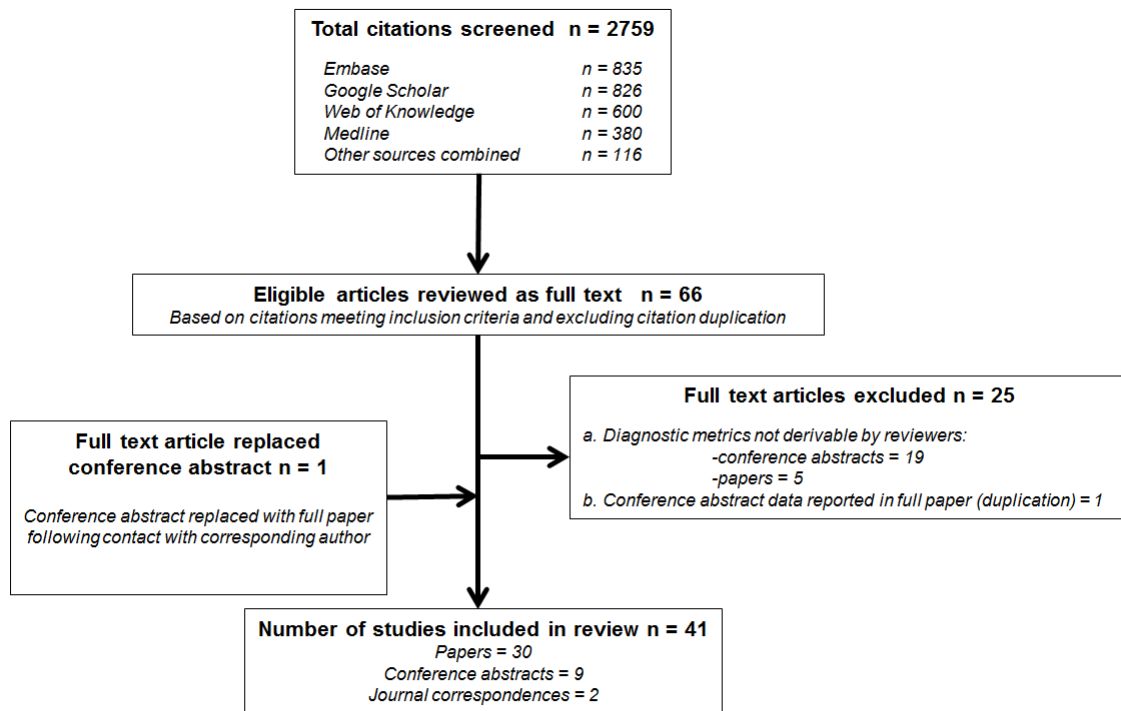| Subgroup	Sensitivity (95% CI)	Specificity (95% CI)	Interaction test for subgroup differences X^2	p
Papers	0.67 (0.62-0.73)	0.88 (0.85-0.90)		
Abstracts	0.69 (0.58-0.78)	0.81 (0.75-0.87)	4.3	0.12
Emergency Department	0.69 (0.52-0.83)	0.93 (0.86-0.96)		
Other hospital setting	0.67 (0.61-0.72)	0.86 (0.83-0.89)	3.2	0.19
ICU	0.64 (0.54-0.74)	0.83 (0.78-0.86)		
Other hospital setting	0.68 (0.54-0.80)	0.89 (0.85-0.93)	5.8	0.06
Haemato-oncology	0.66 (0.61-0.71)	0.86 (0.84-0.89)		
All other patient groups	0.78 (0.64-0.87)	0.88 (0.79-0.93)	2.7	0.26
Stated Roche involvement	0.68 (0.61-0.75)	0.88 (0.85-0.91)		
Not stated	0.67 (0.61-0.73)	0.85 (0.81-0.88)	2.1	0.35

Table 3: Planned sub-group analyses.

Figure 1: Flow diagram of study selection

Figure 2: Summary of independent review of quality of included studies. (Green = yes, yellow = uncertain and red = no)
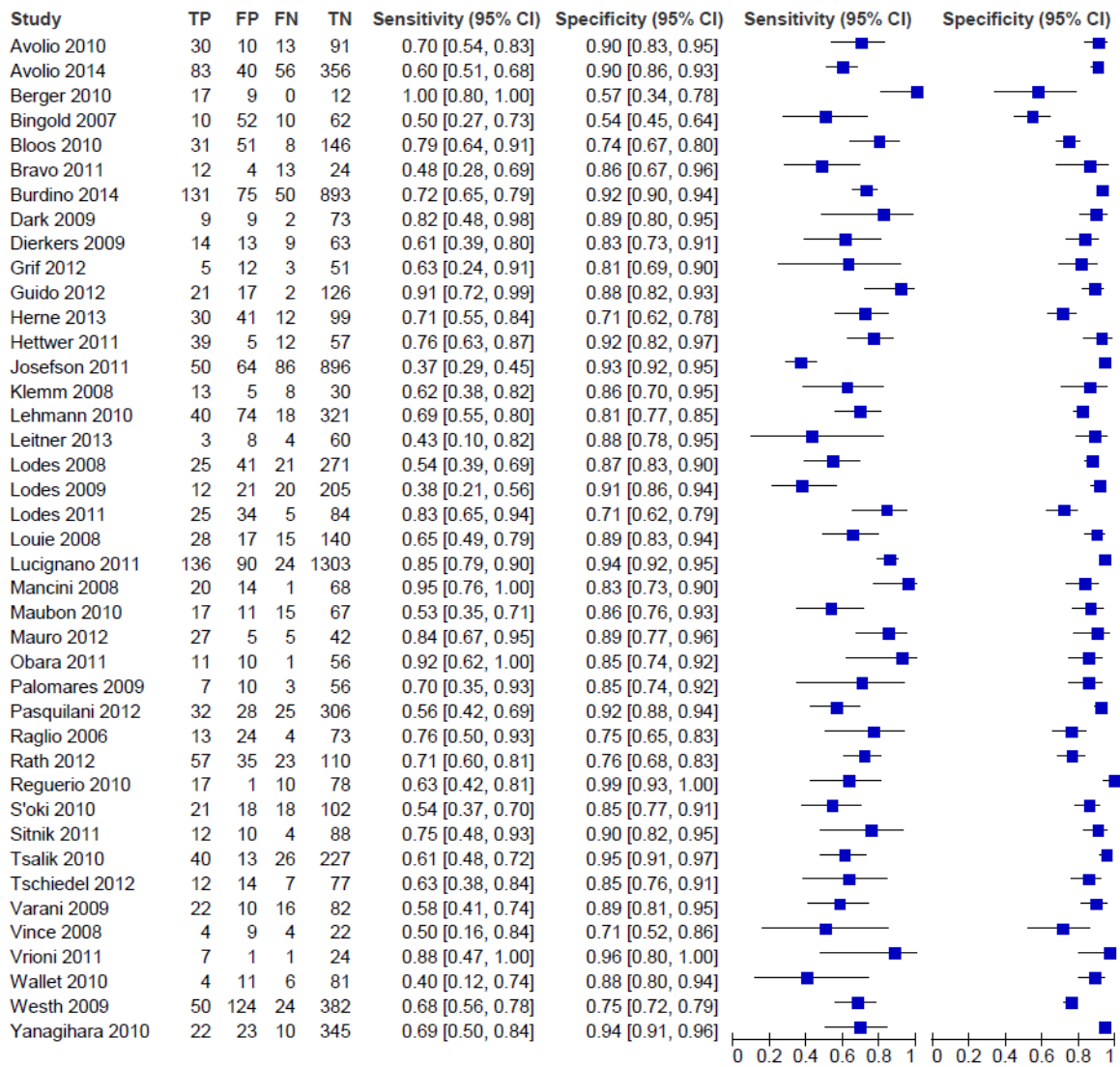
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Avolio 2010	30	10	13	91	0.70 [0.54, 0.83]	0.90 [0.83, 0.95]
Avolio 2014	83	40	56	356	0.60 [0.51, 0.68]	0.90 [0.86, 0.93]
Berger 2010	17	9	0	12	1.00 [0.80, 1.00]	0.57 [0.34, 0.78]
Bingold 2007	10	52	10	62	0.50 [0.27, 0.73]	0.54 [0.45, 0.64]
Bloos 2010	31	51	8	146	0.79 [0.64, 0.91]	0.74 [0.67, 0.80]
Bravo 2011	12	4	13	24	0.48 [0.28, 0.69]	0.86 [0.67, 0.96]
Burdino 2014	131	75	50	893	0.72 [0.65, 0.79]	0.92 [0.90, 0.94]
Dark 2009	9	9	2	73	0.82 [0.48, 0.98]	0.89 [0.80, 0.95]
Dierkers 2009	14	13	9	63	0.61 [0.39, 0.80]	0.83 [0.73, 0.91]
Grif 2012	5	12	3	51	0.63 [0.24, 0.91]	0.81 [0.69, 0.90]
Guido 2012	21	17	2	126	0.91 [0.72, 0.99]	0.88 [0.82, 0.93]
Herne 2013	30	41	12	99	0.71 [0.55, 0.84]	0.71 [0.62, 0.78]
Hettwer 2011	39	5	12	57	0.76 [0.63, 0.87]	0.92 [0.82, 0.97]
Josefson 2011	50	64	86	896	0.37 [0.29, 0.45]	0.93 [0.92, 0.95]
Klemm 2008	13	5	8	30	0.62 [0.38, 0.82]	0.86 [0.70, 0.95]
Lehmann 2010	40	74	18	321	0.69 [0.55, 0.80]	0.81 [0.77, 0.85]
Leitner 2013	3	8	4	60	0.43 [0.10, 0.82]	0.88 [0.78, 0.95]
Lodes 2008	25	41	21	271	0.54 [0.39, 0.69]	0.87 [0.83, 0.90]
Lodes 2009	12	21	20	205	0.38 [0.21, 0.56]	0.91 [0.86, 0.94]
Lodes 2011	25	34	5	84	0.83 [0.65, 0.94]	0.71 [0.62, 0.79]
Louie 2008	28	17	15	140	0.65 [0.49, 0.79]	0.89 [0.83, 0.94]
Lucignano 2011	136	90	24	1303	0.85 [0.79, 0.90]	0.94 [0.92, 0.95]
Mancini 2008	20	14	1	68	0.95 [0.76, 1.00]	0.83 [0.73, 0.90]
Maubon 2010	17	11	15	67	0.53 [0.35, 0.71]	0.86 [0.76, 0.93]
Mauro 2012	27	5	5	42	0.84 [0.67, 0.95]	0.89 [0.77, 0.96]
Obara 2011	11	10	1	56	0.92 [0.62, 1.00]	0.85 [0.74, 0.92]
Palomares 2009	7	10	3	56	0.70 [0.35, 0.93]	0.85 [0.74, 0.92]
Pasquilani 2012	32	28	25	306	0.56 [0.42, 0.69]	0.92 [0.88, 0.94]
Raglio 2006	13	24	4	73	0.76 [0.50, 0.93]	0.75 [0.65, 0.83]
Rath 2012	57	35	23	110	0.71 [0.60, 0.81]	0.76 [0.68, 0.83]
Reguerio 2010	17	1	10	78	0.63 [0.42, 0.81]	0.99 [0.93, 1.00]
S'oki 2010	21	18	18	102	0.54 [0.37, 0.70]	0.85 [0.77, 0.91]
Sitnik 2011	12	10	4	88	0.75 [0.48, 0.93]	0.90 [0.82, 0.95]
Tsalik 2010	40	13	26	227	0.61 [0.48, 0.72]	0.95 [0.91, 0.97]
Tschiedel 2012	12	14	7	77	0.63 [0.38, 0.84]	0.85 [0.76, 0.91]
Varani 2009	22	10	16	82	0.58 [0.41, 0.74]	0.89 [0.81, 0.95]
Vince 2008	4	9	4	22	0.50 [0.16, 0.84]	0.71 [0.52, 0.86]
Vrioni 2011	7	1	1	24	0.88 [0.47, 1.00]	0.96 [0.80, 1.00]
Wallet 2010	4	11	6	81	0.40 [0.12, 0.74]	0.88 [0.80, 0.94]
Westh 2009	50	124	24	382	0.68 [0.56, 0.78]	0.75 [0.72, 0.79]
Yanagihara 2010	22	23	10	345	0.69 [0.50, 0.84]	0.94 [0.91, 0.96]

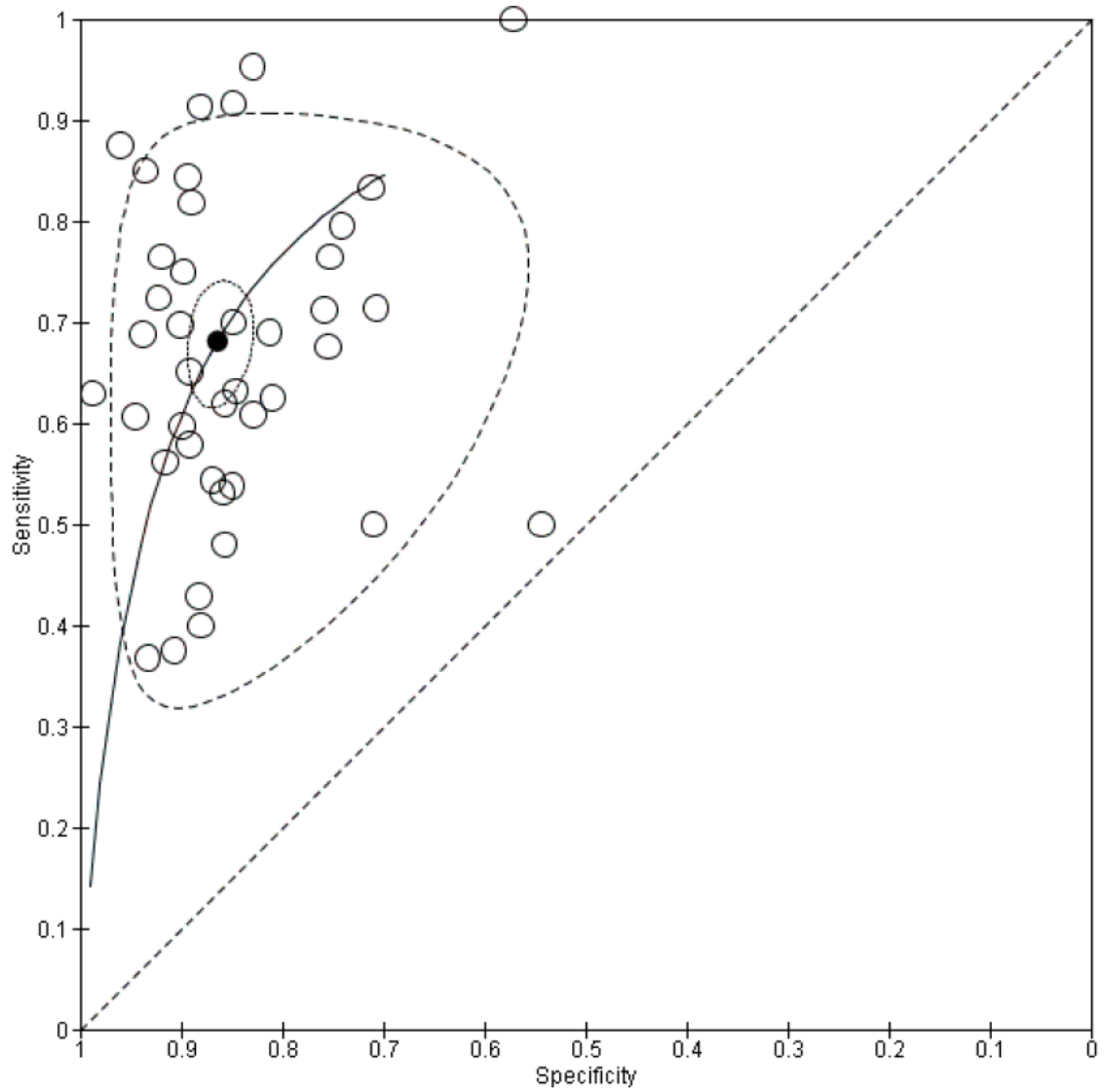**Figure 3:** Forest plot of included studies

Figure 4: Summary Receiver Operator Curve (ROC), with 95% confidence region (dotted) and 95% prediction region (dashed)

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Contributors:
PD and GW initiated the project, BB and CW worked on the initial architecture for the review with specialist molecular diagnostic input from GW, critical care and clinical trial input from PD, DM and GDP, microbiological input from RM and statistical input from SG. CW, DG, KT and PD ran the search strategies and selected the full text manuscripts for review. DM and GDP reviewed the studies with BB and RM. SG designed and conducted the statistical analyses. PD drafted the manuscript and all authors critically reviewed the first draft and contributed to the production of the final manuscript.

Study registration:
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